Advances in Delivery Science and Technology

Michael J. Rathbone
Arlene McDowell *Editors*

Long Acting Animal Health Drug Products

Fundamentals and Applications

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Long Acting Animal Health Drug Products

Fundamentals and Applications

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This book is dedicated to my father George Frederick Rathbone who recently passed away… the man who gave me the wisest of my education

Preface

Long-acting veterinary formulations play a significant role in animal health, production, and reproduction within the animal health industry. Such technologies offer beneficial advantages to the veterinarian, farmer, and pet owner. These advantages have resulted in long-acting formulations growing in popularity in recent years.

The pharmaceutical scientist is faced with many challenges when innovating new products in this demanding field of controlled release. This volume provides the reader with a comprehensive guide on the theories, applications, and challenges associated with the design and development of long-acting veterinary formulations. The authoritative chapters of the book are written by some of the leading experts in the field. It covers a wide scope of areas including the market influences, preformulation, biopharmaceutics, in vitro drug release testing, and specification setting to name a few. It also provides a detailed overview of the major technological advances made in this area. As a result, *Long Acting Animal Health Drug Products* covers everything a formulation scientist in industry or academia or a student needs to know about this unique drug delivery field to advance health, production and reproduction treatment options, and benefits for animals worldwide.

In chapter 1 Sabnis and Rathbone define the current animal health markets for farmed animals and evaluate the opportunities that exist. The chapter provides an outlook for future projections of growth in the livestock industry and the likely resultant demands of the farmed animal health market. In the second chapter Linda Hoorspool conducts a similar analysis (with quite different conclusions) for the companion animal market.

The anatomy and physiology of the farmed and companion animals are provided by Ellis and Sutton, respectively. These chapters describe the large differences between ruminant (cattle and sheep) and monogastric animals (specifically cats and dogs) and highlight the different challenges (and opportunities) faced by formulation scientists in designing and developing long-acting veterinary products for these two physiologically and anatomically different types of animals.

Chapter 5 provides a comprehensive overview of the physicochemical principles of controlled release veterinary pharmaceuticals. In this contribution Fletcher et al. describe the basic physical and chemical properties relevant to drug formulation

which includes the active pharmaceutical ingredient, excipients, and final product. The chapter also highlights the importance of physical and chemical attributes of compounds in the selection of ingredients; development of dosage forms; and their significance with respect to active and final product assessment, characterization, performance, and quality. Sutton describes the basics of biopharmaceutics and its relevance in veterinary drug delivery. This chapter discusses studies that emphasize the similarities and differences in species and routes of administration. From nasal and ocular to transdermal and oral, examples of formulations for veterinary practice are discussed. The main concepts related to analytical testing of veterinary drug products and the development of specifications for critical quality attributes are addressed by Brumfield. This excellent and comprehensive chapter will be of value to anyone working in the industrial setting. Brumfield describes pragmatic strategies for the development and use of analytical specifications throughout the veterinary product development and commercialization life cycle. Also presented are typical analytical testing requirements for quality assessment and registration of selected types of products in major markets (USA, EU, and Japan), and unique challenges related to several veterinary-centric dosage forms including medicated articles for preparation of feeds and drinking waters, and topical parasiticide preparations. The challenges of developing and undertaking in vitro drug release testing of veterinary pharmaceuticals are described in the following chapter by Higgins-Gruber. Long-acting veterinary dosage forms tend to be more complex and varied because of the diversity of species and size of the animals. Therefore, the development of in vitro drug release tests for such products can be challenging and unconventional with respect to the expectations from the regulatory agencies. Higgins-Gruber describes the principles taken into consideration when developing an in vitro drug release test for long-acting veterinary pharmaceuticals that is easy to perform in a quality control environment whilst being discriminating with respect to the impact of critical quality attributes on in vivo behavior.

The remaining contributions of the book describe technological advances in the field of long-acting veterinary products. Chapters devoted to long-acting rumen drug delivery systems (Vandamme), intravaginal veterinary drug delivery (Rathbone), long-acting injections and implants (Cady), intramammary delivery technologies (Alany), veterinary vaccines (Elhay), and delivery systems for wildlife (McDowell) all provide a wealth of information and insight into the current strategies and contemporary research adopted in the development of long-acting veterinary drug delivery systems.

In the final chapter of the book Baird examines the emerging drug discovery technologies in both the human and animal drug delivery fields and discusses the potential for cross over of the learnings, technologies, and outcomes from these areas that result in “spin off” benefits for veterinary medicine. The chapter makes for some interesting reading.

We thank all the authors for their time and effort to put pen to paper to share their experiences and knowledge in this volume. Without their interest and commitment to the area of long-acting animal health products this book would not be the treasury of knowledge that it is.

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Chapter 1
Animal Health Markets and Opportunities: Farmed Animal Landscape

Shobhan Sabnis and Michael J. Rathbone

Abstract This chapter provides an outlook for future projections of growth in the livestock industry and the likely resultant demands of the animal health products market.

It describes the animal health industries landscape that, in recent years, has been dominated by acquisitions and mergers. Mergers have allowed the newly formed companies to expand their portfolios into new areas of prospective growth which include the biotech area. The chapter concludes that such changes have been facilitated by collaborations or licensing agreements with small companies holding patent protected technologies for niche markets. Indeed, the opportunity for partnerships and alliances has become the core of a new business strategy for big pharma.

1.1 Introduction

1.1.1 Livestock Industry

Livestock systems occupy about 30% of the planet's land surface and this sector accounts for 40% of agricultural GDP. The livestock sector is increasingly organized in supply chains that employ about 1.4 billion people globally and livestock products provide one-third of humanity's protein intake [1].

Any views or opinions presented in this document are solely those of the author and do not necessarily represent those of the company

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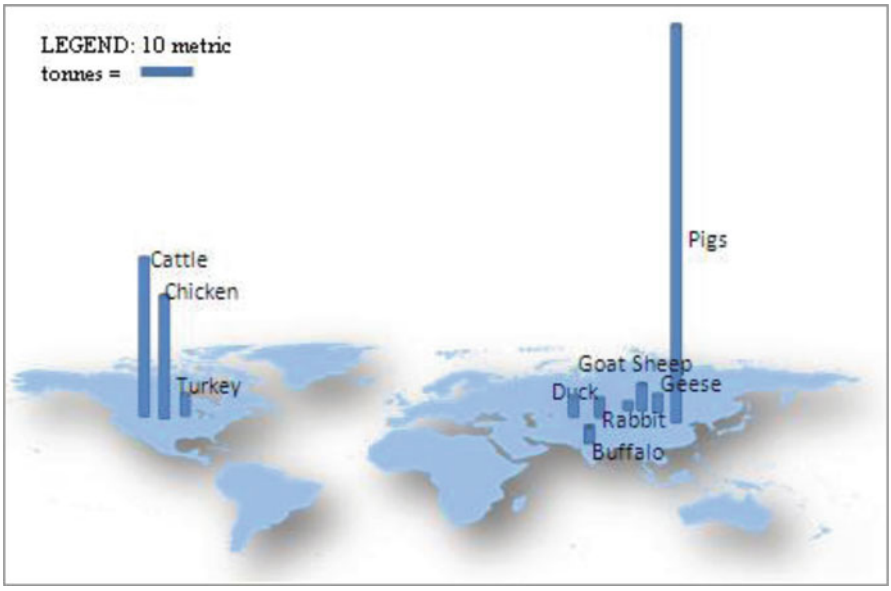

Fig. 1.1 Top producers of meat by species in 2009 (data from FAOSTAT http://faostat.fao.org/site/339/default.aspx)

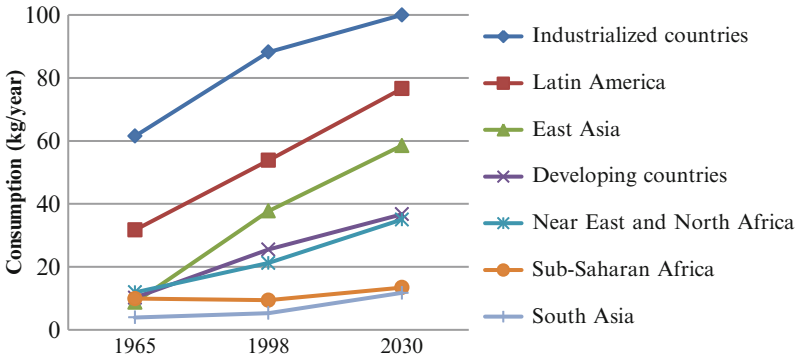

Fig. 1.2 Per capita meat consumption by regions from 1965 to 2030

As countries have become more affluent and the world's population has continued to rise, the demand for meat and other livestock products has grown substantially. Figure 1.1 shows the top meat producing country by species [2].

According to FAO, global meat production is projected to more than double from 229 million tons in 2000 to 465 million tons in 2050, while milk output is set to climb from 580 to 1,043 million tons to meet the demands of the growing population. While the growth in consumption in the developed countries is expected to be steady, the major increase is expected in the countries that have or will experience rapid economic growth. A comparison of per capita meat and dairy consumption by geographical regions is shown in Figs. 1.2 and 1.3, respectively.

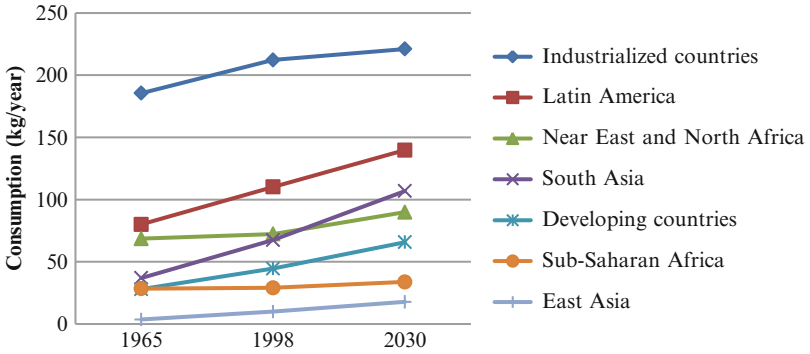

Fig. 1.3 Per capita milk consumption by regions from 1965 to 2030

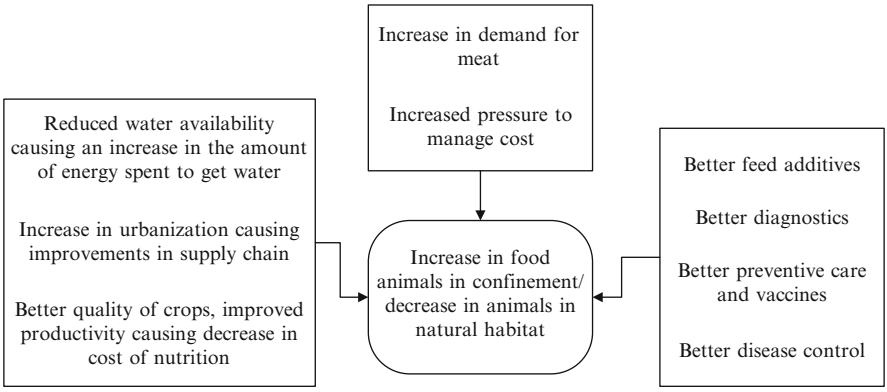

Fig. 1.4 Factors that will trigger the global growth of commercial livestock production in the future

The increase in meat and dairy requirement will be met through direct and indirect pathways. Direct pathways include a higher number of food producing animals per capita, increased weight of meat/carcass, and increased productivity for milk and dairy products. The indirect pathways include a better supply chain for meat products and reduced waste pre and post end user purchase. This increase in demand is also likely to bring about significant changes to the meat production business models. More industrialized and intensive production systems are likely to be the future of livestock industry in many of the countries. Figure 1.4 shows the factors that would be responsible for the shift in global meat production business models [1, 3].

1.1.2 Animal Health Products Market

The overall increase in meat and dairy consumption will continue to be mirrored by a growth in animal health products market. The world animal health market

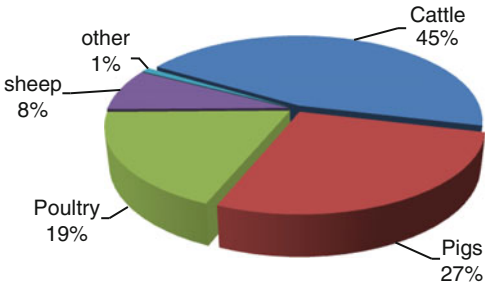

Fig. 1.5 Livestock market by species—2008

was about 17.5 billion US dollars in 2005. It has grown to about 19.2 billion in the year 2010 [4] and if this trend continues, then the world market will surpass 30 billion by 2020.

The animal health market is composed of approximately 60% livestock and 40% companion animal by sales. The livestock market can be further divided by species. In 2008, the share by major species is shown in Fig. 1.5.

The veterinary market for livestock can also be classified in three product groups. They are vaccines or biologicals (25%), pharmaceuticals or small molecules (63%), and feed additives (12%) with market share in parentheses [4–6]. Livestock medicine is becoming increasingly herd based and preventive in approach—a consequence of a shift to mass production business models in the developed and developing countries [7]. This trend is likely to increase the share of biologicals in the future as compared to small molecule pharmaceuticals.

1.2 Animal Health Industries Landscape

The last few years have seen significant consolidation within the animal health industry with acquisitions and mergers. Many of the acquisitions allowed the acquirers to expand into new areas of prospective growth, e.g., aquaculture, genomics, diagnostics, specialty devices, etc. The largest 20 companies based on revenues in 2010 are shown in Fig. 1.6 (data collected from yearly revenue statements where available). Figure 1.6 shows the percent change from previous year on the Y2 axis.

Most of the animal health companies posted positive revenue change from 2009, which was a difficult year for the industry where global economy contracted sharply. Many of these companies have continued to invest heavily in R&D. The Animal Health Institute (AHI, 1325GSt NW # 700 Washington, DC) reports that animal health R&D investment has been 0.6–0.7 billion USD/year for last 5 years, which is only one-fortieth the human health R&D budget on average. There are only a few modified release animal health dosages forms in the market when a comparison is made with human health pharmaceuticals. Fewer than 25 NADA submissions are

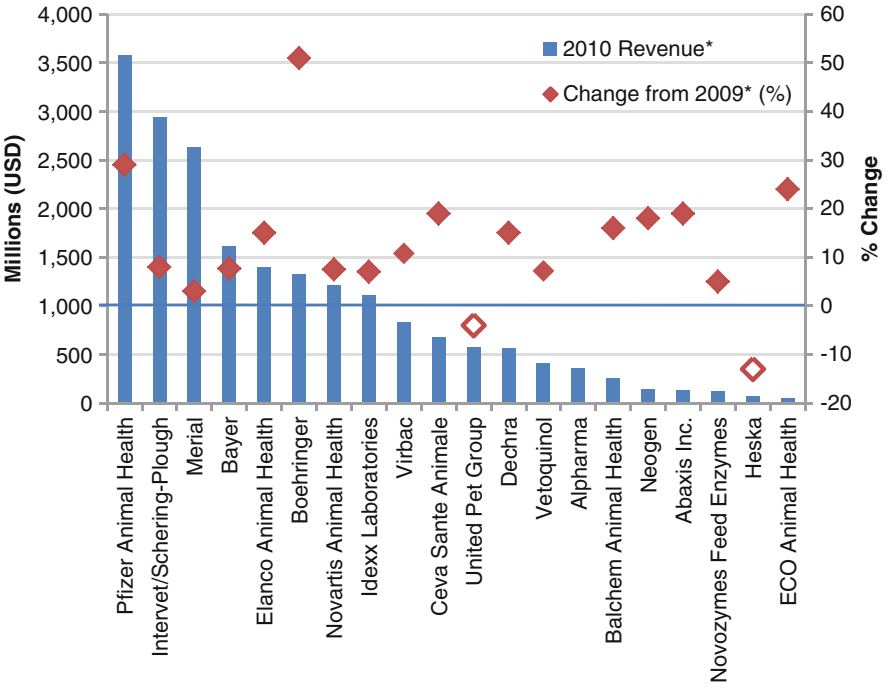

Fig. 1.6 Comparison of yearly revenue of top 20 animal health companies

classified as modified or sustained release. There were 94 NADA submissions (not counting supplementals) made in past 5 years. Out of these, 51 submissions were for farmed animals. However, very few of these were modified release dosage forms. Modified release animal health products registered from 2007 to 2011 for farmed animals are given in Table 1.1 (prepared from data available on USFDA and EPA databases).

Financial factors, which include limited budgets assigned to conduct veterinary R&D, the cost competitive amount that can be charged for the finished product, and the expensive time-consuming product registration process may be some of the factors for this trend [8]. However, there may be a shift in this trend in the coming decades.

1.3 Research Collaborations

According to OECD, comparisons of key indicators across countries suggest a positive relationship between measures of research collaboration and scientific impact. Worldwide, the 50 universities with the highest impact, measured by normalized citations to academic publications across all disciplines, are concentrated in a handful of countries. Overall, 40 of the top 50 universities are located in the United States, and the rest in Europe. A more diverse picture emerges on a subject-by-subject basis.

Table 1.1 Modified release animal health products registered from 2007 to 2011 for farmed animals

Regulated by CVM Trade name	Approval number	Approval date	Active ingredients	Company	Species	Dosage form	Route of administration	Indication
Revalor® XS	NADA 141-269	2007	Trenbolone acetate and estradiol	Intervet/Merck Animal Health	Cattle (steers)	Solid dose implant	SC	Weight gain
Longrange™	NADA 141-327	2011	Eprinomectin	Merial	Cattle (pasture)	Injectable solution	SC	BRD
Excede® Sterile Suspension[a]	NADA 141-209	2008	Ceftiofur crystalline free acid	UpJohn/Pfizer	Cattle and horses	Sterile oil suspension for injection	SC	BRD
EAZI-BREED CIDR Sheep Insert	NADA 141-302	2009	Progesterone	UpJohn/Pfizer	Sheep (ewes)	Solid matrix insert	Intravaginal	Induction of estrus in ewes
DYNAMAX Controlled Release Capsules (Australia)	APVMA:64099/50025	2010	Abamectin, albendazole, cobalt, selenium	Merial	Sheep (adult)	Solid dose tablets in a gastrointestinal retentive device	Oral	anthelmintic
Regulated by EPA								
XP 820	EPA Reg no.39039-17	2009	Abamectin and piperonyl butoxide	Y-Tex Corp	Cattle	Solid matrix	Ear tag	Control of horn flies and ticks
ELIMINATOR® EAR TAGS (for Canada)	NAC No.: 12341562	2006/07	Cypermethrin and diazinon	Vétoquinol Canada Inc.	Cattle	Solid matrix	Ear tag	Control of horn flies and face flies

[a]EXCEDE (Ceftiofur Crystalline Free Acid): A New Sustained-Release Injectable Antibiotic for Horses, Pfizer Technical Bulletin, 2010

There is evidence that some universities in Asia are emerging as leading research institutions. Many of the leading firms in knowledge-intensive industries have emerged in a limited number of regions in the world. The production of scientific knowledge is shifting from individuals to groups, from single to multiple institutions, and from a national to an international scope.

1.4 Patent Landscape

If intellectual property applications are seen as a marker for R&D activity, then the top five animal health companies have made approximately 120 patent applications with the USPTO in last 5 years. This represents only 2–3% of the total worldwide patent applications related to animal health or veterinary topics in those years. Most of the patent applications have been made by smaller entities. Figure 1.7 shows the trend in animal health related patent applications over the last 5 years. Although a major portion of the animal health patent applications had a farmed animal focus, the modified/sustained release category constituted only about 2–4% of the total animal health applications.

The presence of young firms among patent applicants underlines the inventive dynamics of firms early in their development and their desire to develop new activities and products—crucial to their survival and relative growth. During the period 2007–2009, firms less than 5 years old filing at least one patent application

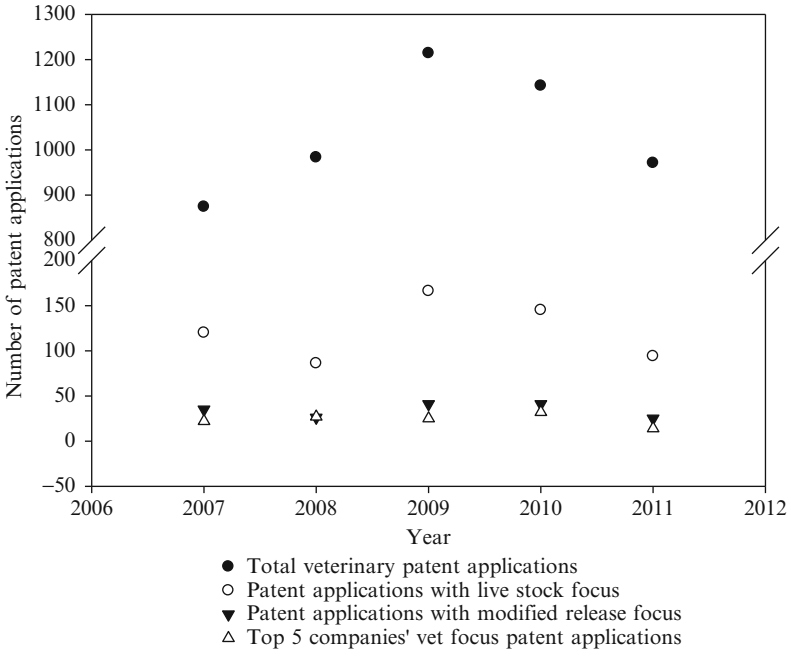

Fig. 1.7 Trend in animal health related patent applications over the last 5 years

represented on average 25% of all patenting firms, and generated 10% of patent applications [9].

1.5 Controlled Release Initiatives for the Future

In today's environment, it is not enough for firms to focus only on short-term technology development based on existing core competencies; they must be able to place long-term technology bets, based on current trends, competition, and stakeholder value proposition [10]. The blockbuster strategy, which involved one drug for large markets, has been gradually observed to decline, leading to narrow product portfolios, a small number of genuinely innovative compounds, and proliferation of life cycle management opportunities. These opportunities will involve significant partnerships and alliances as well as other collaboration networks, including participation of different industry service providers [11]. Resurgence of generic companies

Table 1.2 Niche controlled release technology firms

Institute	Technology	Intellectual property
Iowa State University Research Foundation Inc.	Single dose controlled release vaccine formulations using polyanhydride microspheres	[12]
OctoPlus N.V., Zernikedreef 12 2333 CL Leiden, The Netherlands	OctoDex™ drug and vaccine delivery systems for controlled release of therapeutic proteins and particulate systems and containing dextran hydroxyethyl methacrylate as the polymeric building block	OctoDex company factsheet
Langer Lab, 77 Massachusetts Ave, Cambridge, MA	Development of controlled release systems that can be magnetically, ultrasonically, or enzymatically triggered to increase release rates	[13–15]
MedinCell S.A., Cap Alpha, Avenue de l'Europe 34830 Clapiers France	MedinGel™ in vivo hydrogel forming system constituted of a monodispersed tridimensional of hydrophilic network (PEG) linked with hydrophobic microdomains (PLA) for delivering small molecules, peptides, and proteins	Medincell™ Introductory brochure http://www.medincell.com/medingel/technology
Cytogel Pharma, LLC, 3 Thorndal Circle. Darien, CT	Injectable microspheres are obtained from double bond-functionalized polyhydric alcohol ester or from block copolymer of polyglycerol caprolactone maleate and methoxy poly(ethylene glycol)	[16, 17]

(continued)

Table 1.2 (continued)

Institute	Technology	Intellectual property
Starpharma, Baker IDI Building, 75 Commercial Road, Melbourne, VICTORIA 3004, AUSTRALIA	Macromolecular vehicles such as polylysine dendrimers for enhanced delivery of small molecule and biological drugs to control solubility, half-life, toxicity, and targeting	[18], Starpharma drug delivery summary http://www.starpharma.com/assets/downloads/Starpharma-Drug-Delivery-Summaryv3.0.pdf
DURECT Corporation, 2 Results Way, Cupertino, CA 95014	SABER™ injectable controlled release system uses a high-viscosity base component, such as sucrose acetate isobutyrate and diffusible excipients. DURIN™ technology involves polymers and copolymers prepared from glycolide, DL-lactide, L-lactide, and □-caprolactone. These thermoplastic materials are stable when dry but degrade by simple hydrolysis of the polymer backbone aqueous environment	[19], Durect's SABER fact-sheet http://www.durect.com/pdf/SABER_Brochure_July2010.pdf; [20], Durect's DURIN fact-sheet http://www.durect.com/pdf/DURIN_HRES.pdf
Catalent Pharma Solutions, 14 Schoolhouse Road, Somerset, NJ 08873	OSDrC® technology enables the design of single or multicore tablets, with a variety of core numbers, shapes, sizes, and placement within the tablet for better quality and release control	[21], OSDrC®OPTIDOSE™ Drug Delivery Technology brochure http://www.catalent.com/index.php/offerings/OSDrC-R-OPTIDOSE-drug-delivery-technology/OSDrC-R-OPTIDOSE-Drug-Delivery-Technology
Flamel Technologies, 33, avenue du Dr. Georges Levy—Parc Club du Moulin à Vent, 69693 Vénissieux Cedex—France	Medusa® platform (pGluVE, or PGA A1) is based on a hydrophilic biodegradable polyglutamate chain grafted with hydrophobic Vitamin E. The polymer self assembles in aqueous medium to form a stable solution of nano-sized hydrogels comprising multiple polymer chains and 95% water	[22], Medusa® drug delivery platform description http://www.flamel.com/wp-content/uploads/2011/11/Flamel-Technologies-Medusa-Drug-Delivery-Platform.pdf
PolyPid Ltd., 13 Hamazmera Street, Ness-Ziona 74047, Israel	BonyPid™ is a biodegradable bone void filler that is micro-coated with a PolyPid biodegradable formulation. Its advantage is its ability to treat an infection effectively by controlling the release of the active drug, for 3–4 weeks to successfully eradicate the bacteria that may cause infections	[23]

(continued)

Table 1.2 (continued)

Institute	Technology	Intellectual property
Nanomi B.V., Zutphenstraat 51, 7575 EJ Oldenzaal, The Netherlands	Microsieve™ emulsification technology is applied for the production of precisely defined functional emulsions, and micro- and nanospheres with controlled release, diagnostics, molecular imaging applications. The heart of the microsieve™ technology is a silicon membrane that is fabricated by photolithographic techniques to achieve uniformity of pore size (CV ~ 5%) and shape in a highly reproducible way.	[24, 25]
Trilogic Pharma 331 Perimeter Parkway Ct, Montgomery, AL 36116	TRI-726 system consists of a combination of a triblock copolymer and a natural polysaccharide and is designed to take advantage of body temperature to undergo sol-to-gel transition. It can be a liquid (viscous or dilute), a semisolid (gel/paste), a spray, or a foam to suit the need. TRI-726 has the ability to erode over a period of several hours to several days. This feature allows for slow release of the incorporated drug over this period	[26]

and profit erosion of the small molecule product, advances in the biopharmaceuticals research, and increased trend toward prevention rather than treatment will further give opportunities to niche controlled release technology companies to collaborate and expand the animal health product portfolios. Some such niche controlled release technology firms are listed in Table 1.2.

1.6 Niche Company Considerations

Today, more than ever, the current status of the animal health industry is driving niche companies to develop innovative technologies with the goal for selling them to big pharma. However, such companies should realize that the scope for livestock animal product success is limited and that the competition is fierce. Niche companies develop technology platforms based on perceived technology needs or clinical needs. In-house expertise and/or market savvy determine which track a niche company will pursue.

There is more than one way to crack an egg, and, in a competitive market, the best and most superior technology can be overlooked for one that fulfills the desired

criteria. Those being: cost effectiveness, demonstrable rapid speed to market, robustness, functionality, reliability, and present a point of differentiation. The niche company should bear these criteria in mind, include them in their technology product brief, and build them into their product at every opportunity during the research phase. Big pharma will not invest their time and effort in a niche company's technology just because it is innovative and elegant as these latter features do not enhance sales in a market that is dominated by large numbers of products which promise small revenues.

In recent years, few new drug entities have appeared on the animal health market, numerous existing big blockbuster drugs have come off patent, and there are limited clinical conditions that livestock are treated for (since they are culled well before old age and its afflictions affect the animal). Because livestock have a market defined and fluctuating economic value, technologies must be cheap to manufacture as well as have demonstrable safety, efficacy, and which can be manufactured to a high quality.

In the animal health arena, the opportunities for a niche company to sell or license a technology to big pharma are considerably smaller compared to their human counterparts. To be successful, niche companies need to spend time and effort identifying the next growth segments of the market, identifying the right product candidates to demonstrate feasibility of the technology, and set the goal of being first in line to present their solution to big pharma.

A final consideration is the financial conditions of the deal. All the economic factors discussed in this chapter mean that niche companies need to be realistic when negotiating deals with big pharma. Expectations must be in line with eventual sales revenue and related to the magnitude of risk to turn the technology into a product at the time of negotiation.

Niche companies need to be cognizant of the amount of time and financial investment that is required to assure their technologies are sufficiently along the track to attract big pharma's interest. A great idea is not enough. Indeed, in vivo demonstration of technology feasibility may also not be sufficient to attract attention. The right time to pitch the technology to big pharma is always difficult to determine and should be made on a case-by-case basis. In general, the further along the registration path the technology is, the lower the financial and success/failure risk there is, and the more likely big pharma will be interested in viewing a niche company's offerings. The bottom line is that niche companies should not underestimate just how far they need to take their good idea along the track towards registration before interest by big pharma is forthcoming. This may mean larger and bigger-than-expected investments by the niche company in time, effort, and money to get the technology to that stage. However, the further along the registration track, the greater the financial returns can be negotiated.

A niche company will need to use compounds in their technologies to demonstrate its potential as a delivery platform. Often, when the technology portfolio is presented to big pharma, this results in the delivery system being perceived as being for the treatment of the condition for which the drug treats. In addition, as pointed out earlier, normally a technology will need to be close to registration to attract big pharma's

interest in it (as a marketable product) as this minimizes risk to big pharma. The end result of this need is that the technology loses its identity (as a technology platform) and big pharma cannot see beyond the offering as a product for the delivery of the particular drug that was used to demonstrate the technologies feasibility or flexibility.

Big pharma interested in a technology offering will be willing to negotiate collaborative development deals; licensing of the technology either for clinical conditions, drug types, or market regions; or outright purchase of the technology. Big pharma will be looking for some form of protection of the technology. Therefore, patenting the technology or having some in-house know-how is an important edge. Deals involving cost of manufacture and supply of pivotal batches can be negotiated, as well as upfront, milestone, and final payments, with their magnitude dependent upon the nearness to market registration and financial and success/failure risk considerations.

1.7 Future Directions and Outlook

The biopharmaceuticals area is maturing into mainstream pharmaceutical area. Most of the major animal health companies have implemented biopharma R&D programs. In fact, four of the ten largest human health drugs were monoclonal antibodies and together grossed over 20 billion in 2010. One animal health product has received approval for treatment of canine leukemia (containing CL/mAb 231) [27]. Many of the animal health companies have established active programs and collaborations for developing mAbs and expect to bring product offerings to market within the next few years. Application of modified release technologies for delivering mAbs would be a valuable proposition because of the propensity for highly specific or targeted therapy for a narrow subpopulation. However, mAbs are already being used in many of the diagnostic kits in the animal health industry and this trend is expected to grow in the next decades. In fact, Osborne [28] projects that 80% of all new pharmaceutical products by 2030 would be based on biotechnology. Although such advances may be projected based on trends, this area is still in development in the animal health arena.

Pathways for regulatory approvals need to be clearly defined for new technologies, whether USDA or CVM will act as the regulatory body for such products, or whether a combination of both agencies would be required for the products marketed in the US.

1.7.1 Potential for Niche Companies

The second major challenge for biotech products to enter animal health mainstream is the economics of developing such differentiated products for smaller, targeted subpopulation [29]. There needs to be a value added benefit to make the investment worthwhile. And this makes a case for small niche companies that would provide

specialized, patent protected technologies to big pharmas as collaborations or licensing deals.

1.8 Concluding Remarks

The animal health market is complex and challenged by limited budgets assigned to conduct veterinary R&D, the cost competitive amount that can be charged for the finished product, and the expensive time-consuming product registration process. The last few years have seen significant consolidation within the animal health industry with acquisitions and mergers. Future growth of the livestock industry to meet human demand for food supply presents the opportunity for pharmaceutical intervention to assure high production rates through improved animal health, production, and reproduction. Over the past 50 years, existing small molecules have resulted in relatively few controlled release products appearing on the animal health market (compared to the controlled release products that service the human market). Today the biopharmaceuticals area is maturing into mainstream pharmaceutical area. Major big pharma are hesitant to conduct in-house R&D product development due to the high cost and risks associated with developing such differentiated products for smaller, targeted markets. This offers the opportunity for small companies to develop niche products and provide such specialized, patent protected technologies to big pharmas as collaborations or licensing deals.

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Chapter 2
Animal Health Markets and Opportunities: Companion Animal Landscape

Linda J.I. Horspool

Abstract In the future, the global market for companion animal health products is expected to further grow and become more specialized. The major drivers will be the continued strengthening of the bond between owners and their animal companions, increasing companion animal owner awareness, and increasing companion animal owner demands and expectations for companion animal care. This chapter discusses the background knowledge that is required to enable a formulator to differentiate their drug products through novel delivery technologies which address the future companion animal global market needs with the goal of increasing companion animal owner compliance and thus enhancing efficacy.

2.1 Introduction

Companion animals (dogs, cats, and horses) have come to play an important part in the lives of many people. They provide companionship and a sense of responsibility, demand care and attention, and respond with affection. Although somewhat controversial, a number of studies have shown that owning a companion animal is associated with positive health benefits [1–3], such as lower blood pressure [2], reduced anxiety [4], reduced cardiac arrhythmias [2], greater psychological stability [5, 6], and improved well-being [7]. In addition, animal-assisted therapy has a number of recognized benefits and is becoming mainstream in a number of areas of human health care [8]. It is therefore no surprise that the bond between companion animals

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Table 2.1 Key areas affecting the market for companion animal products

Consumers (companion animal owners)	*Veterinarians*
Economy	Human health advances
Insurance	Regulations
Mobility	Inventory
Travel	Evidence-based medicine
Human–animal bond	Individualization of treatment
Increased diversity of species kept	Emerging diseases
Increasing demands/anthropomorphism	Dispensing/prescription only medicines
Internet	Pharmacies
Pet shops	Technology
Over-the-counter products	Consolidation
Quality of life	Corporate practices
Spend	Practice management
Alternative therapies (herbal, nutraceutical)	Improved medicalization
Disease	*Animal health market*
Collaboration between medical and veterinary sciences/"One health"	Consolidation
	Emerging markets
Altered parasite habitats/pathogen distribution	Increasing costs
Diagnostics	Regulation and harmonization
Resistance	Pharmacovigilance
Prudent use guidelines	Intellectual property
Restrictions on use	Generics
Therapeutic vaccines	Distribution
Chronic disease	Pet food
	Nutraceuticals

and people is continuing to strengthen and with it the market for products, including pharmaceuticals and vaccines, which contribute significantly to the health and well-being of these animals.

Any look at potential opportunities in the companion animal market, particularly with respect to where formulation may play a role, has to be guided by developments that have shaped this segment over the last few decades. Some, but not all, of the advances have followed, at least in terms of active ingredient or in some instances delivery technology, other market sectors, particularly the much larger market for human health products [9]. Finally, the regulatory framework for veterinary products guides and defines product requirements and approvals around the world. Advances have also been driven by companion animal owner (consumer) and veterinary demand for products that make administration simple, with the aim of maximizing owner compliance; to try to ensure fulfillment of the veterinary professional's prescribed course of treatment. The numerous approvals of products for companion animals in the last decade reflect the fact that this market segment continues to be attractive [10].

The landscape of the animal health market for companion animals consists of a number of key areas that are not mutually exclusive (Table 2.1).

2.2 The Market and Its Players

The market for companion animal health products has grown by around 2.5% per annum in nominal terms since 1992 [9] and has been the main driver of growth in the animal health market globally. The market declined in 2009 due to the negative impact of recessionary conditions but can be expected to stabilize again as and when global economic conditions do so [11]. More than three-quarters of the companion animal market is concentrated in the USA and Europe, where (in alphabetical order) five of the top ten world markets are located (France, Germany, Italy, Spain, and the United Kingdom) [9].

The global animal health market is consolidated with the top ten players controlling by far the majority share of the market. For many years the largest players in the companion animal market have been Merial and Pfizer. The market has seen considerable acquisitions and mergers. Proposed mergers and acquisitions are monitored very carefully by governments around the world (such as the Directorate-General for Economic and Financial Affairs in the European Union and the United States Federal Trade Commission) to protect the consumer from reduced competition, price increases, and reduced innovation. A number of the mergers in animal health have been undertaken to meet human health business objectives—by companies that are players in both sectors, such as Pfizer, Merck, and Sanofi Aventis [12]. Merial, formed in 1997 as a joint venture between the animal health divisions of two human health businesses (Sanofi Aventis's Rhone Merieux and Merck's MSD AgVet), became a wholly owned subsidiary of Sanofi Aventis in 2009. Pfizer purchased Pharmacia Upjohn in 2003 and Wyeth in 2009, driven by its human health strategy. Intervet was purchased by Schering-Plough in 2007 and the resultant Intervet/Schering-Plough Animal Health became Merck (MSD, outside the USA and Canada) Animal Health in 2011, following the 2009 purchase of Schering-Plough by Merck. Elanco (briefly known as Lilly for companion animals) entered the companion animal segment strongly in the USA in 2007 and increased their presence in this market outside the USA through their purchase of Janssen in 2011. Consolidation of the players in this segment is likely to continue [13].

2.3 Dispensing and Distribution

The dispensing of medicines has been under discussion for many years. In some countries, such as Italy, veterinarians have a restricted ability to dispense and pharmacies dispense the majority of veterinary prescriptions as well as handling over-the-counter products. The role of the pharmacist has increased in many countries, although training on veterinary species may not have increased in a commensurate fashion [14]. Pharmacists are trained specifically on a number of subjects including

the process of standardizing the dispensing of medicines with, for example, particular focus on length of treatment course [15] where veterinarians focus on the animals under their care and must have a veterinarian–client–patient relationship. With increasing debate and regulation, dispensing may move further into the realm of the pharmacist, which, in many countries, will lead to a potential loss of income for practicing veterinarians.

The internet forms part of the daily lives of many. It has altered business—including the dispensing of medicines and shopping for healthcare products—and communication. Internet pharmacies supplying veterinary medicines to the consumer have been present in a number of countries [16–18] for at least a decade and challenge the veterinarian–client–patient relationship. The internet is here to stay and will continue to confront traditional distribution channels.

2.4 Companion Animal Ownership

Companion animal ownership is continuing to increase. Sixty-two percent of US households now own a companion animal (excluding horses), equating to 72.9 million homes [19]. Many other countries are witnessing higher than ever rates of companion animal ownership, including emerging markets such as Brazil, China, India, Mexico, and Russia. Spending on companion animals is also continuing to increase. In the USA, spending has increased from a total of US$23 billion in 1988 to an estimated US$51 billion in 2011 [19]. Around 25% of this spending was on veterinary care (including medicines) and a further 20% on over-the-counter products and supplies [12], with the remainder on food, accessories, etc. This trend is being mirrored in other countries.

The population of traditional companion animals, especially dogs and cats, has at best increased only marginally in the last decade but the willingness of companion animal owners to spend more on their animals' health and the ability of veterinarians to meet that need have continued to be key drivers of this market. There are differences between, and even within, countries in the attitudes of companion animal owners towards veterinary visits, with some visiting their veterinarian for disease prevention while others only do so for treatment [20, 21]. In many markets, much of the potential for growth in companion animal veterinary care appears to be related to an increase in spend per animal rather than to an increase in patient numbers [21, 22]. However, in times of economic hardship, there may well be limitations in the willingness of owners to spend on veterinary care as opposed to general companion animal care [11]. Thus, many companion animal practices have developed more creative alternatives to allow companion animal owners to pay for treatment [23].

There is a whole range of other animals that people keep as companion animals, such as rabbits, ferrets, guinea pigs, other small mammals, pet birds, reptiles, and ornamental fish. There is a thriving industry around the care and management of these companion animals. The number of so-called exotic companion animals

presented to veterinarians has increased [24]. However, there is far less data on their numbers and few, if any, veterinary products indicated specifically for use in them. For example, around a decade ago, it was estimated that there could be as many as 5 million pet rabbits owned by 2.2 million households in the United States [25] yet there are still few veterinary products indicated for use in this species.

2.5 "One Health"

Collaborative ventures unifying medical and veterinary sciences in areas such as clinical care, disease surveillance and control, education, and research fall under the banner of "One Health" [26]. Climate change, changes in the ecology of parasite habitats, increasing host- and vector-interactions, increased travel by companion animal owners, and importation of animals from endemic areas may be responsible for an increase in the geographical distribution of parasites and an increased risk of vector-borne infection outside traditional endemic areas. This includes not only alterations in the distribution of ticks, such as *Rhipicephalus sanguineus* [27], *Dermacentor reticulatus* (in Europe), and *Amblyomma maculatum* (in the United States) [28] and an increased risk of tick-borne diseases, such as Babesiosis [29], but also changes in the distribution of other vectors and vector-borne diseases, such as Leishmaniasis [30, 31] and heartworm (*Dirofilaria* sp.) [32]. Warm summers suitable for *Dirofilaria* transmission—particularly *D. repens*—in Europe may become the norm [32].

Other parasites, particularly the metastrongyloid lungworms [33, 34] and *Trichuris vulpis* [35] also appear to be spreading and/or may be more prevalent than previously suspected. There are also parasites that pose a significant public health risk, such as *Toxocara* [36] and *Echinococcus multilocularis* [37]. The need for awareness, monitoring, and a good understanding of epidemiology and pathogenesis of companion animals to ensure appropriate year-round control measures has been highlighted by two independent, nonprofit organizations (the Companion Animal Parasite Council (http://www.capcvet.org) and the European Scientific Counsel Companion Animal Parasites (http://www.esccap.org)).

2.6 Resistance

One potential cause of loss of efficacy is resistance. This is inherent to many organisms, such as bacteria and fungi as well as to parasites, and is merely selected for by use, particularly if that use is at concentrations, or in a manner that leads to concentrations, below those that are required to kill sufficient numbers of the target organism. Selection of strains that are tolerant, or able to endure unusually large doses of a poison or toxin, is the first step en route to resistance, heritable genetic adaptation

in the population that results in decreased susceptibility to chemotherapeutic agents, such as antibiotics and pesticides.

There is also concern about the transfer of genetic material coding for multidrug resistance between different species of bacteria [38–41]. For example, in the last 5–7 years, methicillin-resistant *S. pseudintermedius* (MRSP)—an important opportunistic pathogen of companion animals, especially dogs—has emerged, mainly due to clonal spread, as a significant problem [42]. Although reports of colonization and infections of humans with MRSP are relatively rare, the multidrug resistant characteristics of these bacteria mean that they pose a significant risk to animal health but potentially also to human health [43]. There are also methicillin-resistant *Staphylococcus aureus* (MRSA) strains infecting companion animals that are related to clones of MRSA from humans [43]. Many veterinary associations have developed prudent use guidelines promoting appropriate and selective use of antibiotics in companion animals [42]. In future, there may be restrictions on the use of some antibiotics in veterinary medicine and this may extend to companion animals too.

There is evidence that there has been selection for a single-nucleotide polymorphism in the gene encoding a P-glycoprotein transporter in *Dirofilaria immitis*, leading to a homozygous guanosine ("GG-GG") genotype, which is, when present at a high frequency, phenotypically insensitive to high doses of macrocyclic lactone or, in other words, macrocyclic lactone drug resistant [44]. A microfilarial suppression test can be used to identify the presence of resistant parasites and, perhaps more importantly, map the geographic distribution of this issue [44]. If widespread resistance is indeed an issue, alternative strategies and treatment options will be required in the near future to prevent unnecessary suffering of dogs. There may be a simple, cost-effective, and safe tool to manage infected cases. The endosymbiont *Wolbachia* alters the inflammatory and immune responses to *D. immitis* infection and appears to offer a target for conventional antibiotic treatment [45].

Failures in the control of fleas on dogs and cats are common [46] and are frequently due to inappropriate control measures [46, 47]. There is considerable variation in the susceptibility of flea strains to insecticides [47] and differences between colony flea strains (adapted to being kept in the laboratory) and field strains [46]. There are reports of flea strains that are, or appear to be, tolerant to some of the insecticides that have been used for a number of years for flea control [48]. Variation in susceptibility may result in differences in the ability to control flea infestations [47]. Some of these differences may reflect true resistance in field strains [48–51], although this has not yet been documented definitively. Factors that may play a role in the failure to control flea infestations [46, 47] include failure to reapply treatment at appropriate intervals, as well as the complex biology of the flea, including the relationship between it, its host, and the environment [46, 47]. Treatment failures have been documented, and if widespread resistance becomes a reality, then this will pose a significant challenge in the management of flea infestations. Appropriate strategies, such as product rotation, which has already been implemented for equine anthelmintics, and combining active ingredients, as is used currently for extending the spectrum of ectoparasite activity [52–54], may need to be developed and new agents will be of increasing importance.

2.7 Market Segments

Companion animals are living longer [55, 56], and their care often mirrors the trends in human health care. Diagnosis and disease monitoring in veterinary medicine, particularly for companion animals, has also followed trends in human medicine. Diagnostic imaging techniques, such as ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI), have become commonplace and many veterinary practices have in-house analyzers for clinical chemistry and hematology, as well as rapid patient-side tests, e.g., for infectious agents.

There are parallels between certain conditions, such as overweight and obesity and their consequences, between companion animals and their owners [57, 58]. It is therefore no surprise that the companion animal segment is considered to be the animal health segment that is most like the human pharmaceutical sector, with many of the innovations in human medicine, at least in terms of new chemical entities, subsequently adapted and tailored to suit companion animals.

Dogs and cats have been used for many years both in the study of the pathophysiology of human disease and as laboratory animals during the development of products for human health. In fact, there may be some areas where companion animals provide a better model of disease for human medicine than rodents, particularly where a naturally occurring disease in companion animals, such as neoplasia [59–61], is similar to that in humans. Similarly, cats are a good model for human type 2 diabetes [62] but have different lipid metabolism and do not develop the typical metabolic syndrome [63]. In contrast, there are areas where rodents and humans are probably not good models of companion animal disease, such as congestive heart failure and diabetes [64] in dogs.

Infection (viral, bacterial, and parasitic) is still one of the greatest challenges facing companion animals. Chronic, age-related conditions, such as congestive heart failure in dogs, chronic kidney disease in cats, endocrine diseases, such as hyperadrenocorticism in dogs and to a lesser extent in horses, hyperthyroidism in cats and diabetes mellitus in dogs and cats, and osteoarthritis in companion animals, are being diagnosed with increasing frequency. Still veterinary medicine lacks large-scale epidemiological studies that define prognostic endpoints, allowing evidence-based therapeutic decisions. Although there have been research efforts in this area, for example, in chronic kidney disease in cats [65], the drive for properly designed and controlled evidence-based studies is likely to continue and this will ultimately benefit the companion animal population.

There are, of course, many examples where advances in human medicine have been applied to the treatment of dogs, cats, and horses. These agents may be developed for similar indications in companion animals as in humans (Table 2.2). There are also a number of agents where development has been completed for companion animals but not necessarily in human medicine, such as some of the coxibs. Finally, there are also a number of agents used in companion animals that are not available in human medicine. This is particularly notable in the parasiticide arena where many

Table 2.2 Selected examples of agents approved in human health and/or companion animal health

Agents approved for use in human and companion animal health	Angiotensin converting enzyme inhibitors—enalapril, benazepril, ramipril
	Antibiotics—penicillins (e.g., ampicillin, (potentiated) amoxicillin) cephalosporins (e.g., cefadroxil, cephalexin), aminoglycosides (e.g., amikacin, gentamicin), diaminopyrimidines (e.g., trimethoprim)
	Antiemetics—domperidone
	Antifungals—nystatin, azoles (e.g., clotrimazole, miconazole), posaconazole
	Antiprotozoal—miltefosine
	Corticosteroids—betamethasone valerate, hydrocortisone aceponate, mometasone furoate
	Diuretics—furosemide, spironolactone
	Ectoparasiticides—synthetic pyrethroids—permethrin, deltamethrin
	Gastric acid inhibitors—cimetidine, omeprazole
	Hormones—estriol, thyroxine
	Intravenous anesthetics—propofol
	Nonsteroidal anti-inflammatory drugs—meloxicam
	Macrocyclic lactones—ivermectin
	Mercaptoimidazoles—thiamazole, carbimazole
	Phosphodiesterase inhibitors—pimobendan[a]
	Receptor tyrosine kinase inhibitors—masitinib
Agents approved for use in animal health not approved for use in human health	Anthelmintics—emodepside, fenbendazole, febantel
	Antibiotics—cephalosporins (e.g., cefquinome, cefovecin), fluoroquinolones (e.g., enrofloxacin, marbofloxacin, orbifloxacin, pradofloxacin)
	Antiemetics—maropitant
	Ectoparasiticides—imidacloprid, *S*-methoprene, pyriproxyfen, fipronil, indoxacarb
	Endectocides—moxidectin, selamectin, milbemycin oxime
	Intravenous anesthetics—ketamine
	Microsomal triglyceride transfer protein inhibitors—dirlotapide, mitratapide
	Neuroleptics—detomidine, dexmedetomidine, medetomidine, romifidine, xylazine
	Phosphodiesterase inhibitors—propentofylline
	Receptor tyrosine kinase inhibitors—toceranib

[a]Also a calcium sensitizer

agents have been developed for endo- and ecto-parasite control from agents used in crop protection (Table 2.2). This may, in part explain why there are many drugs developed for the treatment of dogs, cats, and horses that are not major franchises in human health. Access to compounds developed for use in humans may not be immediately forthcoming even for animal health companies that have a parent human pharmaceutical company. For example, the introduction of angiotensin II receptor antagonists in human cardiovascular medicine [66] has not yet followed in companion animal cardiovascular medicine.

2.8 Market Evolution

Twenty years ago the landscape for companion animal products was completely different. The key drivers were infectious agents—viral, bacterial, and parasitic, leading to well-developed and established segments for biologicals or vaccines (predominantly against viral and bacterial agents), antibiotics and parasiticides (against internal and external parasites). Nowadays, parasiticides and pharmaceuticals, including anti-infectives and other pharmaceuticals, represent the largest category, followed by biologicals.

Infectious diseases are still very important in companion animals worldwide. Traditionally, modified live and/or inactivated vaccines are available against many viruses and some bacterial pathogens. Technological developments have led to the use of recombinant viral vaccines for companion animals, such as those incorporating canary pox [67] or myxoma [68]. Perhaps more importantly, vaccination of companion animals, particularly dogs, against rabies has reduced not only this devastating illness in this species but also in humans through reduction in rabid dogs bites and wildlife reservoirs, the latter through oral vaccination campaigns over the last 20 years [69]. Protective (sterile) immunity to some pathogens—such as feline herpesvirus [70] and feline calicivirus—remains elusive. More recently, vaccine research has focused on more recently described viruses, such as feline immunodeficiency virus (FIV), other types of pathogen, such as *Borrelia burgdorferi sensu lato* and *Leishmania infantum* [71–74], and emerging diseases such as West Nile virus. One of the important challenges remains the ability to differentiate between infected and vaccinated animals. One possible approach would be the introduction of so-called marker-vaccine type technology, where the immune response to the vaccine is different from that to the infection, allowing the differentiation of vaccinated and infected animals by means of a simple patient-side test. This is something that is already available in the livestock market and will likely emerge within the companion animal arena. Despite the wide availability of effective and safe vaccines there are still large numbers of young puppies that succumb to serious disease due to parvovirus and distemper virus infections which take hold prior to the onset of active immunity, perhaps where maternal derived antibody has interfered with the ability to respond. There are no antiviral drugs approved for use in companion animals.

Vaccines against *Leishmania infantum*, the causal agent of canine leishmaniosis, have been available in Brazil for a number of years [71, 72] and, more recently, in the European Union [73, 74]. This represents a breakthrough and is also a potentially significant step forward in the fight against this zoonotic disease—where the dog forms a true reservoir for the disease in humans—that poses a significant public health concern because it leads to significant human morbidity and mortality on an annual basis [75]. As in human medicine, vector control is also likely to remain of critical importance.

Therapeutic vaccine technology has also appeared. Here veterinary medicine lead the way with the USDA granting conditional approval of the first therapeutic

cancer vaccine for the treatment of melanoma in dogs [76]. The future increase in the vaccines market is mainly due to the positive approach towards preventive treatment rather than curative one.

2.8.1 Compliance

If there is a common theme driving developments in the different segments of the companion animal market it is compliance—the willingness to follow a prescribed course of treatment. When talking about companion animals this means that the companion animal owner has to be willing and able to do this. Although studied extensively in human medicine, few studies have actually focused specifically on assessing owner compliance and many of these have looked at short courses of antibiotic treatment and not long-term medication [77–80]. Interestingly, there is a suggestion that a longer interval between vaccinations (even when this is mandatory rabies vaccination) from 1 to 3 years [81] may actually improve compliance with vaccination. Compliance with heartworm prophylaxis including heartworm testing and recording date of treatment administration has considerable room for improvement [82] and may account for some of the apparent failures of prophylaxis in recent years [44].

Compliance can be improved by reducing treatment frequency or by making treatment easier to administer (and/or remember) as well as by removing the need for the owner to administer follow-up treatment. There are examples of this that utilize the unique interaction between the species and the innate properties of new chemical entities—unique active ingredients. But this can also be addressed through combining active agents (to reduce the number of medications that have to be administered), drug delivery, and value-added services, such as electronic reminder systems.

2.8.2 Enteral and parenteral administration

Oral drug delivery is common and usually straightforward, particularly in the dog. While many of the tablets available are conventional formulations, flavored, often chewable, tablets for oral administration are also available in a number of market segments. For example, the standard approach to heartworm (*Dirofilaria immitis*) prevention has, for many years, been the monthly administration of a low dose of macrocyclic lactone (e.g., milbemycin oxime, ivermectin, or moxidectin) formulated as a flavored, and often, chewable tablet. This capitalizes on two things: the exquisite susceptibility of microfilariae to low doses of these compounds and the relatively long half-life inherent to these lipid soluble molecules. Newer nonsteroidal anti-inflammatory drugs (NSAIDs), such as carprofen [83, 84] and meloxicam, brought better safety profiles through being better tailored, both in strength and formulation, to suit companion animals. The solid, and later flavored, oral dosage

form of carprofen was taken a step further by the honey flavored oral suspension of meloxicam, bringing low dosage, easy to administer, although later there was a move from drop-wise administration to a syringe calibrated in body weight. A number of coxib nonsteroidal anti-inflammatory drugs (NSAIDs) are now approved for, predominantly oral use, in dogs [85–87]. Mavacoxib is particularly interesting because it has an inherently long half-life, meaning that it only requires oral administration once per month [88].

Thyroxine supplementation for the treatment of primary hypothyroidism in dogs has also traditionally been administered as conventional tablet formulations, originally developed for use in humans. One of the drawbacks is that a number of different tablet sizes required to be stocked to allow accurate dosing. Liquid formulations of thyroxine enable the dose to be tailored using a dosing syringe [89]. This formulation not only reduces the inventory veterinarians require to stock but also has been shown to be suitable for once-daily administration to dogs, presumably based on achieving higher peak plasma concentrations following oral administration as a result of the absence of a dissolution phase, thus offering a compliance benefit to the owners of hypothyroid dogs [89, 90].

Another way to reduce the frequency of oral administration is to formulate existing active ingredients in prolonged release formulations. For example, conventional tablet formulations of the mercaptoimidazoles thiamazole (methimazole) or its prodrug carbimazole have to be administered two or even three times daily, at least when starting treatment, for optimal efficacy [91] in the management of feline hyperthyroidism [92]. A sustained-release tablet formulation of carbimazole, with prolonged absorption kinetics resulting in a higher area under the curve and more sustained plasma concentrations of thiamazole has been approved for once-daily administration to cats [93, 94].

Fixed dose combination tablets, such as those used for the treatment of hypertension in humans, have not to date been developed for use in veterinary medicine. These would potentially help to simplify oral administration in situations, such as canine congestive heart failure, where multiple medications are required.

Traditional chemotherapeutic regimens for the treatment of canine leishmaniosis have relied upon pentavalent antimonials that have to be administered by injection [95]. Recently, the alkyl phosphocholine miltefosine, originally developed for the treatment of cutaneous neoplasia in humans and used for the treatment of leishmaniosis [96], has been approved for the treatment of leishmaniosis in dogs [97]. This has the advantage that it can be administered orally, rather than by injection. In addition, the antiemetic dopamine D2 receptor antagonist domperidone, which can be administered orally on a daily basis, has also been shown to modulate the course of leishmaniosis in dogs [98].

There are a number of potential novel approaches to insulin delivery that avoid the need for parenteral injection, such as the oral, nasal, and transdermal routes [99] with technologies such as nanotechnology [100], which, if proven feasible and cost effective, will facilitate owner compliance. Some of these [101] have been investigated in dogs and would simplify treatment considerably but have not yet been achievable outside experimental settings. Although inhalation has been used as a route of administration for insulin in humans [99, 102], this has not been highly

acceptable to patients [99] and is not necessarily easier to administer than subcutaneous injection in veterinary patients. With earlier diagnosis of diabetes in cats, there may also be a role for oral hypoglycemic agents if these can be formulated suitably to permit relatively infrequent and easy administration.

A recent advance in companion animal ectoparasite control is spinosad, a naturally occurring spinosyn from the crop protection field [103]. Spinosad is a systemically active compound that is administered once monthly in oral tablet form to dogs for the control of fleas [104, 105]. This active or more correctly its market dynamics has to some extent shifted the market dynamics within the flea control segment. It is probable, given the success of this innovative approach, that further advances will be seen in this field. Future developments in this field are also likely to come from the crop protection field and will continue to focus on easy application and increasing compliance.

Traditional ectoparasiticide products were applied as powders, sprays, and collar formulations. Many of these formulations suffer from a relatively short duration of action and thus require frequent application to avoid gaps in protection. In the early 1990s, lufenuron, a benzoylurea, was shown to be effective against developing stages of fleas after oral administration once monthly [106–109] and as a long-acting (6-monthly) injectable formulation [110], relying on subcutaneous tissue reservoirs for sustained activity.

Compliance with year-round heartworm preventive administration is poor [82]. The development of a sustained-release injectable active over 6 [111] or 12 months [112] reduces the need for companion animal owner compliance. In human contraception, solid dosage forms for oral administration on a daily basis have in part been replaced by implant-technology releasing progestogens over a period of months [113] to years [114]. In veterinary medicine, gonadotropin analogs delivered using this type of technology have been approved for use in dogs and horses [115–117] and have further advanced this technology further by the utilization of a biodegradable formulation [116], precluding the need for (surgical) removal. This type of technology, which may be applicable to other market segments, is interesting because it provides prolonged action with minimal intervention.

Traditionally injectable antibiotic formulations had to be administered on a daily basis. The inherent long duration of activity of a cefovecin, an extended-spectrum cephalosporin, in dogs and cats [118] has allowed the development of a formulation that maintains concentrations above the ex vivo minimum inhibitory concentration (MIC) of *Staphylococcus pseudintermedius* for around 12 days in dogs [119] and *Pasteurella multocida* for around 14 days in cats [120]. Although the cost of this long-acting injectable agent is higher than a course of potentiated amoxicillin tablets, this may be offset by the lack of need for owner compliance with the former and thus a lower rate of treatment failures [121], at least for uncomplicated bacterial infections. However, there is some concern, particularly for pathogens that are less susceptible, that concentrations may be below the MIC for prolonged periods leading to emergence of resistance [122].

Carprofen and meloxicam injectable has been used for a number of years as a tool in the management of perioperative pain in cats [123, 124] and robenacoxib

[125] has recently been added to this therapeutic armory. However, what is still missing in this field is the ability to reliably detect and monitor pain in this species. The fact that cats either show few signs or fall almost into a rage makes conducting trials in clinical cases particularly challenging. Although models have been developed to reliably measure and assess the effects of this class of agent in the cat, the step from this into clinical efficacy and safety field trials and beyond into routine use in clinical veterinary practice is still in its early stages.

2.8.3 Topical application

Topical application is commonly used for local action in the skin. Corticosteroids are used commonly for topical treatment of skin disease in companion animals, particularly dogs. Many topical formulations are oil-based suspensions for application into the external ear canal or creams and ointments for topical application to the skin. Nongreasy gel [126] and spray [127] formulations are also available. The latter may even provide an alternative for oral ciclosporin (also referred to as cyclosporine, cyclosporin A, or cyclosporine), a calcineurin inhibitor, in the management of allergic skin disease (atopic dermatitis) in dogs [128]. Novel calcineurin inhibitors are available and may facilitate improved treatment outcomes and compliance through their efficacy profiles and improved formulations (less frequent dosing).

The use of corticosteroids in dermatology, allergy, and asthma, particularly in humans, is frequently limited by potentially serious side effects, related to the effects of these agents on carbohydrate, protein, and fat metabolism and the immune system. The anti-inflammatory effects of the corticosteroids are presumed to be mainly due to the inhibition of transcription by the glucocorticoid receptor, while the side effects, in the main, are due to activation of transcription. A number of newer agents, such as hydrocortisone aceponate [127] and mometasone furoate [129], are available for topical use in dogs. These improve on the potency of older agents, such as betamethasone valerate [130, 131], but without necessarily an increase in side effects. For example, mometasone furoate is potent but does not dramatically interfere with allergy tests in dogs, following application into the external ear canal at the recommended dose rate for 7 days [132]. Research efforts in human medicine have focused on developing so-called dissociated steroids, where the anti-inflammatory effects and adverse effects are uncoupled (the so-called transrepression hypothesis), with the delivery of an agent that has anti-inflammatory effects without the major side effects associated with prolonged, high dose use of the current corticosteroids [133]. This area has yet to yield suitable steroidal agents and has led to efforts to develop nonsteroidal agents that are glucocorticoid receptor ligands [133, 134]. It remains controversial as to whether this is in fact achievable [133, 134] and the cost constraints in animal health may lengthen the time before which these agents would be seen in animal health, should such a breakthrough be reached. There could be a significant role for such agents in

veterinary dermatology, particularly in the management of atopic dermatitis, for these agents as and when they become available.

In the late 1970s, it was noted that some candidate herbicides had insecticidal activity. Insecticides used in crop protection suffered from user (and consumer) safety concerns, environmental persistence, and rapid development of tolerance. This led to concentrated efforts to develop custom-designed chemicals that specifically target the insect nervous system bringing with them improved user safety, reduced environmental persistence, and, hopefully, slower development of resistance. These new compounds (imidacloprid and fipronil) were both insect neurotoxins, with rapid effects against susceptible adult insects [135]. Fipronil was developed as a spray formulation for the treatment of fleas on dogs and cats [136]. This was soon followed by the development of low volume spot-on formulations of imidacloprid [137, 138], permethrin [139], and fipronil [140] for companion animal ectoparasite control. Following application the lipophilic active agents persist within the stratum corneum, the viable epidermis, and in the pilo-sebaceous units [141, 142]. These products have changed the small animal ectoparasiticide segment forever. Today, there are many such products, which often have their origins in crop protection. A recent entry to this segment is indoxacarb—the only oxadiazine insecticide available in animal health and the only agent that is a pro-insecticide, requiring enzymatic activation or bioactivation (decarbomethoxylation) in the insect (purportedly by esterases and/or amidases) to produce a highly insecticidal active metabolite [143]. Indoxacarb is only weakly active in its parent form, has short environmental persistence, and is essentially detoxified by mammalian hepatic enzymes (by hydroxylation of the inandone group and hydrolysis of the carbomethoxy group) with the insecticidal activity residing in the *S*-isomer [143].

There are also a number of parasiticides that can be applied topically for systemic activity [144]. The use of these agents solely or in combination with more traditional topical ectoparasiticides has extended the spectrum of activity of the traditional spot-on product to include internal parasites (such as heartworm) as well as other ectoparasites, such as mites [145, 146].

Topical application has the advantage that it can get around the need for oral administration—which may be of particular use in the cat. Anthelmintics have traditionally been administered as oral tablets and more recently as flavored tablets in dogs and cats [147–149]. Emodepside is a semisynthetic derivative of a natural *N*-methylated cyclooctadepsipeptides [150] produced by the fungal microflora *(Mycelia sterilia) of Camellia japonica* leaves that inhibits nematode pharyngeal pumping [151]. This has been developed as a novel spot-on formulation for cats [152], removing the need for oral administration. Similarly, thiamazole, for the treatment of feline hyperthyroidism, can be delivered topically instead of by orally administered tablets. Extemporaneously prepared formulations using pluronic lecithin organogel appear to be associated with fewer gastrointestinal side effects [153] presumably associated with poor skin penetration with the majority ingested orally by the cat during grooming [154]. Recently, a novel lipophilic topical formulation of thiamazole appears to have met the high quality standards required for commercialization [155].

Demodectic mange was traditionally treated with amitraz, a formamidine insecticide, which, despite its efficacy at insect and tick octopamine receptors [156], is fraught with difficulties in producing a user-friendly formulation and user- and patient-related issues due to its agonist effects at alpha-2 adrenoreceptors in mammals [157]. The advent of spot-on products containing amitraz [158] was a step forward, although the risk of systemic side effects and strong chemical smell are still drawbacks. The efficacy of spot-on products versus demodectic mange remains a challenge, with currently marketed products requiring to be administered more frequently than is required to control other ectoparasites (e.g., every week to 2 weeks) [159–161]. Thus, there is still significant room for improvement to ensure compliance with treatment. There are new developments in the formamidine insecticides, but it remains to be seen whether these newer compounds have good efficacy but fewer side effects and drawbacks.

2.9 Delivery Devices

Injection devices, with or without a needle, have been around for decades [162] although only the former have been in routine use for subcutaneous administration in the last 25 years [163, 164]. These devices have helped to improve both patients' quality of life and compliance with therapy [164]. It has only been very recently that this technology has been adapted to veterinary use [165–167].

Percutaneous drug delivery in the form of patches has been used in human medicine since the mid-1980s. Following the availability of opioid patches for transdermal delivery in humans, these agents have been investigated for use in veterinary medicine [168]. Although this type of technology makes drug delivery easy, it is not particularly suitable for use on an outpatient basis in dogs and cats [169]. There have been advances in this area [170], but this means of delivery is not yet in widespread clinical use; however, further advances can be expected as a means of delivering pain control without injection is desirable.

Products for the treatment of otitis externa in dogs generally contain an antibiotic, antifungal, and corticosteroid. Many of the agents have been used for many years, but there are a number of novel agents, such as the triazole antifungal posaconazole and the corticosteroid mometasone furoate [129]. These products are usually delivered in a drop-wise fashion into the external ear canal from a conventional plastic bottle. Recently, an antimicrobial-corticosteroid preparation has become commercially available in a pump [171], which simplifies treatment administration although limited in terms of only being able to administer one dose volume irrespective of the size of the patient. In addition, expandable ear wicks for use with aqueous therapeutic agents similar to those employed in human medicine [172] and ear packing have become popular means of reducing the need for intervention by companion animal owners in the treatment of this chronic, often allergy-based, condition. Many veterinary dermatologists compound their own formulations combined with either an ear wick or ear packing enabling the treatment to remain in situ in the external ear

canal while absorbing exudate for a period of a few weeks following cleaning of the external ear canal. To date, this type of longer acting delivery technology has not yet become widely available commercially. Given the frequency of this indication and its chronicity, it is likely that further advances will be seen in terms of improved delivery perhaps bypassing the companion animal owner, in this field.

2.10 Food Interactions and Diet

One way of simplifying administration for many companion animal owners is the administration of the treatment with food. The potential of food and/or feeding status to alter drug absorption and thus systemic availability has been recognized for a long time. Food can impact the pharmacokinetics of a drug through several mechanisms, including, but not limited, to enhancement in drug solubility, changes in gastrointestinal physiology, or direct interaction with the drug. Some of these effects, such as cation chelation by the tetracyclines, have been long known [173]. Significant food effects complicate the development of new drugs, especially when clinical plans require control and/or monitoring of food intake in relation to dosing. In many cases, little is known about the drug–food interaction. This effect can either be positive [93, 174–179]—absorption is enhanced by the presence of food in the gastrointestinal tract—or negative [85, 176, 180–182]. There are models available that can help predict the qualitative effect of feeding status on drug absorption [183]. Developing specific formulations, such as a self-emulsifying drug-delivery system, so that food–drug interactions are reduced, minimized, or avoided, may offer a possibility in future [184, 185]. But, perhaps more importantly, knowledge about the effects of feeding status on drugs used in companion animals needs to be investigated further.

Appropriate diet has been used to support traditional pharmaceutical therapy for decades in the form of, for example, salt restriction in cardiac disease. So-called prescription diets have been available commercially for more than 20 years. Recently, an iodine-restricted diet has been commercialized for the management of hyperthyroidism in cats. Iodine restriction is not new and is used on a short-term basis for 1–2 weeks prior to radioactive iodine therapy in humans [186]. Long-term dietary iodine restriction is controversial as iodine deficiency has a number of well-documented effects including hyperthyroidism and goiter [187] and may have negative effects on cardiovascular health [188]. That said, similar to diets that, by managing carbohydrate intake for diabetic cats, can potentially alter insulin requirements [189, 190], the iodine-restricted diet presents a potential breakthrough in the sense that approaching therapy by means of dietary control is a likely target for future development. In future, further therapeutic diets may become available.

2.11 Individualized Treatment and Pharmacogenomics

Antibiotic treatment has been targeted to suit the individual for many years through the use of bacterial culture and antibiotic susceptibility testing. More recently, surrogate parameters have been used to establish clinical and epidemiological breakpoints. For time-dependent antibiotics (e.g., penicillins and cephalosporins) the time above the minimum inhibitory concentration (T>MIC) is used to predict clinical efficacy [191]. For time-independent (otherwise known as concentration-dependent) agents (e.g., aminoglycosides, fluoroquinolones) the ratio of Cmax to MIC and/or AUC to MIC is used [191]. Finally, the "mutant selection window" hypothesis postulates that a specific drug concentration zone exists where antibiotic exposure selects for mutant bacterial strains with reduced drug susceptibility [192]. This approach, using the ratio of AUC to the mutant prevention concentration (or MPC—the MIC of the least susceptible mutant in a colony), focuses on maintaining drug concentrations throughout the dosing interval to try to decrease the emergence of resistance [192]. Although there has been some research in this area, to date there is little data on the use of surrogate parameters in veterinary medicine. It is expected that there will be further advances in this area of pharmacokinetic–pharmacodynamic integration to better tailor therapy to suit the individual patient.

The impact of the ATP binding cassette (ABC) transporter protein superfamily on drug pharmacokinetics and pharmacodynamics has been increasingly recognized. P-glycoprotein (P-gp), the product of the ABCB1 (formerly the multidrug resistance or MDR1) gene, is among the most well-characterized drug transporters, particularly in veterinary medicine. P-gp is expressed by a variety of normal tissues, including the intestines, brain capillary endothelial cells, renal tubular cells, and biliary canalicular cells, where it functions to actively extrude substrate drugs [193]. In this capacity, P-gp limits oral absorption and the entry of many drugs into the central nervous system as well as enhancing their excretion from the body. Many drugs used in veterinary medicine are substrates for P-gp, including many chemotherapeutic agents and macrocyclic lactones. A naturally occurring, four base pair deletion mutation in the ABCB1 gene occurs in many herding dog breeds, including collies, Australian shepherds, and Shetland sheepdogs [194–200]. The mutation (ABCB1-1Δ) renders affected animals extremely susceptible to adverse effects of P-gp substrates at doses well below those tolerated by dogs with the wild-type genotype because they are unable to transport these agents from the brain back into the blood [194, 196, 198–200]. Further investigation of this area will likely lead to more targeted individual therapy.

While inter-individual variation in drug metabolism is well recognized in human medicine, there is an ongoing debate on population kinetics in veterinary medicine. Population kinetic studies are not mandatory but provide further evidence to support field efficacy and safety trials based on selection of the correct dosing regimen [174]. It is likely that individualization of treatment will continue to develop and in future there may even be specific product information for subpopulations within a species.

2.12 Generics, the cascade and compounding

Animal health companies invest up to somewhere in the region of 10% of their annual turnover into research and development [9]. In fact, the cost of development has increased by more than 150% [9]. Costs and complexity are likely to continue to increase in line with human health, despite the fact that the animal health market is far smaller than its human counterpart.

2.12.1 Generics

A generic veterinary product is a product with the same quantitative and qualitative composition (i.e., active substance and pharmaceutical form) that has been demonstrated, in appropriately designed bioavailability studies, to be bioequivalent to the reference product. Bioequivalence is a complex area [201]. The assumption is that if two products are bioequivalent then the pharmacological effects, in terms of safety and efficacy, should be more or less the same. However, there are tremendous implications for other important parameters, particularly those related to efficacy, because the pharmacokinetics of the reference and generic product are generally not identical. In addition, there are a number of situations, such as active agents with highly variable pharmacokinetics, prolonged-release formulations, and topical products, where straightforward bioequivalence calculations may not be appropriate [202–205].

In human health, the launch of generic products, after data and patent protection of reference products have expired, has significant financial impact. Thus, it is no surprise that the protection of intellectual property frequently leads to litigation [206]. In animal health, the protection of intellectual property is equally as important as it is essential to recoup product development costs [207], which are in the region of €50 to 200 million (or around US$65 to 265 million) per product [9]. Authorized veterinary generics exist legitimately and can be used by veterinarians in the same way as other authorized veterinary medicines. Human medicines (including generics) that are similar to the authorized veterinary medicines may not be used unless there is no suitable veterinary medicine available.

There are generic products in many segments of the companion animal market as a number of the active ingredients found in key reference brands no longer benefit from intellectual property protection. Generic entrants make market segments increasingly crowded and, although price tends not to fall as dramatically as it does in human medicine, the pricing structure within a segment is generally altered.Some reference brands have still been able to maintain a competitive edge by adding additional claims, changing formulation (e.g., from traditional tablets to flavored chewable tablets or by adding additional active agents [52]). Given the costs incurred in and time required (around 8–12 years) [9] to develop new animal health products, it is likely that intellectual property, not only in and around new chemical entities but also on formulations and manufacturing processes, will remain an important and highly competitive,and potentially litigious, area.

2.12.2 The cascade

European Union legislation is designed to ensure the quality, safety, and efficacy of veterinary medicines. It has, however, been recognized that there are circumstances where the benefits of treatment with unauthorized medicines outweigh the potential risks. This, legal exemption, is known as the "prescribing cascade" and is intended to increase the range of medicines available for veterinary use.

The cascade provides a legal mechanism that allows veterinarians to use their clinical judgment to prescribe a suitable medicine where no authorized veterinary medicine exists. If there is no medicine authorized for a specific condition, the veterinarian caring for the animal may prescribe another product, such as one licensed for use for another condition, another species, in another country or for human use, subject to specific conditions, including where appropriate import requirements. It is only if there is no such medicine available that a medicine can be prepared extemporaneously (by definition provided, made, or adapted as an expedient; makeshift) by a veterinarian, pharmacist, or the holder of an appropriate authorization to manufacture.

The choice of medicine under the cascade should be based on clinical grounds and not on cost. Thus, it is not possible to choose a product approved for use in humans just because it is cheaper than a veterinary product. The cascade not only applies to the choice of medicine but also, for example, to the dose rate chosen. Any variation to what is stated on the license of an approved veterinary product would constitute off label (or extra label) use.

2.12.3 Compounding

The extemporaneous manufacture (or compounding) of medicines for use in animals, frequently by so-called compounding pharmacies, is far more common in the United States [208] than in the European Union [209]. Compounding is generally considered to be an option when the compounded product provides an individual patient with a significantly different product than that available commercially, such as a product compounded to facilitate dosing of an animal with a body weight lower than targeted by the commercially available tablet sizes or one without a coloring agent or excipient to which a particular individual is allergic. In general, since compounded products are exempt from standard approval requirements, including those relating to manufacturing quality [210], it is not intended that they be advertised or promoted.

The responsibility of ensuring that the medicine used is both safe and effective is the responsibility of the veterinarian caring for the animal [208]. Compounding, while it may be appropriate for the optimal treatment of specific cases, is by nature not intended for widespread use as it can be fraught with potential pitfalls, particularly with regard to pharmaceutical quality [208, 211]. Excipients may differ, batch testing may not be performed, long-term testing of the stability of the finished product

is often not conducted, and the shelf life before and after opening [212, 213] and optimal conditions for storage may not be known [212–214]. For example, it has been shown that compounded protamine zinc insulin seldom conforms to the United States pharmacopoeia (USP) [215]. In general, there is a dearth of public or published information on the content and characteristics of extemporaneously prepared products, even those in almost routine use for companion animals in the United States.

There generic products in many segments of the companion animal market as a number of the active ingredients found in key reference brands no longer benefit from intellectual property protection. Generic entrants make market segments increasingly crowded and, although price tends not to fall as dramatically as it does in human medicine, the pricing structure within a segment is generally altered. Some reference brands have still been able to maintain a competitive edge by adding additional claims, changing formulation (e.g., from traditional tablets to flavored chewable tablets or by adding additional active agents [52]). Given the costs incurred in and time required (around 8–12 years) [9] to develop new animal health products, it is likely that intellectual property, not only in and around new chemical entities but also on formulations and manufacturing processes, will remain an important and highly competitive, and potentially litigious, area.

2.13 Regulation and pharmacovigilance

2.13.1 Regulation

The quality, safety, and efficacy of animal health products have to be demonstrated and undergo scientific review by the regulatory authorities before such products can be granted a license. The process ensures that only those products of a defined standard, which have been tested thoroughly and reviewed carefully, reach the marketplace [9]. As part of this process a file or dossier containing all of the data pertaining to all of the studies conducted with a product is submitted to the competent authority.

In the United States, the Department of Agriculture (USDA) is responsible for vaccine registration, at least for those products that are for the prevention of infectious disease [216]. Within the same jurisdiction, the Environmental Protection Agency (EPA) is responsible for the regulation of pesticides, including products with topical activity for the control of ectoparasites on dogs and cats, and the Food and Drugs Administration's (FDA) Center for Veterinary Medicine (CVM) is responsible for the regulation of pharmaceutical products, including pesticides that work systemically and thus require a veterinary prescription [216]. The European Medicines Agency is the central body with groups responsible for the approval of human and veterinary medicines. The centralized procedure is obligatory for medicinal products derived from biotechnology and for other products where a new chemical entity and/or delivery or formulation technology is used [9].

Product approval is not a one-off procedure but ongoing work is required to defend the licenses of existing products, which utilizes around 35% of animal health companies' annual budget for research and development [9]. Not only that, but requirements are, in general, increasing and becoming more stringent [9]. With the increasing complexity of the regulatory constraints, it is expected that, at least for innovative and novel animal health products, the time to market and costs will continue to increase. A number of initiatives have promoted harmonization. Under the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH), the US, European Union, and Japan, observed by Canada and Australia/New Zealand, have worked towards harmonization of the technical data requirements prior to granting market authorization [217]. This has led to a number of guidelines that have been implemented, including one for veterinary medicinal products including vaccines.

Other initiatives have aimed at promoting innovation and increasing the number of approved veterinary medicinal products, such as the EMA initiative for assisting micro-, small-, and medium-sized pharmaceutical businesses [218]. At least 31 companies (16 in animal health and 15 companies developing products for both human and veterinary medicine) have met the strict criteria for the Small- and Medium-Size Enterprise (SME) initiative. However, the success rate of these concerns has been lower than for larger enterprises, mainly due to quality issues [218]. To address a gap in animal welfare, minor use, minor species (MUMS) regulations have been put in place to cover not only these so-called minor species but also less common [minor] use of products in major companion animal species [219–221].

2.13.2 Post marketing surveillance

Pharmacovigilance (or post marketing surveillance) involves the monitoring, researching, assessment, and evaluation of suspected adverse effects of veterinary medicinal products (including lack of expected efficacy and effects in human users). Data is collected not only throughout the development of a veterinary medicinal product but also after a product has been granted approval. Many countries have well-established spontaneous reporting systems that allow veterinary healthcare providers and users to report suspected adverse drug reactions. These reporting systems apply to all medicines used in animals whether within the label for a veterinary medicinal product or relating to off (or extra) label use.

Important lessons can be learned about the performance of agents in a population after a product has been launched onto the market. This is related to the use of the product in much larger numbers of animals and reflects potential variance in pharmacokinetics (e.g., rate and extent of absorption) as well as interactions both with other agents and with the altered physiology found in many disease states. The collation of pharmacovigilance data can lead to adjustment of the product license to take into account effects seen when used in the population intended to be treated.

2.14 Conclusions

In the years to come, the worldwide market for companion animal health is expected to grow further. It will also continue to become more specialized. Major drivers of this are the continued strengthening of the bond between owners and their animal companions, increasing companion animal owner awareness, and increasing companion animal owner demands and expectations for companion animal care. With increasing urbanization, there will continue to be a shift in the species and type of companion animals kept and, while growth in the care of these species is expected, this will of course be influenced by economic, social, and demographic trends. There is a need for evidence-based studies in companion animals and this, along with increasing global regulatory requirements, will continue to increase product development costs. While new chemical entities will continue to emerge, influenced by the much larger arena of human health, and to challenge traditional delivery concepts, there will also be increased focus on differentiating products through novel delivery technology designed to increase companion animal owner compliance and thus enhance efficacy.

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Chapter 3
Anatomy and Physiology of the Farmed Animal

Keith J. Ellis

Abstract Most farmed animals have anatomical and physiological features that are in many ways similar to humans, but there are also important differences. This chapter assumes a basic understanding of mammalian structure and function and attempts to highlight the differences, particularly as they relate to drug delivery and metabolism. There is a conscious bias towards ruminant livestock, as they represent a major sector of the farmed animal population, and are the group that differ most from the monogastrics. More reading will be required for the reader wishing to develop specific bioactive delivery technologies *per se*, but this chapter aims to provide a sufficient understanding of the environment into which the drug is being introduced, and an explanation of the relevance of the (sometimes) unusual modes of administration for the reader more interested in the benefits to be gained from the treatment of livestock.

3.1 Introduction

Ever since man evolved from other life forms, he has relied on animals as a primary source of food and other useful products such as the skin, fiber, and bone. While the gathering of these products was once opportunistic, skills in animal husbandry have been acquired over time and the concept of "farming" animals has evolved alongside farming of various plant (vegetable) resources.

"Farmed" animals can mean many things to different peoples—some cultures rely heavily on pigs, various poultry and aquatic species, and creatures like rabbits, dogs, kangaroos, and many more. These species are often referred to loosely as

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"monogastrics," with added variations such as fore- or hindgut fermentation, or various other terminologies specific to a species.

One of the largest groups of farmed animals is the ruminant. The common members of this class are sheep, cattle, goats, and deer, but there are many other herbivore species which have evolved along similar lines [1]. In simplest terms, ruminants are herbivores that have adapted a foregut digestive system to permit them to utilize the structural carbohydrates in plant materials as an efficient source of energy [2]. One of the major ways to achieve this is by supporting and maintaining a large and very active population of anaerobic microorganisms, together with the resultant enzyme products like hydrolases and cellulases, in a fermentation compartment at the anterior of the gut structure.

It should also be understood that many other animals with differing degrees of fore- and hindgut fermentation [3] can be exploited by humans for food, but fewer of those have been farmed for that purpose in the traditional sense. Examples include various African wildlife, horses, and the camelids.

There is an endless variation in the way in which animals obtain nutritive value from the food that they eat, whether that be of plant or other animal origin, or even in some cases (e.g., the rabbit), their own excreta. There has long been (and perhaps always will be?) considerable speculation regarding the reasons for the relative dominance of the foregut fermenters. However, given the important part they play in the current world, the maintenance of their health is important because of the economic impact of efficient animal production, and because of the health risks to humans of various animal-borne diseases. This in turn leads to the need for "Animal Health Drug Delivery," the title of this book, and will hopefully justify the bias that will be noted towards ruminants in this Chapter.

3.2 Efficient Ruminant Animal Production: Health and Disease

In order to optimize growth and production, the animal must be healthy, and as with any living organism, health relies on access to sufficient quality food to provide for all of the metabolic needs, and on an ability to withstand attack by a variety of pathogens and parasites.

These subjects have been discussed widely in books and the scientific literature to which the reader is directed [4–6], but the purpose in this instance is merely to provide a framework for future thinking and for later Chapters to build upon.

3.2.1 Nutrition

Good nutrition underlies most health issues. Unlike totally free-ranging animals that have relatively unlimited access to a variety of food sources, and often seem to

have an inherent knowledge of what is "good" or "bad," the farmed animal is generally confined to a limited area (paddock, field, feedlot pen), and so can only eat what is provided. With the advances in knowledge over the past century, most farmers now understand the need to, at times, provide supplementary feed to their animals.

By way of example, one situation is the need for trace mineral supplements in the diet of grazing livestock. Clearly, if an essential element is not available in the soil of a region, then it cannot be absorbed into the growing herbage, and so the grazing animal will eventually suffer from a deficiency syndrome. Herein lies one of the most basic opportunities for bioactive delivery to livestock.

But of course there are many other nutrients that could be supplied as a delivered dose, and awareness is increasing of a number of pharmacologically active chemicals that can modify the normal physiological process to lead to (desired) changes in either the efficiency of conversion of nutrients, or to comply with modern consumer demands for (say) lower fat content.

3.2.2 Combating Pathogens and Illness

Any animal is susceptible to illness-causing pathogens, and the constraints and often close-confinement of the farmed animal can lead to enhanced transfer between individuals. Consequently, there is a recognized need for regular treatment of farmed livestock with vaccines, antibiotics, and other moieties to combat disease. In addition, some species of animal have their own peculiar conditions that need controlling. For example, bloat is an illness of ruminants where gas production becomes excessive and the internal pressure can ultimately lead to death, but treatment with specific chemical and biological agents can significantly reduce the impact of the condition.

3.2.3 Parasite Control

Livestock are susceptible to a range of internal and external parasites, most of which have an impact on either product quantity or quality. As a result, there is a need to control these infestations, and while some can be achieved with appropriate management, others need regular or long-acting treatment with chemical or biological agents. As would be expected, the life cycle and living conditions of each parasite species need to be considered in developing an appropriate dosing treatment.

It should also be recognized that a number of parasites that infect humans either reside in livestock, or use livestock as intermediate hosts. Consideration then has also to be given to the safe and effective control of these organisms to benefit the farming community as well as the farmed animal.

3.2.4 Patient Compliance and Modes of Treatment

3.2.4.1 The Need

Unlike the situation in human or even some (companion) animal therapeutic treatments where a single patient is treated for a condition, it is generally the case with farmed animals that all of the herd/flock/group will need to be treated at the same time. However, the value that society places on each individual in the farmed group is usually much less than of a human (or companion animal) life, and so the need arises for means of mass medication at low cost. While a number of farmed animal medicating procedures have arisen from a human application (injectables are a case in point), this situation in turn has brought about some specialized forms of dosing for livestock.

Further unique aspects of dosing animals are that they are not always readily available for treatment (some grazing livestock may only be mustered once or twice a year), and they will not voluntarily comply with a particular dosing regime as one can expect from human subjects! As a result, a range of strategies, particularly those relying on controlled and delayed release technologies, have been developed specifically for the livestock markets.

3.2.4.2 The Processes

Most of the common routes of pharmaceutical administration used for humans are just as applicable to livestock as to any other mammalian form. Some of these are oral, injectable, subcutaneous, nasal, transdermal, rectal, and intravaginal. Each has its own advantages and disadvantages depending on the animal species in question, and the condition that is to be treated.

For example, nasal administration is difficult for livestock, because the subject cannot be instructed to inhale on cue. Similarly, some farmers dislike using injectables because of danger to themselves with uncontrolled animals, and because of tissue damage blemishing the eventual carcass characteristics.

It must also be recognized that the simple ingestion of a small tablet by a ruminant will likely result in the immediate expulsion of the dose in the next regurgitation reflex action! There is no way for the operator to overcome this result.

On the other hand, intravaginal therapy (possibly not widely applied for humans in part because of a social context) is used extensively in the livestock industries for reproductive purposes, and there are a number of other applications that could be pursued.

There are also a range of livestock-specific technologies available which do not involve directly “treating” the animal, including metering an agent into a water source, impregnating an ear-tag with a volatile biological agent, or adding the agent to a self-medicating salt block which will attract the animal to consume it.

One of the obvious routes of dosing that is uniquely available to ruminants is the use of the rumen (the fermentation tank) as a repository for a bolus containing the medicament [7–9] and frequently as a means to also control the release of that material into the animal's gut. The features of the gastrointestinal tract (GIT) that bring this about will be explained in more detail below, and applications discussed in other chapters of this book (Chap. 11).

3.3 The Anatomy of the Ruminant GIT

The reader is asked to recognize the complexity of this subject. The following information provides but a brief outline of aspects important to the oral delivery of bioactives. The anterior section of the ruminant GIT is very different from the human; the sections are described below and shown in Fig. 3.1 [10].

3.3.1 The Reticulo-rumen

This is a large sac (of the order of 5 L in sheep, 50 L in cattle) at the (thoracic) end of the esophagus, sited left of the center of the abdominal cavity, and nestled behind the diaphragm. It has been described [2] as "a tubular diverticulum folded into an S and subdivided by (a number of contractile) muscular folds or pillars" of tissue. The foremost compartment (the reticulum) is directly below the cardia (the entry point of digesta from the esophagus), and has a distinctly different surface texture. The combined sac contains large populations of anaerobic microorganisms, which

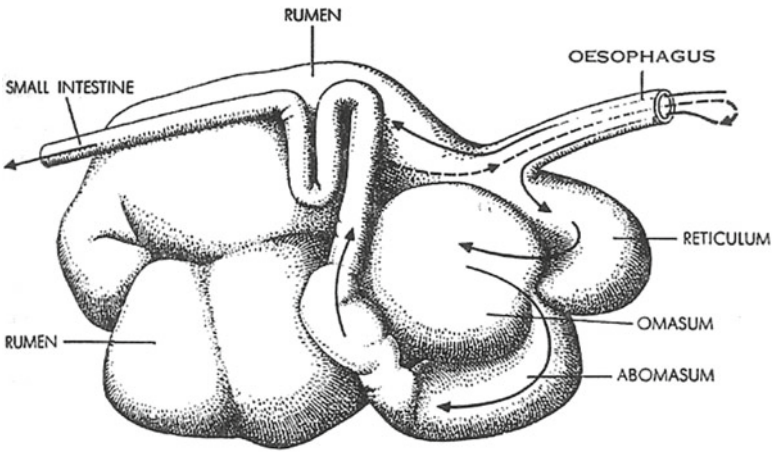

Fig. 3.1 A schematic representation of digestive organs in ruminants (from Annison and Lewis [10])

collectively digest the incoming herbage, breaking down the fibrous and structural cellulose and incorporating nonprotein nitrogen into their own protein-containing cellular components, thereby providing an added source of protein for metabolism lower in the gut. Further, there is a significant amount of carbohydrate fermentation and protein assimilation which results in the production of short chain (volatile) fatty acids, which play a large part in the overall energy efficiency of ruminant digestion. Of interest, in most other animals this type of fermentation occurs more in the lower sections of the gut.

The pillars which compartmentalize the organ are (almost continually) activated by a series of combined responses to nerve stimulation, to bring about a mixing of the contents, the regurgitation of boluses of feed for secondary mastication and the concomitant release (eructation) of the gases produced by fermentation. This phenomenon is known as rumination, and precedes the eventual propulsion of digesta further along the tract. Overall, the movements in this organ are loosely related to, but not the same as the peristaltic motion that propel digesta along the greater length of the intestine.

The siting of the rumen adjacent to the wall of the gut cavity makes surgical modification to exteriorize access to the rumen a relatively simple procedure. Many studies on rumen function, drug delivery technologies, and on the effect of rumen environment on bioactive materials can be performed using such a surgically modified animal. The fistula can be effectively sealed, and the only disadvantage to such a system is that whenever the seal is broken, it takes time before anaerobicity is returned to normal. Apart from that, a fistulated animal may be considered in most respects to be "normal."

3.3.2 The Esophageal Groove and the Reticulo-omasal Orifice

These are specific features or folds of rumen tissue near the entrance from the esophagus, and the point of exit of digesta out of the rumen. In the "preruminant" young animal, this structure appears to propel incoming milk directly from the esophagus to the omasum (see below) allowing it to pass directly down the GIT, but that option is largely lost in adults.

In the adult, these tissues seem to play an important part in the accumulation of the feed bolus for regurgitation during rumination, the control of eructation of gases formed from the fermentation processes, and in the separation of large particulate matter that needs further breakdown and processing from the smaller and more fluid digesta that is ready to proceed through the omasum into the lower parts of the tract.

3.3.3 The Omasum

This is a compact, complex organ which links the rumen to the abomasum. Its size and complexity (and probably also function) vary between animal species (e.g., it is a much larger proportion of the cattle GIT than in sheep). A notable feature of its

structure is the presence of a series of fine laminae or leaves that may act in part as a sieve for material during its transit. However, there may be other absorptive and pseudo-chemical functions (e.g., removal of buffering components) that contribute to the overall efficiency of the total digestive process.

As far as can be recognized, this organ probably has little impact on dosing technologies.

3.3.4 The Abomasum

This region is sometimes referred to as the "true" stomach. In many respects it acts in a similar way to the stomach of many monogastrics, and is the site of considerable secretion of acid and pepsin-like moieties that aid the further breakdown of digesta.

There are two physical features of this organ that deserve mention in relation to drug administration.

The first is the extensive folding of the mucosal lining of the organ, particularly near the omasal end, which tapers to be virtually nonexistent at the pyloric end. These folds (rugae) provide an enlarged surface area for both excretory and absorptive processes, but they can also be used to retain particulate material for extended time periods.

The available evidence suggests that while these rugae do move back and forth with the peristalsis and flow of fluid, small solid particles of higher density can settle into the base of the structure, and remain there undisturbed by the flow of digesta. This feature has been used as the basis of a therapy for copper deficiency in ruminants [11], but the general principle could be applied to a variety of bioactive materials.

The second feature is that because of the general behavior of the animal, and the way in which the abomasum moves and lies when at rest, this organ tends to remain in a relatively constant orientation—that is comparatively "horizontal," with the rugae also maintaining a generally stable orientation. This is clearly different from (say) the human, which rests in an entirely different orientation from its waking activities, and rolls from side to side. One might speculate that the ruminant animal may have an inbuilt mechanism consistent with enhanced gastric retention, and this might present a more simple approach than that required with floating dosage forms in humans [12]?

Fistulation of this organ is also possible, and can be used (for example) to test the effect of direct administration of a drug to this region, without passing through the rumen—however, one cannot be as certain as with the rumen that the surgery does not interfere with vital parts of the peristaltic and secretory processes.

3.3.5 The Lower Gut

From the point of view of bioactive delivery technologies, there is little unique to be said about this section of the GIT. Most of the common knowledge about the role of the intestines, cecum, etc., in other species can be adapted in light of the events that occur before digesta reaches this region.

Perhaps one specific region of the tract that might warrant further investigation for drug delivery is the rectum. There are areas of tissue (Peyer's patches) that may be good receptors for a variety of immune-related biomolecules [13]. Certainly, there are features of the anatomy that make for the reliable sustained retention of an appropriately designed delivery device in this region, but there is a need to identify the appropriate release mechanism to match the biological need.

3.4 The Gut Content and Environment

Modern pharmaceutics have addressed the issue of delivering a bioactive to a specific organ or tissue in a variety of ways, and there are many technologies that can be used to achieve the desired result. Most of these will apply equally to livestock as to any other mammal, but oral administration may have significantly different constraints for livestock, particularly the ruminants, because of their digestive system.

The preceding section of this chapter dealt with the anatomy of the GIT, and explained how the animals have adapted to utilize herbage (often the only source of food available) by a series of alternate processes which include extra chewing, regurgitation and secondary mastication, and fermentation and other attack by microorganisms, before this modified food is passed into the lower gut to be further treated and absorbed in a manner not dissimilar to other animals.

The two main sections of the gut of the ruminant offer a number of options to be exploited by the drug delivery technologist.

3.4.1 The Rumen

The rumen is a highly complex, dynamic, and potentially (chemically) destructive ecosystem and environment.

Specifically, the rumen contains a large amount of harvested herbage. Depending on the nature and quantity of food that is available, and the physiological state of the animal, the daily dry matter intake may be anything between 1 and 3% of the body weight. For the fermentative and digestive processes to function properly, there must be an aqueous environment—as a consequence, the ruminant must have access to water, or be adaptable to reduce its unnecessary excretion of this vital resource. Again depending on conditions, the time after feeding, and the species of animal, the rumen content can vary from a watery broth to a thick, almost solid mass of digesta.

An effective buffering system (fed largely by saliva—see below) maintains the pH of the rumen content at around pH 6, a condition that encourages the microorganisms to work efficiently. There is a high electrolyte concentration, fed continuously and recycled via large volumes (ca. 100 L/day for a cow) of saliva along with a vast array of moieties resulting from the microbial fermentation processes.

As mentioned previously, much of the fermentation and digestion of herbage is a result of a highly complex ecosystem of bacteria and protozoa [14]. However, while this is an essential part of rumen function, its destructive features can also act as one of the most limiting effects on oral dosing of many chemical or biological agents that might otherwise be used to maintain health and/or treat disease.

The fermentation produces large amounts of gas which is belched regularly to avoid excessive pressure buildup. The environment of the whole reticulo-rumen sac is anaerobic (highly reducing), and there is generally a "gas cap" in the dorsal region which is perhaps 10% of the total rumen volume. The gases are predominantly carbon dioxide and methane, with some nitrogen, ammonia, hydrogen sulfide, and hydrogen. The total volume of gas eructated varies over time and is highly dependent on the type of feed and the phase of the feeding and ruminating cycle, but for a cow can be of the order of 50–120 L/h, and may exceed 1,000 L/day.

Regardless of the physical consistency of the material in the rumen, the incessant and regular movements of the rumen pillars ensure there are very few static sections. The herbage particles are being continually mixed, exposed to microbial attack, or being separated into components suitable either for passage through the omasum, or for regurgitation to provide further breakdown by chewing. However, due to this sorting process (apparently based largely on particle size and texture), there are areas within the rumen where there is also particulate stratification. For example, the lower sections of both the reticulum and the main (ventral) sac of the rumen tend to have a highly fluid (water-like) content, while there is often a "raft" of relatively undigested material floating nearer to the surface.

3.4.2 The Abomasum

This region is very different to the rumen. Few large particles pass though the omasum; the material that reaches this organ is relatively uniform and it is quickly acidified to a pH approaching 2 or even less by the abomasal secretions of hydrochloric acid and other materials (gastric juice). Not surprisingly, the activation of these secretions is part of a complex nervous system, where it is thought that everything from feed intake via the mouth to the different rumen movements and the lower gut peristalsis can have an influence. By and large, human intervention is unable to apply any control, so fluctuations need to be accepted as a variable to be lived with when planning drug delivery.

As with monogastrics, this is the gut section in which many of the "normal" digestive functions proceed, preparing digesta either for direct absorption into the bloodstream, or modifying molecules for use elsewhere along the GIT. The bioactive delivery technologist should follow the same guidelines as with other animals. There are no set rules, but commonsense and a detailed understanding of the chemistry of the bioactive under study, and of its expected site and mode of action can lead to a desired outcome.

3.5 Designing an Oral Drug Delivery System for Ruminant Animals

For optimum effect, any bioactive must be delivered at the right concentration, to the right site of action, at the right time, in the right form.

This principle is of special significance in any oral dosage form for ruminants because of the effects the rumen may have on the material before it reaches the "true" stomach.

3.5.1 Properties of the Bioactive Material

Any preconditioning or modification of a molecule that occurs during passage through the rumen will likely affect the way that molecule is metabolized further down the gut. The hostility and variability of the environment tend to preclude any definite prediction regarding the fate of any specific molecule. The alteration can be as simple as adding a ligand or removing a small functional group, to complete destruction of the molecule.

The effects of rumen modification and the consequences of avoiding rumen exposure can be tested in surgically modified animals. It will often be found that there is a need for the use of some form of protection, or effective "bypass technology," to be applied to many drugs.

Rumen Bypass Technology of feed components (particularly protein) is well known [15], and can vary from something as simple as a pretreatment with formaldehyde [16] to a variety of coatings of the feed particles. Similarly, modern synthetics and microencapsulation techniques [17] afford an endless range of possibilities to protect any moiety from degradation or modification.

The significant change in environment on moving from rumen to abomasum then presents alternatives for the efficient removal of that protective coat to allow the "bare" molecule of interest to be made biologically available as intended.

3.5.2 The Timing and Mechanism of Release

Unlike the monogastric where orally ingested material is transferred almost directly to the stomach, there can be a delay of some hours before ingesta or any applied dose reaches the abomasum. While this can be manipulated in part by choosing particles of a specific size and density, it has not been possible to fully engage the movement of the esophageal groove to provide for direct transfer from mouth to abomasum in the adult ruminant.

More importantly, the rumen provides an opportunity to retain a bolus or a depot containing a relatively large amount of any nutrient or bioactive material, and to

have that material released in any desired pattern over time. After mixing with the ingesta that material is then transferred, in diluted form, to the abomasum.

Because of the complex "filtering" mechanisms which control regurgitation and the on-flow of digesta down the GIT, there are a series of considerations to make in respect of any retained bolus. Details will be provided in later chapters, but in essence, any object may be retained by either density (i.e., a heavy object will be retained in the bottom of the reticulum or rumen), or if of similar density to the digesta so that it floats freely, by physical size. Recognizing that any dosage form must be able to be readily administered via the esophagus, a device that relies on size for retention will usually have to employ some form of variable geometry. That is, the shape of the device must first be consistent with the swallowing motion of the target animal, but then on reaching the rumen it must change shape so as to either be too large, or at least be perceived by the sensory components of the rumen to be too large to be passed out of that sac. In essence, such a device can be retained forever, and provided it has no sharp edges to damage tissue the animal functions as if nothing is there! Indeed, as farmers and veterinarians know well, many animals scavenge odd hardware during grazing, and many of those objects remain lodged in the rumen for the lifetime of the animal.

The chemical and physical environment of the rumen as described lends itself to a variety of release mechanisms from these devices. For example, the high water content provides for dissolution and osmosis, the electrolyte concentrations for electrolytic action, the gases may be harnessed for diffusion into expanding chambers, and the turbulence can abrade any surface. What is more, one can envisage a variety of mechanical or electrical devices capable of similar activity.

These release mechanisms can be adapted to virtually any nutrient, bioactive material, or "rumen protected" agent, the only absolute limitation being the amount that needs to be dosed, and its relationship to size or volume.

Similarly, the release profile, whether it be a single dump dose, a slow release, or an intermittent release of several doses over time, can all be designed into a specific device.

And the role of the abomasum in the timing of release, retention, and absorption can also play an important part in developing a drug delivery process. As mentioned previously, small heavy density particles can be retained for long periods in the rugae, and by combining this feature with varying types of protective coatings, or with other "cofactors," one can further control the rate of passage or degree of availability of a particular agent for absorption at this site or at others lower in the gut.

3.6 Conclusion

Most farmed animals will benefit from some type of nutritional or pharmacological intervention at some time. Their behaviors and the environment in which they live, together with their varying anatomy and physiology, present opportunities as well as challenges. Particular attention has been paid in this chapter to ruminants,

because they represent the largest group of farmed livestock, and because they have peculiarly unique features that may not be well understand by the average reader.

A number of drug delivery technologies pertinent to the ruminant animal are described in Chap. 11 of this book.

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Chapter 4
Oral Anatomy and Physiology in the Companion Animal

Steven C. Sutton

Abstract This chapter provides a synopsis of the anatomy and physiology of the gastrointestinal tract of small animals, specifically cats and dogs. The stomach is both a grinding organ and a reservoir, metering foodstuffs into the small intestine for further processing and absorption. The small intestine is the primary site of absorption. On the one hand, the gastrointestinal tracts' acidified stomach contents may result in the precipitation of pharmaceuticals. On the other hand, in response to food, the secretion of pancreatic fluid, bile salts and lecithin in the small intestine may result in a fine emulsion that facilitates the aqueous solubility and subsequent absorption of the pharmaceutical ingredient (API). The large intestine (cecum and colon) completes the processing and absorption of nutrients. Absorption of the API in this region is balanced by its solubility and residence time in this organ. This chapter shows that there appear to be many similarities in the anatomy and physiology of mammals.

4.1 Introduction

The diversity of companion animal species and breeds has exploded in recent years. The Humane Society lists the following categories for pets: dogs, cats, hamsters, gerbils, guinea pigs, rabbits, ferrets, horses, pet birds, rats, mice, and fish [1]. Despite these broad definitions of companion animals this chapter will focus on dogs and

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cats, as these are the most common species seen by veterinarians. But since the American Kennel Association lists hundreds of breeds—from the Affenpinscher to the Yorkshire Terrier [2]—it would not be accurate to generalize physiologic properties to all dogs. For example, canine body weights range from the 2 pound Chihuahua to the 120 pound Great Dane [3].

In some respects, cats and dogs are more different than the differences among dog breeds. While a ten pound Papillon dog may seem the same size as a ten pound Maine Coon cat, their eating habits are quite different. Diversity aside, in this chapter, an attempt will be made to provide some careful generalizations, while keeping in mind the most notable exceptions. As we will see, it is tempting to speculate that the carnivorous cat, accustomed to numerous small daily meals, evolved a smaller stomach and shorter intestine, since that was all that the animal required to process food. In contrast, the omnivorous dog—accustomed to eating fewer, larger meals—would have to use a larger stomach and a much longer intestine for its digestive needs.

4.2 Stomach

Very little absorption of nutrients or pharmaceuticals takes place in the stomach: it is both a mixing/grinding organ and a reservoir for delivery of food to the digestive tract. De Zwart and coworkers [4] have elegantly shown that the grinding function of the stomach is a requirement for breaking down food particles before being squeezed out of the stomach through the muscular pylorus [4]. Food is delivered to the small intestine at a rate that optimizes intestinal absorption of nutrients. Since the stomach in humans is similar in size to that in the dog, the basal volume of water is probably similar in both species, and equal to approximately 24 ml [5]. Based on organ size, the basal volume in the cat stomach would be approximately half that of the dog [6]. Therefore, the basal volume of the cat stomach is probably 12 ml. However, the capacity of the dog stomach is approximately 1 L (similar to that in human) [6], and much larger than that of the 35 ml volume of the cat stomach [7].

The most important digestive enzyme in the stomach is pepsin. In the sham fed dog, secretion of pepsin increases fourfold, whereas in the fed cat this increase is eightfold [8]. A low pH in the stomach facilitates the pre-digestive phase [9]. Sutton and Smith listed various physiologic factors that may influence stomach pH: age, anterior versus posterior, migrating motility complex, disease, stress, location within the stomach, and food [10]. In apparently fasted domestic cats, the stomach pH was reported as (mean ± SD, [range]): 2.5 ± 0.7 [1.5–3.7] [11]. The reported resting gastric pH in beagle dogs has ranged from 1 to 8 [12]. The higher pH can be a problem if this model is used to mimic human. Investigators have used various methods to raise or lower the resting pH in dogs [13]. However, some colonies of beagle dogs have been reported to have a sufficient basal acid secretion to result in a basal low pH. In these dogs, no modification of stomach pH was required [14].

As summarized in an earlier review [15], the migrating motility (or myo-electric) complex (MMC), or the "housekeeper wave," is a periodic gastrointestinal muscle contraction migration that begins in the stomach and duodenum and terminates in the ileum. It is characterized by a period of intense muscular activity following a period of relative quiescence. In the fasting dog, the mean periods of these cycles ranged from 90 to 134 min. As one wave terminates in the distal ileum, another wave would just begin in the stomach/duodenum [15]. During digestion, the pylorus shuts down to prevent gastric emptying of food from overloading the digestive capacity of the small intestine. The pyloris also regulates the size of particles emptied from the stomach. While larger particles are emptied during fasting [16], muscular contractions mix and grind the fed stomach contents until particles are <2 mm in diameter. For non-digestible particles >2 mm, they are retained by the stomach until all the digestible solids have emptied, and the interdigestive MMC has recovered.

Stress, body size, and meal composition (e.g., volume, density, viscosity, particle size, and calories) account for the wide variation in gastric emptying among subjects and studies. Unfortunately, all of these factors are rarely reported for the same study, making comparisons especially difficult [17, 18]. For example, the first-order gastric emptying half-life of *fluid* in the fasted canine stomach was reported as approximately 12 min [19]; the gastric half-emptying of *1.5 mm diameter barium-impregnated polyethylene spheres* (BIPS) in unfed cats was reported as about 24 min [20]. In this case, the twofold longer gastric emptying in "fasted" cats was probably due to the method used (liquid versus small particles), not species.

Wyse and coworkers summarized numerous gastric emptying studies in the cat and dog, measured by radioscintigraphy, radiography, and exhaled $^{13}CO_2$ [17]. Unfortunately, the summary did not include such critical details as the energy content of the food in the study, or the size of the animals tested. Multiple small meals are typical for the domestic cat, while dogs consume most of the daily energy requirements in one meal [8]. Comparing the gastric emptying in cats and dogs after similar meals may be academically interesting, but studies that compare the animal in their most likely domestic conditions are likely to be more informative for veterinarians and owners. In one study, cats were fed 78 kcal of their daily energy requirement with [^{13}C]octanoic acid, and the peak exhaled [$^{13}CO_2$] was detected about 1 h later [15, 21]. In another study, the half-emptying time of 1.5 mm diameter BIPS in cats fed 184 kcal of food (1/4th ration) was 6.43 ± 2.59 h [22]. Either the sieving by the pyloris resulted in retention of the BIPS longer than the smaller food particles on which the marker was adsorbed or doubling the energy content delayed gastric emptying or both.

The gastric emptying time for 1.5 mm BIPs in dogs fed one-quarter of their daily energy requirements (average 319 kcal) was also about 6 h [23]. However, the dogs in this study were crossbreeds ranging in weight from 13.5 to 37 kg. And while there was a wide range of variability, they reported that BIPS emptied faster in larger dogs ($p < 0.05$) [23]. In beagle dogs (10–13 kg), a small snack (210 kcal) resulted in a 4 h delay in the gastric emptying of a telemetric capsule (2.5 cm × 0.6 cm × 0.4 cm), while the full daily rations (1,470 kcal) resulted in a delay in the gastric emptying of

the capsule that exceeded 8 h [15]. The reader is encouraged to peruse the review by Martinez and Papich for a detailed description of the impact of the many physiologic factors on gastric emptying in the dog [18]. Please note that the same physiologic factors are likely to affect gastric emptying in the cat.

4.3 Small Intestine

In some respects, dogs and cats have similar digestive tracks. For example, the absorptive surface area is similar per cm of intestinal length for dog and cats (jejunum: 54 and 50 cm^2, ileum: 38 & 36 cm^2, respectively) [8]. However in other respects, they are different. The National Research Council of the National Academies reported that dogs with a body length of 0.75 m have an intestinal length averaging 4.5 m (small intestine 3.9 m, large intestine 0.6 m) [8]. The length of the small intestine measured in six dogs has also been reported as 336 ± 59 cm [24], and 4.14 m [6]. Cats with a body length of 0.5 m have an intestinal length averaging 2.1 m (small intestine 1.7 m, large intestine = 0.4 m) [8].

Divided into the duodenum, jejunum and ileum, the small intestine is where nutrients and pharmaceuticals are primarily absorbed. Water which enters from the stomach is rapidly absorbed in the duodenum at a rate approximating 12 ml/h/cm intestine [25]. The length of small intestine could easily absorb all water emptied from the stomach. However bicarbonate, water, bile acids, lecithin, and enzymes enter the duodenum in response to the presence of food. This provides the liquid needed to fluidize the chime for transit along the gastrointestinal tract. The duodenum is also the site of abundant active absorptive transport (e.g., calcium, iron, peptides, and penicillin). The jejunum is where emulsification optimizes the chance of fatty nutrients and drugs becoming absorbed, while bile salts are reabsorbed in the ileum.

Due to bicarbonate secretion, the intestinal pH is closer to neutral. Brosey reported that in the cat, the pH for the duodenum was 5.7 ± 0.5 [4.9–6.7], in the jejunum was 6.4 ± 0.5 [5.9–7.6], and in the ileum was 6.6 ± 0.8 [5.1–7.6] [11]. The overall mean pH in the dog small intestine has been reported to be about 7.3 ± 0.09 [12].

Dogs have been reported to secrete bile at a rate of 19–36 ml/day/kg, and the total amount of bile salts (TBS) reported to be secreted in the dog is 1.6–2.9 mmol/day/kg [26]. The biliary secretion rate in cats is reported to be approximately 1.26 ml/h per 3.8 kg cat, or approximately 8 ml/day/kg [27]. The TBS secreted by cats was calculated approximately 2 mmol/day/kg [28]. Therefore, while the volume of bile secreted is different, the total TBS secreted appears to be similar for both species. While the two cat studies were completed in different groups of animals, these data would suggest that cats would have roughly half the bile secretion rate as dogs.

The intestinal permeability of certain probes (3-OMethyl- D-glucose, D-Xylose, l-Rhamnose, 51Cr- EDTA and lactulose) was examined in cats, dogs, and humans. The permeability of the largest probe (lactulose) was greater in cats than in dogs [29]. The somewhat smaller probes were similarly absorbed in both animals. Chiou reported that some compounds were better absorbed in the dog than in human, and attributed this to an intestine that was more permeable in the dog than in the human [30]. Taken together, the data suggest that the cat intestine may be most permeable of the three species.

As summarized earlier in this review, in the fasting dog, the mean periods of the MMC cycles ranged from 90 to 134 min. As one wave terminates in the distal ileum, another wave would just begin in the stomach/duodenum [15]. However, the cat intestine does not exhibit a typical fasting pattern of motility [31].

In one study, the transit of barium sulfate through the small intestine (SIT) in an apparently fasted cat was reported as about 55 ± 10 min [31]. In another study, where cats were fed either a high or low fiber diet, the half-transit through the small intestine of 1.5 mm diameter BIPS was reported as 2.5 ± 2.1 h, $n = 10$ [32].

In the dog, the small intestinal transit (SIT) of the Heidelberg capsule (size is reported as similar to a No. 0 capsule; 21 mm × 7.3 mm × 7.6 mm) was reported as 1.9 h, with much variability (15–206 min) [19]. The large variability of SIT in the beagle has also been reported in a gamma scintigraphic/pharmacokinetic study. The SIT in beagle dogs of a tablet (10 mm × 6 mm) ranged from 23 to 390 min, with the SIT of four of six dogs under 72 min [15].

Species [33, 34] and breed [35] differences in intestinal metabolism can at best lead to a wide degree of variability in pharmaceutical pharmacokinetics, and at worse result in sub-efficacious treatment, or toxicity. The source of this metabolism includes both bacterial and epithelial. Recent publications have confirmed that bacterial phylotypes are numerous and can promote intestinal (and host) health, or contribute to disease (e.g., inflammatory bowel disease) [36]. Although no major review has been published on the subject of intestinal metabolism in cat or dog, reviews on specific compounds and canine intestinal enzymes have been published. Komura published a review on CYP3A and UGT substrates in dog intestines [37]. A species difference between cat and dog glucose metabolism in hepatic enzymes was reported by Tanaka [33]. But Chauret reported no significant differences between P450 hepatic isozyme activities in cat and dog [34].

The small intestine—in its simplest form—is a conduit that carries contents emptied from the stomach and delivers them to the large intestine. However, the variability in transit reported for the dog portrays the complexity that lies in this organ. When one considers the 4 m length of the dog small intestine, it is not surprising that there could be a wide variance in forward or retrograde propulsion, and mixing of the bowel contents, resulting in much more variability in SIT time in the dog than in the cat. However, the SIT in humans is believed to be consistently 3–3.5 h, regardless of meal condition. Compared to humans, the large variability in SIT observed in the dog is puzzling, and remains an area for study (Fig. 4.1).

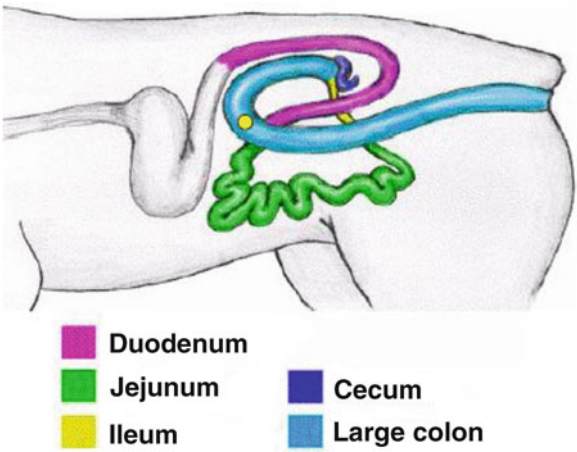

Fig. 4.1 The dog colon [41]

4.4 Large Intestine (Cecum and Colon)

The cecum is a dilated pouch, or "blind gut" that is located between then ileum and the colon, and takes the place of the appendix in the dog and cat. In the dog, the cecum does not participate in the MMC that moves along the ileum into colon [38]. The cecum in the cat is relatively smaller than in the dog, possibly as a consequence of its carnivorous diet. The primary functions of the large intestine are the reabsorption of water, and the formation and storage of feces. However, carbohydrates not absorbed in the small intestine are metabolized by bacteria in the colon into short chain fatty acids, which are absorbed in the large intestine [39, 40]. The cat or dog colon consists of the ascending, transverse and descending segments. The contents of the ascending colon are often somewhat watery, but water is efficiently absorbed, such that the feces are nearly dry by the time they are evacuated. Most of the time undigested food and particles spend in the cat or dog is defined by their residence in the colon [42]. In the cat, the transverse colon contributes to the storage of feces [8] (Fig. 4.2). Similar functions occur in the dogs, and there is additional storage capacity in the sigmoid colon.

The pH in the colon has been reported as 6.5 in the dog and 6.2 in the cat [44]. Because the amount of water is limited and variable, it is often difficult to measure the pH in the colon. No studies on the volume of water in the dog or cat colons were found. However, based on studies in humans [45], the volume of water in the cat or dog probably ranges from 10 to 50 ml.

The colon transit or residence time of radiolabeled food, particles, non-disintegrating tablets or capsules in healthy animals is usually measured by the administration time and the time of a bowel movement which produces the transit marker (dose-collection time). The colon residence time can be calculated by

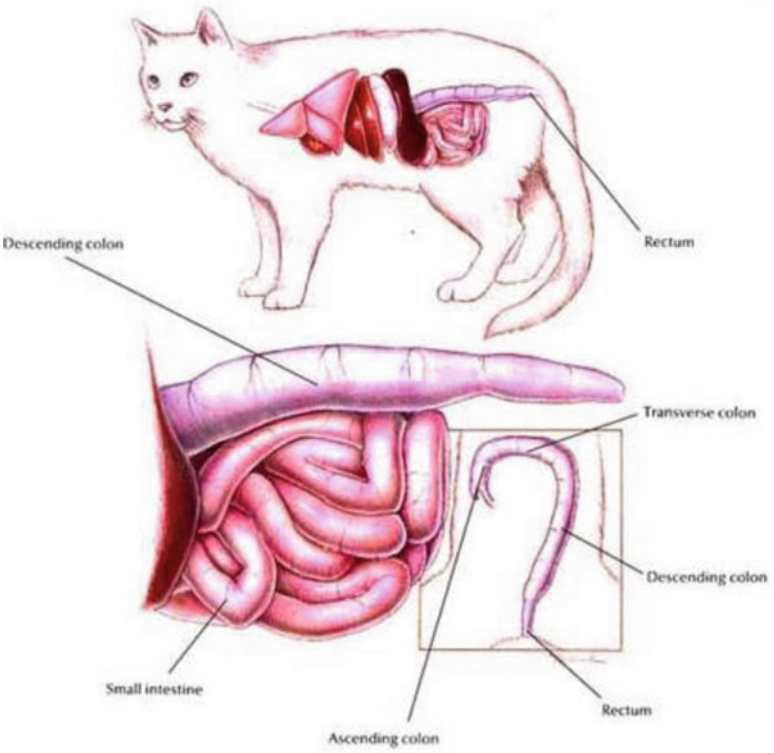

Fig. 4.2 The cat colon Anatomy [43]

subtracting the values for gastric emptying and small intestine transit from the dose-collection time. Peachey and coworkers reported that the total gastrointestinal tract transit time (dose-collection time) of chromium in young cats was (mean ± SD): 26.5 ± 4.8 h, and in older cats was 35.7 ± 14.1 h [21]. The cats were fasted overnight, and then administered the label in 50 g canned cat food (15% of the daily energy requirement). This difference in dose-to-collection time was not due to gastric emptying, and the authors suggested a reduced large intestinal muscle tone in older cats might explain the longer total transit time in that population [21]. Please note that feces may be stored in the descending colon for an extended period of time before defecating. Therefore, as reported for the beagle dog in an earlier review, this value is not comparable to the sum of separately measured gastric emptying, SIT and colon transit. For example, in the healthy adult cat, the half-emptying time of barium sulfate from the right to the left colon, was reported as only 60 ± 5 min [46].

In the dog, the colon transit of a non-disintegrating capsule (35 mm × 10 mm) was (7.1 ± 1.4 h, $n = 3$) [47]. However, the dose-to-collection time of tablets (10 mm × 6 mm) in the beagle dog ranged from 6 h to over 4 days (Fig. 4.3). Based on our experience of 180 tablets (4 tablets per dog), most of the variability in the mouth-collection time was due to storage in feces in the sigmoid colon [15].

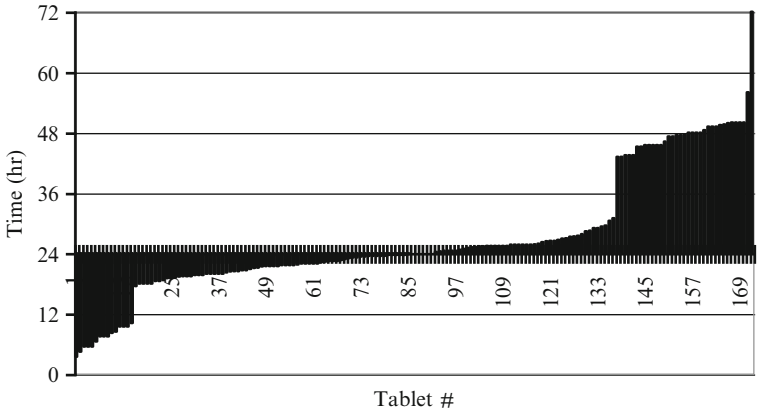

Fig. 4.3 The "dosing-to-collection" time of non-eroding tablets in the fasted beagle dog [15]

4.5 Concluding Remarks

Since most of the canine data cited in this chapter was collected in beagle and mongrel dogs, care must be taken in extrapolating to dogs outside the 10–15 kg weight range. Similarly, most of the feline data was collected in a domestic mix-breed cat with a typical weight of 4 kg. Regardless, one can make several broad generalizations from the published literature in this area (1) cats eat many smaller meals, whereas dogs eat more in one meal; (2) the gastric emptying of 1.5 mm diameter non-digestible particles is similar in both species; (3) the SIT is similar in both species, although much more variable in the dog; and (4) transit and environment of the colon of these animals are similar.

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Chapter 5
Physicochemical Principles of Controlled Release Veterinary Pharmaceuticals

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Abstract This chapter describes the basic physical and chemical properties relevant to drug formulation. It encompasses discussion on the active pharmaceutical ingredient, excipients, and final product and it highlights the importance of physical and chemical attributes of compounds in the selection of ingredients; development of dosage forms; and its significance in active and product assessment, characterization, performance, and quality.

5.1 Introduction

Knowledge of the physicochemical principles of pharmacy is the cornerstone of product research and development. An understanding of the physical and chemical attributes of the active pharmaceutical ingredient, excipients, processing method, and final product underpins the rational selection of ingredients and excipients, facilitates choice of manufacturing parameters, and underpins quality control tests.

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The physicochemical properties of a drug affect its ability to be formulated, dictate selection of excipients, influence the rational selection of incoming raw material tests to assure its suitability for use in the final product, and provide the rationale for its assessment and evaluation during development and post manufacture (i.e., the product quality attributes). The physicochemical properties of compounds play a role throughout the entire product development process from pre-formulation to final product quality control.

The basic physicochemical principles that underpin veterinary product development are the same as those applied to human product research and development. The difference resides in the outcome of the application of that science. Human and small animal (e.g., cats and dogs) product research and development tends to result in products that are similar in size, shape, and drug load. Tablets, capsules, small implants, microparticles, in situ forming gels, solutions, and suspensions are commonly found dosage forms in both these groups. In contrast, products for farmed animals (e.g., cattle, sheep, pigs) are very large, exhibit a variety of shapes not encountered in their human or small animal counterparts, and contain high amounts of active ingredient. In addition, controlled release products for farmed animals often contain drugs that do not possess the same "ideal characteristics" for selection into a controlled release product as those commonly cited in humans (Table 5.1). Nevertheless, the same physicochemical principles apply for all patient groups and form the basis of the science behind the final product.

In this chapter, we describe the physicochemical principles relevant to the development of veterinary pharmaceuticals and which support the rational selection, characterization and assessment of ingredients, their processing, and their evaluation.

5.2 States of Matter

The three states of matter are solid, liquid, and gas. In solids composed of organic molecules (such as drug substances), the individual molecules are held together in fixed positions by noncovalent forces or bonds. These forces include ionic interactions, hydrogen bonding, and van der Waals forces. In each case, there is an interaction between positively charged functional group and negatively charged functional group though one (or both) may be a charge that has been induced and did not exist to begin with. Molecular motion in a solid is limited to vibration and this gives a solid a fixed shape and volume (at a given temperature). In a liquid the molecules move more freely but still interact with one another; hence liquids have a fixed volume, but not a fixed shape. In a gas there is no force of attraction and gases have neither a fixed shape nor a fixed volume; a gas will expand to fill a given volume. Pressure is exerted by the molecules bouncing off the surfaces of the container. Pressure increases as more gas molecules are introduced.

Table 5.1 Ideal characteristics of drugs formulated into controlled release products and rationale for formulating a controlled release product for human and animal use

Element	Characteristic	Human	Small animal	Farmed animal
Drug characteristic	Dose size	Low dose	Low dose	Dose size limited only by the size of the final product
	Potency	Potent	Potent	Not a consideration
	Lipophilicity	Ideal hydrophilic/ lipophilic balance	Ideal hydrophilic/ lipophilic balance	Not a consideration
	Drug utilization	Improve drug utilization	Improve drug utilization	Not a consideration
Rationale	Compliance	Major reason to improve *patient* compliance	Major reason to improve *owner* compliance	Not a consideration
	Convenience	Major reason to encourage patient to adhere to dosage regime	Major reason to encourage owner to adhere to dosage regime	Improve profit through: Decreased contact time with animal Decreased time spent treating animals (herding, yarding, administration) Better utilization of farm staff time
	Cost	Not a major consideration	Not a major consideration	Decrease cost of treatment thereby increasing profit by improving cost:benefit ratio
	Stress	Not a consideration	Decrease stress to the owner	Decrease stress to animal and farmer Improve profit through reduction in stressed animals
	Reason for formulating into a controlled release product	Cure disease Improve health and welfare of the patient Prolong life	Cure disease Improve health and welfare of animal Prolong life	Improve profit—less time spent treating, lower cost of treatment, reduced stress to animals and less contact with animals

5.2.1 Crystallization

Crystallization is a change in state from a liquid to a solid. It encompasses freezing and precipitation and can be considered to be the opposite of melting or dissolution (Sect. 5.3). As there is an associated formation of intermolecular (noncovalent) bonds, energy is given off. During crystallization, the molecules reorientate and organize themselves from the relatively random structures that exist in the liquid form. This organized structure is called the crystal lattice and the repeating unit (imagine the pattern of a fabric) is called the unit cell [1]. So-called "long-range" order is a key component of crystalline materials; noncrystalline materials and liquids may contain "short-range" order, where small groups of molecules are arranged. When one melts a solid, energy has to be put into the system to break the bonds that make up the crystal lattice. The amount of energy required is termed the enthalpy of melt.

Crystallization is most commonly described as precipitation from a solution, though it can occur spontaneously from the amorphous state (Sect. 5.2.3). The production of a supersaturated solution, i.e., the state in which the liquid (solvent) contains more dissolved solids (solute) than can ordinarily be accommodated at a particular temperature, is generally considered to be the first stage in inducing crystallization. The formation of crystal nuclei (nucleation) can be split into two main forms. Primary crystallization occurs when there is a spontaneous coming together of a sufficient quantity of solute molecules that they do not redissolve. Secondary crystallization is the initiation of crystallization by the addition of crystals of solute. The active addition of crystals is called seeding. Impurities such as dust particles or the presence of solid objects (such as a stirrer or the wall of a vessel) may also act as seeds for crystallization. Whether this is grouped with primary or secondary crystallization is debatable, owing to the fact that experimentally it is difficult to distinguish between crystallization caused by impurities or solid objects and spontaneous crystallization.

5.2.2 Polymorphism

Diamond and graphite are both composed of carbon and are both solids under standard conditions. However they have very different properties; they look different; they have a different hardness; graphite conducts electricity whereas diamond does not. These differences exist because the carbon in diamond is organized into tetrahedral structures whereas in graphite there are sheets of hexagons. These different forms are called polymorphs.

Similarly, organic molecules including drugs can be organized into different structures which make different polymorphic forms [1–4]. Unlike carbon with its covalent bonds, these are noncovalent bonds. Since the noncovalent bonds of each polymorph are different, the polymorphs have different melting points and different

enthalpies of melt. Generally speaking polymorphic form is termed the stable form, and as its name suggests it is the most thermodynamically stable form [3]. In the case of carbon, the stable form is graphite. Other forms are termed metastable and given time will convert into the stable form. However, before you throw out your many diamond rings, the process of change from diamond to graphite requires high temperatures (around 1,000°C), a long period of time (millions of years), and a vacuum. For different polymorphs of organic molecules the process may be measured in years, months, or even minutes and hence a clear understanding of the different polymorphic forms and their physical stability is imperative during its formulation into a drug product. This is because changes in the polymorphic form may affect dissolution as will be explained in Sect. 5.3 and may also affect the apparent saturation solubility. It is possible to create different polymorphic forms during the crystallization process by altering the type of solvent, which alters the way in which the solvent and solute interact. It is also possible to create different polymorphic forms by changing the temperature at which crystallization is performed or by altering the rate at which supersaturation is achieved [5].

Pseudopolymorphs or solvates are substances such as drugs that, along with the molecule of interest, contain a solvent as part of the crystal lattice, for example, zinc sulfate monohydrate. Where the solvent is water, the term used is hydrate [5]. Pseudopolymorphs also differ physically, having different densities, melting points, and enthalpies of melt [4].

5.2.3 The Amorphous Form

Since the formation of a crystal is time-dependent, it is possible to create a solid material that lacks a crystal lattice and is essentially random in structure. Such materials are termed amorphous. In many ways, they can be considered to be very viscous liquids and they do not have a melting point. Most polymers are at least partially amorphous; their size limits their ability to organize themselves into repeating structures. Equally volcanic rocks produced by rapid cooling (such as obsidian) may lack a crystal lattice. Organic molecules like drugs can also exist in the amorphous form. The amorphous form can be subdivided into two states: the glassy state and the rubbery state. These names are relatively descriptive; in the glassy state the amorphous material is relatively rigid and brittle; molecules are in fixed positions and the material is relatively dense. When an amorphous material is heated, it passes through what is called the glass transition and the heat capacity (the amount of energy required to heat up a substance by 1°C) increases. This is due to a sudden increase in volume and freedom of molecular movement. The freedom of molecular movement in turn leads to the material becoming flexible and malleable; some materials may be shaped or stretched. This is the rubbery state and it is continuous with the liquid state, i.e., there are no further phase changes. It is in the rubbery state that glass can be blown. As with the metastable polymorphs the amorphous form is unstable and over time

a material will convert into a stable or metastable polymorphic form. Again, this process may take minutes or years depending on the material and the conditions under which the material is stored (this is why there are no obsidian tools predating the cretaceous period). Generally, it is the case that materials are more stable when stored below the glass transition temperature (T_g) [6]. But it has been suggested that for a drug substance, the material needs to be stored at 50°C below the T_g to guarantee stability for the shelf life of the product [7].

However, issues of stability are compounded by the presence of water acting as a plasticizer. Plasticizers are small molecules that are used to reduce the T_g of an amorphous material. The example most people are familiar with is the use of phthalates to reduce the T_g of hard plastics such as PVC (polyvinyl chloride) and make them more flexible [8]. In a similar manner, even a relatively small amount of water will have a deleterious effect on the physical stability of an amorphous material. Since the molecules in an amorphous material (even a glass) are quite widely spaced apart and amorphous drug may contain as much as 5–10% w/w water [4, 9].

5.3 Solubility and Dissolution

The solubility of a material is the maximum amount of solute (material) that can be dissolved in a solvent liquid at a given temperature/pressure. The simplest way to find the solubility is to add an excess of solid material and allow it to come to equilibrium. This will be a dynamic equilibrium and material will be constantly moving into and out of solution. It is possible to make supersaturated solutions. The easiest way is by heating the solvent. With increasing temperature, increasing solute may be dissolved. If the temperature is slowly reduced, then it is not necessarily the case that crystal nucleation will occur (at least initially) [2].

Dissolution is a change in state from a solid to liquid. In order for a drug substance to be absorbed and then exert an effect, it needs to be in solution [10, 11]. As with melting, when a solid is dissolved, the noncovalent bonds holding that solid together need to be broken. New bonds are then formed between the solvent molecules and the solute. Generally dissolution is endothermic; the energy created when the bonds are formed is lower than the energy required to break the crystal lattice of the solid (the bonds that need to be broken between solvent molecules to make a space for the solid should also be considered).

Since different polymorphic forms have different crystal lattice enthalpies, the dissolution rate (see Sect. 5.2.1) and apparent saturation solubility will vary from one polymorphic form to another. However as was stated, solubility is an equilibrium value, and there is only one true equilibrium between the solution phase and the most stable polymorphic or pseudopolymorphic form. So although initially there may appear to be an equilibrium at a higher concentration than exists between the stable form and the solution, over time the system will alter and a true equilibrium will exist.

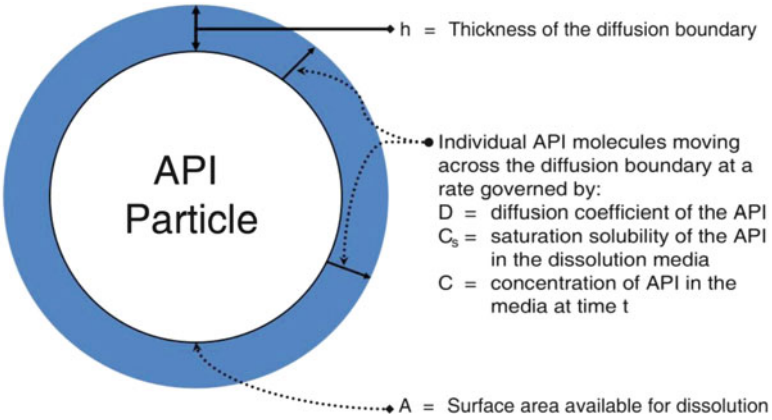

Fig. 5.1 The dissolution of a drug particle

5.3.1 Dissolution Rate

The modified Noyes–Whitney equation (5.1) describes the various contributors to dissolution rate for a solid [12–14]. The model (Fig. 5.1) assumes that dissolution proceeds as follows. At the surface of a particle, there is an interface where a saturated solution exists. Molecules of the solvent passively diffuse down a concentration gradient that exists in the boundary surrounding the particle and into the bulk solution.

Modified Noyes–Whitney equation

$$\mathrm{d}C/\mathrm{d}t = (AD[C_s - C_t])/h, \tag{5.1}$$

where $\mathrm{d}C/\mathrm{d}t$ = rate of dissolution; A = surface area available for dissolution; D = diffusion coefficient of the compound; C_s = solubility of the compound in the dissolution media; C_t = concentration of compound in the media at time t; h = thickness of diffusion boundary.

In the Noyes–Whitney equation, there are two main variables that may be altered: surface area and saturation solubility. Before discussing how these may be altered, the other variables will be excluded. The diffusion coefficient is fixed for a drug in a given solvent. The thickness of the diffusion boundary may only be changed by agitating the solution (hence you stir your coffee or tea).

If we take the paradigm of a solid dosage form or suspension that is taken orally (most common for man and domestic pets) or a device designed to be retained in the stomach of a ruminant beast (rumen), the material dissolves in the GI tract and one has no control over the volume of liquid contained within. Indeed, what is called "sink conditions" may exist (Fig. 5.2). The Noyes–Whitney equation relates to a

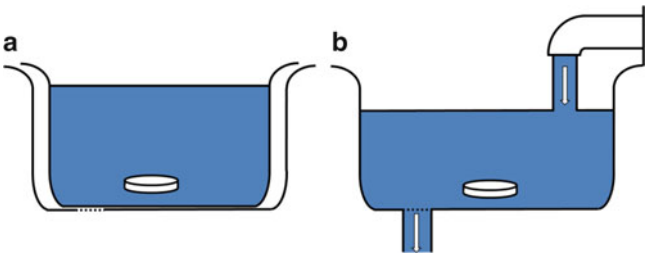

Fig. 5.2 Dissolution in a fixed volume (**a**) versus dissolution under sink conditions (**b**)

fixed volume, as per a sink containing a washing-up bowl that has been filled with water (Fig. 5.2a); as material dissolves in the water, the concentration increases [15]. This results in the concentration gradient that exists in the diffusion boundary becoming progressively shallower. Therefore, dissolution slows down. In Fig. 5.2b, the sink contains a similar volume of water, but the tap is turned on and the plug has been removed; there is a constant turnover of water and so the concentration does not increase and dissolution is not affected.

In the GIT, there is a constant turnover of material; all manner of materials are secreted and absorbed; water is continuously being excreted and absorbed. So sink conditions better reflect the situation found *in vivo*.

The surface area can be increased to improve dissolution rate or limited to provide slow release. A large number of small particles have a greater surface area than a small number of bigger particles; therefore, controlling particle size is important (Sect. 5.4). Different shapes of particle have different surface area to volume ratios (SA:V ratio). A sphere has the lowest SA:V ratio and as shape becomes less spherical the SA:V ratio increases. Particles that are rough or have pores have a higher SA:V ratio than those that are smooth. Most solid dosage forms are manufactured using a degree of compression, so the particles are packed together reducing the surface area. Equally, poorly soluble materials will tend to clump together or be excluded by a solvent. Excipients known as disintegrants can be incorporated into a formulation to counteract these issues [16]. A disintegrant is a hydrophilic material that may or may not be water-soluble but that swells on contact with water; in doing so it pushes apart the formulation and ensures that the surface area is as large as possible. If one desires to retard dissolution, hydrophobic excipients may be used. These reduce the wettability of the formulation; they limit the interaction between the solvent and the formulation, in effect reducing the surface area.

Though the true saturation solubility of a compound is fixed, there are a number of ways of changing the apparent saturation solubility. Changing the polymorphic form or using the amorphous form of a drug or excipient may improve the dissolution rate, as long as the form is stable during the lifetime of the product, and as long as when the dosage form is administered the drug/excipient does not recrystallize [3].

A drug can be chemically modified to produce a more soluble prodrug, where the term prodrug is defined as “a bio-reversible chemical derivative of an active parent drug” [17]. In its strictest sense this can include salt forms and complexes that

are designed to easily disassociate from the drug (Sect. 5.4.1). Regardless of the mechanism of modification, the aim is to induce a higher solubility by reducing the lattice enthalpy of a drug or by introducing an ionizable group. There are a number of issues. In an ideal situation, the prodrug in solution would be degraded to drug and progroup. If this does not occur, passive absorption (which is dependent on the lipophilicity of the drug) may be limited by the hydrophilic progroup or the distribution of the drug around the body.

Solubilization (the use of an inert molecule to improve the solubility of a drug molecule) can improve drug solubility and therefore dissolution. Cyclodextrins are amphoteric molecules that complex with one or more drug molecules and on dissolution this complex should separate [18]. Micelles which self-assemble in solution from amphiphilic block copolymers have a hydrophilic exterior and a hydrophobic interior can also solubilize poorly water-soluble drugs [19].

5.4 Particle Size and Morphology

Particle size is an important characteristic of both a drug substance and the excipients used in a formulation. Particle size correlates with dissolution rate (Sect. 5.4.2), which has an effect on absorption and bioavailability [13, 14]. The particle size of a powder is not a single number, it is distribution. Ideally, the size distribution is narrow and with a unimodal distribution. Broad or bimodal distributions or a very small particle size can all create issues within a formulation [20].

If we take a batch of capsules as an example of a dosage form, each capsule should contain the same amount of active ingredient (and probably excipients). The most obvious way of ensuring this would be to ensure that the content of each capsule weighs the same. However, this is not really feasible and capsules (and most other dosage forms) are not filled by weight. In the case of a capsule, the simplest way is to ensure that the body of the capsule is completely filled. This requires a number of assumptions. The first is that the particles are all the same density. If they all have a similar composition, this is likely to be the case. The second is that there will be the same volume of particle and the same void volume (spaces in between the particles). If the particle size is relatively uniform, then this is likely to be the case. However, if there is a broad particle size distribution, then small particles will tend to settle to the bottom of the powder bed and if samples are taken from the top, middle, and bottom of the powder bed, one will find that the bottom of the powder bed is denser than the top (Fig. 5.3).

5.4.1 Powder Flow

Powder flow is an important factor in the creation of uniform dosage forms. Good particle flow ensures even filling of capsule bodies, tablet dies, and so forth. When

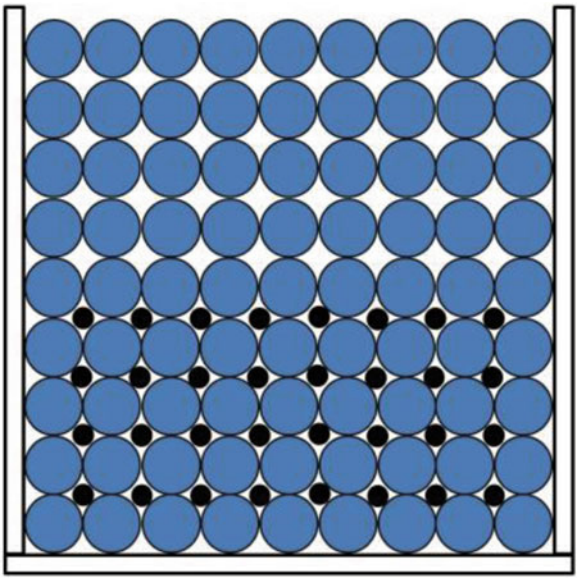

Fig. 5.3 A heavily stylized example of a powder bed containing two different sizes of particle. The smaller particles tend to fill the voids between the larger particles and pack more tightly together and thus the lower half of the powder bed is denser than the upper half

particles display poor flow this is observed as miss-filling; powder either remains static and does not flow or there are sudden movements of powder. There are a number of methods for determining the flow properties of powders. Angle of repose is possibly the most common method. A funnel is clamped above a circular plinth and the base of the funnel is blocked. The funnel is then filled with the powder in question and the block is removed. A heap of powder will form on the plinth and the angle that the powder heap makes with the horizontal is calculated, this is called the angle of repose and the larger the angle the more poor the flowing of the powder. The typical limit for manufacture is about 50°; above this angle some kind of improvement is required. A further test is the compressibility index [21]. Powder is carefully poured into a cylinder mounted on a base that taps the cylinder vertically. The start volume is recorded as is the final volume, following 100 taps. The volume at the start and the volume at the end are used to calculate the compressibility index (5.2) or the Hausner ratio (5.3).

Compressibility index

$$\text{Compressibility index } (\%) = 100 \times (V_0 - V_f) / V_0 \tag{5.2}$$

Hausner ratio

$$\text{Hausner ratio} = V_0 / V_f \tag{5.3}$$

Powders that display good flow will naturally settle prior to the start of the tapping and so little difference is seen. Particles that display poor flow will need to be tapped to settle. A compressibility index of up to about 25% is usually acceptable

(Hausner ratio = 1.34). The speed of consolidation may also be of importance as some powders may compress relatively rapidly, implying better flowability than would be inferred from the compressibility index.

Most flow issues are due to the particles being small, or to the morphology of the particles. Crystal habit is the term used to describe the shape of crystalline materials as they would appear under a microscope. As mentioned previously, crystal habit effects dissolution rate since different shapes have different SA:V ratios. Different shapes also flow differently. A sphere is (as would be expected) the most free flowing shape. A rod is probably the least free flowing shape that is commonly found. The shape of a crystal may be altered by altering the media from which the crystal is grown [3].

If a powder flows poorly, the main way of improving flow is by granulating the powder [22]. Granulation is a process that occurs when a number of particles are stuck together with a binding agent such as polyvinylpyrrolidone. Although one may chose to bind only the drug particles together, it is more common to include other excipients in the granulation process alongside the binder. A disintegrant is often added to break up the particles on delivery (Sect. 5.3.1). A diluent or bulking agent is often added to increase mass [16]. Such a material is also likely to display good flow properties and be easily compressible, if such properties are required. Granulation is a way of gaining the benefit of a larger particle size (improved flow properties), whilst retaining the benefits of a smaller particle (improved dissolution properties).

5.4.2 Particle Size Reduction (Comminution) [23, 24]

A number of reasons that one may wish to reduce particle size have already been suggested, the main one being to improve flow. Generally, some kind of mill is used. Different mills are generally used to generate different particle size distributions.

A cutter mill uses two sets of blades, one stationary set encasing a rotating set. The two pass closely (1–2 mm apart) and fracture the powder. The typical particle size produced is usually measured in mm although particles may be down to 100 μm in diameter.

A ball mill consists of a hollow tube sealed at one end, into which the powder and some ball bearings are added. The open end is then sealed and the tube is placed on a pair of rollers and rotated. As the tube rotates, the powder and ball bearings cascade and collide with one another. Ball mills tend to produce particles 1–100 μm in diameter. Particles are made smaller and more spherical due to the collisions.

An air jet or fluid energy mill uses a current of air which is counterbalanced by a combination of centrifugal force and gravity. The particles therefore rise and fall, colliding into one another. A wide range of particle sizes can be produced with a minimum of around 1 μm in diameter.

One major issue with milling is that it may induce surface disorder (amorphous material) due to the energy imparted during collisions. This surface disorder may be

sufficient to alter the dissolution rate of the formulation and it would be very difficult to distinguish between an improvement of dissolution rate due to surface disorder and one due to particle size reduction. However whilst the latter would be a permanent improvement, surface disorder, which is effectively amorphous material, would change form over time and recrystallize leading to changes in dissolution and other properties [25]. It is also possible for the energy involved in milling to cause a change from one polymorphic form to another. This is particularly problematic when a metastable form converts to a more stable form as a reduction in dissolution rate may be observed.

5.4.3 Particle Size Analysis [20, 23]

Given that particle size has an impact on dissolution behavior, it is important to be able to describe the particle size distribution of a powder. There are a number of methods for analyzing particle size. Generally one is looking at the particle size distribution, since a population of particles is not homogenous. Typically, one will identify one of a variety of means (as will be explained), the median, the mode, and the inter-quartile range.

One of the simplest methods to use is sieve-shaking. A stack of sieves is placed on a platform that is capable of vibrating. The sieve size is largest at the top and smallest at the bottom and follows a $\sqrt{2}$ progression. This allows the resolution from one sieve to the next to remain consistent. The content of each sieve is weighed and used to calculate the particle size distribution or spread and relevant averages.

Microscopy may be used to calculate particle size distributions; however, this is a relatively slow and tedious method. Other techniques include electrical zone sensing which sizes particles based on the degree of electrical resistance they produce between two electrodes. Laser light scattering can also be used to size particles as can the sedimentation rate through an inert liquid.

5.5 Solutions [26]

A solution is generally considered to exist when individual molecules of a solute (such as a drug) are dissolved in a solvent. Solvents can be divided into those that are predominantly apolar and those that are polar such as water. Polar solvents can be further subdivided into those that may donate a proton (such as ethanol) and those that are unable to do so such as dimethyl sulfoxide. It is generally the case that like dissolves like, and so polar compounds tend to dissolve in polar solvents and nonpolar compounds tend to dissolve in nonpolar solvents.

Water is a polar solvent and it dissolves polar molecules. A nonpolar solvent like hexane will tend to dissolve nonpolar molecules. This is because in order to be dissolved the attractions between the solute molecules must be overcome by the attraction

between solvent and solute molecules. It is typically said that like tends to dissolve like, hence polar solvents dissolving polar solutes. However, it is also the case that polar interactions tend to be stronger than nonpolar interactions and so regions of polarity on a relatively nonpolar molecule may be sufficient. Some molecules act as surfactants; they have a region that is polar and a region that is nonpolar. Phospholipids that make up the lipid bilayer of cellular organisms are surfactants. Proteins tend to have hydrophobic and hydrophilic regions and act as surfactants. The ionic and nonionic surfactants in detergents and washing-up liquid are present so that we might clean grease and fat materials from clothes and plates. Surfactants will be covered in more detail in Sect. 5.7.

5.5.1 Ionization and pK_a [26–28]

Water is polar because the oxygen atom is more electronegative than the hydrogen atoms and therefore the shared elections are pulled closer to the oxygen atom. The oxygen therefore carries a slightly negative charge, and the two hydrogen atoms, a slightly positive charge. Sodium chloride (NaCl) dissolves in water because it freely disassociates into positively charged sodium ions, which are surrounded by slightly negative oxygen atoms and negatively charged chloride ions which are surrounded by slightly positive hydrogen atoms.

pH is a logarithmic scale that approximately equates to the negative logarithm of the concentration of protons (or hydrogen ions) in solution. Water, which has a pH of 7.0, disassociates to give positively charged hydrogen ions (H^+) and negatively charged hydroxide ions (OH^-). Adding an acid such as hydrochloric acid (HCl) decreases the pH and makes a solution more acidic as the hydrogen and chloride disassociate. To be truly precise, hydrogen ions are taken up by water molecules to give H_3O^+. Carbon dioxide (CO_2) acts as an acid as it reacts with water to form bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) liberating one or two hydrogen ions. Adding a base such as sodium hydroxide (NaOH) causes the pH to increase as the number of hydrogen ions is reduced. This is because the hydroxide (OH^-) ions take up a proton to give water. Equally and more commonly, a basic substance like ammonia (NH_3) will in solution take up a proton and become charged (NH_4^+; ammonium). This is the Lowry–Brønsted theory of acids and bases. Whilst it is not the case that a molecule must carry a charge or become charged in order to dissolve in water. Sucrose, for example, dissolves because, like water, it has hydroxyl groups which again carry a slight charge.

A significant number of drug substances are either weak acids or weak bases (or possibly both). However, unlike the salts mentioned above only a percentage of these molecules become charged in solution. The aqueous solubility of a weak acid or base is dependent on the degree ionization and this in turn is dependent on the pH of the solution and the properties of the molecule. The acid disassociation

constant (K_a) is used to determine the degree of ionization at a given pH (Equation 5.4 and 5.5.

Equation 5.4 gives the relationship between the log acid disassociation constant (pK_a), pH, and the concentration of uncharged (AH) and charged (A^-) species of an acid where square brackets denote concentration:

$$pK_a = pH + \log_{10}([AH]/[A^-]). \tag{5.4}$$

Equation 5.5 gives the relationship between the log acid disassociation constant (pK_a), pH, and the concentration of uncharged (B) and charged (BH^+) species of a base where square brackets denote concentration:

$$pK_a = pH + \log_{10}([BH^+]/[B]). \tag{5.5}$$

Equation 5.6 gives the relationship between the overall solubility at a given pH (S), the solubility of the uncharged moiety (S_0; a constant across pH), pH, and p*K*a for a weak acid.

$$pH = pK_a + \log_{10}(\{S - S_0\}/S_0). \tag{5.6}$$

5.5.2 *Partition Coefficient (and Distribution Coefficient)*

In order for a drug to reach systemic circulation or a site of action, it is likely that it will need to be absorbed across a cell membrane. All cell membranes are composed of a phospholipid bilayer. The phospholipids making up the bilayer spontaneously organize themselves so that the hydrophilic phosphate heads face outwards and the diglyceride "tails" face inwards. In order for a drug to enter a cell or be absorbed it will need to cross this boundary. Similarly, in order to be excreted it will need to cross this boundary, usually via transcellular passive diffusion. A drug therefore needs to be lipophilic in order to cross the bilayer. However, it is generally the case that only individual molecules are absorbed; therefore the drug needs to be in solution first, usually in water. It is therefore also broadly true that a drug will need to possess a degree of hydrophilicity.

Whilst drug solubility in a variety of solvents is important, it is also helpful to know how a drug partitions between two solvents. For example, oil and water are immiscible; they form separate layers. If we introduce a molecule like benzene into this system some will end up dissolved in water but the vast majority will end up in the oil phase. A molecule like glucose, with its many hydroxyl groups, will largely reside in the water phase (Fig. 5.4). If we divide the concentration in the oil by the concentration in the water, we get the partition coefficient (P). Since P can vary widely, it is most commonly converted into the log P by taking the logarithm to base ten ($\log_{10}$).

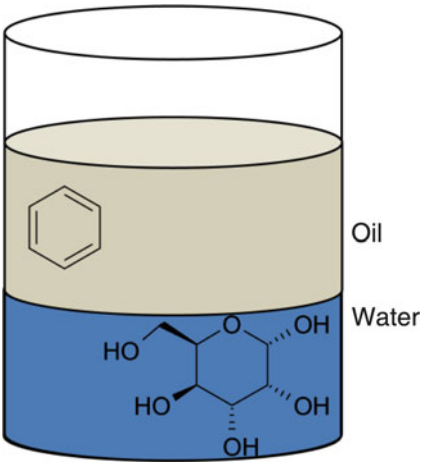

Fig. 5.4 Benzene and glucose partitioning in oil and water

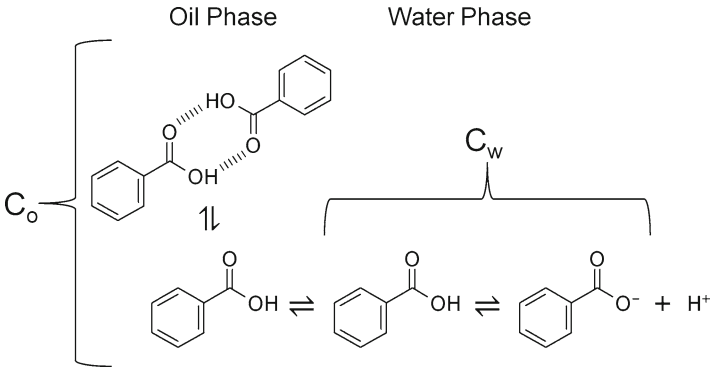

Fig. 5.5 The partition of benzoic acid between oil and water

The situation becomes more complex when a weak acid or base becomes involved. Take the partitioning of benzoic acid between oil and water (Fig. 5.5). In the oil phase, the benzoic acid may exist either as individual molecules or as dimers held together by hydrogen bonding between the carboxylic acid groups. This is a constant and so is of little concern; the concentration in the oil phase can still be calculated. In contrast, in the water phase benzoic acid may become ionized with the degree of ionization being dependent on the pH of the solution. Therefore if one alters the pH, more of the benzoic acid will partition into the water phase and what is observed as the partition coefficient will alter. This observed value is called the

distribution coefficient (D or log D) and is usually stated for a particular pH [29]. The log D at pH 7.4 is quite commonly quoted as this is the approximate plasma pH of man. Furthermore, a number of mammals such as cats, dogs, cattle, and horses have a broadly similar plasma pH so the value may have merit in veterinary formulation.

If the pK_a is known, log D can be converted to log P using one of the two equations (5.7 and 5.8). In practical terms, for an acidic drug log D will be effectively the same as log P where the pH of the aqueous media is more than 2 less than the pK_a. For a basic drug, the log D and log P will be the same if the pH is more than 2 more than the pK_a as in each case the drug will be effectively unionized (99%).

Equation (5.7) gives the relationship between log D at a given pH, pK_a, and log P governing acids:

$$\log D_{(\mathrm{pH})} = \log P - \log\{1 + 10^{(\mathrm{pH} - \mathrm{p}K_a)}\}. \tag{5.7}$$

Equation (5.8) gives the relationship between log D at a given pH, pK_a, and log P governing bases:

$$\log D_{(\mathrm{pH})} = \log P - \log\{1 + 10^{(\mathrm{p}K_a - \mathrm{pH})}\}. \tag{5.8}$$

Typically, log P/log D measurements are performed using 1-octanol and water. 1-octanol exhibits hydrogen bonding acceptor and donor properties typical of many biological membranes and its partial polarity allows the inclusion of water, which is also a feature of biological lipid membranes and leads to a more complex partitioning behavior than would a less polar, essentially anhydrous solvent [30, 31]. Three other solvents have been further suggested: propylene glycol dipelargonate (PGDP), which acts as a proton bond acceptor; chloroform which acts as a proton bond donor; and cyclohexane which is essentially inert [32, 33]. Isobutanol has been used to characterize the binding of drugs to serum proteins as drugs tend to bind through lipophilic interactions, therefore leading to partitioning-like behavior between protein and solution [34].

Low log P (<0) values tend to lend themselves to injectable dosage forms as these drugs will display poor absorption. Intermediate (0–3) values are helpful for oral drug delivery for humans and small animals as the drug will need to dissolve in the gastrointestinal tract and then be absorbed. Higher values (2–4) are helpful for transdermal drug delivery since the outermost layer of the mammalian epidermis, the stratum corneum, is composed of what are essentially dried, dead cells. If the log P is very high (>4) there is a risk that if the drug reaches systemic circulation the drug will tend to build up in fatty tissues and may be toxic [35].

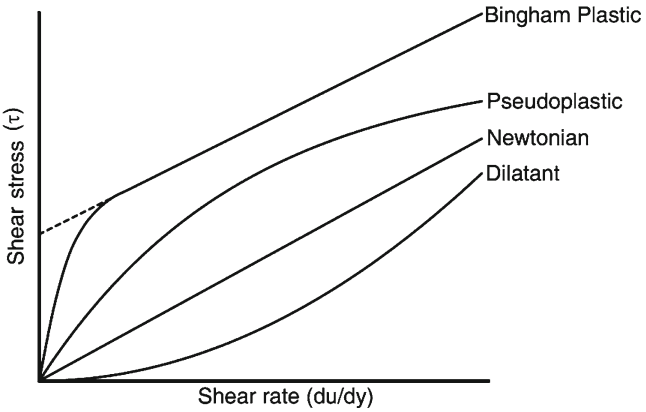

Fig. 5.6 Flow curves (rheograms) for different fluid behaviors

5.6 Rheology and the Flow Properties of Liquids

Viscosity is the thickness of a liquid; its resistant to flow; the antonym being fluidity. Rheology covers both flow and the deformation properties of liquids. These are important properties with implications for dissolution and diffusion of solid particles in liquids as well as for the quality assurance of semi-solid and liquid formulations. Understanding the viscosity of liquid and semi-solid dosage forms is important as it impacts on a number of quality aspects of pharmaceuticals including drug release and stability.

5.6.1 Newtonian Fluids [36]

Newtonian fluids are fluids that exhibit a rate of flow (du/dy also called shear rate) that is proportional to the shear stress (τ) and the two are related via the dynamic viscosity (μ). All gases and simple liquids like water, ethanol, olive oil, glycerol, or molten metals follow Newton's law of viscosity (5.9):

$$\tau = \mu(du/dy). \tag{5.9}$$

The measurement of viscosity is most often performed using one of the two methods. A falling-sphere viscometer uses Stokes' law (Sect. 5.6) to calculate the viscosity from the terminal velocity of a sphere moving through the liquid. Another option is to use a capillary viscometer where a fluid is pulled through a capillary tube usually by gravity and the rate at which the fluid moves can be related to the viscosity.

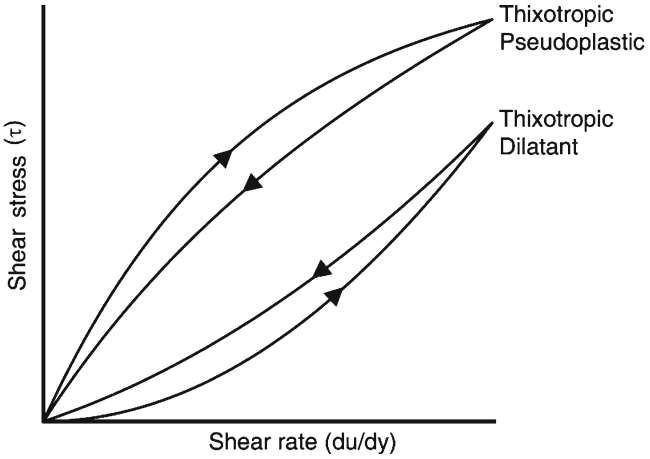

Fig. 5.7 Rheograms produced by thixotropic pseudoplastic and dilatant materials

5.6.2 Non-Newtonian Fluids [36, 37]

Non-Newtonian fluids are those that deviate from the equation outlined above and are shown in Fig. 5.6. Newtonian flow is depicted as a straight line passing through the origin. Bingham (plastic) flow requires a minimum shear stress to be achieved before the material begins to flow, below which the material will not flow and instead behaves as an elastic solid. Its flow properties can be described using a modification of the Newtonian equation (5.10), which includes the Bingham yield stress (τ_y).

The flow of a Bingham plastic:

$$\tau = \tau_y + \mu(du/dy). \tag{5.10}$$

Equation 5.10 implies that the flow of a Bingham plastic can be depicted by a straight line rheogram. However, flow will occur below the Bingham yield stress prior to the straight line that makes up most of the shear rate. Some semisolids tend to behave as Bingham plastics with common examples being toothpaste and mayonnaise.

In contrast to mayonnaise, ketchup displays pseudoplastic flow, as do many aqueous pharmaceutical formulations that contain polymers such as polyvinylpyrrolidone, cellulose, or polyacrylic acid. Pseudoplastic flow differs from both Newtonian and Bingham plastic in that the rate of flow cannot be described by a straight line. As the shear rate increases the rate of increase in shear stress slows, or, as you stir more quickly, the viscosity goes down. One reason for this is that the shearing acts to untangle the polymer strands and aligns them in the direction of the shear, increasingly counteracting the Brownian motion that causes them to entangle.

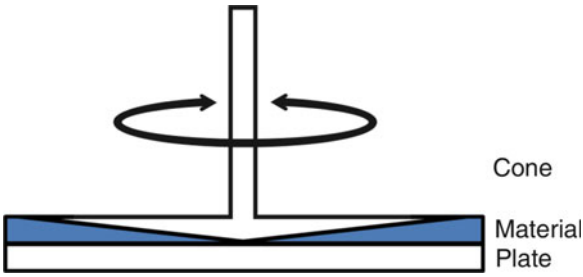

Fig. 5.8 A cone viscometer

Dilatant flow can be considered to be the opposite of pseudoplastic flow; as the shear rate increases the viscosity increases. This is most commonly seen with suspensions that contain deflocculated particles (Sect. 5.6). Flocculated suspensions tend to display plastic or pseudoplastic behaviors.

The length of time for which a non-Newtonian liquid is subjected to shear may also impact on the viscosity. When a pseudoplastic material is subjected to shear, viscosity decreases as has been noted. When the shear ceases and even where the change that has led to this decrease in viscosity is reversible, the material is unlikely to return to its initial condition instantly. Such materials are termed thixotropic materials. Both pseudoplastic and dilatant materials may display thixotropic behavior. If a pseudoplastic or dilatant material is exposed to increasing and then decreasing shear rates, the curve of the rheogram will differ between the two stages (Fig. 5.7).

For non-Newtonian liquids, it is important not only to measure viscosity over a wide range of shear rates but also to have a firm understanding of the history of the fluid. The most common method of measurement is to use a rotational viscometer. A rotational viscometer uses a cone or cylinder that rotates in or on the surface of the test liquid. An example of a cone viscometer is presented in Fig. 5.8. A stationary plate has a rotating cone above and the cone just touches the plate; the gap between the plate and cone is filled with material. This gap is typically much smaller than depicted as the angle between cone and plate can be less than 1°. Torque transmitted to this cone in contact with the liquid is measured from which the viscosity is obtained. The cone can continuously rotate in one direction or can oscillate back and forth [38].

5.7 Colloids and Suspensions

Disperse systems are systems that consist of two phases. A suspension consists of a liquid continuous phase and solid particles that make up the disperse phase. An emulsion consists of two liquid phases. The term colloid is normal, used to describe systems where the dispersed (colloidal) particles are between 1 nm and 1 μm in diameter, although larger particle sizes may on occasion display similar behaviors. Where the continuous phase is water, the term hydrocolloid is used.

Milk is a hydrocolloid; fats make up the colloidal particles of the dispersed phase whilst water makes up the continuous phase. Phospholipids and proteins act as surfactants, stabilizing the colloidal system. However, given 12–24 h milk separates into two layers: cream (containing a high concentration of fat) and milk. The process of homogenization is used to stabilize the milk in a single layer. There are a number of pharmaceutical colloidal systems including suspensions, creams, and aerosols all of which, like milk, may be unstable and may require homogenization.

5.7.1 Colloidal Systems [39, 40]

Colloidal systems can be separated into two main types: those that are predominantly formed due to exclusion from the continuous phase (lyophobic colloids) and those that form thanks to interactions with the continuous phase and self-assemble (lyophilic colloids).

A fine suspension of a poorly soluble hydrophobic drug in water would be an example of a lyophobic system as the water and the drug are repulsed by each other. Moreover, if two particles of drug come together there is no incentive for them to separate again. This is called flocculation. Synonyms include agglomeration and aggregation, with the latter tending to be applied to proteins in solution. Coalescence is the name for the coming together of liquid particles. Ostwald ripening is a second phenomenon that results in the formation of larger particles. The drug in our suspension in water is actually in equilibrium with the solution form. This is a dynamic equilibrium and drug molecules are constantly dissolving and precipitating. Larger particles tend to grow and smaller particles shrink because the larger particles are thermodynamically more stable; the surface area to volume ratio is lower and since the drug and water are mutually repulsed the smallest surface area will be the most stable form.

Strictly speaking, lyophilic colloids include solutions; however, only more complex systems will be dealt with in this section. Surfactants form a subset of lyophilic colloids called association colloids. If one dissolves a surfactant in water, at low concentrations lone molecules will exist in solution; the simple lyophilic colloid. At higher concentrations above the critical micelle concentration (CMC), the surfactants (50+ monomers) will aggregate and self-organize into a spherical shape; the hydrophobic/lyophilic tails will be internalized and hydrophilic heads will face outwards. This is the association colloids. It is the formation of micelles by detergents that dissolves fats to clean clothes. Micelles and other vesicles such as liposomes (which have a bilayer similar to a cell) have attracted considerable interest as drug delivery systems as a hydrophobic drug may, in effect, be dissolved in the center of the micelle or in the bilayer of a liposome.

Colloidal particles move in a random erratic motion named Brownian motion. As a result, the particles will diffuse spontaneously from an area of high concentration to an area of lower concentration. This diffusion will be in line with Fick's first law (5.11):

$$dm/dt = -DA(dC/dx), \tag{5.11}$$

where dm is the mass of substance that diffuses over time (dt), D is the diffusion coefficient, A is the area over which diffusion is taking place, and dC/dx is the concentration gradient for diffusion.

The rate of sedimentation of particles greater in size than 0.5 μm is given by Stokes' law (5.12). Particles smaller than 0.5 μm will tend to be more affected by Brownian motion:

$$v_s = 2/9\{\rho_p - \rho_f)/\mu\}gR^2, \tag{5.12}$$

where v_s is the particles' settling velocity (m/s), ρ_p and ρ_f are the mass density of the particles and the fluid, respectively (kg/m^3), μ is the dynamic viscosity (N s/m^2), R is the radius (m), and g is the gravitational acceleration (m/s^2).

5.7.2 *Emulsions [40]*

An emulsion consists of two immiscible liquids, typically an oil and water, in which one is dispersed finely through the other. They are generally considered to be lyophobic systems and over time the disperse phase will coalesce. The term for irreversible coalescence is cracking. Emulsions tend to separate by creaming, so called because milk that is not homogenized separates into milk and cream, the latter being a layer that is oil rich. Whilst Stokes' law (5.12) is usually used to assess the rate of sedimentation it can also be applied to the process of creaming. Creaming is generally reversible by mixing or shaking. The rate of creaming can be slowed by reducing the radius of the disperse phase droplets; this occurs when a homogenizer is used.

The main way in which an emulsion is stabilized is the use of surfactants which act as emulsifying agents. Mayonnaise is an emulsion of oil and vinegar stabilized by the egg proteins and a phospholipid called lecithin. Pharmaceutical surfactants include those that have an anionic (negatively charged) head-group and tend to be toxic, so are only used for topical applications: those that are cationic and those that are nonionic. Combinations of cationic and nonionic surfactants tend to be used for preparations that are to be administered internally to an animal. Amphoteric surfactants such as the aforementioned lecithin can also be used particularly for intravenous delivery of fat emulsions.

Surfactants are selected based on their hydrophile–lipophile balance (HLB). Each nonaqueous component (oil/fat/wax) of an emulsion is assigned a HLB value based on relative proportions of hydrophilic and lipophilic parts of the molecule.

A weighed average HLB for these components is calculated. This value is then matched by the HLB for the surfactants.

Externally applied emulsions tend to be divided into ointments, creams, and gels. Ointments, which are usually quite oily occlusive semisolid formulations, are useful for local delivery of actives rather than transdermal delivery. Creams tend to be oil in water emulsions and as such require a preservative of some description. They tend to spread more easily and are better for systemic delivery, they are considerably less occlusive and so do not hydrate the skin as well as ointments do. Gels are semisolids that consist of a liquid (generally water) that is thickened by a (hydrophilic) polymer.

5.7.3 Suspensions [40, 41]

Suspensions are subdivided into colloidal dispersions (particle size <1 μm) and coarse suspensions (particle size >1 μm). Suspensions are often produced instead of solutions due to the difficulty of producing a suitable vehicle that will dissolve the active ingredient. This is why neomycin is formulated as an otic suspension. Suspensions are also useful as oral delivery systems for domestic pets and to treat bloat and deliver anthelmintic drugs to ruminant animals.

Particle size is important for stability as noted in Sect. 5.4. Large particles (>5 μm) may also cause irritation and myotoxicity when injected or delivered to the eye. However, as noted in Sect. 5.3.1, particle size may be used to slow dissolution rate which in turn may slow absorption into the bloodstream. It is therefore possible to create slow release parenteral formulations by increasing particle size. Oral suspensions are common, as are externally used suspensions. Lotions are thin suspensions (some lotions are solutions) containing little solid material which may be designed to dry rapidly and pastes are thick occlusive suspensions containing a large amount of solid material which may be used to absorb exudates. Suspensions tend to be lyophobic and are therefore relatively unstable; the risk of Ostwald ripening and flocculation have already been introduced (Sect. 5.7.1) [41].

It may prove difficult to create a suspension initially, particularly if the active ingredient is poorly soluble in the continuous phase; it is likely to be poorly wettable and there will be a limited interaction between the two. Surfactants are often added to act as wetting agents. Materials such as xanthan gum, alginates, and celluloses may be used in low concentrations to act as colloids that will coat the solid particles. In higher concentrations, these will act to thicken the suspension which will slow the rate at which particles settle (5.12). Ideally they will thicken the suspension to give rise to thixotropic pseudoplastic or plastic flow (Sect. 5.6.2); when stored the liquid would be viscous; however, on applying a shear force (pouring or injecting for example), the network would be reversibly broken so such actions become easier.

It is also important to decide whether a system is flocculated or deflocculated as each has different drawbacks. In a deflocculated system, there is limited interaction between particles and so sedimentation is dependent on individual particle size. Larger particles tend to sediment first whilst smaller particles remain suspended. The system may not appear to change as this is occurring. Then the small particles

will eventually sediment. A clay or cake is formed at the base of the suspension and this may prove difficult to resuspend. In a flocculated system, larger and smaller particles interact and sediment together. The sediment layer is formed more rapidly but is less tightly packed and consequently will be easier to disperse.

5.8 Stability

The stability, or indeed instability, of a drug substance or product can generally be considered in one of three ways: chemical, physical, or microbial. A fourth term, biological, is often used when discussing protein or nucleotide product, but biological changes are usually related to physical or chemical changes in the active [42–44]. Changes to the chemical structure of an active ingredient are often the main limitation for shelf life. Physical stability is most commonly an issue in colloidal systems and coarse suspensions as described in Sect. 5.7.3. Microbial contamination is most common in formulations that are at least partially aqueous. It tends to be less of an issue in correctly stored solid dosage forms as these tend to desiccate microbes and are a poor growth medium. Microbial contamination is most critical in any parenteral drug delivery system where the animal's defenses against pyrogens and bacteria are being bypassed.

5.8.1 Chemical Stability

There are a number of routes by which a drug (or excipient) may undergo chemical degradation. Hydrolysis is the degradation of a molecule by water. It most commonly occurs at sites where a carboxylic acid has been functionalized into an amide or an ester. Esters tend to be more susceptible than amides as oxygen is more electronegative than nitrogen (see Sect. 5.5.1 for a brief explanation of electronegativity) [45]. Solvolysis covers the effect of nonaqueous solvents. Oxidation is the degradation of a molecule involving the loss of electrons. For organic materials like drugs, this is synonymous with the loss of hydrogen or an increase in the number of oxygen–carbon bonds at the expense of oxygen–hydrogen bonds. Light, trace metals, oxygen, and oxidizing agents may all play a part. Many drugs have a chiral center, they therefore exist in two (or occasionally more) forms that are mirror images of each other like your left and right hand; *cis*- and *trans*- or *dextro*- and *levo*- are the prefixes most often used. Generally, the two forms will have different levels of activity or toxicity. Cis-permethrin is 10 times more toxic than trans-permethrin. Dextromethorphan is used as an antitussive in cats and dogs yet its enantiomer (levometorphan) is an opioid narcotic [46]. Some drugs may spontaneously convert from one form to another (racemization) leading to changes in activity [47]. Epinephrine may change in aqueous solution; either via acid or base. Maillard reactions between reducing sugars and amine groups are a common compatibility issue.

There are a number of ways of improving chemical stability; storage temperature can be reduced leading to an improvement in stability; this is crude and is not

particularly helpful in veterinary medicine where cost is an important factor. Changing from water to a nonaqueous solvent may improve stability. If this is not possible changing pH may help. Hydrolysis is often catalyzed by protons in solution. Similarly hydroxyl ions may catalyze a reaction. Making a neutral buffered solution may therefore aid stability. UV radiation and the blue end of the visible spectrum may be blocked by amber glass. An opaque container or cardboard box may help. Aluminum foil is comparatively expensive but will block light very effectively. Plastic is not completely impervious to moisture and so aluminum foil or glass is often used to prevent hydrolysis. Equally one may package with a desiccant such as silica.

5.8.2 Physical Stability

Physical stability has largely been covered in previous sections. However, there are a number of further points worth considering. Since plastic packaging is permeable to water vapor, aqueous products packaged in plastic containers may become more concentrated on storage; a particular problem for prefilled syringes designed to give a variety of measured doses [48]. Plastics used in the pharmaceutical industry are rendered more malleable by the use of plasticizers (Sect. 5.2.3). These may leach into liquid formulations leading to changes in the container or medicine, a particular problem for polyvinyl chloride [43]. Protein formulations have a huge issue with stability; an active protein is very often not just a long list of amino acids, it is a properly folded three-dimensional structure. On storage, drying, or freezing, this structure and with it the activity of a protein may be lost [43, 49].

5.8.3 Microbial Stability

Microbial contamination and resultant spoilage of products pose less of a problem for pharmaceuticals than they do for food products. For parenteral formulations, it is not just viable organisms that need to be controlled but all pyrogens [50]. For other delivery routes, it is common to expect a maximum of 100–1,000 colony forming units per g or mL. This is generally not sufficient to cause spoilage and so water must generally be present to allow the number to grow on storage. Where the formulation is at least partly aqueous say a cream or a suspension, it is common to use a preservative such as parabens, benzyl alcohol and the like. Multiuse otic preparations are typically produced sterile but contain a preservative such as benzylkonium chloride to prevent contamination by the person administering the preparation or the patient animal. Benzylkonium chloride is also used in some dermal sprays as a disinfectant and cleanser. Choice of preservative is not always straightforward; changes in pH may change the activity of the preservative and a preservative that partitions into the oil phase of an emulsion would be unlikely to have much of an effect on microbial growth that would occur in the aqueous phase.

5.8.4 Stability Testing [42–44]

Stability testing of human medicines has to a large extent been harmonized across the three largest markets (Europe, USA, and Japan); as it is time-consuming and expensive, most other nations have generally been happy to take their cue from the harmonized guidelines, with only hot and dry or humid climate zones differing. Harmonization of the guidelines for veterinary drugs and products has followed on from this.

Stress testing of drug substance and product is designed to break the active ingredient in order to aid the identification of degradation products and assess stability. It includes the effect of temperature (incremental increases of 10°C from 50°C upwards), humidity (75% relative humidity; RH or higher), oxidation, photolysis, and hydrolysis in solution or suspension across a range of pH. Stability testing is then performed under long-term and accelerated conditions. Long-term conditions are usually either 25°C/60% RH or 30°C/65% RH depending on whether the product is for market in the more temperate areas or the hotter areas. In climate zone IV countries (hot, humid) human medicines are tested at the more stringent conditions of 30°C/75% RH; it is therefore likely that this will be most acceptable for veterinary medicines. A minimum of 12 months data is usually required. Accelerated conditions are also used (40°C/75% RH) as may be intermediate conditions (30°C/65% RH). For items stored in the fridge, the long storage conditions are 5°C with a range 2–8°C. The accelerated conditions are as per the normal long-term conditions for a drug or product stored at room temperature.

5.9 Concluding Remarks

This chapter has described a range of physical and chemical attributes relating to the active pharmaceutical ingredient, excipients, processing method, and final product that underpin the rational selection of ingredients and excipients, facilitate choice of manufacturing parameters, and underpin quality control tests. A sound understanding of the physicochemical properties of the drug is fundamental to its formulation. The physical and chemical properties of the drug dictate its ability to be formulated, the rational selection of excipients, the chemical stability of the active, and the physical stability of the formulation and define the final product characteristics and quality attributes.

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Chapter 6
Biopharmaceutics and Veterinary Drug Delivery

Steven C. Sutton

Abstract Biopharmaceutics operates at the interface of pharmaceutics and physiology. This chapter discusses observations and studies that emphasize the similarities and differences in species and routes of administration while providing references for notable reviews. The quintessential requirement of drug dissolution is discussed for immediate release formulations, and the use of biodegradable microspheres is reviewed for controlled release injectable formulations. From nasal and ocular to transdermal and oral, examples of formulations for veterinary practice are presented. If the formulation and the biopharmaceutics results in a well-absorbed drug product in one species, would it will behave similarly in other species? We can predict this when we have a sound understanding of the sometimes specie-specific physiology, and pharmaceutical knowledge of the formulation. With numerous routes of administration and preclinical studies already public knowledge, we may be on the brink of the golden age of veterinary pharmaceuticals.

6.1 Introduction

According to Mosby's Medical Dictionary,[1] biopharmaceutics is the study of the chemical and physical properties of drugs, their components, and their activities in living organisms.[2] Specifically, the field operates at the interface of pharmaceutics and physiology. With respect to veterinary drug delivery, this chapter reviews observations and studies that emphasize the similarities and differences in species and

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[2] http://medical-dictionary.thefreedictionary.com/biopharmaceutics

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routes of administration. However, the chapter's focus is in the contribution of the animals' physiology to the performance (or lack thereof) of the drug product and the API (active pharmaceutical ingredient). The delivery of medicine to animals can be via nearly every route of administration imaginable. However, the majority of reports on the administration of drugs to animals consist of preclinical studies of drug products intended for use in humans. Since a thorough treatment of biopharmaceutics and veterinary drug delivery could fill a book in its own right, this chapter will attempt to summarize the most popular routes of administration and animal species.

6.2 Routes of Drug Administration

6.2.1 Injectable Routes

Aside for emergency use, the intravenous (IV) route of administration is rarely used in small animal health. Also, aside from selecting the appropriate blood vessel, there are little differences in this route among species. The subcutaneous (SC) route of administration in small animals appears limited to the administration of insulin [1]. The intramuscular (IM) route of administration has been recently reviewed by Shah and coworkers [2].

6.2.2 Nasal Route

The nasal route of administration has been successfully demonstrated for acute therapy with poorly absorbed molecules. The bioavailability of the 100 ul of a 30 mg/ml bisphosphonate alendronate in saline was 50% following instillation into the nasal cavity of the awake dog—a 50-fold improvement over oral administration [3]. The enhanced bioavailability was due to three factors: high concentration of API possible from a favorable aqueous solubility, ability of the API to act as a self promoter (permeability enhancer), and the absence of significant dilution from bodily fluids. Nasal administration was also successful for fentanyl in the cat [4]. While similar improvements of bioavailability have been demonstrated for larger molecules, the requirement of a permeability enhancer renders the use of such a formulation unlikely for chronic application [5].

6.2.3 Ocular Route

Aside from the occasional drops and ointments, the ocular and otic routes of administration are seldom used in veterinary practice. Drug delivery to the eye in animals—as in humans—requires the API to be in solution, nonirritating (isotonic and neutral

pH), and viscous enough for some retention. The administration of a single drop (approximately 50 ul) was as effective as several drops, since the excess overflowed the conjuctival sac, and was wasted. A report by Proksch et al. showed that the penetration of a 50 ul instillation of 300 μg fluoroquinolone antibiotic into the conjuctival sac of each eye was sufficient in monkey and rabbit to combat most infections [6]. The interested reader should study an excellent recent review by Mitra's group [7].

6.2.4 Joints

The local delivery of the API to the precise site where it is needed has been the premise for joint therapy in humans [8] and recently has been applied in veterinary practice [9]. However, challenges persist in methods to maintain pharmacologic concentrations of the drug in the joint. The API must be in solution to be effective, yet as a solution the API rapidly diffuses away from the intended site. Formulation advances, such as microencapsulation and microspheres designed to stay in the joint as a reservoir for the release of the API hold promise for extended delivery [10] (see Sect. 6.3).

6.2.5 Transdermal Route

Transdermal drug delivery has had limited success in human pharmaceuticals due to the requirements of a small dose, a small patch, an optimum partition coefficient of between 1 and 3, and a small molecular weight [11]. Transdermal administration for animals is further complicated by the requirement of close-clipping (not shaved) of fur prior to application. Shaving before application has been shown to enhance permeability by 500%, due to the resulting abrasion compromising the skin barrier [12]. Finally, there is a need to prevent the animal from removing the patch or formulation. Removing the formulation may result from licking, rubbing, or burrowing into bedding. The category of transdermal administration has historically included the so-called pour-on products popular in farmed animal care. In a review of ivermectin, the bioavailability of the pour-on was reported as 15% that of the SC route, and substantial absorption of API following licking likely contributed [13]. In companion animal care, there are reports comparing IV or oral routes of administration to the transdermal application in cats of methimazole [14, 15] and atenolol [16, 17], and fentanyl in dogs [18]. Penetration enhancers have improved some of these transdermal preparations, but with the risk of irritation [19].

Methimazole availability has been highly variable after application of transdermal preparations in cats [14]. For example, 2.5 mg methimazole in 0.05 ml pluronic lecithin organogel (PLO) formulation was applied to the inner pinnae of the ears (alternating each administration) of cats. After 4 weeks, 67% of the cats treated with

transdermal methimazole were euthyroid (compared to 82% with oral methimazole) [20]. However, the absolute bioavailability of transdermal methimazole was poor and variable (mean ± SD): 11.4 ± 18.7% [15].

In cats, after 1 week of twice-daily transdermal application of 1.1 mg/kg atenolol in a carbomer gel with 2.5% tween-20 to the inner pinnae of the ears, the peak median plasma concentrations were 24% of those after oral administration: (135 ng/ml [range: 25–380 ng/ml]) and (569 ng/ml [range: 242–854 ng/ml]), respectively [16]. If we can relate the peak median plasma concentrations in this study to RBA, then bioavailability relative to oral administration was quite good (24%), but highly variable. The transdermal route provided a max peak median plasma concentration that was 15-fold the min peak median plasma concentration.

Also in cats, following application of 30 μg/kg fentanyl in a PLO formulation to the inner pinnae of the ears, fentanyl concentrations were less than 0.2 ng/ml. In contrast, 2 μg/kg fentanyl orally administered resulted in plasma fentanyl concentrations of approximately 1 ng/ml [4]. If the concentrations are correlated to the relative bioavailability, then fentanyl in this formulation was approximately 1% as bioavailable as oral.

Significant transdermal bioavailability in the cat was achieved using the commercially available Duragesic® patch (25 μg/h fentanyl, Janssen Pharmaceuticals, Inc., Titusville, NJ). For example, after only 12 h, the patch resulted in steady-state plasma fentanyl concentrations of (mean ± SEM) 2.05 ± 0.89 ng/ml, corresponding to an absolute bioavailability of 36% in cats. Following removal of the patch, plasma fentanyl concentrations decreased slowly [21]. In contrast, while these levels were also seen in dogs following application of the patch, plasma fentanyl concentrations decreased precipitously following removal of the patch [18]. Evidently in these "overweight" cats, fentanyl partitioned into fatty tissue in close proximity to the patch, and provided some additional drug input after the patch was removed. This is not likely a species-specific effect, but rather a lifestyle consequence of the sedentary adult cat. In our experience, a 13.5 kg beagle dog is likely to have little adipose tissue.

6.2.6 Buccal and Sublingual Routes

Bioavailability of an API from the oral cavity may be superior to the oral route in some cases: i.e., when the orally administered API is subjected to a large first pass extraction by the intestinal and/or liver enzymes, resulting in a significant portion of the dose rendered inactive by metabolism. Another example might be if the API was prone to acid degradation (due to stomach) or instability due to pancreatic enzymes. However, this route of administration has its challenges. While the human may be able to hold the dissolved API in the mouth, this cannot be expected of the veterinary patient. Therefore, formulation alone must retain the API in the oral cavity until it is dissolved and absorbed [22]. Drugs with a larger logD at the saliva pH tend to be absorbed faster. In the case of some drugs, 5 min was sufficient for absorption of

from the oral mucosa [23]. After transmucosal application of buprenorphine, absolute bioavailability was reported as 38–47% in dogs [24], and essentially complete in cats (due to pH 9 saliva) [25].

6.2.7 Oral Route

As discussed in Chap. 4, the stomach is both a reservoir and processing organ. The review by Martinez and Papich on the stomach in the dog focused on the various pharmaceutical and physiologic factors that would influence gastric retention of the drug product [26]. The effect of the stomach on the drug product and the API can be reduced to the effects of acid, crushing, retention, disintegration, and dissolution. The environment in the stomach ranges from highly acidic in the fasted state, to a mixture of food and water in the fed state. If the drug product exposes the API to the stomach acid in the fasted state, degradation of the labile API is possible.

If the drug product has an enteric coating—which prevents water from penetrating at low pH—then the drug product may be emptied from the fasting stomach in the next 2 h via the migrating myoelectric current (MMC, housekeeper wave) [27]. If the enteric coating of the product developed a defect, allowing water to penetrate the tablet, then the crushing forces of the stomach may be sufficient to break the tablet apart. However, if the drug product is small (<2 mm diameter pellets), then the gastric emptying of the drug product will be more rapid [28]. Noncoated tablets will imbibe water and be crushed as well. The combination of the some fluid and the crushing, mixing forces of the fasted stomach will result in the disintegration of the formulation. For most API, the volume of water in the fasted stomach would be insufficient to support complete dissolution. However, rapid emptying of the small particles will deliver the API to the duodenum, dissolution will continue, and the API will be absorbed.

The effect of food on the gastric physiology is a function of (1) osmolarity, (2) viscosity, (3) caloric density, and (4) volume (including any water consumed) [26]. Therefore, a cat that eats a small, semisolid proteinaceous meal may exhibit all the same physiologic changes observed in a fed dog, but on a smaller scale. One can peruse the literature and find numerous examples of food effects. Some of these studies may even appear to be in conflict to others, but if the four characteristics listed above are equalized, then the effects should be similar. With the exception of recent publications, most food effect studies do not provide enough information for such a comparison. Unfortunately, it is because of the earlier reports that there continues to be much consternation over the food effect.

The interested reader might review the effects of food on numerous drug products summarized by Martinez and Papich [26], including:

1. The contribution of food to the formation of a film surrounding an IR tablet, thereby slowing its disintegration and dissolution [29]
2. The expected slowing of the gastric emptying of acetaminophen powder and therefore slowing the rate but not the extent of absorption [30]

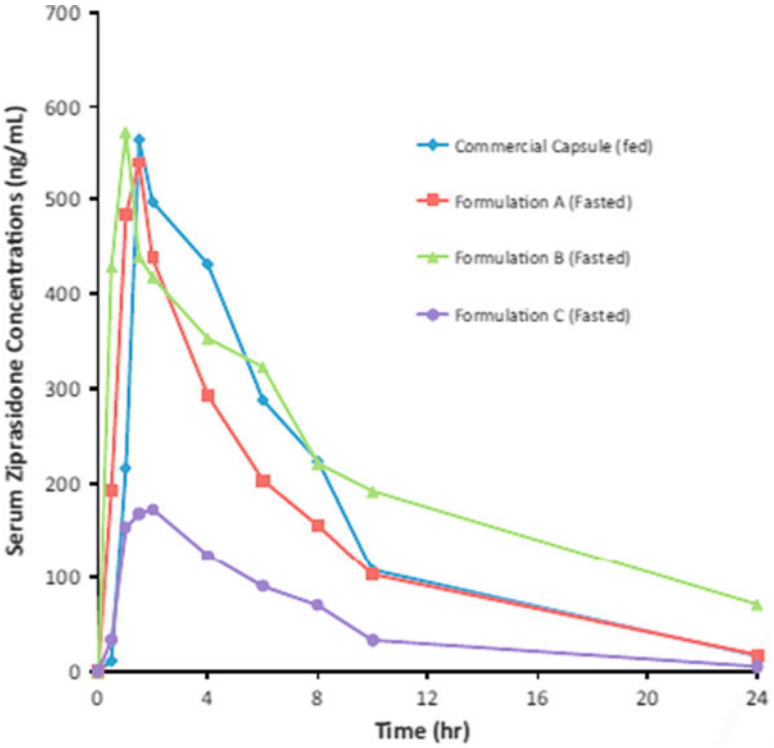

Fig. 6.1 Serum ziprasidone concentrations following oral administration of a conventional (immediate release) capsule formulation in fed beagle dogs, and the nanosuspension formulation in fasted beagle dogs (formulation B). From [35], with permission, John Wiley and Sons

3. The expected slowed gastric emptying of 3 mm enteric-coated particles containing aspirin, presumably because of the sieving property of the pylorus [30]
4. The gastric retention of an enteric-coated tablet with dimensions 8 mm × 3.4 mm [30], not unexpected based on the observations of Fix and coworkers [31]
5. The expected slowed gastric emptying and increased duodenal solubility of the approximately 1 g cefadroxil [32], based on water volume in the dog small intestine, cefadroxil solubility (17 mg/ml, [33]), and calculations in Sutton [34] (adjusted for dog)

As frustrating as the food effect can be for some API and formulations, there is at least one easy and practical work around. While trying to develop a formulation to avoid the food effect for ziprasidone in humans, we demonstrated precisely a nanosuspension of ziprasidone in beagles [35]. As shown in Fig. 6.1, the nanosuspension of ziprasidone gave a similar exposure in fasted beagle dogs, as the commercial

capsule formulation did in fed dogs. The meal in the fed dogs was selected to mimic the food effect for the commercial capsule formulation observed in the clinic [36].

6.3 Controlled Delivery and Future Trends

The impetus for controlled release drug delivery in animals—as in human therapy—is the relief of adverse effects and/or improved compliance. There apparently is room for improvement in compliance among veterinary treatment. For example, only about a third of dog owners were compliant with a 5- or 10-day antibiotic treatment [37, 38]. One can extrapolate that the compliance would be even worse for cats, farmed or wildlife animals (including exotic and zoo species). The controlled release implant or depot formulation has been successfully employed in farmed animals (e.g., antibiotics and growth hormones), although the issue of residual API or scarred muscle when brought to market continues to be challenging [39, 40]. Using the poly (lactic-co-glycolic) acid (PLGA) microspheres, injectable controlled release formulations are widely applied in human pharmaceuticals [41]. This technology is ripe for the application to veterinary pharmaceuticals. Taking advantage of the unique anatomy of ruminant animals, delivery via a device retained in the ruminant has been successful (e.g., treatment of parasites), but carry their own list of challenges [42].

The pursuit of gastric retention to prolong the delivery of an API to the entire small intestine is likened to draw of the Sirens to Ulysses. Despite over 30 years of trying, there is still no reasonable method for significantly extending residence in the stomach [43]. On the other hand, the use of conventional controlled delivery in veterinary care is starting to appear, as the market begins to produce a segment that may be cost-effective for the investment. While the challenges are many—see a review by Rathbone [44]—the application of human drug products to companion animals should be quite natural and straight forward for dogs [36]. It has been nearly 20 years since a controlled release diltiazem formulation in beagles was demonstrated [45]. And a controlled release formulation of sertraline—a treatment for compulsive behavior in dogs [46]—was described in beagles 8 years ago [47].

Oral controlled release formulations have also been developed for cats. And a once-daily oral administration in cats of a controlled release carbimazole (prodrug of methimazole) formulation resulted in an extended release profile (Fig. 6.2) [48]. However, another "controlled release" formulation of carbimazole in cats did not demonstrate any appreciable extended release profile [49]. In the latter study, the half-life of carbimazole after the "controlled release" formulation was similar to that of the IV administration from the former study. The so-called "controlled release" character of the formulation was not evident. This was likely due to "soft" tablets breaking apart in the stomach.

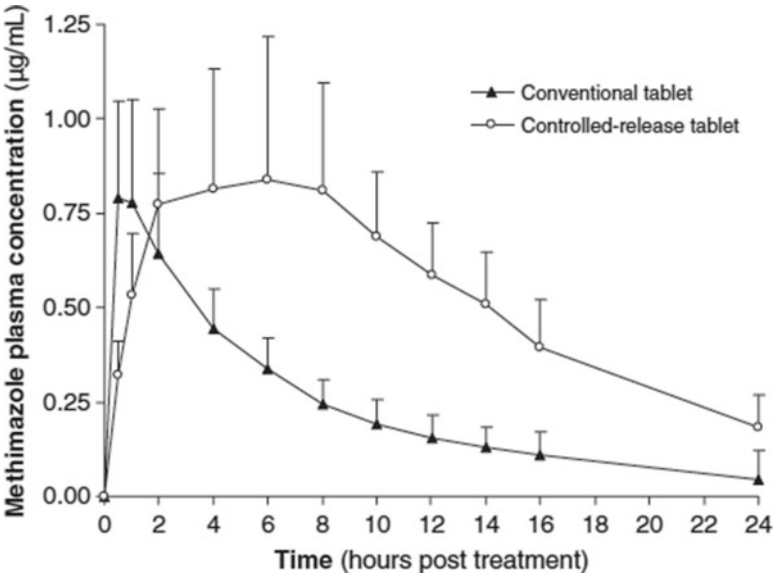

Fig. 6.2 Plasma methimazole concentrations following oral administration of a conventional (immediate release) and a controlled release tablet in the cat. From [48], with permission, John Wiley and Sons

6.4 Concluding Remarks

Veterinary drug delivery is at a crossroads of sorts. While the human pharmaceuticals market has experienced flat earnings over the past decade or so, the earnings from veterinary pharmaceuticals market has been increasing. Most human pharmaceuticals have already been evaluated in animals as part of their preclinical development and as stated previously, the development of controlled release formulations has also been largely demonstrated. Furthermore, the tools for demonstrating the controlled release nature of formulations (e.g., scintigraphy) and for working around poorly water soluble API (e.g., nanosuspension) are readily available. The literature and the workforce have become replete with the experience and knowledge that had once been trade secrets of "Big Pharma." These are the tools for the expanding veterinary pharmaceutical industry.

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Chapter 7
Quality by Design and the Development of Solid Oral Dosage Forms

Raafat Fahmy, Douglas Danielson, and Marilyn N. Martinez

Abstract The intent of this chapter is to provide a high-level overview of the various options available within the QbD tool chest and to describe the benefits derived from adopting a QbD approach to drug product development. This chapter also presents a set of terms and definitions that are consistent with ICH Guidelines, practical examples of how QbD can be applied throughout a product life cycle, and the interrelationship between the multiple factors that contribute to product understanding and to the development of product specifications.

Abbreviations

API	Active pharmaceutical ingredient
CPP	Critical process parameter
CQA	Critical quality attribute
DOE	Design of experiment
FMEA	Failure modes effect analysis
ICH	International Conference on Harmonization
ISO	International Organization for Standardization
QbD	Quality by design
QRM	Quality risk management
QTPP	Quality target product profile
MCC	Microcrystalline cellulose
MSPC	Multivariate statistical process control

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MST	Material science tetrahedron
NIR	Near-infrared spectroscopy
PAR	Proven acceptable ranges
PAT	Process analytical technology
PIP	Process-ingredient product diagram
PQS	Pharmaceutical quality system
RPN	Risk priority number
RTRT	Real-time release test
SPC	Statistical process control

7.1 Introduction

Quality by design (QbD) has been defined as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" (ICH Q8). Despite the innovative aspects of QbD approaches in pharmaceutical product development, the concept of QbD has long been widely applied within such industries as automotive, aviation, petrochemical, and food production.

A fundamental goal of drug product development and regulation is to ensure that each marketed lot of an approved product provides its intended in vivo performance characteristics. Globally, regulatory agencies are encouraging the pharmaceutical industry to adopt the QbD concept when developing a new drug product or when improving their legacy drug products. Within the past several years, experts from regulatory authorities within the USA, Europe, and Japan have worked together under the umbrella of International Conference for Harmonization (ICH) to establish a basis for applying these concepts.

Ultimately, the use of QbD provides an understanding of the drug product based on science and risk. It provides a mechanism whereby scientists utilize their understanding of material attributes and of manufacturing process and controls to assure that the product performs in a manner that is consistent with its targeted in vivo performance [i.e., its quality target product profile (QTPP)]. It also affords drug sponsors and regulators greater flexibility in terms of product specifications and allowable postmarketing changes. To this end, the ICH has published several guidance documents related to product quality. The documents that are the most relevant to QbD are the ICH Q8(R2) [Pharmaceutical Development], ICH Q9 [Quality Risk Management], and ICH Q10 [Quality System].

This chapter provides a high-level overview of the various options available within the QbD tool chest and describes the benefits derived from adopting a QbD approach to drug product development. This chapter also presents a set of terms and definitions that are consistent with ICH Guidelines, practical examples of how QbD can be applied throughout a product life cycle, and the interrelationship between the multiple factors that contribute to product understanding and to the development of product specifications.

7.2 Quality by Design

As stated in ICH Q8, "Quality cannot be tested into products." In other words, quality should be built into the design of the product. Therefore, process understanding is a vital component of the overall product development scheme.

Within the QbD paradigm, the critical quality attributes (CQAs) of the drug product or raw materials (i.e., the physical, chemical, biological, or microbiological properties that need to be controlled to assure product quality) and the critical process parameters (CPPs) (i.e., the various process inputs that affect product quality) need to be understood. To achieve this understanding, all of the independent variables associated with the manufacturing process and raw materials should be studied (i.e., equipment, locations, parameters, and sources or grade of excipients). In so doing, the CQAs and CPPs can be identified and controlled, thereby reducing the variability in product performance, improving product quality, reducing the risk of manufacturing failure, and enhancing productivity.

In contrast to the QbD approach, when specifications are derived from data generated on up to three clinical batches (not necessarily at production scale), it is not possible to obtain the necessary mechanistic understanding of the formulation and the manufacturing process. The impact of this lack of understanding is the development of product specifications that may either be more rigid than necessary to insure product performance or, alternatively, may not be strict enough to insure batch-to-batch consistency in patient response. This lack of understanding may be of particular concern during scale-up, which can lead to altered product performance.

When manufacturing a product in accordance with the recommendations described in ICH Q8(R2), the critical formulation attributes and process parameters can be identified through an assessment of the extent to which their respective variations can impact product quality. In general, when developing a new product using QbD, the following steps may be considered (Fig. 7.1):

1. Define the quality target product profile (**QTPP*)*** as it relates to quality, safety, and efficacy.
2. Design the formulation and the appropriate manufacturing process using an iterative procedure that involves the identification of the material Critical Quality Attributes (**CQAs**) and the manufacturing Critical Process Parameters (**CPPs**).
3. Develop a **Process Understanding** strategy, defining the functional relationships that link ***material*** CQAs and CPPs to the ***product*** CQAs through risk and statistical models. The selection of the raw materials and manufacturing process depends on the QTPP and process understanding:
   (a) The CQAs of the drug product relates to the Active Pharmaceutical Ingredient (API) and determine the types and amounts of excipients that will result in a drug product with the desired quality.
   (b) The CPPs of the drug product relates to all processing associated with the API and the excipients.
4. Combine the resulting product and process understanding with quality risk management to establish an appropriate control strategy. This strategy is generally

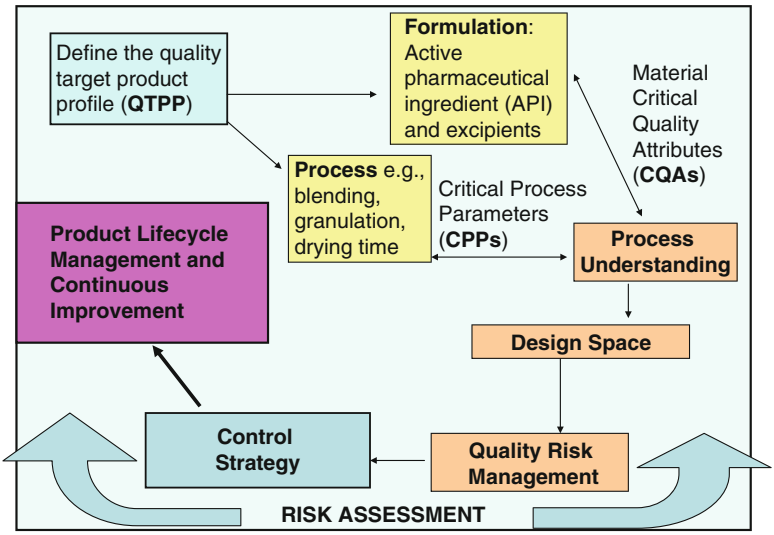

Fig. 7.1 The QbD approach to product development and life cycle management

based on the proposed **Design Space** (derived from the initial targeted conditions of product manufacture).

5. Based on the design space and the identification of the high risk variables, define procedures for minimizing the probability of product failure (**Risk Management**).
6. Based on the quality risk management tools established for a product, establish a method to implement risk management strategies (i.e., define the **Control Strategy**). These control strategies become the basis for continuous product quality and performance.
7. Through process and product understanding, formulators have greater flexibility to manage and improve their product throughout its marketing life cycle.

Global understanding of product formulation and manufacturing process establishes design space and subsequent control strategies. As long as the process remains within a design space, the risk is minimized that product quality and performance will be compromised. Thus, the QbD approach can benefit both the patient and the manufacturer, and may lessen regulatory oversight of manufacturing changes that often occur over the course of a product's marketing life cycle.

7.2.1 The QTPP

Pharmaceutical quality is characterized by a product that is free of contamination and reproducibly delivers the labeled therapeutic benefits to the consumer [1].

The QTPP process is a starting point for identifying the material and product CQAs, guiding the scientist in designing a suitable formulation and manufacturing

process. This is akin to a drug development strategy based on a foundation of "planning with the end in mind" [2], providing an understanding of what it will entail to ensure the product safety and efficacy. QTPP includes such considerations as (ICH Q8(R2), 2009) [3]:

- The intended use and route of administration
- The dosage form, delivery system and dosage strength(s)
- The container closure system
- The pharmacokinetic characteristics of the therapeutic moiety or delivery system (e.g., dissolution, aerodynamic performance
- Drug product quality criteria such as sterility, purity, stability, and drug release

Because the QTPP includes only patient-relevant product-performance elements, it differs from what is typically considered product manufacturing specifications. For example, the QTPP may include elements of container–closure system (a critical component of developing injectable solutions) and information about pharmacokinetics and bioequivalence. These considerations would not be typically be included as part of the product release specifications [3]. Conversely, product specifications may include tests such as particle size or tablet hardness, which are not typically part of the QTPP.

To establish QTTPs, the in vitro drug release should be linked to the desired clinical outcome [4–6]. Ultimately, such information can be used to integrate product design variables with the kinetics of the drug, the drug uptake characteristics, and the intended pharmacodynamic response in the targeted patient population.

7.2.2 Critical Quality Attributes

A CQA is a physical, chemical, biological, or microbiological property or characteristic that should be maintained within an appropriate limit, range, or distribution to ensure the desired product quality. For example, assay, content uniformity, dissolution, and impurities are common CQAs for an immediate release tablet formulation. There are two specific CQAs that are associated with the QbD paradigm: (1) CQAs can be associated with the drug substance, excipients, intermediates (in-process materials); (2) CQAs can be defined for the finished product (ICH Q8).

Risk assessment, knowledge of material physicochemical and biological properties, and general scientific insights are all components integrated into an identification of the potentially high-risk variables that need to be studied and controlled [7]. The final product CQAs should be directly related to the safety and efficacy of a drug product [3].

7.2.2.1 Selecting the Appropriate Manufacturing Process and Determine the Critical Quality Attributes of the Drug Substance, Excipients

Product development is an interdisciplinary science. For example, the formulation of solutions is guided by rheology (i.e., the study of material flow characteristics)

and solution chemistry. The development of tablets involves powder flow and solid state interactions. To manufacture an optimal drug delivery system, preformulation studies should be conducted to determine the appropriate salt and polymorphic form of a drug substance, to evaluate and understand the critical properties of the proposed formulation, and to understand the material's stability under various process and in vivo conditions.

To obtain a better understanding of the characteristics of pharmaceutical formulations, a product development scientist can benefit from applying the concept of the material science tetrahedron (MST). For example, let us examine the three different techniques for preparing a tablet mix prior to the compression stage: direct compression powder blend, dry granulation, and wet granulation. Direct compression is ideal for powders which can be mixed well and do not require further granulation prior to tableting. Dry granulation refers to the blending of ingredients, followed by compaction and size reduction of the mix, to produce a granular, free flowing blend of uniform size. This can be achieved by roller compaction or through "slugging." Finally, wet granulation involves the production of granules by the addition of liquid binders to the powder mixture.

To deliver a stable, uniform and effective drug product, it is important to know the solid state properties of the active ingredient, both alone and in combination with all other excipients, based on the requirements of the dosage form and processes. Simply relying upon USP monographs for excipients do not address issues pertaining to their physical characteristics and their relationship (interaction) with other components of the formulation or with the manufacturing process itself.

As will be discussed later, one of the excipient functionalities that can be understood through the MST is the dilution potential of an excipient. Dilution potential is a process-performance characteristic that can be defined as the amount of an active ingredient satisfactorily compressed into tablets with a directly compressible excipient. Since the dilution potential is influenced by the compressibility of the active pharmaceutical ingredient, a directly compressible excipient should have high dilution potential to minimize the weight of the final dosage form.

The MST facilitates an understanding of solid state material characteristics such as excipient work hardening. Work hardening, whether of the excipients or of the API, is important in roller compaction. Roller compaction depends on the excipient's ability to exhibit deformation without loss of flow or compressibility. On recompression, the excipient should exhibit satisfactory performance.

Powder segregation is a problem during the transfer of dry powder material from the blender to the tablet press. Direct compression is more prone to segregation due to the difference in the density of the API and excipients. The dry state of the material during mixing may result in static charge and lead to segregation, leading to problems such as variation in tablet weight and content uniformity.

If a powder blend's properties are not suitable for direct compression and if the drug is neither heat nor moisture sensitive, then wet granulation processes may be appropriate for generating granules with the desired flowability. Flowability is

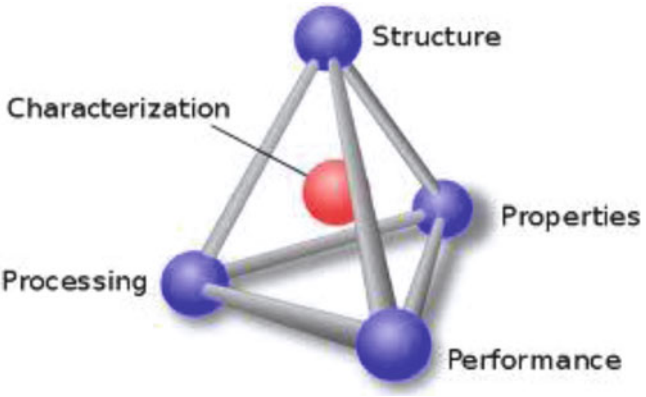

Fig. 7.2 The material science tetrahedron [8]

important for minimizing tablet weight variations, for ensuring a high density for high tablet filling weight, and for ensuring material compressibility. Wet granulation narrows the particle size distribution of a tablet formulation's bulk powder, thereby eliminating segregation problems. Superior compressibility permits the use of higher quantities of the API, which promotes better distribution of the API within the tablet.

7.2.2.2 Material Science Tetrahedron

The term "material science tetrahedron" has been coined to describe the interplay between the following four basic elements [8]:

- *Performance*: the efficacy, safety, manufacturability, stability, and bioavailability of the drug product.
- *Properties*: the interactions of the API with the biological targets in the body, the mechanical and physiochemical properties of the excipients, API, and the finished product. Drug substance physiochemical properties include water content, particle size, crystal properties, polymorphism, chirality, and electrostatic charge.
- *Structure*: a description of the geometric configuration of the product constituents such as consideration of the molecular, crystalline, bulk powder, and the granular characteristics of the various components.
- *Processing*: the chemical synthesis, crystallization, milling, granulation, and compaction of the product.

The interaction of these four elements (the six edges of the tetrahedron) is as important as the identified basic four elements (Fig. 7.2). Both the basic elements and the interaction of these elements must be well understood.

Examples of the use of the material science tetrahedron in the QbD framework are as follows:

a. Structure–Property Relationships: In a QbD approach to formulation development, there is an understanding of how the components of the formulation relate to the product CQAs identified in the QTPP. This understanding can be mechanistic or empirical (derived from experiments). Formulation development should focus on an evaluation of the highest risk attributes as identified in the initial risk assessment model. The following are examples of structure–property relationships:

   - The structure of drug crystals can profoundly influence crystal properties. Polymorphs of an API differ in their solubility, dissolution rate, density, hardness, and crystal shape [9].
   - Many excipients are spray dried because amorphous material is more plastic than crystalline material and can therefore compress into harder tablets.
   - An excipient may be described as multifunctional because it has physicochemical features that may give it diverse useful properties.

b. Structure–Processing Relationships: The physicochemical properties of the excipients and the API can influence the manufacturing process. For example:

   - Many tablet excipients are available in grades that are distinguished by differences in their structure. Each grade of excipient possesses a unique set of properties that offers an advantage in the manufacturing process.
   - The shape and particle size distribution of a granulation affects its tabletability. Properly processed tablet granules should have a spherical shape to optimize powder flow during mixing and tablet compression. The presence of fines helps to fill the inter-granule space, thereby promoting uniform die fill. However, an excessive amount of fines can be detrimental to powder flow, leading to inconsistent die fill and tablet capping.

c. Structure–Performance Relationships: Direct compression excipients are materials where desirable performance is achieved through their structure. A good example is modified starch, where the amylose structure can be modified to make it perform as either a binder, a disintegrant, or as a combination of both. Other relationships that fit within this category include:

   - The molecular weight and substitution type (structure) of the various grades of hydroxypropyl methylcellulose (hypromellose) is a unique performance characteristic that makes it useful for specific types of applications.
   - The hydrous or anhydrous forms of excipients can also differ in their formulation performance characteristics. For example, the hydrous form of an excipient may compress into harder tablets, whereas the API may be more stable with the anhydrous form.

d. Performance–Properties Relationships: Common tablet and capsule excipients have internal and surface properties that give them desirable mechanical

performance. Similarly, an API may exhibit different performance characteristics within a formulation. Examples of this type of relationship include:

- Relationship between the particle size distribution of the API (property) and the content uniformity of the tablets (performance) in a direct compression formulation.
- Moisture content (property) and tablet granulation compression performance to determine the hardness and friability of a tablet.
- Acid reaction kinetics (property) that indicate whether a mineral antacid provides rapid or slow acting neutralizing functions.
- Starches, when used in oral dosage forms, stabilizing hygroscopic drugs and protecting them from degradation [10].

e. Processing–Performance Relationships: The effect of equipment operating parameters (processing) on the performance of intermediate and final drug product is the processing–performance relationship. Examples of this type of relationship include:

- A wet granulation can compensate for difficulties associated with the compressibility of certain APIs, providing a means to prepare a robust tablet formulation from a poorly compressible API. Conversely, over-processing (in the form of over-mixing a wet granulation) can delay tablet dissolution performance.
- Some filling line tubing can absorb preservatives contained in a solution.

f. Processing–Properties Relationships: The effect of equipment operating parameters (processing) on the properties of intermediate and final drug product is the processing–properties relationship. For example:

- During wet granulating, the spray rate and mixing speed affects the size, friability, and density properties of the granules.
- When used in a wet granulation, the water-absorbing properties of microcrystalline cellulose (MCC) help promote a uniform distribution of water throughout the wet mass, preventing the creation of over-wetted regions. Subsequently, during the wet granulation sizing operation, MCC prevents extrusion and aids in consistent batch-to-batch processing.

As seen in the above examples, information derived from the MST can be invaluable in helping the formulator predict product performance over a wide range of material attributes, processing options, and processing parameters. Once identified, the relationships between the drug substance, the excipients, the manufacturing process, the equipment design features, and the operating parameters can be identified in relationship to the performance of the drug product. The contribution of each of these variables to the product CQAs can therefore be described. By showing the relationship between the processing parameters, the excipient properties, and the properties of the intermediate and final drug product, the manufacturing scientist can monitor and control the appropriate CPPs.

7.2.3 Process Understanding and Design of Experiment

The design space and relevant CQAs can be identified through an iterative process of QRM and experimentation. The outcome of these iterations can be used to assess the extent to which each variation will impact the QTPP [11].

Traditional pharmaceutical product development uses factorial (full and/or partial) statistical designs and response surface methodologies to systematically evaluate formulation/process variables and to relate them to product CQAs. These designs provide comprehensive process understanding. As such, they are invaluable for evaluating the manufacturing process and formulation factors as they influence the QTPP. There are however practical limitations imposed by the exponential increase in the number of experiments needed to address each additional factor. Therefore, the availability of a mechanism for reducing this experimental burden is essential to support the practicality of using this systematic approach within product development [11].

When developing a product using the QbD, the number of experiments can be optimized through the use of risk methods that identify those variables that need to be studied (i.e., those that present the greatest risk for product failure). To this end, a well-defined design of experiment (DOE) approach to study manufacturing variables provides a mechanism that allows the scientist to reduce the number of experimental factors to only those that may significantly influence product quality and performance. Once identified, the effect of each variable can be explored both individually and in combination through a series of separate experiments.

In general, there are three types of experiments that may be employed: (1) screening experiments to select the critical variables, (2) interaction study for interactions between variables of interest, and (3) optimization study for developing a more carefully planned design space.

- *Screening experiments* are often used to limit the initial number of parameters that need to be studied through the use of existing scientific literature and previous experimental outcomes. This narrows the number of variables and attributes for future studies to those recognized as having the greatest potential effect on the CQAs. Screening experiments offer a high-level, global (not in-depth) overview of the formulation variables. As such, they generally require fewer experiments than do interaction or optimization studies. In this regard, models such as central composite design or Plackett–Burman (PB) may appear to share some attributes with factorial experiments. However, PB models cannot describe the importance of specific interactions but rather describe the importance of each manufacturing process and formulation variable that can impact the product CQAs.
- The advantage of the PB design is that multiple factors can be screened with relatively few trials. The disadvantage of these designs is that interactions between variables cannot be determined. A failure to replicate these interaction studies renders it impossible to readily characterize their inherent variability. Despite these limitations, when a large number of studies are needed to implement a higher resolution factorial design, the PB design can be the more pragmatic solution.

- *Interaction experiments* usually involve fewer factors than do screening studies. As compared to screening studies, interaction studies provide a richer understanding of the relationships between independent and dependent variables. The presence of a significant interaction implies that the magnitude of the effect of one of the variables is a function of the "level" of the other. For example, the effect of compression force on dissolution and disintegration can depend on the amount of one or two of the excipients (such as microcrystalline cellulose or magnesium stearate). Interaction studies can be designed as fractional or full factorial experiments.
- *Optimization studies* provide a complete picture of the variables affecting the QTPP. The goal is to limit the number of investigated parameters so that a full factorial design can be used. The results of these optimization studies enable the formulator to estimate the quadratic (or higher order) terms needed for mapping a response surface. In so doing, one can identify the effect of the material CQAs and CPPs on product performance. Maximum and minimum parameter constraints can be defined, and parameter combinations leading to optimum process and product performance can be identified and included in the design space. Common designs used for optimization studies are Box–Behnken, three-level factorials, or mixture designs. Optimization studies can be performed using scale up or production batches.

7.2.4 Design Space

Once process understanding has been demonstrated, the scientist can begin the studies to establish the boundaries of the design space.

The ICH Q8R2 defines design space as: "*The multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post-approval change process. Design space is proposed by the applicant and is subject to regulatory assessment and approval*."

The design space is dependent on formulation, equipment, and batch size. Within a design space there is a region, termed the control space, which is bounded by the upper and/or lower limits for the material CQAs and the CPPs. If the control space is much smaller than the design space, then the process is robust. In other words, if the limits within which a product is manufactured is within the limits where there is no risk of altering product quality and performance, the process presents little risk of product failure. However, when this is not the case, stringent process control may be needed to assure that the product is consistently manufactured within the design space [2]. This is illustrated in Fig. 7.3.

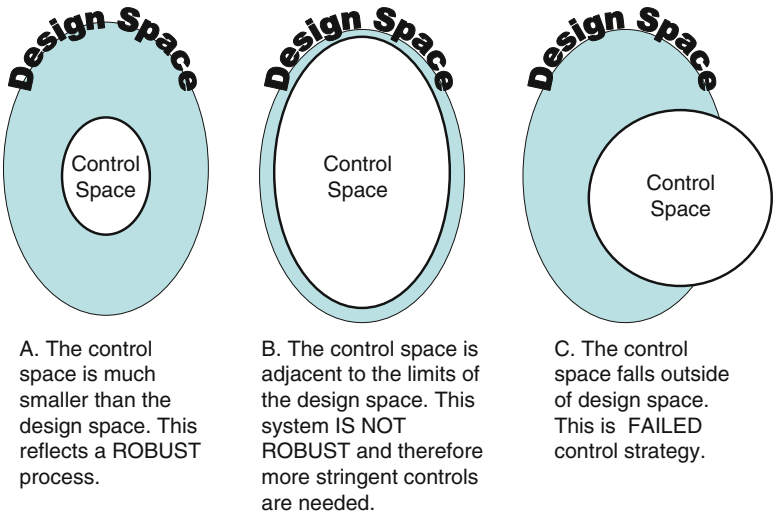

Fig. 7.3 The relationship between control strategy and design space

Design space should be linked to the QTPP, to the product CQAs, and to the manufacturing control strategy. Although not a regulatory requirement, the development of the design space demonstrates product and process understanding, thereby enhancing manufacturing and regulatory flexibility.

Generally, design space is developed on a small scale, and control strategies are developed to manage any residual risk that may occur. The development of the design space can be based on first principles (i.e., fundamental knowledge of the individual manufacturing variables) and/or on the use of empirical models. A statistical analysis of historical data can contribute to the establishment of the level of confidence that describes the design space, including the edges that will insure consistent product quality and performance. When developing a design space for a single unit operation, special consideration should be given to the overall manufacturing process, particularly the immediate upstream and downstream steps.

Factors contained within a design space include (but are not limited to) the sources of excipients or drug substance, the manufacturing processes, and/or the variations in manufacturing facilities. Design space can be implemented by using such techniques as:

- Proven acceptable ranges (PARs), which is the upper and lower limit of the material CQAs and CPPs that assures that the product meets the QTTP. The PARs can be determined based on prior knowledge, scientific judgment, and experimental data
- Conventional testing or feedback/feed forward mechanism for parameter adjustments
- Process Analytical Technology (PAT)
- Mathematical expressions

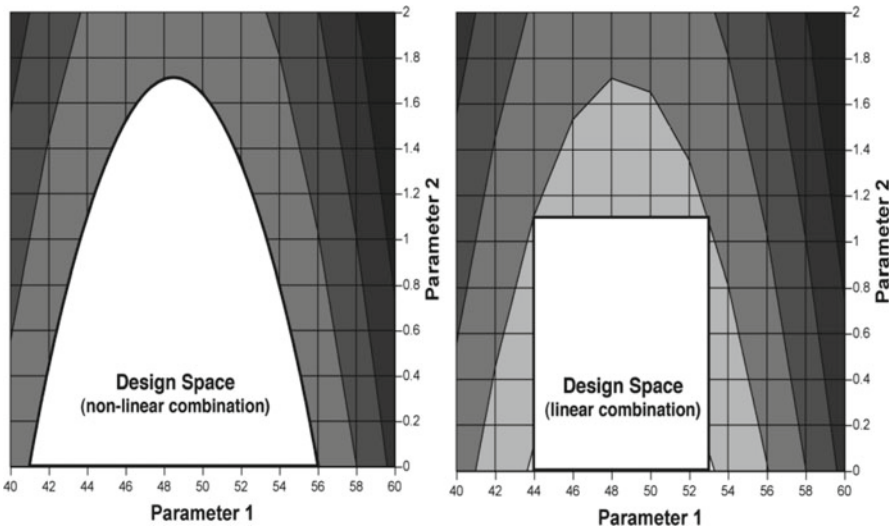

Fig. 7.4 The design space (represented as a contour plot) for two parameters (parameters 1 and 2) as they relate to their combined effect on in vitro dissolution. The response surface is described by a white area and by the various shades of gray such: the white area has the targeted dissolution of 85–90%, and the percent dissolved progressively decreases up to the *black* zone, which is represented by the region shaded in *black*. In this example, the acceptable range for one parameter is dependent on the value of the other parameter. The acceptable variation for the parameter combination is defined by the region in white (ICH Q8(R2)) [3]

The edges of the design spaces do not necessarily need to be validated. However, it might be possible to conduct verification studies of the performance of the design space scale-dependent parameters as part of process validation. Design space verification includes monitoring or testing of CQAs that are influenced by scale-dependent parameters. Additional verification of a design space might be triggered by changes, e.g., site, scale, or equipment. Additional verification is typically guided by the results of risk assessments of the potential impacts of the change(s) on design space [12].

Examples of such changes include modifications in the level or grade of excipients, changes in the manufacturing site, scale-up, and the use of new equipment. Design space verification should be guided by insights generated during product development and by an assessment of the potential risks associated with changes in the design space. While shrinking the design space is considered to be a minor change, design space expansion constitutes a major manufacturing change and ICH –Q8 notes that this may require additional regulatory interaction.

An example of the design space established for the relationship between two parameters (Parameter 1 and Parameter 2) as it impacts in vitro dissolution (Y axis) is provided in Fig. 7.4 (ICH Q8(R2)).

Additional data gained through manufacturing experience with the design space should be retained by the manufacturer. In the case of changes to the design space, appropriate filings should be made to meet regional regulatory requirements.

7.2.5 Risk Assessment and Risk Management

Qualitative risk assessments are often performed early in the pharmaceutical development process and should be repeated as knowledge is gained during the development and life cycle of a product. It is useful to identify and rank the variables from formulation and process parameters on key product CQAs [7].

A risk-based approach can be applied to the assessment of control strategy suitability across different scales. It should be considered a component of the control strategy development. This assessment might include risks from such variables as the raw materials, processing equipment, facility, environmental controls, personnel capability, experiences with technologies, and historical experience (prior knowledge).

While quantitative risk-analytical techniques have been applied to engineering design space problems for many years, its application within the pharmaceutical industry is relatively new.

As part of risk assessment, risk analysis, as defined by ICH Q9, is: "*the qualitative or quantitative process of linking the likelihood of occurrence and* severity of harm. *In some risk management tools, the ability to detect the harm (delectability) also factors in the estimation of risk*." Thus, based on the results of the risk assessment, QRM schemes should be established. The QRM is a control strategy that includes the parameters and attributes linked to the excipients, the drug substance, process parameters, and the facility. The risk control strategy should combine in-process controls (such as online or at line monitoring), finished product specifications, control of all analytical methods, frequency of monitoring, system feedback/feed-forward control mechanisms, and the associated preventive and corrective actions.

The importance of QRM is growing rapidly, both in its theory and application, in the pharmaceutical arena [2]. QRM tools are used for building the structural and quantitative models that support selection of the product CQAs used when defining the design space and it is incorporated with increasing frequency as a component of the QbD paradigm [13]. Although quantitative approaches to optimizing design space parameters are not new, the recent QbD efforts are novel in their application of QRM as the framework for the "risk-based thinking" that links manufacturing product quality to patient risks (the QTPP) throughout the product life cycle [7].

The rapid growth in desktop computational power has revolutionized the area of QRM. The ability to perform Monte Carlo simulations on the desktop has enabled QRM to come within the realm of routine risk management and DOE-based process design [14]. Numerous desktop software applications are now available as add-ons to spreadsheet software, sophisticated statistical packages or as stand-alone software applications [13, 15, 16].

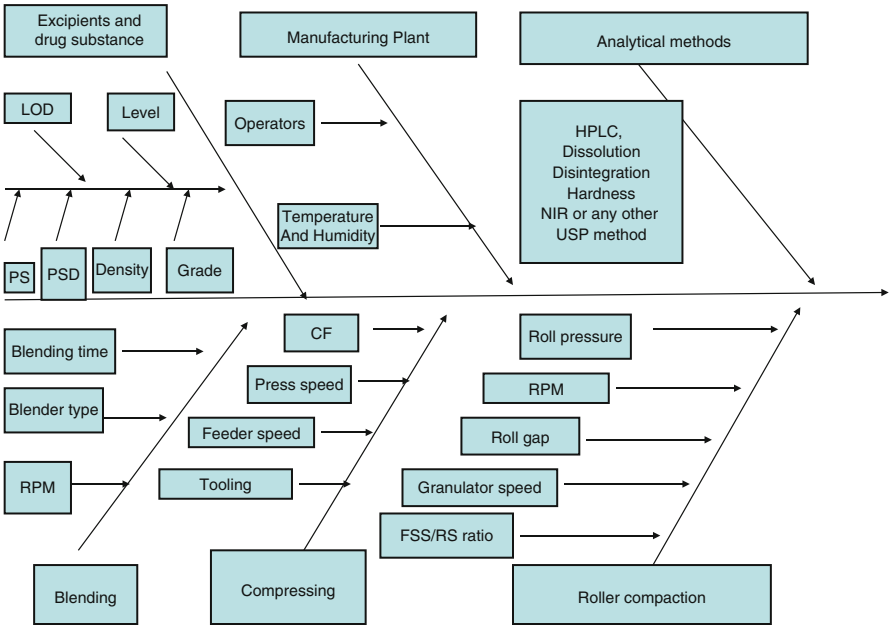

Fig. 7.5 Ishikawa fishbone diagram for tablet production via roller compaction. *Source*: FDA and The University of Maryland, Contract number HHSF223200810030C

7.2.6 Risk Assessment Tools for Establishing the Design Space and Control Strategies

To support the establishment of the design space and control strategy, an Ishikawa diagram can identify the independent variables (the material and process parameters) that can affect the QTPP. For example, the control strategy for an immediate release tablet using direct compression should focus on upstream control of material characteristics such as particle size, shape, and density, loss on drying, grade, and source. These attributes would then be incorporated in the excipient and API specifications. In contrast, if the model is for roller compaction, we may need to focus on the process parameters that influence particle size and shape, material density, and the Carr index.

The following risk "cause and effect" diagram shows an example of how to organize and present all variables that can affect the QTPP when using roller compaction to manufacture immediate release tablet dosage form (Fig. 7.5).

The parameters outlined in the Ishikawa chart help the scientist decide the parameters that need to be studied and controlled based on prior knowledge, experience, and the outcome from the initial visibility studies. The diagram includes all formulation and manufacturing variables, as well as the analytical methods and the online control systems. A risk analysis, in accordance with ICH Q9, is used to identify

those variables and unit operations that are likely to have the greatest impact on the desired quality attributes.

Screening studies and the outcome variables can be further assessed through the use of failure modes effect analysis (FMEA) (i.e., a method for identifying potential problems in the design or process). The FMEA is a mechanism for classifying risk as being high, medium, or low with respect to factors that can compromise product quality and performance. This analysis enables the various modes of failure to be prioritized (for risk management purposes) in accordance with the seriousness of their potential consequences, their frequency of occurrence, and their ease of detection. Once this assessment is complete, the DOE (or other experimental approaches) can be used to evaluate the impact of the high ranking variables, thereby facilitating process understanding and the development of appropriate control strategies. Based on this information, the formulator can identify the product CQAs that need to be further examined and controlled.

Another important aspect of FMEA, especially for larger organizations, is that this analysis facilitates a systematic gathering of current knowledge within the organization. This knowledge management system allows the risk information to be stored for future use (enhancing institutional memory) [11].

Through the FMEA, a risk priority number (RPN) can be generated for each combination of failure mode severity, occurrence probability, and likelihood of detection. The RPN is mathematically described as follows:

$$\mathrm{RPN} = \begin{bmatrix} 5 \\ 4 \\ 3 \\ 2 \\ 1 \end{bmatrix} O \times \begin{bmatrix} 5 \\ 4 \\ 3 \\ 2 \\ 1 \end{bmatrix} S \times \begin{bmatrix} 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{bmatrix} D,$$

where

O is the occurrence probability or the likelihood of an event occurring. *O* is ranked as:

5, likely to occur

3, 50:50 chance of occurring

1, unlikely to occur

S (severity) is a measure of the seriousness of the impact of failure mode on product performance. *S* is ranked as*:*

5, severe effect

3, moderate effect

1, no effect

D is the detectability, or the ease of identifying a failure mode. The greater the detectability of the failure mode, the lower the risk it presents to product quality. *D* is ranked as:

1, easily detectable

3, moderately detectable

5, hard to detect

The following table demonstrates the use of a FMEA to rank the risk of each variable (Table 7.1). All high-risk factors should be studied through a DOE developed by a subject matter expert [17].

An identification of the tools for parameter attribute measurement and analysis is an essential component of the design space and for its potential use during online/in-line process control. One example of a model supporting analytical procedures is an empirical (i.e., chemometric) model based on data generated by various PAT-based methods (see below for additional discussion).

Several statistical models can be employed for describing the components necessary for process monitoring and controls. These include models based on Univariate Statistical Process Control (SPC) and Multivariate Statistical Process Control (MSPC). Such models are generated by studying multiple batches that have been manufactured within prescribed targeted limits of the manufacturing variables so that the design space boundaries can be defined.

7.2.7 Control Strategy

Traditionally, process control has been achieved through the tight regulation of key process parameters at predetermined set points or ranges. It did not account for any sources of variation caused by raw materials or by the manufacturing process. Consequently, these control strategies did not allow for flexibility within or between production batches. However, by using methods that allow for the use of in/at line monitoring and control, these limitations can be remedied.

A control strategy is a planned set of constraints, derived from current product and process understanding, which assures product quality and performance. As such, it is integral to assuring that the QTPP is realized for each marketed lot. The controlled parameters can cover material attributes (as related to drug substance and other product materials) and the operating conditions of the facility and its equipment. It can include the regulation of product manufacture via the use of in-process controls, finished product specifications, and the method and frequency of monitoring and managing the product and its production [17]. Types of control strategies may include (1) controlling the CQA for all the raw materials, (2) online or at-line monitoring systems for the CPPs and process endpoints, (3) a monitoring program (intermittent testing) for verifying multivariate prediction models, (4) the development and implementation of product specifications, (5) in-process or real-time releasing testing, and (6) feedback/feed-forward mechanisms for corrective and preventive actions.

The identification and linkage of the CQAs to the raw materials and to the CPPs should be a goal when designing a control strategy. The control strategy will reduce risk but will not change the criticality of the various attributes. Initially, control strategy is developed and implemented for the production of the clinical batches, and it can be improved to be more efficient for use in commercial manufacture as new knowledge is acquired. Additional emphasis on process controls should be

Table 7.1 Application of a FEMA model to describe the risk of failure in raw materials or manufacturing processes

Failure mode and effect analysis

Variable	Failure mode	Impact of failure	*S*	Potential cause	*O*	Suggested detection methods or current controls	*D*	RPN
Excipients	Different sources	Physical properties	5	Physical variation (particle size, shape)	5	Visual inspection	3	75
	Grade	Dissolution, hardness	5	Poor development or wrong grade	5	Disintegration, dissolution, hardness tester	5	125
	Level	Dissolution, hardness	4	Operator error, poor development	5	Disintegration, dissolution, hardness tester	5	100
Blending	Blender speed	CU	5	Operator's error, equipment failure	5	NIR	1	25
	Blending time	CU	5	Poor monitoring	4	NIR	2	40
	Fill level	CU	5	Operator's error	4	NIR	2	40
	Humidity	CU	5	Poor air handling	3	NIR/Hygrometer	4	60
Dry granulation	Feed screw speed	Granule uniformity	4	Machine failure, poor development	4	HPLC/NIR	2	32
	Mill speed	Particle size, hardness	5	Machine failure,	5	Malvern	1	25
	Roll pressure	Particle size, hardness	4	Machine failure, operator's error	5	Malvern/hardness tester or Carr index	4	80
	Roller texture	Ribbon density	3	Machine failure, poor development	4	Carr index	4	48
Tableting	Compression force	Hardness, dissolution	5	Operator's error, machine failure, or poor granulations	5	Disintegration, dissolution, hardness tester	3	75
	Compression speed	CU	5	Operator's error, equipment failure	2	NIR/HPLC	4	40
	Humidity	CU and hardness	4	Poor air handling	2	Hygrometer	2	16
	Tooling	CU, tablet weight	3	Operator's error	5	NIR/HPLC	5	75

61–125 high risk; 31–60 medium risk; 1–30 low risk

S, Severity of excursion *S* = 1 (low) 5 (high); *O*, occurrence of the excursion = 1 (low), 5 (high); *D*, Detection of excursion *D* = 5 (high), 1(low)

Risk priority number (RPN) = SXOXD

Source: FDA and The University of Maryland, Contract number HHSF223200810030C

considered when products cannot be well-characterized and/or when the quality attributes cannot be readily identified (*ICH-Endorsed Guide for ICH Q8/Q9/Q10* Implementation) [12].

An important component of the control strategy is the integration of an effective monitoring system to assure product consistency during (rather than after) batch production. It can also be used to identify aspects of the manufacturing process that are in need of improvement. The process performance and product quality monitoring system should conform to the principles set forth in ICH Q10. An example of such a strategy is Process Analytical Technology (PAT), which, through the use of real-time monitoring and control techniques, can be used to manipulate process parameters (process inputs, X1, X2, X3, …) within specified limits in order to insure that the drug product attributes (process outputs, Y1, Y2, Y3, …) are contained within some prescribed range. PAT can include such simple tests as the monitoring of pH, humidity, or temperature. It can also include more complicated "process analyzer" techniques such as Raman, Laser-Induced Fluorescence (LIF), and near-infrared (NIR) spectroscopy. NIR, in conjunction with chemometric modeling techniques, provides a fingerprint of the raw materials. PAT can be used to control manufacturing parameters such as blending time, particle size, the end coating time of controlled release tablets, and the amount of moisture in a granulation (to determine the drying endpoint). It can also be used to test content uniformity, assay, hardness, dissolution, and the disintegration time. Its advantages include speed, efficiency, and its nondestructive nature.

7.2.8 Developing a Plan for Real-Time Release Test and Process Improvement

RTRT provides a roadmap for applying the results of the FMEA to risk assessment strategies that ensure the continuous quality of the in process procedure and/or of the final finished product [3]. Through the use of real-time release test (RTRT) procedures, processes are continuously monitored, providing far greater control than do strategies where samples are taken only at the beginning, middle, and end of a run. The inclusion of feedback on product quality (both from internal and external sources, such as a certificate of analysis, product rejections, nonconformances, recalls, deviations, audits and regulatory inspections and findings) provides a mechanism whereby a QbD paradigm can be employed to promote process improvement.

Pharmaceutical development studies enhance the knowledge of product performance over a wide range of material attributes, processing options, and process parameters. It enables manufacturers to define the limits within which materials and processes can vary without comprising product quality and performance. The outcomes of these studies facilitate the establishment of a design space, providing greater flexibility in allowable variations in both process and materials.

7.2.9 *Product Life cycle Management and Continual Improvement*

Throughout the life cycle of the drug product, there is a continual need for modification due to changes in excipients or excipient suppliers, new manufacturing equipment or location, scale-up, or even formulation changes. When these events occur, having established a well-defined relationship between material CQAs, CPPs, and product CQAs is invaluable. Such product understanding enables the manufacture to identify whether or not the intended changes can be implemented without compromising product performance. Thus, employing a QbD paradigm supports process improvement and, potentially, expansion of the design space. Together, the implementation of product and process understanding supports efforts to improve production efficiency without compromising product quality.

7.3 International Consensus on QbD: The ICH Guidelines

Pharmaceutical development (Q8) in combination with QRM (Q9) and Pharmaceutical Quality System (PQS) (Q10) provide a systematic, risk- and science-based approach to pharmaceutical manufacture and development, improving the assurance of drug quality and manufacturing efficiency.

The following guidelines serve as the international foundation for applying the various aspects of QbD as described in this chapter:

ICH Q8 (R2) Pharmaceutical Development [3]: This guidance defines QbD as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and QRM." It recognizes that ***quality*** cannot be tested into products, but rather that quality should be built in by design. This guidance also identifies those areas where the demonstration of greater understanding of pharmaceutical and manufacturing sciences can create a basis for regulatory flexibility. The QbD approach involves (1) the identification of product attributes that impact the product's safety and efficacy, (2) the selection of excipients and the process for delivering these attributes, (3) the development of a robust control strategy to ensure consistent process performance, (4) the establishment of the design space, and (5) validation and filing of the process demonstrating the effectiveness of the control strategy and the proposed ongoing monitoring method. Although the Q8 guidance is intended for the drug product, some of the concepts are applicable to raw materials and should be taken into consideration when creating a raw material QRM program.

The ICH Q9 Quality Risk Management guideline [7]: This guidance defines QRM principles and describes QRM tools that can facilitate the development of effective and consistent risk-based decisions. These principles and tools are intended to be integrated into product manufacture throughout its life cycle. This guidance can be

applied to drug products involving small molecules, biologics, and biotechnology products. Processes covered include those associated with product development, manufacturing, distribution, inspection, and submission/review processes.

The ICH Q10 Pharmaceutical Quality System [17]: This guideline applies Drug substance and drug products, including biotechnology products and biologics. ICH Q10 describes a comprehensive approach to an effective pharmaceutical quality system based on International Organization for Standardization (ISO) concepts, including applicable Good Manufacturing Practice (GMP) regulations. ICH Q10 is intended to complement ICH Q8 and ICH Q9, serving as a model for a pharmaceutical quality system that can be implemented throughout the different stages of a product life cycle.

In addition to these ICH guidelines, specific guidances have also been provided by the U.S. Food and Drug Administration, European Medicinal Agency (EMA), and the Japanese Ministry of Health, Labor, and Welfare (MHLW). Although these documents provide regulatory expectations from the different jurisdictions, they all share the foundational principles set forth in the ICH guidelines described above.

7.4 The Role of QbD Today and in the Future

Figure 7.6 provides an overview of the QbD approach and the various components that are needed to insure effective control strategies and to support continual product improvement.

As we move forward towards the development of novel drugs, dosage forms and delivery systems, it is clear that this science-based approach to pharmaceutical manufacture will be an essential component of the drug product life cycle. It is only through identification of the QTPP and CQAs that control strategies can be optimized that will assure that each product batch will have the intended quality and performance [14]. This is particularly important in view of the challenges that will be facing some of the novel drug delivery systems in the future, including parenteral formulations and biologics.

Ultimately, the goal of this approach is reduced product variability and defects (advantage to the patient), reduced lead time, and lower inventory (manufacturer savings), and improved cost efficiency, improved manufacturing efficiency, and better adaptive process control to facilitate and implementation of process improvements (a benefit to everyone). Moreover, this science-based approach facilitates interactions between drug manufacturers and regulators such that the most appropriate product specifications to insure in vivo product performance can be more efficiently defined.

An excellent overview and discussion of the QbD approach from an international perspective (presented by regulators and manufacturers of human pharmaceuticals) can be found at http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/AdvisoryCommitteeforPharmaceuticalScienceandClinicalPharmacology/UCM266751.pdf.

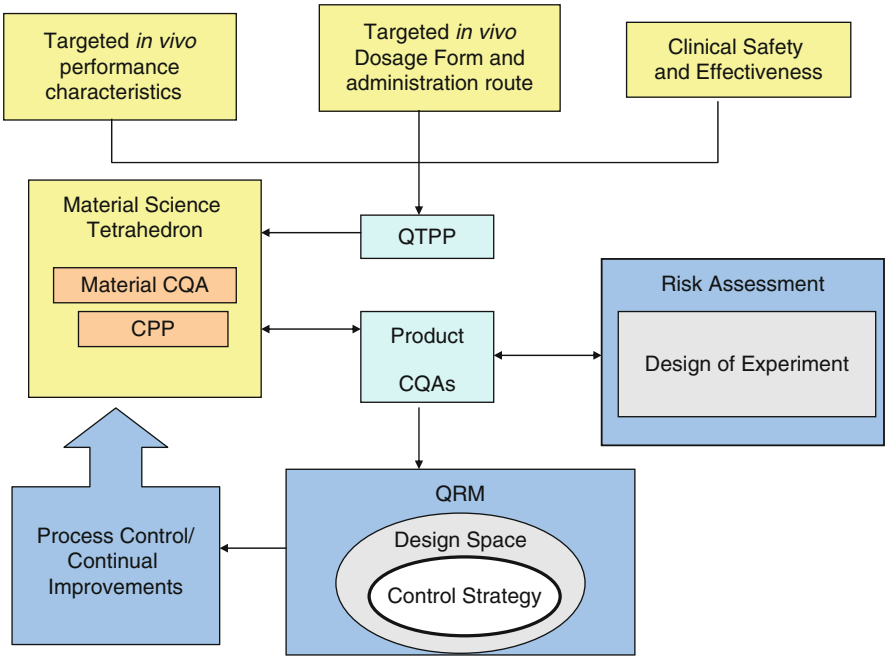

Fig. 7.6 An overview of the QbD process as integrated from the beginning of a product development (identifying the QTPPs) to the final set of process control and ongoing product improvements. *Yellow boxes* represent the in vivo and material variables are set for the product. The *orange boxes* in the material science tetrahedron are based on the selection of materials and processes. The *light blue boxes* are the in vivo and product performance characteristics that the product is intended to achieve. In order to insure that each marketed lot of product meets these goals, the risk assessment/risk management strategies are implemented (represented in the *dark blue boxes*). As product improvement is implemented over the course of the product's market life, the process recycles through the QbD paradigm

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Chapter 8
Final Product Testing and the Development of Specifications for Veterinary Pharmaceuticals

Jay C. Brumfield

Abstract This chapter reviews the main concepts related to analytical testing of veterinary drug products and the development of specifications for critical quality attributes. Pragmatic strategies for the development and use of analytical specifications throughout the veterinary product development and commercialization life cycle are discussed. Also presented are typical analytical testing requirements for quality assessment and registration of selected types of products in major markets (USA, EU, and Japan), and unique challenges related to several veterinary-centric dosage forms including medicated articles for preparation of feeds and drinking waters, and topical parasiticide preparations.

8.1 Introduction

This chapter reviews the main concepts related to analytical testing of veterinary drug products and the development of specifications for critical quality attributes (CQAs). Pragmatic strategies for the development and use of analytical specifications throughout the veterinary product development and commercialization lifecycle are discussed. Also presented are typical analytical testing requirements for quality assessment and registration of selected types of products in major markets (USA, EU, and Japan), and unique challenges related to several veterinary-centric dosage forms including medicated articles for preparation of feeds and drinking waters, and topical parasiticide preparations.

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In this chapter, analytical testing refers to the nonclinical testing of physical, chemical, or microbiological quality attributes as typically accomplished using wet chemical, instrumental, or microbiological methods. As per the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH), "a specification is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria, which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a drug substance or medicinal product should conform to be considered acceptable for its intended use. 'Conformance to specifications' means that the drug substance and/or medicinal product, when tested according to the listed analytical procedures, will meet the listed acceptance criteria. Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities as conditions of approval" [1, 2]. Several critical points are captured in this definition. First is the fact that a specification is the sum of a test (i.e., a tested characteristic), a test method (a.k.a. a test procedure), and the acceptance criteria:

Specification = (tested characteristic) + (test method) + (acceptance criteria)

Or, more simply:

Specification = (what is being tested) + (how is it tested)
+ (what test result is acceptable or unacceptable)

Hence, the development of specifications includes the identification of characteristics that need to be tested, the development of appropriate analytical test methods, and the development of acceptance criteria proven to yield an acceptable product. Second is the fact that a test method and the associated acceptance criteria are inherently linked such that changes in the test method may require changes in the acceptance criteria and vice versa. For example, if it were determined necessary to tighten the specification for an impurity (i.e., lower the maximum amount allowed in the drug product) due to toxicological concerns, it may be necessary to modify the method to improve its sensitivity, achieving a lower limit of detection and limit of quantitation, and to revalidate the method.

Testing of a product for compliance with specifications is only one part of an overall product quality control strategy [1–3]. Product batch release testing serves as a final confirmation of selected key quality attributes of the product rather than a complete characterization and assurance of quality. The testing strategy is based upon the wealth of product and process knowledge (the knowledge space) gained during development. This knowledge space underpins the narrower design space of the product, process, and specifications to which the commercial product must routinely comply. As per the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guideline on Pharmaceutical Development, "A comprehensive pharmaceutical development approach will generate process and product understanding and identify sources of

variability. Sources of variability that can impact product quality should be identified, appropriately understood, and subsequently controlled" [3]. Furthermore, comprehensive quality strategies include compliance with current Good Manufacturing Practices (cGMPs), which introduces the need for appropriate facilities, qualified equipment, trained personnel, validated processes, etc. Quality cannot be tested into a product; it must be built into the product by design [3, 4].

Because analytical testing and specifications development are very broad subjects, it is impractical to provide a fully detailed and exhaustive discussion of all pertinent topics within this chapter. Selected literature references are provided for more detailed reading. Where possible, the cited literature is specific to veterinary product applications; however, it should be noted that the volume of literature related to human health product development and commercialization far outweighs that available and specific to veterinary products. The human health literature is very pertinent though given that there are many more similarities than differences when comparing human and veterinary products for these particular topics. Indeed, the Chemistry, Manufacturing, and Controls (CMC) information required to support registration of veterinary pharmaceutical products (formulated with small, non-immunological molecules), which is the culmination of much of the testing and specification knowledge accumulated during product development, is nearly identical to that of human health products. The science of human health and animal health CMC development is identical, and the regulatory requirements are very similar, with some minor differences (e.g., tolerable levels of organic impurities) and special requirements related to certain veterinary dosage forms (e.g., medicated feeds) as discussed herein.

8.2 Scope and Noteworthy Related Topics

The focus of this chapter is analytical testing and specifications for the final formulated drug product of small molecule (nonimmunological) actives. Whenever someone hears the term "product," they usually think about the formulated material. However, for analytical testing and specifications purposes, it is useful to think of the product in a broader perspective—as the combination of the formulated material and the primary packaging, including the container closure and any labeling materials in direct contact with the container (e.g., printed adhesive labels). This broader perspective is important because the quality of the product throughout shelf life is often directly impacted or maintained by the selected packaging materials and container design. Hence, formulation and packaging design are intertwined. This chapter therefore includes a brief discussion of testing considerations for primary packaging materials (those in direct contact with the formulated material) and dosing devices.

Beyond the packaging, additional primary factors in the overall quality of the drug product are the quality of the active drug substance and the excipients. Many physical and chemical parameters of the drug substance can directly influence the

manufacturability, quality, and performance of the drug product. For example, the crystal habit/morphology, crystal structure/polymorphism, particle size, and intrinsic solubility of the drug substance can influence the product's disintegration and dissolution/drug release properties, and stability. These properties can in turn impact the bioavailability and hence bioequivalence between drug products. Hence, drug substance testing considerations will also be briefly discussed.

Other topics that are noteworthy but generally excluded from discussion in this chapter include:

Immunologic products based upon small molecules (vaccines) and biologically derived actives such as peptides or biomass products: Many of the principles discussed herein are applicable to these types of products, but there are often other special tests required that go beyond this discussion [5–7]. Products based upon actives that are pure, homogeneous isolates from fermentation processes or semi-synthetics (i.e., fermentation-derived molecules that undergo subsequent chemical processing to yield a pure active) are generally covered by the discussions presented herein.

Excipients: Specifications and test methods for common excipients may be found in the current editions of the respective licensing countries pharmacopeias such as the United States Pharmacopeia/National Formulary (USP/NF), The European Pharmacopoeia (Ph. Eur.), and The Japanese Pharmacopoeia (JP). In addition, it is common for veterinary products to include raw materials that are approved as food or feed additives (e.g., as described in the Food Chemicals Codex (FCC) and the Official Publications of the Association of American Feed Control Officials (AAFCO), respectively). Formulation with novel, noncompendial excipients that are not generally regarded as safe (GRAS) or approved as food additives is often avoided because it may require a more extensive characterization similar to that performed for a new chemical entity (NCE) drug substance, potentially including toxicological qualification to support its use [8]. Some consideration will be given to functional excipients such as antioxidants and microbial preservatives as special studies may be needed in development to support their selection and use.

Quality-by-Design (QbD): Certain principles of Quality-by-Design (QbD) factor into the development strategies presented herein, but a more comprehensive discussion of the role of QbD in veterinary product development is presented in Chap. 8 of this book.

In-Process Testing: Testing of a product up to the point of it being a final dosage form, i.e., in-process testing, is often an important part of the quality control strategy. Certain tests when conducted during manufacturing may yield data that is sufficient to satisfy specification requirements when the acceptance criteria are equal to or tighter than the registered specification and the test is listed in the specification [1, 2]. A subtopic of In-Process Testing that is also of importance to QbD is Process Analytical Technologies (PAT). The initiative, "Pharmaceutical cGMPs for the 21st Century," was launched by the United States Food and Drug Administration (FDA) in August 2002. QbD is a key part of the initiative and PAT

is an important element within QbD [4]. Implementation of QbD and PAT may reduce the testing required on the final drug product, and in its ultimate embodiment may facilitate real-time release (*vide infra*). As per ICH Guideline Q8(R2], "Understanding sources of variability and their impact on downstream processes or processing, in-process materials, and drug product quality can provide an opportunity to shift controls upstream and minimize the need for end product testing" [3]. PAT and QbD have been the subjects of considerable discussion between the regulatory authorities and industry in recent years; however, at the present time, "any decision on the part of the manufacturer to work with the [US Food and Drug] Agency to develop and implement PAT is a voluntary one." Because comprehensive statistical modeling is often needed to establish the reliability of predictive mathematical models used in PAT, this technique may lend itself best to post-approval implementation for animal health products once a wealth of batch data is available [4]. For example, Near-Infrared Spectroscopy (Near IR) is a very useful PAT technique which may be employed for the identification and analysis of excipients, intermediates, and finished products. Near IR has several significant advantages in that it is an instantaneous, nondestructive test that can provide data on a variety of quality attributes simultaneously (e.g., identity, assay, water content). Nonetheless, the development and validation of this test is complex in that it requires an extensive training/calibration data set to ensure that it will correctly discriminate between materials of acceptable quality and those which are unacceptable without generating α (Type I) or β (Type II) errors, i.e., accidentally generating an out-of-specification (OOS) result for an acceptable batch or a passing result for an unacceptable batch [9, 10]. Based upon private discussions with industry colleagues, it appears that few veterinary products today employ PAT, possibly because of the significant investment required to implement this technology when the guarantee of—and timeline for—a return on investment may be unclear.

8.3 The Drivers for Specifications Development

Specifications development is driven by many factors including quality control, customer needs, plus scientific, regulatory, legal, and economic considerations.

8.3.1 Quality Control

The primary role of QC testing is to provide a final verification that a batch of product meets required quality parameters, supporting its release to the commercial market. This "batch release" verification is achieved when the results of all tests comply with the acceptance criteria set forth in the registered specifications, plus any more stringent internal targets established by the manufacturer to provide increased confidence of specification compliance at the end of shelf life. Hence, the utility

of drug product testing in QC may be viewed as a risk mitigation role, where the assurances of product quality cumulatively established through the overall quality control system of cGMPs, raw material process controls, validation, training, etc. are subject to one final confirmation before the product is released to the market. Furthermore, a sampling of representative commercial product batches is typically placed on long-term stability studies each year as part of a marketed product stability commitment to the licensing authorities. Such studies provide periodic verification that the product complies with the shelf life specifications through the assigned expiration date. Additional discussion on batch release testing and stability testing is provided elsewhere in this chapter.

8.3.2 Customer Needs

When selecting a veterinary product, the customer (i.e., the veterinarian, pet owner, rancher, etc.) has several primary concerns: efficacy, cost or value, and safety. Secondary considerations include availability of supply, reputation of the product or manufacturer, product referrals from trusted sources, etc. These factors and others combine to impact the customer's *perception* of the product, which is essential for commercial success. Major factors influencing customer perception of efficacy are the rate and magnitude of obvious improvements in the condition of the animal. Perceptions of the product's safety (or lack thereof) are based upon any observations of side effects. The product must also perform consistently over time. Hence, the potency and purity of the product throughout its labeled shelf life are of critical importance to the customer, and this must be achieved on all batches in order to provide the desired product consistency.

Beyond the impact that the product has on the animal, the customer's perceptions of product quality are also heavily influenced by those characteristics that are easily discernable through sight, smell, or touch of the product in its packaging or during product administration (e.g., viscosity of an injectable product which may impede syringability). Visually discernable characteristics might include consistency of appearance of the formulated product including color, size, volume of product in the container, homogeneity (phase segregation, particulate matter, or precipitation), and dissolution rate (for products requiring reconstitution or soluble powders for administration in drinking waters). Packaging observations would typically note any leaks, bloating or concavity of the package (paneling) over time, blemishes, or improper cap removal torque (cap too loose/leaking or too tight to remove). Tactile observations might include the texture or viscosity of a product. For a frequently administered product, the customer may also notice if a batch has an uncharacteristic odor. Because these matters of pharmaceutical elegance, consistency, and ease of administration impact the customers satisfaction of the product, all the relevant parameters need to be anticipated and controlled by the manufacturer through the appropriate specifications. For commercial success, the customer's expectations need to be met and managed, either by providing a product that is consistent and acceptable for all of these parameters or through labeling. In some cases, there may

be unavoidable variation or changes over time in a characteristic that have been proven to have no impact on product safety or efficacy. In such cases, it may be necessary to advise the customer of this through the product labeling (e.g., indicating in the package insert that the product may turn a darker color over time and that this does not impact product quality). In other cases, it may be necessary to advise the customer on how to administer the product when changes are noted. For example, it may be appropriate to include in the labeling that an injectable product may be more difficult to syringe on cold days (due to increased viscosity) and that warming the product is an appropriate way to make it easier to administer.

8.3.3 Scientific Drivers

The product development scientist should consider what tests are needed to fully characterize the product's physical, chemical, and microbiological quality. Any properties which might change over time for a given batch that are indicators of product stability or which might vary on a batch-to-batch basis (e.g., due to drug substance or excipient batch variation or process variability) typically need to be characterized, and the need for long-term control via specifications considered. Beyond batch release and stability evaluation, a huge amount of analytical testing is performed during development to build a knowledge space for the product and its manufacturing process. These studies are an essential part of the overall design process that is captured as part of the developmental pharmaceutics sections of product registration dossiers. These studies help to identify potential sources of variability and to develop strategies for minimizing their impact. Such studies may help to determine the criticality of a test or to gauge appropriate acceptance criteria for future specification setting purposes. Many of the tests conducted during development may only need to be performed once, or a limited number of times during development, and may never be implemented as routine QC batch release and stability specifications. Examples include spectroscopic and mass spectrometric tests to elucidate the structure of the active as a part of primary reference standard characterization or the structure of impurities as part of an identification or qualification strategy [11–13]. Other developmental studies might include performing antimicrobial effectiveness testing on product formulated with various levels of preservative for preservative content assay specification development, studies to explore drug substance/excipient compatibility, or to establish an upper limit for moisture content of a nonaqueous formulation.

8.3.4 Regulatory Considerations

Regulatory considerations can be categorized as related to either compliance or to product registration. From a compliance perspective, the requirement to test active ingredients, excipients, packaging materials, and final products is captured in

government regulations as a part of GMPs, for example in the US Code of Federal Regulations 21CFR 211 and in the European GMP requirements detailed in the Eudralex. Many of the concepts and practices discussed herein such as batch release testing, the conduct of stability studies, and investigation of out-of-specification results have significant basis in cGMPs and are therefore captured in the manufacturer's standard operating procedures (SOPs).

From a product registration perspective, before a veterinary pharmaceutical product can be marketed, a compilation of information that fully describes the CMC aspects (the product, the manufacturing process, the specifications, etc.) plus evidence of efficacy, target animal safety (TAS), human food safety (HFS, when applicable), environmental impact, etc. must be reviewed and approved by the local licensing authority. Specifications are always a key part of this registration dossier, and once approved, they become a legal obligation for the drug product manufacturer. Data submitted as part of the CMC dossier supports claims regarding product shelf life (i.e., the data underwrites that the product will comply with specifications throughout the expiration date), how long the product can be used once first opened, storage conditions, the impact of temperature and humidity excursions experienced during shipping, and the steps for preparation and administration by the customer. Requirements and suggestions for parameters to be tested for product registration may be found in many places, including the VICH guidelines and guidances for industry of the appropriate governments and licensing authorities including the European Medicines Agency (EMA), the United States Food and Drug Administration (FDA), FDA-Center for Veterinary Medicine (CVM), and the US Environmental Protection Agency (EPA, for topical ectoparasiticides) to name a few. Furthermore, the FDA recently introduced a science- and risk-based list of questions for consideration in the preparation and review of regulatory dossiers. This question-based review (QbR) process asks many questions that require test results and specifications to address. Drug product manufacturers should consult the pharmacopeia of the intended markets for general tests based upon the product type, and specific tests and specifications if the product is described in an existing monograph. VICH guidelines and guidance documents of the intended licensing country authorities should also be consulted.

8.3.5 Legal Considerations

The registered specifications of a product are a legal contract between the manufacturer and the licensing authorities of the countries in which the product is approved for sale. Specifications may also be a part of the legal agreement between suppliers of excipients, actives, and packaging materials and their customers. Production of pharmaceutical products is often outsourced to contract manufacturing organizations (CMOs). In these cases, the specifications are part of a contract between the CMO and the company for which they manufacture the product. Active ingredients or other components that fail registered specifications are generally unsuitable for

use in the manufacture of the drug product. It should, however, be noted that not all attributes controlled through the supplier's specification tests may be critical to the performance and acceptability of the drug product. There may be a subset of tests performed by the supplier that are critical, and others that are irrelevant. Also, the drug product manufacturer may identify additional specifications for CQAs not tested by the supplier but which must be met before the material can be deemed suitable for product manufacture. Obviously, this practice introduces some supply risk where the vendor is not obligated to supply a material that meets all of the manufacturer's requirements. It is therefore in the drug product manufacturer's best interest to negotiate the specifications of the incoming materials with the vendor as part of a legally binding "quality agreement," ensuring that all quality attributes of importance to the drug product manufacture are suitably controlled and guaranteed by the vendor.

In most countries, a product that fails one or more of the registered specification tests cannot be legally distributed for sale. In the European Union, the Qualified Person (QP) has a critical role in ultimately determining the suitability of the product for distribution. The QP has final authority to release a product to the market and holds some personal legal accountability for the products acceptability.

Product test results are often critical to the creation and defense of intellectual property, and debunking of patent claims. For example, patent claims may include the synthetic or manufacturing process or composition (which can often be verified by the presence or absence of trace ingredients or impurities), conformation of the active to a specific polymorphic form (which can be substantiated through a variety of analytical techniques), and the drug release profile of a modified release dosage form (as evaluated with an in vitro drug release test).

Compliance with compendial requirements is also a legal driver of specifications. When products are sold in a market and the product is described in the national (e.g., USP/NF), regional (e.g., Ph. Eur.), or international (e.g., World Health Organization) pharmacopeia, compliance with the standards set forth in the compendia is mandatory unless explicitly justified by the manufacturer. Applicable requirements may include the contents of general chapters, product-specific monographs, or monographs on excipients or the drug substance used in the product.

8.3.6 Economic Considerations

In order to maximize profitability, pharmaceutical manufacturers are particularly sensitive to the cost of goods, which includes the costs of raw materials, production, packaging and application devices, testing, and distribution. Specifications need to be developed with necessity, risk/benefit, and practicality all in consideration. Practicality includes analytical testing: cost and time required to perform the test, whether the test can be performed within the company (in-house) or if it needs to be outsourced for a fee. Practicality also includes considerations of the probability of failing a test and rejecting a product batch. Sufficient testing should be conducted

during development to ensure that the process is capable of routinely producing product that meets or exceeds all quality parameters. It would be far too costly to produce a product with an out-of-control process that routinely yielded unacceptable batches that would be rejected and discarded, not to mention the fact that this would be inconsistent with cGMP expectations. Lastly, it should be noted that when multiple methods are available to test the same quality parameter, the most scientifically rigorous test may not always be the best QC method, especially when the data quality and information is nearly equivalent but the cost of performing the test is excessive.

8.4 Similarities and Differences in the Analytical Development of Veterinary versus Human Health Products

The development, optimization, validation, technology transfer, and implementation of analytical test methods for pharmaceutical materials and processes, including the development of specifications, can be cumulatively considered as the "analytical development" portion of the drug development process. Analytical development is largely identical for animal health and human health product development. The same drivers exist for creation of human and animal product specifications, and the same analytical technologies are employed in both cases. Regulatory similarities are considerable, as can be seen from a comparison of ICH and VICH regulatory guidances on test procedures and acceptance criteria [1, 2, 14]. Where minor differences exist, they are largely driven by safety considerations or guidances specific to dosage forms not used in humans. In human products, patient safety is of primary concern. In animal health, target animal safety is still of concern, but human food safety is most important. An example can be seen in a comparison of ICH vs. VICH thresholds for impurities reporting, identification, and qualification, where higher levels of impurities are typically allowed in veterinary products. Also, some data requirements are relaxed to encourage manufacturers to register their products for use in minor or exotic species (ones for which the development of species-specific products would not be economically feasible due to the small number of animals treated).

The main differences to the analytical scientist working in animal health stem from the pace of development, unique dosage forms, drug dose and range of doses that must be delivered, and the frequent use of complex or natural ingredients. Arguably, the challenges faced by a scientist working in animal health product development are often greater than those encountered by their human health colleagues.

In both human health and animal health, analytical development begins early in the research phase with characterization of the active compound and continues throughout the product development and commercialization lifecycle. The development phase of this lifecycle is different and typically much longer for human vs. animal health products. Human product development entails years of preclinical studies (in nonhuman species), followed by years of human clinical trials, first in a

limited then increasing number of healthy subjects (Phase I and IIa), followed by studies in symptomatic patients (Phase IIb) and large pivotal clinical trials (Ph III) [15]. This entire process often spans 10 years or more, allowing ample time for the advancement from rudimentary formulations in early trials to commercial image product in Phase III. In contrast, in animal health product development, it is possible to quickly evaluate the drug substance in the target species and generate a clinical proof-of-concept (PoC) for safety and efficacy, at which point the drug candidate may enter full development (Phase III). Hence, Phases I, IIa, and IIb are greatly compressed in animal health product development. This also means that the time to develop a commercial image formulation is compressed. It is desirable to have the final formulation that will be commercialized available as soon as possible after the clinical PoC is achieved so that the pivotal (Phase III) clinical trials (efficacy, TAS, and HFC) may begin. Hence, the timeframe for analytical development is greatly compressed, and the CMC-related activities are frequently on the critical path to commercialization of the product.

Analytical development in animal health is also complicated by the huge array of unique and challenging product types [16]. While some formulation types are unique to human health (e.g., respiratory delivery device based-products such as dry powder inhalers and metered-dose inhalers), there are just as many or more unique product types in animal health, each one bringing its own unique set of challenges for specifications development, such as spot-on pesticide products, medicated feeds, and products for administration in drinking water.

Due to body weight extremes and differences in drug metabolism and physiology across veterinary species, the payload of drug that needs to be delivered can vary significantly. For an active that has relevant indications across a variety of species, the dose delivered to a large animal like a horse or cow may be many times that delivered to a small dog or cat. Even within a given species, it may take several strengths or sizes of drug product to effectively treat animals of different body weights and remain within the therapeutic and safety margins of the active. The veterinary product analyst is challenged to develop the appropriate test methods and specifications to characterize all strengths of the product [15]. Physiologic and metabolic differences may also lead to differences in pharmacokinetics across species. Dose and drug release requirements, including design of in vitro tests may therefore be impacted. The species and body weight differences in veterinary drug development are in some ways similar to the considerations in special populations of human patients, e.g., pediatric, geriatric, pregnant, enzyme, or immunocompromised individuals.

In terms of the complexities introduced by product composition, veterinary products for oral delivery often rely upon natural food or feed ingredients to make them palatable. Such ingredients are typically complex in composition, containing many compounds which can create matrix issues which may cause poor recovery of the analyte, and interferences.

Lastly, it is undeniable that the development of veterinary pharmaceutical products faces tremendous time and cost constraints. Such considerations, when paired with the rapid development timeline discussed earlier means that fewer product batches are typically produced and characterized during veterinary product development

than in human product development. This increases the specifications development challenge because data available to support the specifications at the time of registration may be quite limited.

8.5 Selection of Critical Quality Attributes

Veterinary product development is often justified and initiated with a product proposal capturing assumptions regarding the active ingredient, the indications, anticipated outcomes, formulation attributes based upon the intended target species and desired route of administration, and any established details regarding the market, key competitor products, etc. Once these most basic concepts are established, the process of product development begins with simultaneous development of formulations, analytical methods, and packaging. The veterinary product analyst is challenged to develop the test methods while simultaneously applying them to characterize prototype formulations and packaging, assessing their relative quality and stability. Ideally, the commercial product, its primary packaging, and all specifications would be available simultaneously and as early as possible in the development program to support the batch release of clinical trial materials to support pivotal safety and efficacy studies.

It is important that all physical, chemical, and microbiological attributes needed for comprehensive product characterization are identified as early as possible so as to allow maximum time for method development and collection of data to support development and registration. Such attributes are often called critical quality attributes (CQAs) or key quality attributes (KQAs) [3]. A strategy for the process of identifying CQAs might entail:

1. Identify tests known to be required plus any other characteristics that can be tested that might impact quality or indicate changes over time.
2. Collect data on a variety of product batches where formulation and processing variables are intentionally varied. This often includes stability data for product stored under the intended and accelerated stability conditions.
3. Based upon a review of the data, identify any tests that are required or that are critical quality or stability indicators. Tests for these attributes should be further refined, validated, and implemented as QC tests.
4. Document and discontinue nonessential tests.

In Steps 1 and 2 it is best to cast a broad net, exploring as many potential quality parameters as possible to build the knowledge space of the product and process. A costly error in product development is to assume that a parameter is unimportant and that it will not need to be controlled via a specification for registration purposes, only to find out later that it is critical and that there is no data available to develop the specification. The CQA selection process begins with the identification of all quality attributes that are *known* to be important, as well as those which *might* be important but require data to gauge their importance. Several of the critical properties requiring

characterization are readily apparent and ubiquitous: visual appearance, identification, assay of the active ingredient content, and determination of impurities or degradants. Such tests are considered General or Universal Tests [2]. Other tests are considered Specific Tests and may be important given the dosage form type, the physical and chemical properties of the active ingredient, and intended product administration/usage details (e.g., uniformity of dosage units for tablets or liquid products in single-use containers). The drivers of specifications detailed previously should be thoroughly considered when building the list of tests.

Once the parameters to be monitored have been determined, the next steps are to collect and analyze data. The goal is to simultaneously evaluate formulation prototypes (to determine which has the best quality, performance, and stability), process capability (means and variability), and suitability of the test methods. These studies ultimately lead to an understanding of the impact of variables, including their criticality (and therefore need to be controlled via specifications), and what acceptance criteria should be established. Multiple product batches should be tested, including batches manufactured with preferred suppliers' materials and nominal processing conditions, and batches manufactured with any alternate suppliers' materials, grades of excipients, or processing variables. It may also be beneficial to test batches manufactured using extreme conditions for the purposes of setting specification acceptance criteria; for example, batches could be manufactured with prolonged hold times prior to packaging, or with intentionally high levels of moisture (e.g., water spiking studies) for specification setting and developmental pharmaceutics purposes. It may also be useful to collect data using orthogonal methods for confirmatory purposes or in an effort to select the best analytical method to measure a certain parameter. In some cases, more than one test method may be required to fully characterize a given parameter. Furthermore, the data may indicate some tests to be independent, where the result of that test has little or no discernable link to that from other tests (e.g., microbiological enumeration test results often have little connection to other quality test results). The results of other tests may show the quality attributes to be linked or dependent in some way upon each other. Consider the impact that particle size of the drug substance in a suspension or solid oral product might have upon its drug release properties.

The data may also indicate certain quality attributes to be irrelevant or sufficiently controlled through other qualities that are already monitored. Such attributes would not be considered CQAs, and it may at some point be justified to discontinue testing of that quality attribute.

8.6 Considerations in the Selection and Use of Test Methods

8.6.1 Identification and Development of Test Methods

Once the quality attributes that need to be monitored have been identified, the next step is to select appropriate test methods. The methods needed may be readily available from work done on similar dosage forms, other products using the same active

ingredients, or from literature or compendial sources. If methods for the specific drug product and active ingredient are not available, methods used for similar dosage forms or structurally similar actives may be appropriate starting points for method development. When products are listed in the pharmacopeias of the licensing countries, it is expected that the product will, if tested, comply with the specifications set forth in the compendia at a minimum. Compendial requirements for all countries in which the product will ultimately be sold will need to be met or justified in some way [1, 2]. This is simplified in cases where the compendial test methods and specifications are harmonized across the licensing countries or regions. When new products are developed that are not listed in the pharmacopeias, there may still be certain general compendial tests that can be applied to test certain quality attributes. When pharmacopeial methods are applied to drug products not described in a monograph, the method needs to be validated for use with that specific product. Even when a product is listed in a pharmacopeia, the use of noncompendial (alternative) methods is typically allowed if the drug product composition does not allow the compendial method to be validated (e.g., due to interferences with one of the excipients) and if an equivalent or superior method has been developed. If an alternative method is employed, it is important that the method is capable of demonstrating that the product passes compendial requirements [17]. To test other quality attributes, the innovator company needs to develop tests and specifications that are specific to the new active substance and drug product. References [1, 2] discuss typical requirements for General and Specific tests in greater detail.

When a new method must be developed, the analyst should begin with the end in mind, i.e., the analyst should consider the validation parameters that the method will ultimately be required to achieve, and design the method so as to meet these requirements. Methods need to be characterized or validated such that they are fit for the intended purpose. In the early stages of product development, it is not necessary or practical to fully validate analytical methods to the extent described in the VICH guidelines or the compendia. Once prototype formulations are to be used in clinical studies, the amount of method validation required typically increases so as to provide better assurance of clinical material quality. By the time the product is registered, full validation according to VICH [18, 19] and compendial requirements is necessary. However, due to the financial and time risk of conducting pivotal clinical trials (efficacy, TAS, HFS) and VICH registration stability studies, a significant amount of method validation is typically performed prior to releasing the batches used in these studies.

When considering if a method is “fit for purpose,” it should be noted that the suitability of a method is inherently linked to the formulation composition, manufacturing process, packaging materials, etc. for which it was developed and characterized. A change in any one of these product-related parameters requires re-evaluation of the methods, and the methods may need to be modified and/or revalidated to accommodate such changes. Method changes may bring improvements (in sensitivity or specificity, for example) which introduce the potential to discover new information related to product quality. Specifications may need to be adjusted, and product stability may need to be reassessed for any changes that would require a different shelf life assignment.

8.6.2 Method Validation Considerations

Detailed discussions of method development and validation go beyond the scope of this chapter, but it is worthwhile mentioning at a high level the validation criteria typically recommended in the VICH Guidelines [1, 18] and various pharmacopeias:

8.6.2.1 Specificity

Specificity is defined by VICH as, "…the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc." [18]. Given the complexity and variability of the matrix for certain veterinary products (e.g., Type C medicated feeds formulated with natural ingredients), development of a method that can be assured of routinely achieving specificity despite batch-to-batch variation can be challenging. This parameter is typically evaluated by applying the test to nonmedicated (placebo) formulations of both fresh and stressed matrices to evaluate the potential for interference.

8.6.2.2 Linearity

Linearity is the ability of a method to (within a given range) give a response that is proportional to the concentration of the analyte. Certain tests may yield nonlinear responses. In those cases, the relationship between sample concentration and test result needs to be fully understood and expressed mathematically and/or in some manner so as to allow accurate determination of concentration when compared to a reference signal or standard. Linearity is typically evaluated by testing samples of the analyte prepared at various known concentrations above and below the nominal sample concentration. The concentration found is expressed as a function of the known sample concentration through a linear regression. Slope, intercept, and correlation coefficient are typically reported.

8.6.2.3 Accuracy

Also known as "trueness," accuracy is the closeness of agreement between a test result and the expected result. Accuracy for formulated product tests is often evaluated as a "recovery" experiment where a known concentration of the analyte is spiked into the product matrix, typically at the specification or label claim concentration, and the amount found is expressed as a percentage of the amount added. Bias may occur when the matrix contributes to or suppresses the signal, or causes incomplete recovery of the analyte due to binding.

8.6.2.4 Precision

Precision is the closeness in agreement of results between a series of measurements made on a homogeneous sample. Several types of precision are typically evaluated: System precision reflects the precision capabilities of an analytical instrument when a single sample preparation is analyzed multiple times. It excludes the impacts of sampling error and analyst error in sample preparation. Repeatability, however, is a measure of precision that includes the impact of the analyst and sample preparation. Repeatability expresses the precision when the same homogeneous matrix is sampled, prepared, and analyzed multiple times by the same analyst on the same instrumentation. Intermediate precision, also known as "second analyst precision," evaluates the within-laboratory variation attributable to performance on different days, different analysts, different instrumentation, etc. Reproducibility is the measure of precision between different laboratories and is typically evaluated when the methods are transferred between laboratories, e.g., from the development lab to a quality control lab at a manufacturing plant. Precision is typically expressed in terms of standard deviation and relative standard deviation (RSD).

8.6.2.5 Robustness

Robustness is the ability of an analytical method to remain unaffected by small and deliberate variations in method parameters and provides an indication of method reliability and critical variables. For example, in a chromatographic technique, variations explored might include mobile phase pH or percent organic composition, detection wavelength variation, flow rate variation, column temperature variation, column lot variation, etc.

8.6.2.6 Sensitivity

A test method needs to have adequate sensitivity to detect the analyte and quantify it with acceptable accuracy and precision at the lowest expected concentration or as required by the specifications. Sensitivity is usually expressed as the Limit of Detection (LOD) or Detection Limit (DL) and Limit of Quantitation (LOQ) or Quantitation Limit (QL). An acceptable LOD is typically considered to be three times the signal-to-noise ratio of the method, and an acceptable LOQ is ten times the signal-to-noise ratio.

8.6.2.7 Discriminating Ability

Ultimately, analytical test methods need to be capable of discriminating between product batches of acceptable and unacceptable quality. One type of test in particular that is expressly evaluated for its discriminating ability is the in vitro dissolution,

disintegration, or drug release test. Discriminating ability is demonstrated by preparing and analyzing products that are formulated or manufactured in a way so as to give acceptable and unacceptable drug release performance (i.e., drug release is too fast or too slow), and may be linked to in vivo evaluations of product performance.

8.6.2.8 Considerations of Sampling Procedure, Sample Size, and Point of Collection

Some tests may be designed or applied specifically to evaluate the homogeneity of the product prior to any mixing so as to gauge the need for mixing, and the appropriate instructions to apply to the product labeling. However, for most tests, appropriate mixing or homogenization of the product prior to sampling is desired. Sample size needs to be taken into consideration so as to be appropriate for the attribute being assessed. It may be appropriate to evaluate some properties on larger bulk samples taken at random from the product batch or composites of random samples. In other cases, samples representative of the dose that the smallest target animal would receive may be appropriate (for example, when evaluating content uniformity). In general, a good rule is to target a sample size of 1–3 times the dose that the smallest animal would receive.

The point in processing and location of sampling points also needs to be determined. For example, full characterization of uniformity of a dry blend for a solid oral dosage formulation may necessitate many small samples taken from various locations in the mixer at various mixing times. Some tests may be performed on bulk (prepackaged) goods, and others may be essential to perform once the final goods are packaged in the primary container, especially identification testing.

Sampling instructions including location, number, quantity, storage container, and conditions need to be specified for the individuals responsible for this task. The number of samples to test and the number of replicate tests to be performed on each sample need to be detailed for the analyst in the written test procedure or specification. In some cases, it may be desirable to test 2 or more individually prepared and analyzed replicates, e.g., to reduce variability.

8.6.2.9 Reference Materials

Reference materials may include pure standards for comparative testing or mixtures of materials (e.g., mixtures of drug-related impurities for demonstration of chromatographic method specificity). Reference materials need to be identified, prepared (through additional purification if necessary), and characterized in a manner to support their intended use. Many analytical tests are performed by comparison of the sample to a well-characterized reference material. Identification testing by spectroscopic methods or comparison of chromatographic retention time, and the assay of content of active substances or preservatives are good examples.

8.6.2.10 Reporting of Results

If unspecified and left to the preference of individual analysts, test results may be reported in many different ways. Some may choose to record, "Complies" or "Conforms to Specification." Others may document numerical values to various degrees of precision. In order to allow comparison of test results over time, across different analysts and laboratories, it is best to standardize the format for data reporting. This is often detailed in the written test procedure or through the wording and format implied by the acceptance criteria in the specification. Whenever numerical results are generated, it is best to report the numerical result instead of "complies" so that trends may be detected. Proper use of significant figures and compliance with the data reporting conventions set forth in the pharmacopeia and VICH guidelines should also be considered. For example, unless requiring particularly stringent controls (like in the case of potentially genotoxic impurities), impurities in drug products are typically reported in terms of percent of the label claim of the active ingredient to one decimal (0.7%, 1.2%).

8.7 Batch Release Testing

8.7.1 Batch Release Testing in Quality Control

As previously discussed, a primary driver for testing and specifications is the need for a final confirmation of product quality prior to use of the product for clinical or commercial purposes. In the USA, 21CFR211.165 subpart I states, "For each batch of drug product, there shall be appropriate laboratory determination of satisfactory conformance to final specification for the drug product, including identity and strength of each active ingredient prior to use." This final confirmation is typically referred to as "batch release testing" and involves the testing of all CQAs using methods that have been demonstrated to be "fit for purpose." The test results are then compared to the predetermined acceptance criteria that are a part of the product specifications. When all test results comply with the acceptance criteria, the batch may be acceptable for use and may be approved by the responsible laboratory manager or quality authority within the company. The test results may also be atypical in that the results of one or more tests is either out-of-trend (OOT) with respect to historical results or out-of-specification (OOS) by failure to comply with the predefined acceptance criteria. In either case, the result in question needs to be thoroughly investigated to determine if the result is an anomaly (e.g., a false result due to laboratory error), or credible, and the root cause of the unexpected result should be determined. In order to investigate an OOT or OOS result, it may be necessary to invoke analytical methods not typically utilized for batch release testing (e.g., forensic microscopy and spectroscopic tests to identify the composition of unexpected foreign matter observed as part of the visual Appearance test). Once the underlying cause(s) have been determined, corrective and preventative actions (CAPAs) are

typically established so as to remedy the cause and prevent it from impacting future batch production or testing. If the result is determined to be an anomaly, it may be possible (depending upon the laboratory's operating procedures) to invalidate the original result and replace it with the results of a retest of the original sample, or results of a resampled aliquot from the batch. When the result is determined to be credible and OOS, the impacted batch is usually determined to be unacceptable for use. Clearly, it is important that the pivotal clinical trial batches, registration stability batches, and validation batches all comply with the final registered product specifications for batch release (and shelf life), and any deviations or OOS/OOT results are thoroughly investigated, documented, and that the batches are suitably controlled (e.g., recalled or restricted from further distribution if deemed necessary).

QC testing also provides an indication that the manufacturing process is under control, i.e., performing within expected routine variation to yield a consistent product. Monitoring batch release and stability data of many batches over time allows the manufacturer to develop an understanding of overall process capability and variability. This in turn allows the manufacturer to identify batches that are statistical outliers as indicated by out-of-trend test results at batch release and to identify a trend that may be an indicator of product or process drift. Outlier batches may bring an increased risk or uncertainty of compliance with specifications throughout shelf life. Some manufacturers also utilize laboratory control samples to assess long-term analytical method variability. In this practice, a sample of a reference product batch is routinely tested along with the batch release or stability samples, and the results of the reference batch are plotted on a control chart to look for trends in method performance.

8.7.2 Certificates of Analysis

Batch release results are usually summarized in a document called a "Certificate of Analysis" or CoA. A complete CoA contains the full specification (i.e., description of the tested parameter, reference to the analytical method used, and the acceptance criteria) to which the material was tested, plus the reportable test results and a statement of whether the batch passes or fails. CoAs also clearly state the product name, strength, batch identifiers, and typically the date of manufacture, dates of testing, and several compliance or conformance statements such as a declarations that the batch was manufactured and tested under current good laboratory practices (cGLPs) or cGMPs, that the product complies with compendial requirements for residual solvents (e.g., USP<411>) and bovine spongiform encephalopathy/transmissible spongiform encephalopathy (BSE/TSE), etc. Finally, CoAs must bear a handwritten or electronic signature of a responsible testing authority such as a laboratory manager or quality assurance professional and the approval date. While the CoA may seem like the final testament of product testing prior to release to the market, it may be required to completely repeat the CoA testing (or selected tests such as identification testing) depending on the country in which the product was manufactured and the countries to which the final product is shipped for distribution.

CoAs, batch release specifications, and test method procedures are cGMP controlled documents and as such are maintained in a controlled document system that prevents accidental modification.

Test procedures and acceptance criteria frequently change throughout the lifecycle of a product as new data is taken into consideration and test improvements are implemented. For this reason, CoAs need to clearly reference the version of the methods and specifications that were in place when the testing was conducted. Ultimately, the product manufacturer needs a change control system that provides traceability of the methods used throughout product development, stability programs, and those methods used for process validation and product commercialization. The method and specification history, and sample CoAs for pivotal product batches are typically submitted as part of the marketing application dossier.

8.7.3 Reduced Testing

In some cases, the amount of final product testing required for batch release can be reduced significantly. One example, called *parametric release*, is where processing parameters, e.g., time, pressure, or temperature when used in conjunction with the knowledge collected during sterilization process development and validation, may be used in lieu of sterility testing on injectable products. Hence, in parametric release, processing parameters are measured instead of the actual CQA (sterility). The assumption is that if the validated process conditions are achieved, the harmonized compendial test for product sterility (Ph. Eur. 2.6.1 Sterility, JP. 4.06 Sterility Test, and USP<71>Sterility Tests) should pass if tested [1, 2, 20]. Extending this concept to other tests beyond sterility and to the measurement of other in-process materials and conditions, it may be possible to achieve "real time release." "Real time release is the ability to evaluate and ensure the acceptable quality of in-process and/or final product based on process data" [4]. With ample process/product understanding (at a mechanistic level), "on-, in-, or at-line measurement of critical attributes that relate to product quality provides a scientific risk-based approach to justify how real time quality assurance is at least equivalent to, or better than, laboratory-based testing on collected samples."

Another way in which product testing for batch release can be reduced is through a concept known as "skip lot testing" or "periodic testing" in which not every batch of product is tested for a defined parameter. A key assumption though is that any batch, if tested, must pass. It is important to define the frequency of testing, to have confidence that all batches will pass, and to have manageable risk in the event that an untested but OOS batch were inadvertently released for use in animals. Therefore, this practice is typically implemented postapproval once sufficient data is in place to assure routine conformance with the specification. It may be implemented as part of a "sunset clause" where the testing frequency will decrease from every batch to every n^{th} batch at some time point, e.g., after process validation. This practice may be useful for tests such as residual solvents or inorganic impurities [1, 2]. The testing plan should also describe the conditions on which the test would be resumed, for

example, in the event of a manufacturing process or site change [17]. Additional assurance of compliance for untested batches may be provided in some other way such as through parametric release or other in-process test results [21].

8.8 Stability Testing

8.8.1 Regulatory and Scientific Drivers for Stability Testing

Similar to batch testing, the need for stability testing is firmly rooted in cGMPs. In the USA, 21CFR211.166 Subpart I on Laboratory Controls establishes the expectation and minimum requirements for a documented stability program. The Expiry Dating section in 21CFR211.137 Subpart G on Packaging and Labeling Control states, "To assure that a drug product meets applicable standards for identity, strength, quality, and purity at the time of use, it shall bear an expiration date determined by appropriate stability testing described in Sec 211.166." Hence the primary purposes of a stability program from a regulatory perspective are to establish and verify the shelf life during which the product will comply with specifications. This includes the determination of the appropriate storage conditions, which need to be indicated along with the expiry date on the label of commercial product. VICH GL3 establishes the stability data requirements for registration of veterinary drug products [22]. Beyond requirements set forth in the regulations, stability studies are a critical science-driven part of the pharmaceutical product development process. Stability studies are used in formulation development to screen prototypes and identify the best candidates for further optimization. Products used in pivotal clinical studies are typically evaluated in stability studies which may be the registration stability studies or supplemental studies of a similar design. Other studies may be designed specifically to screen and select appropriate primary packaging for clinical supplies and final market image products. Forced degradation studies, which can be viewed as a specialized type of stability studies in which the product or active are exposed to severe stresses to purposefully degrade it, are very useful during method development. Forced degradation studies help to determine if analytical methods are truly capable of detecting changes that might occur in CQAs under routine storage conditions (i.e., demonstrating that the test methods are "stability indicating"). These studies also provide a way of determining degradation pathways and generating degradation products for isolation and identification. Stability studies are often conducted to support hold times during manufacturing where the product is held in bulk in nonfinal packaging, or excursions in temperature and humidity that might be experienced during shipping. Other studies are designed to support the stability of the product once the primary packaging has been opened (e.g., broached closure studies for parenterals) or while it is being used over a prescribed dosing period (in-use studies). All of these different permutations of stability studies provide data that should be considered for specifications development purposes. Further discussion on selected stability topics is provided below.

8.8.2 Forced Degradation Studies

Forced degradation studies provide a wealth of very useful information regarding how susceptible a pharmaceutical material is to various stresses, decomposition pathways and products, and test method capabilities. Studies are often performed during development to explore forced degradation of the drug substance, drug product prototypes, products stored in various packaging options, and even placebo formulations. Drug substances are typically studied under extreme heat, humidity, acidic, alkaline, oxidative (e.g., in a hydrogen peroxide solution or in the presence of a free-radical initiator like Azobisisobutyronitrile (AIBN)), and photolytic conditions versus unstressed control samples to determine the stresses to which the substance is most sensitive. For stresses found to be extremely detrimental to the substance, it may be desirable to identify the primary degradation products so as to elucidate the chemical pathway, which may in turn provide insights as to appropriate ways of stabilizing the molecule, e.g., through the selection of an appropriate antioxidant. Forced degradation studies on drug products are typically performed only under those stresses that are most relevant. Hence, all products are generally exposed to heat, humidity, and photo-stresses, but only liquid products might be exposed to acid, base, and oxidizers. Forced degradation studies are usually performed in inert containers such as glass or quartz so as to eliminate the impact of any package incompatibilities. However, product stability studies under extreme conditions when packaged in various prototype containers may be useful to demonstrate compatibility and select appropriately protective materials. Subjecting a placebo to forced degradation studies may be useful to identify the origin of unknown peaks observed in the drug product assay and related compounds chromatographic methods (e.g., if attributable to the placebo, the primary packaging, or the labeling materials). Furthermore, analysis of the forcibly degraded drug substance and product on these methods with determination of mass balance, specificity via peak purity algorithms, or application of mass spectrometric detection and/or orthogonal separations provides assurance that the method is stability indicating.

8.8.3 Developmental (Nonregistration) Stability Studies

The challenge to the formulations scientist is to develop a robust manufacturing process and an intrinsically stable product that does not require costly formulation technology, expensive packaging, or refrigerated or frozen storage to achieve stability. To identify a stable product in a short period of time, it is often useful to simultaneously evaluate CQAs for various formulation prototypes stored under long-term storage conditions (e.g., 25°C/60% Relative Humidity, RH) and accelerated storage conditions (e.g., 40°C/75%RH, or higher temperatures) that can be used to infer product performance over longer periods of time at the nominal storage condition.

Evaluation of formulation prototypes in various packaging options versus control samples stored in glass at frozen conditions helps to gauge packaging options and relative stability of formulation prototypes. In cases of drug substance/excipient incompatibilities (evident through instability of the formulation vs. the pure drug substance when stored under similar conditions), it may be necessary to perform simple compatibility studies of the drug substance plus one or two excipients at a time to identify the source of the incompatibility. To support process development, it is usually desirable to evaluate the stability of in-process materials, which includes the final product before it is packaged in its primary packaging. Such studies conducted on bulk goods help underwrite a hold time prior to packaging.

Developmental stability studies are also routinely conducted to underwrite the quality of clinical supplies. In order to comply with cGLP and cGCP (good clinical practices) requirements of pivotal clinical trials, it is typically necessary to ensure the quality of (cGLP or cGMP) clinical supplies throughout the in-life dosing period of the trial. Sometimes this is accomplished via the analysis of samples taken before and after the dosing period. Such "entrance and exit testing" is particularly useful for cGLP studies of limited in-life dosing duration. For longer clinical trials, especially cGCP studies using cGMP clinical materials, it is common to perform stability studies that mimic those performed for registration purposes where product is tested at various intervals and various long term and accelerated storage conditions. If no change in test results for CQAs is observed over the course of the stability study, then the quality of the supplies was maintained. If significant changes were observed, impact on clinical trials should be evaluated. If significant changes in product quality prove to be unavoidable over the long term, it may be necessary to underwrite the observed changes through the evaluation of aged supplies or those manufactured to mimic the characteristics observed upon aging to ensure safety, efficacy, and performance is maintained. For example, changes in in vitro drug release characteristics or increases in degradation product levels are often observed for solid oral dosage forms during long-term storage. Clinical in vivo trials may be necessary to ensure that the drug release of aged goods provides acceptable pharmacokinetics, and that the degradation products do not have adverse safety effects. Last, it should be noted that in some cases, the clinical studies performed to underwrite clinical trial material may be supportive to the registration stability program (and may be submitted as part of the registration dossier). It is also common that batches manufactured for registration stability studies find their way into clinical trials; hence, the stability study of such batches serves multiple purposes.

8.8.4 Registration Stability Studies

Registration stability studies for veterinary products are conducted in accordance with the VICH guidelines [22, 23]; however, other local, regional, and international compendial or government requirements should be considered when designing the

studies. Confirming the appropriateness of study design with the relevant regulatory authorities, through meetings or protocol review if offered, is valuable, particularly where a reduced or partial testing strategy is employed (*vide infra*), or a single study is intended to support registration in markets of dramatically different climates.

Registration stability studies are performed according to a written protocol that describes the number of batches, storage conditions, test intervals, test methods, and acceptance criteria, and often additional details regarding data analysis plans and how deviations or investigations will be addressed. Selected details may also be managed in a documented fashion outside of the protocol, such as sample pull dates and grace periods to allow early/late withdrawal and testing of samples which may be managed through the manufacturer's SOPs or laboratory information management system (LIMS). Typically, 3 unique drug product batches, at least 2 of which are at 1/10 of commercial scale or larger are tested. Testing includes all CQAs at the inception of the study, and any CQAs that are expected to be stability indicating or that might change are tested throughout the study. In a full study design, all strengths and package permutations of the product would be tested. Orientation of the product also needs to be considered, and it may be necessary to evaluate product stored both upright and inverted so as to incorporate potential long-term effects of product contact with closure systems. Selection of conditions for long term and accelerated storage depends upon background knowledge regarding the physical and chemical stability of the drug product gained through developmental studies, plus considerations of customer needs and the regulatory requirements of the markets in which the product will be sold. Customer need considerations for veterinary products may drive the manufacturer to evaluate more stressful conditions than would be done for human health products, given the potential for the veterinary product to be stored in a barn or outdoors under uncontrolled conditions. Common storage conditions for products distributed to veterinarians or pet owners for use in clinical or home settings might include long-term storage at 25°C/60%RH, accelerated storage at 40°C/75%RH for 6 months, and an intermediate condition of 30°C/65%RH for 12 months as a backup to be tested in the event of significant changes at the accelerated condition. By contrast, products intended for use in food producing animals which might be stored outdoors for prolonged periods of time might be stored at 30°C/65%RH for the long term, and the accelerated condition (40°C/75%RH) may be evaluated for 12 months or more. It may also be desirable to study refrigerated conditions (5°C/ambient RH) as a part of the study for production animals so as to bracket an intended product labeling of "Store between 2 and 30°C." In cases where liquid products are stored in semipermeable plastic containers, reduced humidity conditions may be necessitated in order to simulate worst case storage for weight loss, such as 25°C/40%RH or 30°C/35%RH for long term and 40°C/≤25%RH for accelerated storage [24]. Refrigerated and frozen storage is less convenient and less common for veterinary products than for human products. Such storage is mainly possible for clinically or home-administered products. For refrigerated product studies, 5°C/ambient RH is used for long-term storage, and 25°C/60%RH is used for accelerated storage. For frozen storage products, long-term storage is conducted at −20°C/ambient RH, and there is no defined accelerated storage condition.

The WHO defines the stability requirements for world markets based upon five climate zones (I, II, III, IVa, and IVb) with varying mean kinetic temperatures and humidities to be considered in stability study design [25]. For example, Australia has climatic conditions encompassing Zones II–IV, i.e., long-term storage conditions are 30°C/65%RH and accelerated conditions of 40–45°C/75%RH are run for at least 12 months [26]. The Association of Southeast Asian Nations (ASEAN) which includes Thailand, Singapore, Vietnam, Philippines, Malaysia, and other countries also requires more stressful storage conditions than needed to register products for the US and European markets [27]. During veterinary product development, it may be difficult to anticipate or fund all of the stability studies needed to achieve simultaneous global product registration. Often, stability is done for major (US and EU) markets initially, and supplemented postregistration with any additional studies needed for specific markets which may require additional studies under different storage conditions. References [25, 27–29] provide additional information that may be useful when designing stability studies for global markets.

Other areas in which stability studies for veterinary products differ from those for human health products evolve from the speed of product development and product size and permutation considerations. Because overall product development programs including clinical trials can be quite rapid for veterinary products, it is undesirable to have CMC related activities such as stability studies delay the overall registration dossier submission. For this reason, it is common for veterinary product submissions to be submitted with only 6 months of long term and accelerated stability data. Additional stability data (e.g., through 12 months) is typically available and provided to the regulatory authorities during the review cycle to support the desired shelf life dating period. By comparison, human health product submissions contain at least 12 months of long term and 6 months of accelerated stability data.

Chamber space and the size and permutation of packaging presentations often presents storage space issues in veterinary stability studies. Veterinary products may be supplied in many doses and package sizes to accommodate animals of different sizes. Furthermore, some veterinary products for use in food producing animals are supplied in larger containers which may not fit in reach-in (refrigerator) style stability chambers, therefore necessitating the use of larger, walk-in chambers.

In addition to the stability studies initiated prior to submission of the registration dossier, commercial product batches are typically placed on stability through the full term of the approved shelf life. Such studies are typically performed only at the highest temperature supported by the labeled storage condition, i.e., the long-term storage condition. The first 3 batches of commercial product (usually corresponding to the process validation batches) are placed on stability, along with a certain number of commercial batches each year the product is produced. The exact requirements are usually set forth in a marketed product stability commitment which is submitted to the regulatory authorities of the countries in which the product is licensed. Furthermore, it may be necessary to perform additional studies, including those at accelerated storage conditions, when needed to support changes in formulation, process, or packaging changes. Stability data typically needs to be submitted for regulatory review to support such changes [30].

8.8.5 Reduced Testing

In the previous section on batch release, several opportunities to reduce the test burden using parametric, "real time" release, or skip-lot strategies was discussed. Even if such practices are used to reduce the batch testing performed for product release, it will still be necessary to prove the stability of the product throughout shelf life, so the stability study burden is not necessarily reduced by the implementation of QbD principles. Stability studies are performed on each strength and container size of the drug product unless bracketing and matrixing study designs are justified and employed [24].

A full study design is one in which all tests performed for all samples at all conditions at all time points. A reduced study design can be accomplished through bracketing or matrixing [31]. Bracketing is typically applied to multiple strengths or package sizes of identical (or very similar) formulations and involves only testing those permutations which represent the upper and lower extremes. The differences in drug products of different strengths and containers of different sizes need to be discussed and a justification presented as to why the presentations under investigation represent the extremes in the critical variables [31]. Factors such as surface area/volume ratio, headspace volume, closure type, container size, materials of composition, and wall thickness are typically considered for such an argument. Matrixing is defined by the VICH as, "…the design of a stability schedule such that a selected subset of the total number of possible samples for all factor combinations would be tested at a specified time point" [31]. Bracketing is used quite frequently in veterinary product submissions, but matrixing is much less common. Due to the rapid development timelines and limited stability data availability for veterinary products at the time of regulatory submission, employing matrixing designs may lead to a deficiency of data to confidently establish shelf life dating.

8.8.6 Photostability Studies

In addition to the effects of temperature and humidity, regulatory guidelines and prudent practices require the investigation of product exposure to ultraviolet (UV) and visible radiation [32]. As per the VICH guideline, drug substances and drug products are to be examined fully exposed to a course of not less than 1.2 million lux hours of visible light (400–700 nm) and 200 watt h/m^2 of UV (320–400 nm). Subsequent layers of protective packaging are added to the point that acceptable stability is achieved. The VICH photostability guideline is, for all intensive purposes, identical to the ICH photostability guideline for human products. The exposure conditions set forth in the VICH photostability guideline support storage of products in a hospital or pharmacy where it would receive 500 lux of illumination for 24 h per day for 3 months (about 100 days):

$$[500\ \text{lux}) \times [24\ \text{h/day}) \times [100\ \text{days}) = 1.2\ \text{million lux - h}$$

Typical indoor lighting delivers 500–1,000 lux, but direct sunlight can approach or exceed 100,000 lux [33]. The UV wavelength requirement of the VICH guideline is based upon radiation filtered through window glass where the wavelengths below 320 nm would be attenuated, and the irradiance was set arbitrarily. In outdoor conditions where UV radiation is unfiltered, wavelengths below 320 nm may be relevant, and the guideline's UV irradiance requirement may also be rapidly exceeded. Hence, for veterinary products that may be stored outdoors routinely or may see direct sunlight for greater than 1 day during use, the anticipated exposure should be carefully considered vs. the exposure requirements set forth in the VICH guideline.

8.8.7 In-Use and Temperature Cycling Studies

In-use stability studies are conducted to demonstrate that the product will remain compliant with registered specifications once the primary packaging has been opened and throughout the labeled use period. In-use stability studies for veterinary products may be more extensive than for human pharmaceuticals given the unique conditions of administration for selected veterinary products, especially those which are administered in the field such as medicated articles for the preparation of feeds and drinking waters. Additional considerations regarding in-use testing for these special products are discussed later in the drug products testing section.

In-use studies are typically performed on two batches of drug product, at least one of which is near the end of its shelf life [34]. The studies are designed to as closely as possible mimic the conditions of use and to insure that the last dose still meets specification. This is accomplished by testing the last dose withdrawn or the remaining sample in the bottle once all other aliquots have been removed. An EMEA Guideline from 2002 presents the European requirements for in-use stability testing of veterinary products in multidose containers [35]. Unless all container sizes are to be evaluated in the in-use study, it is important to select the container that represents the worst case scenario (e.g., maximum product exposure, headspace, exposure duration, etc.) and to justify the selection. The applicant should think of the worst case scenario that needs to be supported from a product use and storage perspective. For example, for a daily use product known to be sensitive to oxidation, this might entail daily sample pulls to replenish the headspace. In-use studies are important to establish the maximum use period for parenteral products after first opening (and reconstituting or diluting the product as needed). Restrictions in the EU limit parenterals to be stored and used for a maximum of 28 days after first opening, and the actual use period needs to be underwritten by in-use stability studies [36]. Temperature cycling studies, also known as "freeze–thaw studies" are performed to demonstrate that the product is stable with respect to brief temperature excursions that might be encountered during shipping and storage. Typical conditions might include three cycles of exposure to −20°C followed by 50°C for periods of 24–48 h each. All stability-indicating specification tests would be performed to examine for potential adverse effects. If any adverse effects such as phase separation, precipitation, or packaging failures were noted, special labeling may be required,

such as to avoid storage above a certain temperature, or "do not freeze." The drug product ultimately needs to be capable of complying with the registered shelf life specifications following the cumulative effects of long-term storage at the labeled conditions *plus* any changes incurred in photostability, in-use, and temperature cycling studies.

8.8.8 Interpretation of Stability Data

Significant change in a stability study is defined as a 5% change from initial values of assay, any degradant exceeding its acceptance limit, or failure to meet acceptance criteria for other tests such as appearance, in vitro drug release (i.e., failure of final stage testing for multistage specifications), microbial limits, etc. Typically, significant changes in one or more quality factors are responsible for the limitation of a product's shelf life. Often, this is caused by assay drop due to instability, assay increase due to loss of vehicle through semipermeable packaging, growth of degradation products due to instability of the active, degradation of microbial preservatives leading to the potential for microbial growth, or changes in drug release properties as a function of time. Certain parameters which show change with stability storage typically follow zero-order kinetics, such as assay, degradation products, and preservative content [27]. In these cases, the product's shelf life can be estimated statistically by extrapolating the available data at the long-term storage condition to the point at which the one-sided 95% confidence interval of the mean exceeds the shelf life specification acceptance criteria (see Appendix A of ref. 37]). As the amount of data decreases or the variability of the data increases, the breadth of the confidence intervals increase, and the shelf life is reduced. Hence, given the very limited amount of data typically available at the time of registration, statistical data analysis is often of limited use for shelf life prediction of veterinary products. Statistical prediction of shelf life is often better facilitated once additional (postregistration) data is available. In cases where no significant changes in CQAs are observed, the shelf life established at the time of marketing authorization is typically determined by the amount of data available (in months, x) at the long-term storage condition, with the maximum allowable shelf life at product launch typically being $2x$. Additional extensions of shelf life beyond this initial dating may be possible based upon real time data for commercial product batches.

8.9 Testing of the Product Packaging

8.9.1 Regulatory and Scientific Drivers

In the USA, 21CFR211.84, Subpart E sets forth basic expectations for the control of drug product components, containers, and closures, requiring a test for confirmation of the identity of the packaging materials and that, "Each component shall be tested

for conformity with all appropriate written specifications for purity, strength, and quality." Furthermore, 21CFR211.94 requires that drug product containers and closures not be reactive, additive, or absorptive so as to alter product safety, quality, purity, or strength, and to provide adequate protection for the drug product. Justification of materials of composition is usually provided in marketing application dossier [3]. Testing of containers and closures typically includes verifying the identity of the materials using a spectroscopic method such as Fourier transform infrared spectroscopy (FTIR) plus physical measurement of critical dimensions vs. the dimensions and tolerances specified in the component specification or drawings.

Selection of appropriate primary packaging materials (i.e., those in direct contact with the formulated product) is a critical element of product development. Accelerated storage stability studies are often performed on formulations filled into several prototype packages so as to select the best material and package design on the basis of compatibility and protection afforded to the formulation. The drug product is tested by the stability-indicating methods to assess product quality, including weight gain or loss due to water or solvent transmission, and the packaging is carefully scrutinized for evidence of leakage, swelling, paneling, etc. The impact of the labeling system also needs to be evaluated. This is especially important where liquid products are stored in semipermeable packaging systems where it is possible for trace product transmission to impact label adhesion or print clarity, or for label components to leach into the product. Labeling systems may include direct printing to the container, or printed adhesive labels that are applied to the primary package. Printed adhesive labels can be quite complex compositionally, including multicomponent adhesives, substrates, inks, and varnishes. The potential for various compounds to leach from packaging components, including labels, into the product has become the subject of increased scrutiny by regulatory authorities and industry in recent years.

8.9.2 Extractables and Leachables

Although geared to bioprocess materials, several articles by Colton et al provide good overviews of basic concepts related to leachables and extractables, and further references to relevant FDA and EMEA guidances on this subject [38, 39]. Colton et al defines:

Extractables: Chemical compounds that migrate from any product-contact material (including elastomeric, plastic, glass, stainless steel, or coating components) when exposed to an appropriate solvent under exaggerated conditions of time and temperature.

Leachables: Chemical compounds, typically a subset of extractables, that migrate into a drug formulation from any product contact material (including elastomeric, plastic, glass, stainless steel, or coating components) as a result of direct contact under normal process conditions or accelerated storage conditions. These are likely to be found in the final drug product [38].

Today there is no regulatory guidance for extractables and leachables specific to veterinary products, so manufacturers adopt strategies used for human health products. The essential first step is to develop a leachables and extractables strategy for the product and packaging system. Depending upon the nature of the product, the composition of the packaging, and the intended route of administration, it may be deemed sufficient to use packaging materials which comply with compendial requirements (e.g., USP<381>, <661>, <660>, <87>, or <88>) without further evaluation for extractables and leachables. Extractables and leachables studies may be needed when the route of administration, product volume dosed, or uncertainty of chemical compatibility between the formulation and packaging material pose increased risk. Colton et al. also describes a risk-based ranking where parenterals > ophthalmic products > topicals > solid oral dosage forms [38]. Physical state, surface area of contact, chemical composition of the formulation vehicle, and packaging component composition all factor into the risk. Beyond small organic molecules and oligomers, it is possible for packaging components to leach elemental (inorganic) impurities. One strategy that could be employed for veterinary products is to begin with an extractables study where the packaging components are forcibly extracted (e.g., via Soxhlet extraction) in water and several organic solvents of varying polarities, potentially also including the formulation vehicle. The extracts would then be analyzed by gas and liquid chromatographic techniques with mass spectrometric detection so as to identify the extracted compounds. If either no extractables are found, or toxicological assessments of all compounds found indicate that there is negligible risk due to exposure to the compounds, then the study might be concluded. It may also be advisable to investigate the extractable study samples via the test methods that will be used for batch release and stability testing so as to verify that there is no interference from the extracted compounds. If however one or more of the extracted compounds may pose a safety risk, then it may be necessary to determine if the compound(s) will leach into the product under normal storage conditions, and to what concentration. Commonly referred to as "leachables studies," such studies are performed on placebo or active product formulations filled into the target packaging system and stored under long term and accelerated stability conditions. The product or placebo is then analyzed using an analytical method that is validated for the extractable compound(s) of interest, and the compound(s) found are quantified. The levels found are then re-assessed from a toxicological perspective, and either the packaging is determined to be adequately safe, or a new packaging material may need to be evaluated.

8.9.3 Container Closure Integrity Testing and Studies to Simulate In-Use Conditions

Packaging material selection and design can also have significant impact upon the uniformity of doses delivered from the package or device, and also the undeliverable volume of product that is retained in the package. The uniformity of doses

delivered may also depend upon product related attributes such as resuspendability, viscosity, and particle size distribution [40]. Conditions of the package or device use also need to be considered. This may include cap removal force, plunger actuation force for a syringe or dosing gun, etc.

Beyond visual observations of packaging integrity, other types of packaging tests performed as a part of batch release or stability testing include studies to evaluate the integrity of the closure before and following use. A good example is the case of injectable products where measures of closure re-sealability following successive punctures are performed. In these "coring" studies, the product may be visually examined for the presence of particulate debris introduced from the closure, and the re-sealability of the closure may be investigated through the use of a tests such as vacuum/pressure decay, trace gas permeation/leak tests, or a dye ingress test where the product package is immersed in a high contrast dye, and the product is examined visually and/or spectroscopically for signs of leakage. Container closure integrity testing (CCIT) may also be used as a surrogate test on stability in lieu of sterility testing. For products labeled as sterile, the sterility test is obviously a critical quality parameter, but because of the high product volume demands of sterility testing, it is not preferred as a routine component of stability testing. Once performed to support batch release, it may be possible to perform CCIT as an alternative for continued assurance of sterility throughout the shelf life of the product [41].

8.10 Drug Substance Testing

Analytical testing and development of specifications for drug substances (active pharmaceutical ingredients, or APIs) for use in veterinary products are typically performed in much the same manner as for actives used in human products (e.g., by comparison of ICH [14] and VICH [1]). Indeed, the specifications may be very similar, with a few minor exceptions. Organic impurities that are related compounds of active ingredients are controlled slightly differently for substances used solely in veterinary products vs. substances that are used in human health or human and animal health products [42]. For drug substances used solely in veterinary products, the impurity reporting, identification, and qualification thresholds are 0.10%, 0.20%, and 0.50%, respectively. If the drug substance is also used in human products, then the identification and qualification thresholds default to the ICH levels which vary depending upon the dose of substance delivered daily. For a substance dosed at ≤2 g/day, the ICH reporting threshold is 0.05%, the ID threshold is 0.10% or 1.0 mg/day, whichever is lower, and the qualification threshold is 0.15% or 1.0 mg/day, whichever is lower [43]. Toxicological considerations for impurities, especially potentially genotoxic impurities (PGIs), may also be different in food animals vs. companion animals vs. humans. Further discussion on PGIs is provided later in this chapter. See also Gangadhar, Saradhi, and Rajavikram for a recent discussion of genotoxic impurities control for drug substances for human health products [44].

Drug substance tests and specifications often serve as very good starting points for the development of methods and specifications for drug products. For example, specifications for an impurity that is both a process-related impurity and a degradation product cannot be tighter for the drug product than for the drug substance. The contributions of the drug substance to the overall residual solvents, moisture, and heavy metals load of the formulation (along with excipients) need to be factored into drug product specification development. The assay/impurities method for the drug substance is often a great starting place for development of the drug product assay/impurities method. VICH Guideline GL39 Decision Tree #1 provides guidance for the development of impurity specifications for drug substances, and Decision Tree #2 in the same document provides guidance for drug product impurities specifications [1]. Further considerations for drug substances that are fermentation derived may be found in a draft CVM Guidance [45].

During development, additional tests may be performed to characterize the drug substance, and these tests may not be run routinely as a part of batch release/recertification testing or drug substance stability programs. Examples include bulk or tap density, salt selection studies, polymorph screening, partition coefficient determination, solubility as a function of pH or organic solvents, melting range, and tests performed to unequivocally identify the molecular structure and conformation of the active such as Nuclear Magnetic Resonance (NMR), high-resolution mass spectrometry, Raman spectroscopy, and elemental analysis.

Batch release and recertification specifications for drug substances used in veterinary products often include tests for the following parameters. Special remarks are captured where warranted. This is not intended to be a comprehensive list; instead, the synthetic process used to produce the active plus the intended formulation and use of the product needs to be considered when establishing the CQAs for the drug substance to ensure that all quality and safety considerations are addressed.

Appearance (Visual Description): A common flaw in establishing visual appearance acceptance criteria is to include a descriptor that cannot be discerned visually, such as references to the material being crystalline or amorphous. Such characteristics should be separated from the appearance test and addressed through orthogonal methods. Furthermore, it may be appropriate in certain instances to allow for the presence of small amounts of extraneous matter or off-colored particles where it has been determined to be intrinsic to the drug substance quality and found not to pose any safety/quality risks for use in the drug product. In such instances, it is best to state what is acceptable or not through the acceptance criteria so as to improve the objectivity of this otherwise most subjective of tests. In cases where drug substance color variation is observed and the color may result in variations in drug product appearance, a colorimetric method may also be needed.

Color/Clarity in Solution: These tests may be needed if the active will be used in solution formulations, especially injectables.

Identification (ID): ID testing for actives is typically performed using one or more methods, at least one of which is usually FTIR.

Polymorphic Control: If the drug substance has been shown to exist in more than one crystalline form, or to exist as a solvate or hydrate, then its polymorphic form should be confirmed with a capable method such as Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), or Powder X-Ray Diffraction (PXRD). It may be necessary to establish a quantitative limit for the content of an undesired polymorph.

Counterion Identification and Stoichiometry: This is important if the drug substance is produced as a salt.

Assay: This test quantifies the content of the active ingredients, typically by comparison to a well-characterized reference standard. To establish reference standards, purity is determined by subtracting the contributions of all impurities (organic, inorganic, metals, solvents, water) from 100%. For chiral actives that are a pure enantiomer, an enantioselective method may be needed to quantify the content of the desired enantiomer. Assay tests should be stability indicating.

Impurities (a.k.a. Related Substances or Related Compounds): This test quantifies organic impurities content of the drug substance, including residual raw materials, intermediates, or process by-products and any degradation products observed in the substance. This test should be stability indicating for the formation of any degradation products.

Residual Solvents: Residual solvents used in or formed by the drug substance manufacturing process are quantified and controlled in the substance, and possibly also in the formulated drug product. Additional discussion is provided in a later section.

Sulphated Ash: This test, also known as "Residue on Ignition" is used to gravimetrically determine the inorganic impurities present in the active following acid digestion and pyrolysis. This test is harmonized across the USP, Ph. Eur., and JP (Ph. Eur. 20414 Sulphated Ash; JP 2.44 Residue on Ignition Test, and USP<281> Residue on Ignition).

Heavy Metals: Traditionally tested using a wet chemical technique, this is a test for residual content of metals which may be introduced as catalysts or metallic reagents in the drug substance synthesis. The current industry and regulatory trend is to use a wet chemical method as a general screening method, plus additional methods specific to any metals of toxicological significance which may be present due to their use in the synthesis [46]. Techniques such as Atomic Absorbance (AA) or Inductively Coupled Plasma–Mass Spectrometry are appropriate for this purpose.

Moisture: Water content is typically determined by Karl Fischer titration. With appropriate justification, Loss on Drying (LOD) may be used, e.g., where there are no residual solvents toxicological issues (e.g., only Class 3 solvents are likely to be present).

Microbiological Enumeration Tests: It is common for drug substances to be tested for microbiological quality according to compendial microbial enumeration methods, including the absence of specified harmful organisms. Endotoxins and sterility may also be required depending upon the formulation to be produced.

Particle Size: Particle size of the active is a particularly important quality attribute in that it may impact manufacturability, content uniformity, dissolution, bioavailability, and stability. Particle sizing of drug substances may be accomplished by a variety of analytical sieving, microscopy, image analysis, light obscuration, or laser light diffraction techniques; however, it should be noted that these tests are not interchangeable and the results of the test are often technique and instrument dependent, and tend to be more variable than many other tests (e.g., chromatography). The different analytical technologies available have differing abilities to measure particles of nonspherical geometry and to detect or break up agglomerates. Caution should be taken when switching between technologies, e.g., during method transfer across laboratories.

Where crystallization or precipitation performed in the synthetic process is inherently unable to provide adequate particle size control, and where it has been found to affect drug product production, it may be necessary to do a particle size reduction step like milling, micronization, or de-lumping/de-agglomeration. Such techniques reduce and typically narrow the particle size distribution, and necessitate repeating the surface area or particle size test along with other CQA tests. Additionally, surface area measurements may be used in conjunction with or as a substitute to particle size. In vitro drug release may in some cases correlate better with drug substance surface area.

Developing specifications for particle size for actives used solely in veterinary products is often limited due to the minimal number of drug substance batches produced via the commercial process at the time of registration, and the difficulty in producing drug substance batches of different particle size for clinical evaluation. Specifications may therefore be based off of the particle size distribution of a few pivotal batches used in the clinic instead of being set as broad as might yield product of adequate performance. Hence, if particle size is known to be critical, one approach is to resort to routinely micronizing the material rather than conducting extensive studies to determine the range of sizes that give adequate performance.

8.11 Drug Product Testing

This section presents quality attributes typically tested for batch release and stability purposes for selected veterinary dosage forms. Special considerations and challenges for dosage forms unique to veterinary medicine are discussed. The lists of quality attributes to be tested are not intended to be comprehensive; additional tests may be warranted depending on the unique composition, characteristics, and intended use of the product. Hence, appropriate tests need to be considered on a case-by-case basis for each product taking into account the drivers listed in Sect. 9.3 of this chapter. Several CVM Guidance documents provide additional examples for consideration [47, 48].

In addition to the dosage forms listed below, there are many different types of controlled release dosage forms used in veterinary medicine [49]. Other chapters of

this book provide greater detail on the design and application of certain controlled release dosage forms. The quality control tests listed herein for solid oral dosage forms provide very good starting points for consideration in the development of rumenal boluses, implants, and engineered or injection-molded devices such as intervaginal delivery devices, ear tags, and collars. The tests listed for parenterals should be considered when developing rumenal injections, long acting injections, and intermammary products.

8.11.1 Solid Oral Products

In addition to conventional solid oral tablets, this section is applicable to chewable tablets including palatable chewable tablets of hard or soft textures. Many of the tests required for solid oral products are also applicable to implanted (injectable) tablets, injection-molded products such as ear tags and collars, and rumenal boluses which may be designed as immediate release (IR), sustained release (SR), or pulsed delivery dosage forms. Some bolus designs leverage osmotic pressure to deliver a paste or solution of drug over an extended period of time, and others are homogeneous matrices designed to deliver the drug through an erosive mechanism [50]. Boluses may incorporate a holder, coating, or weight that aids in retention in the rumen, and that may be a functional part of the release mechanism. Long acting rumenal boluses are discussed in greater detail in Chap. 11 of this book.

For solid and semisolid oral products, the quality attributes tested are very similar to those tested for corresponding human health products, and include:

Appearance.

Identification: Typically two tests (primary and secondary) are performed for all drug products. Common approaches include the match of chromatographic retention time of the drug substance peak to that of a reference standard and spectral match by UV/Visible spectroscopy to that of a reference standard or spectrum. Data for both of these approaches can be collected concomitant with Assay and Degradation Products.

Assay: Grinding or extensive extraction procedures may be needed to achieve comprehensive recovery of the active from polymeric systems such as ear tags, collars, implants, etc.

Degradation Products: Process-related impurities from the active that are known not to be potential degradation products are typically excluded.

In vitro Dissolution, Disintegration, or Drug Release: This topic is discussed extensively in another chapter of this book. Testing of large rumenal boluses is particularly challenging due to the large physical size and drug payload [51]. The same challenge exists for polymeric devices and may require unconventional dissolution media or agitation conditions as discussed in Chap. 10 of this book.

Uniformity of Dosage Units: This may be done by content uniformity testing or by mass variation if certain requirements are met. This test is harmonized in the USP, Ph. Eur., and JP (see Ph. Eur 2.9.40. Uniformity of Dosage Units; JP 6.02 Uniformity of Dosage Units; and USP General Chapter <905>Uniformity of Dosage Units).

Residual Solvents: Residual solvents may be adequately controlled at the drug substance and excipient level. However, if control at the raw components level is not feasible, or solvents are used in the drug product manufacturing process, then residual solvents may need to be tested on the final drug product. This applies to all veterinary dosage forms.

Moisture or *Loss on Drying.*

Microbiological Enumeration Tests: Microbiological Enumeration Tests are harmonized across the USP, Ph. Eur. and JP. See for example Ph. Eur. 2.6.12 Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests, and Ph. Eur. 2.6.13. Microbiological Examination of Non-Sterile Products: Tests for Specified Microorganisms.

Hardness, *friability*, *and tablet weight:* These additional tests are often performed as in-process tests. If the drug product has a functional score, the ability to section tablets and achieve dose uniformity on the individual pieces may need to be evaluated. Texture Analysis or melting range may be useful for soft chewable tablets so as to establish that the dosage form is soft enough to be chewed by the animal, or broken up in the animals' stomach if swallowed intact, yet hard enough at room temperature to easily be handled by the pet owner. For drug substances that are chiral, it is typically sufficient to demonstrate during developmental stability and forced degradation studies that the active does not undergo changes in enantiomeric composition, obviating the need for chiral assay of the drug product. If the drug substance is prone to polymorphism, it may be necessary to demonstrate that it does not undergo form conversion in the dosage form. Often, it is very technically challenging to measure the drug's polymorphic form and to quantify the content of an undesired form with sufficient sensitivity due to drug product matrix effects. A better approach may be to demonstrate retention of polymorphic form in developmental stability studies using simple binary- or ternary-mixtures of the active and excipients stored under accelerated conditions. Such compatibility studies may use sufficiently high concentrations of active to yield improved sensitivity, and while they may not unequivocally rule out the potential for conversion in the final drug product, any susceptibility to form conversion observed in simple studies could be assumed to carry over to the product. Where in vitro dissolution or drug release testing is known to be discriminating of polymorphic form, this test may be employed as a surrogate to direct measurement of polymorphic form in the drug product.

Often, ingredients are added to veterinary dosage forms for oral administration to make them more palatable to the animal, improving dosing compliance. "The term 'palatability' refers to the voluntary (free choice) acceptance or ingestion of a pharmaceutical composition by companion animals, as measured by a standard palatability test, such as an acceptance, preference, or consumption test" [52].

"Heartgard® Chewables (ivermectin) and Heartgard Plus® (ivermectin/pyrantel) are beef-based chewable once-a-month products for heartworm disease and for the treatment and control of certain gastrointestinal parasites in dogs and cats. In general, because they are meat based, these products are highly palatable and are designed to be readily accepted by dogs when proffered to them, thus obviating the needs to insert the medication into the dog's mouth" [52, 53]. Where encapsulation technology for controlled release or taste masking is employed, it may be necessary to demonstrate through in vitro drug release testing that there is ample time before release of the active (breakthrough) so as to maintain palatability. Such a test may be a component of batch release and stability testing. Unless the dosage form is delivered in a way so as to force the animal to swallow it whole without chewing, typical coating approaches used for taste masking of tablets for humans may not be applicable for veterinary products. The use of coated granules or particulates may help address this chewing concern [52].

Palatability in veterinary species is typically evaluated through clinical studies during development [54]. Clinical testing of aged product or product that has been stored under accelerated stability conditions may be useful to confirm the stability of the flavoring agent. It may also be possible to develop analytical laboratory tests to monitor flavor profiles, e.g., gas chromatography (GC) with headspace analysis or other sensor-based techniques such as the "electronic nose" or "electronic tongue" may be used to gauge palatability for batch release or stability purposes [55, 56]. Also see [15, 16] for additional, discussions on palatability.

8.11.2 Parenterals

This category pertains to solution, emulsion, or suspension products delivered via intravenous, intramuscular, and subcutaneous routes. Intrarumenal injections, intramammary products, and more engineered systems such as long acting parenterals using suspension, gel, or microsphere technologies would also require similar testing. For parenteral products, the quality attributes tested are very similar to those tested for corresponding human health products, and include:

Appearance: For solution products, especially those intended for intravenous administration, absence of visible particulate matter is expected.

Subvisible Particulate Matter: In human health, this compendial test is performed for all injectable solutions. This is also true in most countries for veterinary parenterals; however, in the USA, this requirement has been indefinitely postponed for veterinary preparations for IM and subcutaneous administration [57]. Intravenously administered veterinary products must still comply. Compendial subvisible particulate tests are harmonized (except for products filled in 100 ml volumes where the JP is more stringent). See Ph. Eur. 2.9.19. Particulate Contamination: Sub-visible Particles; JP 6.07 Insoluble Particulate Matter Test for Injections; and USP<788> Particulate Matter in Injections.

Identification.

Assay: In the case of encapsulated (microsphere) products, it may be desirable to quantify the "free" or unencapsulated fraction of the active to distinguish it from that contained within the microspheres, or the total fraction.

Degradation Products.

In vitro Drug Release: Although typically associated with solid or semisolid oral dosage forms, this test may be needed if the injectable product is a suspension, or if there is a controlled-release mechanism designed into the formulation. This test may also be useful to demonstrate a lack of form conversion for suspension products using actives that are subject to polymorphism.

pH.

Uniformity of Dosage Units: This applies to parenteral products provided in single-use containers.

Fill Volume: The test for determination of volume of injectables in containers is harmonized with Ph. Eur. 2.9.17. Test for Extractable Volume of Parenteral Preparations; and JP 6.05 Test for Extractable Volume of Parenteral Products; and USP<1>Injections General Chapter test entitled "Volume in Containers."

Residual Solvents.

Moisture: If the formulation is nonaqueous, the water content may be critical to the physical and chemical stability of the product and may need to be closely monitored.

Microbiological Qualities: These include sterility and bacterial endotoxins

Osmolality.

Functional Excipient Assay: If antioxidants or microbiological preservatives are added, it may be necessary to ensure their concentration in the product is of a sufficient level to ensure activity. Testing of antimicrobial agent content may be done as a surrogate to Antimicrobial Effectiveness Testing (AET, also known as "Preservative Effectiveness Testing" or "Preservative Challenge"). AET is typically performed during parenteral product development to establish the minimum concentration of preservative that is necessary to maintain microbial quality. Such data supports the formulation justification and also the lower specification established for the antimicrobial agent content.

Particle Size: This is evaluated on stability for suspensions to determine if there are any changes in particle size over time due to dissolution or Ostwald ripening.

Density.

Viscosity.

Redispersability: Also known as resuspendability, this test for suspensions involves agitation of the product as per the labeled instructions followed by visual or

instrumental inspection to ensure product homogeneity. For suspension products containing multiple actives, chemical testing of an aliquot may be necessary to ensure all actives are adequately resuspended.

Reconstitution Time: This is done for products that must be diluted prior to administration.

8.11.2.1 Liquids

This section is applicable to solution and suspension preparations for oral, topical, transdermal, otic, or ophthalmic use, including products labeled as "oral drenches" or "pour-on" products for topical or transdermal delivery, and viscous liquids/semisolids such as pastes or ointments. Small volume topical or transdermal pesticide products, known as "spot-on" products are covered in greater detail due to their unique challenges.

Typical tests performed include:

Appearance.

Identification.

Assay.

Degradation Products.

In vitro Drug Release (For orally administered suspensions or pastes).

pH.

Uniformity of Dosage Units: This applies to products supplied in single-use containers.

Fill Volume.

Residual Solvents.

Moisture: If the formulation is nonaqueous, the water content may be critical to the physical and chemical stability of the product and may need to be closely monitored.

Microbiological Enumeration.

Osmolality (or Tonicity): May be important for ophthalmic products.

Functional Excipient Assay: If antioxidants or microbiological preservatives are added, it may be necessary to ensure their concentration in the product is of a sufficient level to ensure activity.

Particle Size: This is evaluated on stability for suspensions to determine if there are any changes in particle size over time due to dissolution or Ostwald ripening.

Density.

Viscosity: It may be useful to perform additional rheological characterization beyond viscosity measurement, particularly for viscous suspension, ointment, or paste products. This may include assessment of properties such as syringability, or the texture of the material as measured via texture analysis or penetrometry.

Redispersability: Performed for suspension products.

Reconstitution Time: This is done for products that must be diluted prior to administration.

8.11.2.2 Spot-On Products

Small volume topical or transdermal pesticide solutions, emulsions, or suspensions are known as "spot-on" products and are frequently developed as flea and ticks remedies for companion animals. These products are often nonaqueous in composition so as to achieve solubilization of the active, plus the desired spreading, drying, duration of activity, and appearance characteristics on the target animal. The volumes applied to the animals are usually minimized in order to reduce the potential for product "run off" or dripping off the animal. In order to accommodate animals of different sizes and maintain appropriate therapeutic margins, the products are usually supplied in multiple fill volumes of single-dose containers. Spot-on products are particularly challenging to develop due to the small volumes of product which must be accurately filled and quantitatively delivered to the animal, plus the need to avoid product/package interactions and solvent evaporative loss or moisture uptake, and differences in global regulatory requirements. In most countries including those throughout Europe, spot-on products are registered through the same processes as any other pharmaceutical product. A recent guideline in Europe provides additional requirements specifically for these products [58]. In the USA, the registration process depends upon whether the activity of the drug requires systemic exposure in the target animal. If the drug acts locally (topically), then the product is registered through the US Environmental Protection Agency. If the drug acts systemically (i.e., via transdermal absorption), then the product is registered with the FDA. The US EPA's pesticide registration data requirements differ from those of typical pharmaceutical licensing authorities in several ways and are summarized in 40CFR158.

Many of the tests listed for liquids apply to spot-on products, with a few exceptions. The first major difference for spot-on products concerns the measurement and reporting of potency. For most liquid pharmaceutical products, potency is equivalent to the assay of the solution concentration which is reported as a percentage of the nominal w/v concentration expressed on the label. This practice is followed for FDA registered pesticide products. For EPA registered products, specifications for solution concentration of the active are known as the "certified limits" and typically the width of the acceptance criteria window depends upon the nominal strength of the drug product, N, expressed as a percentage by weight of the product (w/w%):

When $N \leq 1.0\%$, the certified limits are $N \pm 10\%N$
When $1.0\% < N \leq 20.0\%$, the certified limits are $N \pm 5\%N$
When $20\% < N \leq 100\%$, the certified limits are $N \pm 3\%N$

Hence, dilute products that use potent actives are subject to ±10% limits which are common assay limits for pharmaceuticals in the USA and selected other countries, but broader than typically approved in Europe. Intermediate concentrations are subject to the ±5% limit which is common for assay of other types of pharmaceutical products in Europe, and the most concentrated of spot-on products require very tight ±3% limits, which are tighter than required for most other types of pharmaceutical products registered anywhere in the world. The applicant may propose other limits, but the requirements cited earlier are the starting point or benchmark for EPA products.

As per the EMEA guideline, assay for spot-on products is not simply the solution concentration of the active. Instead, the guideline defines assay in terms of the dose of active that is likely to be delivered to the animal. Assay is expressed as milligrams per container of average delivered mass, which takes into account the solution concentration and deliverable volume from an average container. Limits for this test are set as 95–105%. Hence, to determine this assay, at least two measurements are needed: solution concentration of actives and average deliverable volume. Due to the small volumes that need to be accurately determined, this test may be performed gravimetrically with a conversion to volume based upon the product density. It is therefore important during product development to design a container that delivers the appropriate dose with minimal and reproducible residual volume so that the product is expressed quantitatively and precisely. A slight overfill is typically added to compensate for the residual volume held up in the container when the contents are delivered. The 95–105% specification required by the EU guideline is challenging to achieve as the measured assay depends upon many factors including:

Accurate determination of required overfill.
Filling accuracy and precision.
Accuracy and reproducibility of the delivered volume test.
Accuracy and reproducibility of the solution concentration test method.
Solution concentration of the drug product.

The EU guideline suggests that the manufacturer measures the solution concentration of active for the bulk drug product prior to filling and adjust the fill volume so as to achieve the target mass delivered per container of average deliverable volume. This practice is not intended to compensate for solution concentrations that exceed the typical requirements for solution concentration of 95.0–105.0% of label claim as these requirements must also be met. Even if this adjustment practice is used, filling tolerances and precision of the deliverable volume and solution concentration tests need to be exceptional to comply with this test. For example, a fill volume of 97.5% and a solution concentration of 97.5% will yield an assay of 95.1%, barely complying with the guideline. A product at 102.5% of the nominal solution concentration that is filled at 102.5% of the target volume would yield an assay of 105.1%, which is out of specification. In the end, the practical ramifications of the guidance are that the solution concentration and fill volume cannot vary simultaneously in the same direction by more than ±2.5% of nominal, which is much tighter than for other veterinary dosage forms. Indeed, this is even tighter than for human products which may simply be controlled by the UDU requirement. Adjustment of

fill volumes based upon solution potency may not be practical in the manufacturing environment, and if the product contains multiple actives, it may not be possible to compensate in cases where the solution concentration is subpotent for one active and superpotent for the other. The EU guideline also requires that, "A test for uniformity of dosage units should be included." "The liquid should be removed from the individual containers in a manner likely to be used by the person treating the animal." Hence, the packaging system must also be capable of very precise delivery.

The US EPA requires other tests that further differentiate spot-on products from other veterinary liquids. For instance, 40CFRPart 158 discusses the need to characterize the product's vapor pressure, corrosion characteristics, and explodability among other parameters. The EPA Office of Prevention, Pesticides, and Toxic Substances (OPPTS) Guideline on Stability Storage recommends stability test conditions different than typically done for pharmaceutical drug products [59]. EPA stability storage conditions include 20 or 25°C/50% RH, warehouse conditions, accelerated conditions (40–54°C), and cold extremes (−20 to 0°C). EPA does not require that the product be tested under all of these conditions. Stability storage conditions should be selected and justified by the applicant. At a minimum, EPA stability protocols include tests for assay, impurities, weight loss, and an assessment for corrosion of packaging.

8.11.3 Medicated Feeds

While parenterals or traditional oral products are an attractive option for the medication of individual animals or small groups, such individualized treatment is undesirable for larger groups of animals. Therefore, the addition of medication to the animals' diet either through their feed or drinking water is a standard method for treating larger groups of animals. Such products are very popular for cattle, swine, poultry, and fish. This chapter discusses special testing considerations for feeds, including Type A medicated articles, Type B and C medicated feeds, blocks, licks, and liquid feed supplements (LFS). Depending upon the solid or liquid nature of these products, many of the testing considerations already presented for solid oral and liquid products may be appropriate.

8.11.3.1 Feed Types and Composition

In the USA, there are three types of medicated feed products [60]:

Type A Medicated Articles: Sometimes called "premixes," these products consist of API with or without excipients. These products are intended solely for use in the manufacture of another Type A medicated article or Type B or C medicated feed (i.e., they are not directly fed to animals).

Type B Medicated Feeds: Sometimes called "concentrates," these products are used solely for the manufacture of other Type B or C feeds. Type B medicated feeds contain nutritional components at a level of not less than (NLT) 25% by weight.

Type C Medicated Feeds: Type C medicated feeds are consumed by the animal, either as a complete feed, or as a component of the diet that is top dressed or offered free choice with other types of feed.

Free choice feeds are a subcategory of Type C medicated feeds. "Free choice medicated feeds are those products which contain one or more animal drugs and are placed in feeding and grazing areas but are not intended to be fully consumed at a single feeding or to constitute the entire diet of the animal" [61]. This category includes dosage forms such as medicated blocks (licking blocks), mineral mixes, and liquid feeds.

Feed products are different from other veterinary pharmaceuticals because of the way in which they are administered. Most other pharmaceuticals are typically used "as is" directly from the primary container with no further dilution. Hence, in-use stability studies for those products focus on demonstration that the product will comply with its shelf life specifications through the end of the dosing period, and the test methods used for batch release and stability testing usually suffice for this purpose. On the other hand, feed products are often blended with complex mixtures of natural feed ingredients prior to use (and may contain such ingredients as sold). Hence, the analytics of feed products, both "as is" and postdilution may be complicated by matrix effects, and the stability and homogeneity of the final diluted feeds needs to be demonstrated for feeds of varying composition, common methods of manufacture, and labeled dosing levels. Different analytical test methods may be necessary to test Type C feeds (which are fed "as is") vs. Type A medicated articles (which are always further diluted prior to use). Whenever Type A or B feeds are marketed, manufacture of the corresponding Type C medicated feed needs to be demonstrated, along with suitable assay, stability, homogeneity, and shipping segregation data.

A significant level of analytical complexity is often encountered for feeds due to the use of natural ingredients such as clay agents, molasses, and various whole/partial grains or meals (alfalfa, wheat, etc.), brewer or distiller's by-products, animal protein, fats, and oils. Inert ingredients used in feeds are typically controlled according to the specifications set forth in the Official Publications of the American Association of Feed Control Officials (AAFCO), also known as the "AAFCO Manual" [61]. Testing of feed ingredients often involves characterization of nutritional value, including "proximate analysis," i.e., testing of fiber, fat, and protein content. Proximate analysis or other aspects of feed ingredient quality is usually a consideration for feed mills, ingredient vendors, and state or contract labs doing routine quality checks on Type C feeds, but it may also become an issue for manufacturers of medicated articles where such ingredients are used in the product. In addition, the AAFCO Manual discusses the concept of Analytical Variations (AV), which is a measure of typical test variability based on the AAFCO Check Sample Program. The AV section describes typical variation around the specification limits for various feed component tests to allow for the inherent variability in sampling and analysis. Test results that are within the AV tolerances of the specification are considered acceptable. See also refs. [62, 63] for additional information on the testing of feed components.

Natural ingredients can cause analytical interferences impacting feed method specificity or recovery of the active substance. Because of these effects, analysis of

feed products often requires complex extraction schemes involving grinding, solvent extraction, sonication or heating, and sample cleanup steps such as solid-phase extraction to lessen the effects of interfering compounds. Guidance regarding the validation of methods for analysis of Type C medicated feeds is provided by CVM Guidance for Industry #135 [64]. Assay methods for feeds also need to have adequate sensitivity and range to deal with concentrations of active that may range from parts-per-million (ppm) levels to many orders of magnitude higher. Accuracy (recovery) needs to be evaluated in at least two different typical feed matrices. The types of feed that may be produced should also be considered, and it may be necessary to examine dry vs. moist feed, mash versus pelleted feed, steam pelleted versus extruded feed, etc. Feed composition and processing may vary significantly depending upon the target species and age of the animals being treated. For example, tilapia are naturally vegetarian, catfish are omnivorous, and salmonids are carnivorous. Tilapia and catfish feeds are grain based and are produced as floating feeds and have lower fat content (6–8%) than salmonid feed (22–36%). Salmonid feed sinks and contains animal-based protein and fat (e.g., fish oil, poultry fat, fish meal) and the size of the pellets and fat/oil content is varied significantly depending upon age of the animal, with small crumbles to pellets for fry, fingerling, and smolts (50–80 g in animal weight) and large pellets for adults (up to 4–5 kg in animal weight) [65]. The impact is that it is difficult to have one single method for all types of feeds that may be produced. Even though the same active and Type A or B product is used, different methods may be required for different matrices of Type C feeds [66]. Furthermore, the composition of fish feed is constantly evolving as producers seek to minimize cost while maintaining or improving nutritional value.

Feeds are often used to administer multiple medications and dietary supplements simultaneously. For this reason, the product development scientist needs to consider the other items that may be potentially co-administered and should demonstrate that the analytical method maintains suitable specificity and recovery in the presence of these ingredients. The potential for drug–drug interactions and chemical incompatibilities should also be considered, and where necessitated may require contraindication on the product labeling.

Feeds may also contain microtracers, which are small particles, typically metallic, but also potentially of mineral or organic composition, typically with a highly visible dye added [67]. Microtracers are added to confirm adequacy of cleaning in a manufacturing facility and to allow quick and easy identification for the presence or absence of certain feed additives. This practice is particularly useful where drug–drug interactions or contraindicated usage may present safety or efficacy concerns. Microtracer content may be confirmed by visual inspection or may require physical or chemical methods for detection and quantification.

8.11.3.2 Stability Testing of Feeds

In the USA, stability guidance for medicated articles and feeds is provided in CVM Guidance for Industry #5 [48]. Stability studies for Type A medicated articles generally follow the same VICH stability study design as for other pharmaceuticals,

and these products are assigned expiry dates based upon long term and accelerated stability data. Type B medicated feeds may be evaluated using various stability study designs, sometimes as rigorous as performed for Type A medicated articles, but minimally as performed for Type C medicated feeds. Type C medicated feeds are typically evaluated on stability under long-term storage conditions for 90 days, with testing at 0, 1, 2, and 3 months. Type B and C medicated feeds may or may not have expiry dates; it is determined on a case-by-case basis. Both are typically supported by at least 1 month of accelerated stability, with testing at 0, 2, 3, and 4 weeks. Typically, such data is generated on at least three production scale batches manufactured over a range of medication levels, feed types, manufacturing processes, etc. For example, where Type B and C feeds are medicated at multiple dose levels, minimum and maximum levels need to be tested. If multiple methods of preparation may be employed (e.g., "top coated" vs. incorporated, different types of mixers, etc.), representative batches of each type should be tested. Feed characterization typically also involves homogeneity testing, which is an assessment of uniformity of assay (active ingredient content) across a batch, and within individual containers (typically bags) if partial container use is allowed. Feeds may be trucked over long distances from feed mills to the site of product use. Therefore, homogeneity is also evaluated on bags that have been shipped to evaluate the potential for segregation of the active. European guidance also requires the assessment of homogeneity, shipping segregation, compatibility with other potentially co-medicated articles or minerals, and stability [68]. References [16, 23, 47, 48] provide additional discussion on feed products.

8.11.3.3 Liquid Feed Supplements

Viscosity may be important for liquid feed supplements as it has the potential to impact mixing and positional stability (i.e., settling of the active). Stability studies are often used to determine if product settles (stratifies) after sitting. Storage for 8 weeks without agitation at 30°C and under refrigeration (2–8°C) is typically sufficient for CVM to consider a product as positionally stable, but verification in at least one field study is required [48]. Additionally, a short duration freeze–thaw study of at least 5 days is recommended to show homogeneity (by testing top/middle/bottom samples) and stability for liquid feed supplements.

8.11.3.4 Blocks

Medicated blocks are considered Type C medicated feeds and, "…are compressed feed material (which contain medication) that is shaped into a cubic form for animal free-choice use" [48]. Unique to blocks is the requirement that they be stability tested under conditions of warehouse storage (25°C/60%RH and 37–40°C) and field use for 14–90 days outdoors on the range. Block surface and core samples are assayed using stability indicating and validated methods. Mash used in the manufacture of the block is also tested.

8.11.3.5 Additional Considerations for the Analysis of Type C Medicated Feeds

As a part of the product development and registration process, manufacturers of Type A medicated articles or Type B medicated feeds are required to test typical Type C medicated feeds that may be produced from the products they sell. Evaluation of Type C medicated feeds for homogeneity, segregation, and stability requires the development and validation of an assay method. Furthermore, the assay method needs to be suitable for field use by state or contract laboratories for the analysis and control of the Type C medicated feed. The US FDA-CVM requires that the practicality and transferability of the method to external laboratories is demonstrated as part of the approvals process [69]. The feed method is transferred through a multilaboratory evaluation commonly called a "method transfer study" or "method trial." Method trials for new Type C medicated feed methods typically involve 3 labs, including both contract and state feed labs; although in certain cases, only a single lab is required to participate in the trial [70]. As described in CVM Guidance for Industry #136, method trials are multistep processes [69]. First, the sponsor develops, validates, and documents the method, and generates batch data as a proof of performance [71]. This background information and a protocol for the method trial are then submitted for CVM review. Once the CVM accepts the method and protocol, the trial participants gather for an in-person demonstration of the method. The sponsor walks the participants through the conduct of the method, including any critical or technique-sensitive steps. The method and protocol documents are revised to incorporate any comments from CVM and participating labs to improve clarity. Method participants then perform the method in their respective laboratories to become more familiar with its performance before conducting critical phase of the trial, which is an inter-laboratory proof-of-performance test of control (unmedicated), fortified (spiked), and medicated feeds. Data for each of these samples from each participating lab are then documented and provided to CVM for analysis [69]. If data from the participating labs shows good agreement, the method is deemed to be under control and the participating labs are considered qualified to perform the feed method.

8.11.4 Drinking Water Products

This section discusses analytical considerations for soluble powders, granules, or liquid concentrates intended to be diluted in drinking water. Such products are used for treatment of larger groups of swine, poultry, and sometimes cattle. Many of the quality control tests listed for solid oral or liquid products would in general be applicable to these types of products; however, the unique way in which they are administered brings special considerations for in-use stability studies. The wide range of ways in which the products could be used needs to be anticipated and underwritten or contraindicated based upon data from laboratory and/or field in-use stability

studies. Factors to be studied include impact of water of different pH and hardness (calcium and chlorine content), materials that the product or diluted product may contact, and dissolution time as a function of temperature [72].

Stability of the product in the market image container is demonstrated as per the VICH guidelines as for any other pharmaceutical, but the in-use stability studies on the diluted drinking water need to explore the materials of different composition the product may encounter in the field such as piping and troughs made from galvanized steel, poly(vinyl chloride) (PVC) plastic, rusty iron or steel, etc. Drinking water stability is usually evaluated at 25°C and 37–40°C, possibly higher for certain climatic zones, using water prepared at the lowest labeled dose concentration. One fresh batch of product and one batch at least 6 months old (stored at 25°C/60%RH) is used to demonstrate that old product still performs acceptably. Each batch of product is used to prepare two different batches of medicated water using water of different hardness and pH [72]. In Europe, the permitted (maximum) use period for medicated drinking water is limited at 24 h. Hence, in-use stability studies for products distributed in the EU only need to cover this period [73]. Dissolution of solid products is critical and should be evaluated at a variety of temperatures to ensure that it is sufficiently rapid. Also, if a measurement device is provided, dosing accuracy, precision, and suitability regarding chemical and physical compatibility with the product should be demonstrated.

8.12 Specifications

This section presents general concepts regarding the development of specifications for veterinary drug products, including enhanced detail on some topics requiring special consideration.

8.12.1 The Specifications Development Process

The establishment of specifications for pharmaceutical components and products is a data-driven, evolutionary process. It is not a one time, “set it and forget it” event. As discussed earlier in this chapter, the process begins with identification of all of the physical, chemical, and microbiological properties that might be important to the quality of the product and identification of appropriate test methods to monitor these attributes. As data is collected throughout development, the list of tests that need to be performed, and the corresponding acceptance criteria, may be refined. Certain tests may be found to be of little value and may be discontinued. Data may indicate that certain attributes are more or less critical than originally thought, thus driving the tightening or loosening of acceptance criteria. Also, it may be discovered that some additional attribute needs to be monitored, requiring the addition of a new test method and acceptance criteria. The evolution of tests, test methods, and

acceptance criteria means that specifications are *developed* throughout the lifecycle of a product. The specifications development process has several natural milestones, e.g., when specifications are established for the release of supplies to be used in pivotal clinical studies, for use in registration stability studies, and when "finalized" for product registration and commercialization. However, the process does not end when the product is commercialized. Additional changes may be needed to address postapproval regulatory commitments, new compendial or regulatory requirements, and future changes in excipients, packaging components, or manufacturing processes. Improved analytical method capabilities may lead to additional findings (e.g., discovery of previously undetected impurities) that need to be accounted for in the specifications.

In some cases, the specifications required for a certain product are straightforward, for example when there are standard criteria required by the pharmacopeias or regulatory guidelines. In other cases, the manufacturer must develop appropriate specifications based upon the drivers listed in Sect. 9.3 of this chapter, batch data, industry conventions, and the input of regulatory authorities. Ultimately, it is up to the manufacturer to justify the selected specifications through reference to the applicable guidances, compendia, and data on pivotal product lots used in clinical and stability studies and supportive (prototype) batches. Per VICH Guideline GL39, "When a specification is first proposed, justification should be presented for each procedure and each acceptance criterion included. The justification should refer to relevant development data, pharmacopeial standards, test data for drug substances and medicinal products used in toxicology, residue (when relevant) and clinical studies, and results from accelerated and long term stability studies, as appropriate. Additionally, a reasonable range of expected analytical and manufacturing variability should be considered. It is important to consider all of this information" [1]. Specifications of commercial products are tied either directly or through a well-understood relationship to those of the drug product batches evaluated in the pivotal clinical safety and efficacy studies performed during product development. In this manner, it is assured that the commercialized product's safety and efficacy are supported by and consistent with the clinical studies that serve as the basis for the product's marketing authorization. Indeed there are many decision factors to consider in developing specifications, including:

Manufacturing process capability.

Analytical methods capability.

Batch Release data.

All stability data (developmental, forced degradation, registration, photostability, in-use, etc.).

Clinical data related to drug product safety, efficacy, and pharmacokinetics.

Regulatory requirements.

Current industry practices and trend.

Dialogue with regulatory authorities. Input from regulatory authorities is extremely valuable when developing specifications and is particularly important when proposing an unconventional acceptance criterion or strategy, e.g., if the registration stability program will leverage matrixing or if commercial product release will leverage skip-lot testing, PAT, parametric release, or "real time" release.

Risk management considerations often play a considerable role in the specifications development process [3]. For example, commercial product specifications for modified release dosage forms often control in vitro drug release tightly around the data from pivotal clinical batches to minimize the chance of unacceptable pharmacokinetics. Likewise, impurities specifications may be conservatively established based upon the exposure achieved in clinical safety studies. In both cases, the product may actually be capable of acceptable PK or safety if the specifications were much broader; however, the limited knowledge space due to a small number of similar product batches being tested in development leads to a narrow specification design space. The limited number of batches studied in development and limited amount of stability data available at the time of registration (typically only 6 months) are common challenges in the establishment of specifications for veterinary pharmaceuticals. The estimated process capability at the time of filing may differ significantly from the true process capability, which may only become evident after a larger number of commercial batches have been produced. The implication is that specifications filed at the time of registration may need to be revisited once additional data is available [1, 2, 21]. Some tests that are not driven by process capability (e.g., UDU and other compendial tests) may be set unambiguously at the time of market application and may not require subsequent adjustment [21].

8.12.2 Establishing Acceptance Criteria

Specification acceptance criteria may be expressed as discrete and text-rich statements of quality requirements (e.g., "A colorless to slightly yellow solution" for an Appearance test) or as continuous numerical requirements (e.g., "95.0–105.0" for Assay as % of Label Claim). Tests such as Appearance and Identification often have text-rich acceptance criteria. Appearance acceptance criteria are based upon the range of observations from batch release and stability testing, and anticipation of expected variations that would be perceived by and acceptable to the customer. Identification tests might list acceptance criteria such as, "The absorbance spectrum of the sample preparation conforms to that of a reference standard preparation," or, "The retention time ratio of the major component peak of the sample preparation to that of the standard preparation is 0.98–1.02." Acceptance criteria for many compendial tests are straightforward and stipulated in the pharmacopeia, e.g., for microbiological enumeration tests, sterility, subvisible particulates, and UDU. Tests which have numerical specifications typically include assay (of actives and functional excipients), degradation products, pH, moisture, viscosity, density, particle size,

and residual solvents. Furthermore, acceptance criteria may be centered about the nominal or theoretical value, or may be asymmetric, with broader tolerances in one direction of departure from the mean than in the opposite direction. For example, an assay specification of 95.0–105.0% is symmetric around a theoretical mean of 100.0%, but if the active is known to degrade, and no source of potency increase is possible (e.g., no chance of evaporative concentration), then an appropriate specification might be 90.0–105.0%, which is asymmetric.

8.12.3 Statistics in Setting Acceptance Criteria and Analyzing Stability Data

When setting specification acceptance criteria, one should consider the results of average and extreme batches, plus the contributions of analytical variance, sampling variance, process variance, etc. [5, 14, 21]. Limited data does not lend itself to a robust statistical estimation of process capability, and it may be impractical to generate meaningful confidence intervals [21]. It may be useful to determine if stability data on multiple individual batches can be pooled (e.g., via ANCOVA analysis to establish that the data sets have comparable slopes) or else the worst case batch should be used to establish the shelf life and/or shelf life specifications. Indeed, in such cases, additional reliance is typically placed on the extreme values observed in batch data plus a rough guess allowance for analytical variability, and this is quite common in the development of specifications for veterinary products.

As per the WHO, "Where the [stability] data show so little degradation and so little variability that it is apparent from looking at the data that the requested shelf-life will be granted, it is normally unnecessary to go through the statistical analysis" [25]. A stable product for which no appreciable change is registered is indeed the ideal case for veterinary products. In this event, the shelf life that can be assigned with the limited data available at the time of registration is maximized. Otherwise, if a trend is evident in the stability data, statistical approaches may be useful in predicting and justifying a shelf life and acceptance criteria for shelf life specifications. "An approach for analyzing the data on a quantitative attribute that is expected to change with time is to determine the time at which the 95% one-sided confidence limit for the mean curve intersects the acceptance criterion" [25]. Other reports justify the use of 99% confidence intervals to set specifications [74].

Beyond setting specifications and establishing the expiry dating, statistics may be useful to predict with some level of confidence that the measured parameter will remain within its registered limit through the end of shelf life [75]. Process capability may be used to express the ability of a product and process to achieve compliance with the established specifications [40]. Process Capability Index (CpK) is defined as the (process mean—specification limit/3 × process standard deviation), where values less than 1.0 indicate diminishing degrees of acceptability, and values greater than 1.0 indicate increasing levels of robustness [76]. Statistical prediction of the probability of passing registered limits coupled with the use of internal release

limits (*vide infra*) can provide greater routine confidence of compliance with shelf life specifications. Also, Wang presents a method for statistical estimation of the probability of passing the USP dissolution test [77]. When no internal release limits are used or where very limited data sets are available that may not be conducive to statistical analysis, it is important that the registered shelf life limits are set broad enough to have confidence that normal batches will remain within specification throughout shelf life. However, specifications must not be set so broad that quality, safety, or certainty of product performance is compromised.

See Dukes and Hahn [78] for additional discussions of specifications development including the use of the Arrhenius equation and tolerance intervals, and Shao and Chow [79] for an approach to estimation of shelf life for products which may have two-phase degradation slopes (like lyophilized products for reconstitution).

8.12.4 Acceptance Criteria: Too Broad, Too Tight, or Just Right?

Setting specifications is a balancing act between two types of risk: 1] the risk of rejecting an acceptable batch (α or Type I error), and 2] the risk to the patient of releasing a product that is unacceptable (not pure, safe, or effective) (β or Type II error). Hence, specifications that are too loose provide insufficient discrimination between acceptable and unacceptable batches, and specifications that are too tight increase the probability of unnecessary OOS results and rejected or recalled batches when quality and performance may not actually be a concern. Several practices may help to manage the risk of batch failures due to tight specifications:

Run Additional Studies to Support Broader Specifications: Decisions regarding the acceptability of commercial batches (when compared to specification) are based upon individual test points, and there is little opportunity to wait to see what future (stability) test points bring if results at batch release or a stability test point are out of specification. Hence, setting the specification acceptance criteria as broad as defensible is important to prevent batch recalls. Nonetheless, there are always limits as to how broad the specifications may be set, and this is influenced by the availability of data, the range and variability of the data, prudent practice, and what is acceptable from a regulatory perspective. Consistent with QbD principles, the release and shelf life specification acceptance criteria should be both within the knowledge space and design space such that product quality is acceptable and product performance is assured. Tight acceptance criteria should be set for critical parameters, and broader criteria set for less critical measures. It is important that the manufacturer understands the impact (if any) that formulation and processing variables have on the ability to comply with specifications and on product stability. During development, studies should be performed to explore extremes of formulation and processing variables so as to underwrite broader ranges in acceptance criteria if acceptable. An example of the studies that could be conducted would be the spiking of water at various levels to a nonaqueous system to explore the impact on chemical and

physical stability. The water specification would then be established at some point slightly below the maximum acceptable value as determined from this study.

Establish More Restrictive In-House Specifications: In-house specification limits (sometimes called "alert limits") may be used in conjunction with registered specifications for batch release and shelf life. Typically, in-house specifications are set more conservatively than the registered specification and are used to flag batches that are statistical outliers or out of trend and that may have increased risk of generating an OOS result throughout the remaining shelf life. In-house specification limits are established on the basis of process capability and stability data, but it often takes considerable data on multiple batches to employ this practice [75]. A batch which complies with both the in-house specification and registered specification provides maximum confidence of compliance with specifications throughout shelf life. A batch which complies with registered specifications but is outside of the in-house limits may still be suitable for release to the market, but the risk of future OOS results should be carefully evaluated. Of course, batches which fail to comply with both in-house and registered specifications are unacceptable and are usually rejected.

Establish Different Acceptance Criteria for Shelf Life vs. Batch Release: In Europe and selected other global markets, it is commonly accepted that specifications may be different at the time of batch release testing vs. at the end of shelf life [1, 2, 17]. This allowance is typically leveraged for tests such as assay, related compounds, preservative content and moisture where changes over time may be expected, demonstrated through stability studies, and are predictable. In markets where separate release and shelf life specifications are not allowed, the specifications must obviously be broad enough to describe the product both at the time of release and at the end of shelf life, plus any additional effects on the quality that might result during the in-use period.

Improve test method precision: This may involve changes regarding sampling or how the method is performed. One of the most common approaches to improving precision is to test replicate sample preparations. Written procedures or test methods must clearly state the number of replicates to be tested and how the data is to be treated for comparison to the specification. For example, an assay method might require duplicate preparations for each sample, with each replicate being assayed once. Procedures might then stipulate an agreement factor for the raw data such that the data must be within some predetermined agreement in order to be considered a valid data set. Data that complies with the agreement factor might then be averaged and compared to the specification acceptance criteria.

Other approaches: Finally, it should be mentioned that as more extensive data becomes available and the process capability is better understood, specification acceptance criteria may need to be adjusted. Changes may include re-centering acceptance criteria around a new nominal value, or tightening or loosening the acceptance criteria. If the acceptance criteria cannot be modified (e.g., due to safety or efficacy concerns, or regulatory requirements), then it may become necessary to modify the drug product

or its manufacturing process to re-center the product/process performance within the existing acceptance criteria or to shorten the assigned expiry dating to improve confidence of compliance throughout shelf life.

8.12.5 Global Specifications

Achieving one set of batch release and shelf life specifications that is harmonized globally is very desirable because it reduces burden and complexity for the manufacturer's regulatory affairs professionals, QC laboratories, QA professionals, etc. However, achieving this can be quite complicated due to the different expectations of licensing authorities in different markets. For example, in Europe, EU Directive 75/318/EEC states "Unless there is appropriate justification, the maximum acceptable deviation in the active substance content of the finished products shall not exceed ±5% at the time of manufacture. On the basis of the stability tests the manufacturer must propose and justify maximum acceptable tolerance limits in the active substance content of the finished product to the end of the proposed shelf life" [17]. Hence, unless significant change is documented in the registration stability studies, active substance assay limits for drug product batch release and shelf life are typically 95.0–105.0% of label claim. However, in other markets including the USA, limits of 90.0–110.0% at release and shelf life are normal.

Furthermore, specifications are frequently negotiated with licensing authorities as part of the dossier review process; hence, even if the manufacturer sets forth with a plan for globally harmonized specifications, divergence is often required to obtain global product approval.

8.13 Special Considerations in Specifications Development

In addition to the items presented below, see also reference 80 for selected special considerations in the pharmaceutical development of veterinary dosage forms.

8.13.1 In Vitro Dissolution Testing

In vitro dissolution or drug release tests and specifications are often among the most challenging to develop, and the challenge is amplified by the dose range, dose payload, physical size, and complex composition of many veterinary drug products, plus the different gut conditions of various species. The use of long acting dosage forms in veterinary medicine is particularly attractive due to the potential to reduce animal handling, which translates to less stress on the animal and economic savings [81, 82]. The European Medicines Agency requires in vitro dissolution testing for

modified release dosage forms for veterinary use [83]. Dosage forms such as boluses, implants, intervaginal devices, etc. all present unique in vitro test method challenges and may inherently require exorbitantly long drug release tests, driving the exploration of conditions that promote accelerated release to achieve a test of reasonable duration. Specifications may be single point, e.g., "Not less than 80% released at 30 min," or may be multipoint in order to define a release profile. Single point dissolution measurements are typically acceptable for immediate-release dosage forms, but multipoint measures may be needed for modified release dosage forms. Under certain conditions, dissolution may be replaced by disintegration testing [1, 2]. When multipoint specifications are used for long acting dosage forms, an early test point is used to assure that there is no dose dumping, a middle test point is used to define the release rate, and a later test point is used to provide assurance of comprehensive drug release. Acceptance criteria are typically set with using a "window" of NMT 20% (i.e., mean ± 10%) for acceptance criteria (% released) at each test point [1]. Furthermore, the specifications may be single stage (i.e., pass or fail) or may have multiple stages where failure to comply with the acceptance criteria of Stage 1 may not be an overall failure, but instead may require additional testing and evaluation vs. Stage II and potentially Stage III acceptance criteria. Where multistage specifications are used, the product should not routinely require Stage II testing, and should rarely require Stage III testing. All dissolution or drug release tests are performed on multiple dosage units to assess dose-to-dose variability in performance. Typically six dosage units are initially tested, with an additional 6 tested at Stage II and an additional 12 units tested at Stage III, if necessary.

The in vitro drug release test ties the performance of commercial batches to that of clinical batches evaluated during the product development process. Beyond its utility as a QC method, in vitro drug release is also useful to support formulation, process, and site changes, including batch size scale-up and may avoid the need to perform in vivo bioequivalence studies [84].

Because of the importance of this topic, a more detailed discussion is presented elsewhere in this book (see Chap. 10). Other useful references include the FDA guidance on Dissolution of Immediate-Release Dosage Forms and Decision Tree #7 in VICH Guideline GL39 which provides guidance for the development of dissolution specifications, and Brown et al which discusses dissolution challenges for unique dosage forms [1, 85, 86].

8.13.2 Functional Excipients

For the purpose of this discussion, "functional excipients" is limited to antioxidants and microbiological preservatives. Antimicrobial preservatives may need to be added to multidose products that are not self-preserving [80]. Certain nonaqueous formulations of exceptionally low water activity may not require preservatives, but their ability to maintain microbial quality still needs to be demonstrated during development. Antioxidant use is typically dictated by the instability of the active ingredient or that of another excipient, which could then lead to reactivity with the

active. Antioxidants may be sacrificially degraded during the manufacture or storage of the product throughout its shelf life [80] and the levels of the antioxidants need to be established such that there remains a sufficient amount to ensure product quality throughout shelf life, and that the product remains within its registered limits for assay, degradation products, etc. Special studies are typically required to select the appropriate functional excipients and the amount needed, which defines the specification acceptance criteria. The selection process may include the preparation of prototype formulations with a variety of functional excipients present at different levels, and assay of their content plus other CQAs following storage at accelerated stability conditions. Selection of appropriate microbiological preservatives and levels often requires Antimicrobial Effectiveness Testing (AET) on formulation prototypes prepared at nominal and reduced levels of the preservative. Specifications at the time of release might be 90.0–110.0% of label, but shelf life specification for the preservative could be considerably lower and are established at some level slightly above the minimum amount needed to maintain microbial quality as per the AET data. The shelf life specification for an antioxidant may be quite low, especially if it is sacrificed in protection of the formulation. Developmental studies for antioxidant selection should also consider the excipients or conditions that are contributing to oxidation, along with the expected variability and "worst case" conditions likely to be encountered. This may include the testing of excipients from both primary and secondary vendors and extremes in manufacturing process variables.

8.13.3 Impurities and Degradation Products

Impurities present in drug substances and drug products may originate from organic sources (process impurities, degradants), inorganic sources (e.g., metallic catalysts used in the synthetic process), and residual solvents from the process [42]. Impurities in the drug product may be contributed by the drug substance, excipients, solvents, or the drug product manufacturing process (e.g., if spray drying from solvents).

Organic Impurities: Organic impurities that are degradation products need to be monitored in drug products, and organic impurities that are only formed as a result of the drug substance synthetic process do not need to be monitored in the drug product. Typical drug product impurities specifications for commercial products include individual impurities which may be specified or unspecified, and of known or unknown identity, plus total impurities, which is the sum of all individual impurities exceeding the VICH reporting threshold. In general, unless there are toxicological concerns, individual impurities in veterinary drug products may be controlled with a reporting threshold of 0.3% and an acceptance criterion based upon batch data but not more than (NMT) 1.0%. Impurity identification and qualification would be performed if the level exceeds 1.0%. If the degradant is uncommonly observed, it may be controlled as an individual unspecified impurity with a level of NMT 1.0%. If it is commonly observed, then it may warrant designation as a specified impurity with a corresponding acceptance criterion based upon its toxicological qualification data and likely levels as determined from stability studies. In general,

the quantification limit of the impurities test method should be not more than the reporting threshold according to the VICH guideline (i.e., ≤0.3% for drug products and 0.1% for drug substances). VICH Guidelines GL10 and GL11 provide decision trees for impurities identification and qualification [42, 87]. Note that the VICH Guideline on Impurities in New Veterinary Drug Substances, "…is not intended to apply during the clinical research stage of development…," but compliance with the guideline, or scientific justification for alternative approaches, is required at the time of market application [42].

As mentioned earlier, typical VICH impurities limits do not apply to compounds of known toxicity. Potentially Genotoxic Impurities (PGIs) are generally drug substance process impurities and in such cases may be best controlled at the drug substance stage. If however PGIs are shown to arise from forced degradation conditions or on stability studies, they would need to be monitored in the drug product. PGIs may need to be monitored and controlled at ppm or parts-per-billion (ppb) levels, presenting significant analytical challenges. The manufacturer should identify proper process controls to eliminate or minimize the content of these impurities, including tight analytical monitoring. A general approach for the identification of PGIs is provided in Gangadhar, Saradhi, and Rajavikram [44]. Once the structure of impurities has been identified, an approach for addressing PGIs might begin with a search to see if the compound is a known PGI or possesses functional groups that are similar to those known to be of toxicological concern (i.e., observed on other PGIs). Further confirmation that the molecule is indeed a PGI would be gained through conducting either a genotoxicity assay, an in silico analysis using one of the commercially available SAR programs such as (MultiCASE's Mcase, Accelrys's Topcat, or LHASA's DEREK), or lastly performing an Ames bacterial mutagenicity test (if the in silico analysis suggested the compound was a PGI). The Ames test is considered more definitive than the in silico analysis. Currently, there is an emerging interest in potentially genotoxic impurities in veterinary drug products, but little information specific to veterinary products can be found in the literature. Most literature is borrowed from human health, e.g., ICH Final Concept Paper M7 [88] and the EU Guideline on the Limits of Genotoxic Impurities [89]. Roy provides a high level overview of pharmaceutical impurities testing and identification, and includes references to articles on impurity isolation and identification [11]. Further discussion of impurities identification can be found in literature by Martin and Görög [12, 13].

Modern analytical technology is continuously reducing the levels at which impurities can be detected and quantified. With the exception of PGIs, impurities detection at levels lower than stipulated by the VICH guidelines may be irrelevant for veterinary applications and may indeed raise more questions than can be answered.

It is also worth mentioning that a guideline on the development of specifications for impurities in antibiotics is under development by the EMA at the time of preparation of this book chapter [90].

Inorganic Impurities: Inorganic impurities may be of mineral or metallic composition and typically originate from catalysts or reagents used in synthetic processes. Historically, such impurities have been controlled at the drug substance or raw

material stage via gravimetric techniques (sulfated ash/residue on ignition tests) or wet chemical heavy metals testing. More recently, the perspective on metallic impurities has started to shift toward monitoring using elemental specific methods like ICP-Mass Spec or ICP-Optical Emission Spectroscopy and to recognize the potential for contributions to the drug product from a variety of sources [46]. Most drug substances still require compendial sulphated ash and heavy metals tests, but in cases where specific inorganic impurities are known to potentially be present (due to their use in the synthetic process), a validated elemental-specific method is also added to the release test battery. The strategy outlined in the EMEA guideline considers the relative toxicities of various metals, grouping them by their potential to cause safety issues and by the routes of product administration which can have toxicological implications. Testing is performed for batch release but not on stability since inorganic impurities levels should not increase as a function of time.

Residual Solvents: Historically, drug substances and other raw ingredients were tested for residual solvent content. In recent years, a broader approach has evolved where the whole drug product is controlled for solvent content. This control may still be performed at the drug substance and excipient level, summing their respective contributions. Otherwise, the final drug product may be tested. The drug product manufacturer needs to develop a control strategy for solvents that demonstrates that they have adequate knowledge and control of solvents likely to be present, and that the product will comply with the regulatory requirements. The total solvents content of the drug product must comply with the VICH Guideline GL18 [91] and, in the USA, with USP<467>. Additional useful guidance is provided in CVM Draft Guidance for Industry #211 [92]. VICH Guideline GL18 categorizes solvents into three groups using a risk-based approach based upon available toxicological data [91]:

Class 1: Solvents to be avoided: Class 1 solvents are known or suspected human carcinogens and environmental hazards. This category includes benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethene, and 1,1,1-trichloroethane. Where their use is unavoidable, the permissible levels are very low.

Class 2: Solvents to be limited: Class 2 solvents are nongenotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity. This category also includes solvents suspected of other significant but reversible toxicities. Examples include acetonitrile, hexane, toluene, and xylene. Permissible levels are low, and the manufacturer has several options for control of Class 2 solvents including complying with the concentration (ppm) limits in the guideline for each active and raw material in the formulation, complying with the guideline's permitted daily exposure (PDE) requirements for the whole drug product, or justifying higher limits based upon considerations such as toxicological data, route of administration, dosing regimen, etc.

Class 3: Solvents with low toxic potential: Class 3 solvents are known to have low potential for toxicity to man (e.g., acetone, ethanol, 2-propanol, tetrahydrofuran). Class 3 solvents have PDEs of 50 mg or more per day. The concentration limit is 5,000 ppm or 0.5%.

Although the Class 1, 2, and 3 solvent lists provided in the guideline cover most solvents of pharmaceutical interest, there are a number of noncategorized solvents for which no adequate toxicological data can be found, such as isooctane, methylisopropyl ketone, and trichloroacetic acid. The manufacturer needs to provide a justification for the use and level of such solvents if they are likely to be present.

Acceptance criteria for residual solvents tests are typically expressed as not more than the concentration listed in the guideline for each solvent of interest, or a concentration calculated to ensure compliance with the guideline. Testing is performed at batch release and not on stability since the solvent levels should not increase over time.

8.14 Conclusions

From this chapter, it should be evident that drug product testing and specifications development are essential and challenging major components of the overall process of developing innovative veterinary pharmaceutical products. Development of the tests and specifications is inseparable from the processes of selecting the appropriate formulation and packaging, and once developed, these tests play a critical role in the ongoing quality assurance of commercial products. In many ways, the analytical testing and specifications development of veterinary pharmaceutical products are very similar to that of human health pharmaceuticals. Specifications development for veterinary products is driven by many of the same scientific, regulatory, legal, and customer needs considerations. Nonetheless, as can be seen from the examples provided herein, veterinary products present many unique analytical development challenges due to the huge array of product types, dose ranges, and the frequent use of natural ingredients. Further complexity is added by the continuously evolving regulatory guidances, pharmacopeial requirements, philosophies such as QbD, and enhanced capabilities afforded by emerging technologies such as Ultra High Pressure Liquid Chromatography.

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Chapter 9
In Vitro Drug Release Testing of Veterinary Pharmaceuticals

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Abstract In vitro drug release testing for veterinary pharmaceuticals is a regulatory requirement to aid in the understanding of the in vivo performance of a dosage form. The in vitro release test is used throughout development for formulation and process characterization and post-approval to ensure product quality and therapeutic effect. The current recommended in vitro test equipment and conditions are better suited for mimicking human gastric system physiology but not that of the patients being dosed with veterinary pharmaceuticals. Veterinary dosage forms and delivery systems tend to be more complex and varied because of the diversity of species, size of the animals, and prevalence of unconventional excipients often not used in human health drug products. Therefore, the development of in vitro release tests specific for use with veterinary medicines can be challenging and unconventional with respect to the expectations from the regulatory agencies. Regardless of the analytical approach to development, the final in vitro release test is expected to be discriminating with respect to the impact of critical quality attributes on in vivo behavior and easily performed in quality control environments.

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9.1 Introduction

In vitro drug release testing is used to characterize release of a drug from a dosage form. The most commonly used technique for in vitro drug release testing is dissolution. According to the United States Pharmacopeia (USP), disintegration, dissolution, and drug release testing is required for "…dosage forms in which absorption of the drug is necessary for the product to exert the desired therapeutic effect" [1]. Disintegration is most often used as a quality control test with immediate release drug products that contain a drug substance that is highly soluble, highly permeable, and dependent solely on disintegration of the dosage form for absorption. While dissolution was originally intended for use with solid oral dosage forms with an immediate release profile, its use has since been extended to include controlled release and other novel dosage forms such as transdermal patches, semisolid topicals, chewable tablets, suppositories, suspensions, and vaginal implants [1–4]. For most solid oral dosage forms, both immediate and controlled release, the test is often referred as "dissolution." However, for novel dosage forms, the term "drug release testing" or "in vitro release testing" is preferred to differentiate it from the more standardized "dissolution" test [3, 5]. The intent and often the test conditions of in vitro testing for veterinary products (including unique dosage forms) are equivalent to that for human solid oral dosage forms.

Dissolution or in vitro release testing measures the time required to solubilize a given amount of active pharmaceutical ingredient (API), ideally in a physiologically relevant media. The solubilization is dependent on the release of the API from the dosage form and the solubilization of the API in the media [3]. In addition to the dosage form, the physicochemical properties of the API as well as specific test parameters such as apparatus, speed, temperature, and media can impact the release profile [4].

In vitro release testing is an important part of the drug development process and product release. The objectives of in vitro release testing include [5]:

1. Understanding the availability of drug from a given dosage form and the time associated with drug release
2. Evaluation of excipients, formulation changes, and the manufacturing process during development
3. Predicting in vivo performance
4. Ensuring product quality upon batch release and between batches
5. Demonstrating that changes to the manufacturing process, site of manufacture, and increase in scale of manufacturing do not impact product performance

The in vitro release test may satisfy all of the above objectives or may be developed with the intention of achieving only one or two specific objectives.

There are many sources available that provide guidance for in vitro release testing, the majority from the perspective of the development of the human health medicine. There are many similarities between the development of veterinary and human medicines. Success in both is driven by extensive research and development, global

presence, regulatory compliance, and focus on the customer. However, there are key differences, specifically with regard to in vitro release method development. This chapter discusses the importance of in vitro release testing, the unique aspects of veterinary medicine that present challenges to developing a biorelevant in vitro release test, and suggestions for science-based method development, validation, and specification setting.

9.2 Relevance of In Vitro Testing

In vitro release testing is used throughout the drug development process and post-regulatory approval. The development of an in vitro release test should be initiated early in development because its role evolves from initially providing critical drug release profiles to aiding in formulation development, excipient selection, and an understanding of in vivo studies. It is the only test available to assess the release of a drug substance from a dosage form over time, which may be correlated to the physiological absorption characteristics of a dosage form. Post-approval, in vitro release testing ensures a consistent manufacturing process, satisfies regulatory requirements, and supports scale-up and post-approval changes (SUPAC). If there is an in vitro/in vivo correlation (IVIVC), the in vitro data can be used in lieu of in vivo absorption data to justify bioequivalence and biowaivers.

9.2.1 Physiological Relevance

Many drugs are orally administered, and the permeability and absorption of the drug can vary greatly depending on the physicochemical properties of the drug substance and the nature of the dosage form. Ideally, the in vitro release test should be designed to account for these variables. On a basic level, the gastrointestinal tract (GI) of a human and most animals consists of the stomach, small intestine, and large intestine. Each of these components varies greatly in size, function, bacterial flora, enzymatic activity, and pH. Most of the nutrients from food are absorbed during the process of digesting food and moving it through the GI tract. Most dosage forms, when administered orally, are disintegrated and absorbed through the digestion process.

A nonionized drug substance is more likely to be absorbed than a weak acid or base because of the lipophilic nature of the GI tract. The absorption of a charged drug substance, however, is impacted by the pH of the local environment. The pH of the GI tract is dependent on the region, presence, and type of food [6]. The pH of the stomach tends to be 1–4, but the small intestine can be as high as 8. Gastric emptying will also impact the amount of time a dosage form will spend in the GI tract and can directly impact drug absorption, ultimately resulting in being the rate-limiting step for bioavailability [6].

The Biopharmaceutics Classification System (BCS) was developed for categorizing APIs based on their solubility and gastrointestinal permeability and consists of four classes, Class I, II, III, and IV [7]. Class I drug substances are highly soluble and highly permeable. The rate-limiting step for these APIs is drug dissolution, which exceeds the rate of gastric emptying. Class II drug substances have low solubility but are highly permeable. The bioavailability in vivo is often dissolution-limited. Class III drug substances are highly soluble but have low permeability. The absorption of the drug is permeability-limited, even when dissolution is rapid. Class IV drug substances have low solubility and permeability. Class IV drug substances are difficult to dissolve and have limited permeability through the gastrointestinal mucosa [8].

Although the BCS classification was developed and intended for use with human pharmaceuticals, it can be applied to veterinary pharmaceuticals because it is a measure of certain physicochemical properties of a drug substance [7]. Although the BCS as described can be used in veterinary drug product development, it may be more relevant to consider altering the definition of certain BCS categories for different species based on physiological differences versus humans in an attempt to improve the relevance. These properties can be fully characterized for a given drug substance irrespective of its intended use, either in human or veterinary medicine. The prediction of the BCS class for an API can aid in the prediction of in vivo pharmacokinetics. In addition, it can be used for the development of accurate and predictive in vitro release tests to support bioequivalence without the need for additional clinical studies [9]. This decreases development time, increases speed to market, and reduces the cost of clinical studies. This is especially important given the rapid and aggressive development timelines of many animal health products.

One unique challenge of veterinary medicine is that the use of a given API will often extend beyond one species, frequently in multiple dosage forms or delivery systems. Interspecies product bioavailability differences are common. Therefore, an understanding of drug substance physicochemical properties, including BCS classification, must be used in conjunction with animal physiology, formulation development, and in vitro release testing to predict formulation performance and bioequivalence from one species to another [7, 10]. An understanding of bioavailability and bioequivalence is particularly important for post-approval product line extensions across species as well as to support the approval of generic products.

9.2.2 Regulatory Requirements

In vitro release testing is critical for demonstrating product performance, quality, and batch-to-batch reproducibility. Historically, dissolution has served as a quality control release test, but with increased knowledge, its role has evolved to include the demonstration of IVIVC, bioequivalence/bioavailability, and the justification of biowaivers, i.e., for proof of bioequivalence on the basis of in vitro data without additional in vivo clinical trials. The increased emphasis on biorelevant dissolution testing has allowed companies and researchers to minimize the amount of in vivo testing that is required [11, 12]. The method should be developed with the ability

to discriminate the critical quality attributes of the formulation or manufacturing process that can impact the in vivo performance of the dosage form. In addition, the method should be stability-indicating for the duration of the shelf life of the drug product.

From a regulatory perspective, the United States Food and Drug Administration (FDA), European Medicines Agency (EMA), and International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) have guidances related specifically to dissolution testing. These guidances include the following:

1. Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, August 1997 (FDA)
2. Guidance for Industry: Extended Release Solid Oral Dosage Forms: Development, Evaluation, and Application of In vitro/In vivo Correlations, September 1997 (FDA)
3. Guidance for Industry: Waiver of In vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System, August 2000 (FDA)
4. Guidance for Industry: Bioequivalence Guidance, November 1996 (United States FDA, Center for Veterinary Medicine, CVM)
5. Note for Guidance on The Quality of Modified Release Dosage Forms for Veterinary Use, February 2004 (EMA)
6. Guidelines for the Conduct of Bioequivalence Studies for Veterinary Medicinal Products, July 2001 (EMA)
7. Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Dissolution Test General Chapter Q4B Annex 7 (R2], November 2010 (ICH)
8. Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Disintegration Test General Chapter Q4B Annex 5 (R1], September 2010 (ICH)

Collectively, these guidances describe the role and requirements for in vitro release testing of new dosage forms. There is an expectation from the regulatory agencies that an in vitro release method will be developed for any dosage form that relies on the release of an API and its subsequent absorption in vivo, regardless of the type of dosage form or delivery system [4]. It is expected for formulation development, batch-to-batch quality control, and for post-approval changes and investigations. A scientifically sound assessment of the in vitro dissolution test should be used to determine the validity and appropriateness of the test.

9.3 In Vitro Test Conditions

The desire to have an in vitro test that predicts in vivo performance of a dosage form is the main driving force behind the development of a method that reflects physiological conditions, if possible. However, this may not always be possible nor is it a

requirement for routine testing. The final test conditions should be based on the physical and chemical properties of the drug substance and the dosage form as well as the environmental conditions the dosage form is exposed to after administration [13].

9.3.1 Apparatus

Dissolution testing can be performed using a variety of apparatus [14]. The apparatus available and the human dosage form typically analyzed using that apparatus are summarized in Table 9.1 [5, 15].

The United States Pharmacopeia (USP), European Pharmacopoeia (Ph. Eur.), and Japanese Pharmacopoeia (JP) have harmonized the general chapter on dissolution testing of the majority of solid oral dosage forms. The Ph. Eur. describes the dissolution test requirement by dosage form. The USP, however, has a general chapter on dissolution [2] that encompasses a variety of dosage forms. The Ph. Eur. has a dissolution test-specific chapter for transdermal patches [16], medicated chewing gums [17], and lipophilic solid dosage forms [18], while neither the USP nor the JP has these. USP recognizes the use of Apparatus 4, 5, 6, or 7 for dissolution testing of transdermal patches. In addition, not all apparatus are recognized by each pharmacopeia. The JP does not recognize Apparatus 3, and the USP does not currently recognize the masitification apparatus used for medicated chewing gum described in the Ph. Eur.

Apparatus 1 and 2 were primarily designed for solid oral immediate and controlled release dosage forms. Typical operating conditions are 50–100 rpm for both baskets and paddles [14]. Qualification and validation of these apparatus is well-characterized and widely recognized [15]. Because of this, Apparatus 1 and 2 are preferred for testing unless their use has been shown to be unsatisfactory [13]. Sample containment within the media can be challenging when using Apparatus 2. To overcome this, sinkers may be used. Apparatus 3 may be used for small, bead type controlled release dosage forms or when a media change is needed [19]. Apparatus 4 is often used for dosage forms with limited solubility when sink conditions cannot be obtained. Apparatus 4 has a reservoir and a pump for the dissolution

Table 9.1 Dissolution apparatus classification according to the harmonized dissolution test [5, 15]

Classification	Description	Typical use
Apparatus 1	Rotating basket	Immediate or modified release
Apparatus 2	Paddle	Immediate or modified release
Apparatus 3	Reciprocating cylinder	Modified release
Apparatus 4	Flow-through cell	Modified release or transdermal
Apparatus 5	Paddle over disk	Transdermal
Apparatus 6	Cylinder	Transdermal
Apparatus 7	Reciprocating holder	Non-disintegrating oral modified release or transdermal

media, and a flow-through cell where constant fresh media flows over the dosage form. It can be set to provide either laminar or turbulent flow and operate as either an open or closed system. It allows for pH changes that are often needed with controlled release dosage forms [20]. Apparatus 5, 6, and 7 are most often used with human transdermal patches [15, 21].

9.3.2 Media Choices

Various parameters, including pH, solubility, and stability of the drug substance, should be taken into consideration when selecting a dissolution medium. In addition, the physical properties of the dosage form may impact the release profile, i.e., immediate versus modified release. Sink conditions (the volume of medium at least three times that required to form a saturated solution of a drug substance) are preferred but not required as long as the method can be demonstrated to be discriminating. Dissolution medium for oral dosage forms and suppositories is heated to approximately 37 ± 5°C prior to the start of the test and is maintained throughout the test to mimic in vivo body temperatures. However, for topical dosage forms, the temperature may be as low as 25–30°C. Temperature can be varied but must be scientifically justified [22].

In general, aqueous buffered dissolution test medium is preferred with a pH range from 1.2 to 6.8, typically not to exceed 8.0, to mimic physiological conditions [1, 13]. The volume of the media is typically 500, 900, or 1,000 mL, but may be increased to 2 or 4 L with justification. USP recognizes the use of the following media: dilute hydrochloric acid, buffers in the pH range of 1.2–6.8 (7.5 for modified release), simulated gastric or intestinal fluid (with or without enzymes depending on the dosage form), water, and aqueous media with surfactants (with or without acid or buffers). Acceptable surfactants, commonly used for poorly soluble or wettable APIs, include polysorbate 80, sodium lauryl sulfate, and lauryldimethylamine oxide at approximately 1% [23]. The Ph. Eur. recommends similar dissolution media. However, the Ph. Eur. monograph describes specific pH values to be used and provides detailed preparation. For example, at a given pH, the monograph recommends specific buffers to be used [24]. The use of organics with aqueous media is discouraged by both the USP and Ph. Eur. but can be scientifically justified.

In addition, there are biorelevant gastrointestinal media that can simulate the fasted and fed state, as modeled from humans. These media are in addition to the biorelevant simulated intestinal and gastric fluid described in the USP and Ph. Eur. monographs, which mimic the fluid present in each region but not the presence of food. These media have been characterized to simulate in vivo behavior, taking into account food effects on the dissolution and bioavailability of the dosage form [25].

The need for deaeration of the medium should be determined. Air bubbles can alter dissolution profiles, cause buoyancy if they cling to the dosage form, or result in particles clinging to the walls of the apparatus. Various techniques are used to

eliminate air bubbles in media, including heating, filtering, and drawing a vacuum. The composition of the media should be taken into consideration when deaerating. For example, during deaeration, surfactants may foam and organics may evaporate [14].

9.4 Challenges of In Vitro Release Testing for Veterinary Medicine

Veterinary dosage forms and delivery systems tend to be more complex and varied because of the diversity of species, animal body weights, and prevalent use of natural ingredients and excipients often not used in human drug products. The cost of goods is also carefully considered during API and formulation development. The animal health industry manufactures products for a variety of species, including (but not exclusively) companion animals (dogs and cats), ruminants (cattle, sheep, and goats), swine, fish, and horses. Therefore, dosage forms are often tailored to the target species, resulting in multiple dosage forms for a product line and multiple doses of a given dosage form to accommodate the wide range of animal body weights.

In vitro release testing was devised for use with human health medicines and dosage forms. The recommended apparatus and test conditions as described in the USP and Ph. Eur. monographs were developed with the intent of mimicking the release and absorption of drug products in humans, and were modeled based on human physiology [5]. Therefore, in vitro release testing conditions for veterinary medicine may not correlate well with the physiology of the animal for which the dosage form was developed. From a regulatory standpoint, an in vitro release test is expected for novel veterinary dosage forms, minimally to support formulation development, quality control release test, and SUPAC. The uniqueness of the dosage forms, the complexity of the physiological environment of the animals, and the design of the in vitro apparatus can result in an in vitro test that may be too discriminating or completely lacking in biorelevance [3]. The recommended conditions described by USP or the Ph. Eur. may be modified to take into account the physiology of the target species, with appropriate scientific justification. This is especially true for ruminal digestive systems, which are completely unique from that of most other animals and remarkably different from human physiology. To further complicate in vitro release testing for animals, there can be specific breed differences within a species. Because of the lack of biorelevance with in vitro test conditions for veterinary medicine, IVIVC in animals is challenging and not often pursued.

9.4.1 Gastrointestinal Physiological Differences

Dogs, cats, and pigs are monogastric species. Dogs and cats are carnivores while pigs are omnivores. Their physiology of digestion and drug absorption are similar

to each other and are not unlike humans (but there are distinct differences). Horses are herbivores with monogastric digestive systems, unlike ruminants that are herbivores with a multi-component gastric system [26]. Interestingly, dogs and pigs tend to eat their meals at specific time intervals while cats, horses, and ruminants tend to graze throughout the day. Because of the difference in diets and preferred eating styles, the pH of the gastric system of herbivores and carnivores differs [26]. Collectively, these differences often make the in vitro release testing of dosage forms difficult to correlate to in vivo performance without modifying the test conditions described by the USP and Ph. Eur.

9.4.1.1 Monogastric Systems

The GI system of dogs is relatively simple but has distinct characteristics from humans. In addition, the variation in breeds of dogs extends to the gastrointestinal (GI) system. For example, the size of dog impacts the size of the GI tract. For a larger breed, the GI tract is 2.8% of their total body weight while for smaller breeds it is 7% [27]. The dog GI is believed to be at least one pH unit higher than humans, which is typically between a pH of 1–2 in a fasted state for humans. Humans, after eating, exhibit an increase in pH to approximately 3–5 due to the buffering effect of the food [28]. However, multiple studies have shown great variation in the gastric pH for dogs. Sagawa et al. conducted a study to understand the pH of fed and fasted beagle dogs. The results of their study showed that the pH value for fasted dogs was 2.05, higher than for humans, while for fed dogs it was between 1.08 and 1.26. Other studies have shown a fed pH of 0.5–3.5 [29]. Based on these results, fed dogs do not appear to experience the same increase in pH as humans after eating.

GI transit time for a companion animal may impact the ability of a drug to be completely absorbed prior to gastric emptying. This is particularly important with controlled release formulations. A thorough study was conducted by Martinez and Papich that described the factors that impact gastric residence time between dogs and humans. These differences include the crushing force of the dog stomach, which exceeds that of humans, the greater restrictiveness of the pylorus in dogs, variable food effects between species, and anatomy [28].

There are also differences in the physiological characteristics of monogastric systems between species, not just from that of humans. Cats tend to have proportionally smaller stomachs than dogs. Studies have shown that the stomachs of cats and dogs empty at approximately the same rate when fasted but is slower for fed cats [30]. In addition, it has been shown that cats have greater small intestine permeability compared to dogs and humans [31]. The stomach lining for pigs contains the same three mucosal tissue layers as humans but differs in the relative area of each [26]. It has been noted that these differences can inactivate certain drugs. The pH of the stomach for horses (pH 5.5) is much higher than that reported for dogs. The relative capacity of the stomach in horses (8.5%) is much lower than that reported for pigs (29.2%) and dogs (62.3%) [26].

9.4.1.2 Ruminants

Ruminants have a unique digestive system that contains four chambers for digestion versus the single stomach for most mammals. They have a fore-stomach that includes a rumen, reticulum, and omasum, all three of which contain bacteria for digestion [31]. After the fore-stomach, they have an abomasum, which functions similarly to the stomach of humans. After bacterial digestion in the fore-stomach, any remaining fibrous material that was not completely broken down is returned to the mouth of the animal for further chewing. All other digested material is moved to the abomasum. The rumen is the largest segment of the digestive system and is the site of fermentation and degradation of cellulose by anaerobic bacteria and protozoa that naturally flourish in this anoxic environment. The pH of this region is typically 5.5–6.5. However, the pH after the rumen decreases with each successive compartment, from approximately 6 in the reticulum to 2–3 in the abomasum [31].

GI transit time for a ruminant is much longer than that of most other animals. The rumen retains foods for approximately 18 h [32]. Afterwards, a portion may be regurgitated for additional chewing and digestion. Once food has moved passed the fore-stomach it spends approximately 30 min in the abomasum [32]. A dosage form developed specifically for use in ruminants needs to be designed to avoid regurgitation and survive the conditions of the rumen for extended periods of time.

9.4.2 Dosage Forms and Examples of In Vitro Release Test Conditions

The selection of a dosage form is most often driven by the target animal for which the dosage form is intended. For a veterinary medicinal product to be successful, the physiological characteristics of the target species, physicochemical properties of the API, and pharmacological influences must be taken into account during drug product development. Successful development of an in vitro release test for veterinary medicines must also take these factors under consideration. The test conditions described by the USP and Ph. Eur. are not always applicable to the dosage form of interest. Deviations from the test conditions described in the monographs may be made but scientific justifications must be provided. The justifications may include the physiological conditions of the target species, novel formulation technologies to which conventional methodology cannot be applied, and intended use of the in vitro release method. Ideally, the approach should be shared with the regulatory agencies early in development to gather their insight and thoughts. At that time, supportive in vivo and in vitro data should be shared to bolster the use of a non-compendia method.

As a result of the unique physiology and broad spectrum of veterinary patients, the dosage forms in veterinary medicine vary greatly, from the more traditional oral dosage forms such as tablets, suspensions, and solutions to the more obscure such as gel parenterals, boluses, and implanted devices. Contributing factors to the use of novel dosage forms in veterinary medicine include: high drug load and large size of

the dosage form, desired long-term duration of activity, ease of use, patient and owner compliance, and administration route. In this section, brief descriptions of a select population of the novel veterinary dosage forms available are described along with successful approaches for in vitro release testing. This is, of course, not an exhaustive review of the novel dosage forms in development or on the market.

Boluses are solid-oral dosage forms that are most often administered to ruminants and horses. Boluses can be formulated to have immediate or modified release properties. In vitro release method development is challenging because often the boluses are extremely large in size with a high quantity of API. Examples of boluses on the market range in size from 5 g to almost 20 g with approximately 500 mg to 16 g of API. Method development challenges include the size of the bolus and insoluble excipients that may cloud the media or make filtering a challenge [33]. Conventional dissolution test conditions (Apparatus 2, 900 mL of media) have been demonstrated to be suitable for use with specific types of immediate release boluses [34, 35]. However, alternate in vitro testing has been described for osmotically controlled boluses intended for use for extended periods of time [36]. Rather than using compendial apparatus, the bolus was placed in a container with an exit port. Water was added to the container until the bolus was almost completely submerged, and the container was stored at 40°C for a week. Extruded material from the bolus was collected weekly and the output levels were determined. An in vivo study was performed and the results correlated well with the in vitro release test [36].

Chewable tablets or moist chewable tablets are becoming increasingly popular in veterinary medicine, especially with companion animals because of the ease of administration and palatability. At first glance, the chewable tablet or moist chewable might be considered a conventional solid oral dosage form. However, in vitro release test development can be challenging. Although the intent is to have the dosage form chewed, this cannot be guaranteed. Therefore, the assumption should be made that the dosage form could be swallowed intact, in which case the API must be released from the intact dosage form for a therapeutic effect [37]. If the chew is non-disintegrating, then compendial method conditions may need to be modified to ensure release of the API, including increased agitation rates, longer durations of testing, or use of surfactants and/or organics to improve the disintegration and API release. Mechanical breaking or cutting of the chew can be used to mimic chewing which is more reflective of the intended administration [3]. However, ensuring that the dosage form is evenly broken or cut might be difficult, depending on the size of the dosage form, the formulation, and manufacturing process. In addition, insoluble material may impede the ability to visually screen the disintegration and make in-line filtering difficult. Alternatives might be the mastication apparatus that is currently accepted by the Ph. Eur. [17] for use with medicated chewing gums, although this is not recognized by the USP, or the use of complementary or surrogate techniques to ensure that the product is conducive to drug release. For example, techniques to monitor texture (hardness) or melting point may be relevant.

Implants are solid, often polymeric, devices containing a drug and release mechanism that ensures the proper dosing of drug over time during residence in vivo. Some require medical assistance for insertion and removal (if removal is required as

some implants are not biodegradable). Implants appeal to veterinary medicine for their long-acting capability, eliminating the need for repeated dosing of a large number of animals. The in vitro test should take into account the in vivo release mechanism of the drug, the physiological environment, and the interaction between the host and the implant. The release rate can be influenced by the delivery system design, excipients, polymer, and water content [38]. The duration of an in vitro release test of an implant can be days to months. The test has to be designed to ensure stability of the active ingredient. Preservatives may be added to the media to minimize microbial growth [3]. The use of USP Apparatus 4 has been successful for implants with in vivo correlation [39]. Accelerated release test conditions, e.g., using media of high organic content and fast agitation, might be suitable as a quality control test, but the method should be shown to be discriminating to modifications in the formulation and manufacturing process. A correlation between accelerated in vitro and in vivo data is preferable but not always feasible [37].

Oral suspensions contain solid, insoluble particles uniformly dispersed throughout a liquid phase. The liquid phase can be aqueous, organic, or oily in nature. For some oral suspensions, drug release may be the rate-limiting step for the absorption of the active ingredient . In vitro release methods for suspensions often use Apparatus 2. The rate of agitation may need to be increased, depending on the viscosity of the suspension, to prevent accumulation at the bottom of the dissolution vessel [3]. Suspensions with higher viscosity require a greater agitation speed than lower viscosity formulations. The choice of media and additives should ensure release and solubilization of the active ingredient. The method of re-suspension of the dosage form should be explored and standardized with regard to speed, frequency, and time of shaking to ensure a homogenous sample before introduction into the dissolution vessel [3]. The method of sample introduction should also be explored and described for accurate, precise, and reproducible introduction. For sample introduction, geometric considerations of surface area to volume of sample may need to be standardized. The sample size (in volume or weight) should reflect that of a typical dose [38].

The diversity of dosage forms in veterinary medicine often results in the use of alternative analytical approaches to in vitro release methods. Modifications to the in vitro test conditions described by the USP and Ph. Eur. are acceptable with sufficient scientific justification. However, if the desired outcome of the in vitro release test is the power to discriminate critical quality attributes in the formulation or manufacturing process to guarantee batch-to-batch reproducibility, alternate test methods may be more informative and simpler to develop and implement than conventional dissolution testing. The experimental conditions, instrumentation, qualifications, and validation steps should conform to the requirements discussed by the regulatory agencies. Alternative analytical tests other than dissolution could be used collectively or individually to assess the relevant critical quality attributes that could impact the drug release profile from a dosage form. One example of this is the use of a texture analyzer to replace conventional disintegration. A texture analyzer provides a constant force to a solid oral dosage form, such as a tablet or chew, over time providing a plot of the distance traveled by the probe as a function of time. From this plot, the disintegration time can be inferred [40].

9.5 In Vitro Release Method Development, Validation, and Specification Setting

As described earlier, the role of in vitro release testing varies greatly throughout product development. An understanding of the intended use of the method is desirable prior to initiating method development to ensure that critical quality attributes are explored early in the development process. Deliberate changes to the formulation and manufacturing process that could impact in vivo performance should be studied during development to demonstrate that the method is discriminating with respect to these changes. Alternatively, methods developed for IVIVC or bioequivalence studies may require multiple formulation iterations to achieve the desired in vivo relationship. They may also require in vivo clinical studies to understand biological absorption and the impact of formulation modifications, and statistical analysis of the results to understand the correlation. Methods developed for IVIVC may be too complicated for use as a routine quality control test. If the intent is to have one in vitro release method for use as both a quality control release test and to establish IVIVC, then the method should be discriminating with respect to the impact of critical manufacturing or formulation parameters on in vivo behavior and be robust in quality control environments. An understanding of the goals of the method, the formulation characteristics, and in vivo performance throughout the development of the drug product is essential for setting meaningful specifications that ensure the quality and efficacy of the drug product.

9.5.1 Method Development

In vitro release test method development begins with a thorough understanding of the physicochemical properties of the drug substance, including pK_a, solubility and stability as a function of pH, particle size, ionic strength, crystal form and salt form. In addition, during the early stages of product development, the in vitro release test often supports formulation development and excipient selection. Therefore, the properties of the dosage form should also be considered, including excipients, manufacturing process and release mechanism.

When screening potential dissolution media, the pK_a, solubility and stability as a function of pH will have the most impact. Every attempt should be made to achieve sink conditions to ensure that the solubility of the drug substance in the medium is not rate-limiting. Stability of the drug substance in the medium, including the impact of any additives such as a buffers or surfactants, should be demonstrated [14]. The use of surfactants is permitted in small quantities when the drug substance has poor aqueous solubility or wettability but should be justified. The use of hydroalcoholic or organic solvents is not recommended. In most cases, it is expected that the dissolution medium be biorelevant and representative of physiological conditions [41]. A biorelevant medium is selected based on the BCS classification of the

drug substance, absorption site (if known), and whether absorption is limited by permeability of the drug substance or dissolution of the dosage form. This is of particular importance when the goal of the method is to establish IVIVC, bioequivalence or bioavailability [14]. The use of media to distinguish between the fed and fasted state is typically used for IVIVC only and not commonly as part of a routine quality control test.

The dosage form most often determines which dissolution apparatus is suitable. The USP and Ph. Eur. make recommendations as to which apparatus is appropriate for a variety of dosage forms, as described earlier. In addition, the size of the dosage form and the quantity of drug substance it contains are characteristics to consider when selecting an apparatus. Compendial apparatus and methods should be the starting point during method development. However, if the dosage form is novel and not described in a pharmacopeia, or if the results obtained using compendial conditions are not meaningful or relevant, then alternate approaches may be utilized with sufficient scientific justification, including evidence that the compendial method was inadequate. Non-compendial test methods need to include both the apparatus and conditions. Conditions can often be justified based on physiological differences or issues related to the dosage form itself, including the size, drug load, formulation, or incompatibility with traditional test conditions. A non-compendial apparatus, however, should meet the requirements of compendial apparatus. The non-compendial apparatus should be easily fabricated but rugged, provide a controlled test environment with respect to temperature, media loss, and speed control, and be described with precisely measured dimensions for each component [42]. Regardless of the variations, the non-compendial method should be stability-indicating, reproducible and discriminatory with respect to changes in the formulation, the manufacturing process, or the dosage form following exposure to stress conditions such as light, humidity, and temperature [23].

Selection of a quantitative technique is based on the chemical properties of the drug substance and the complexity of the sample matrix of the dosage form. Quantitation of the drug substance is often performed using ultraviolet/visible (UV/VIS) detection, high performance liquid chromatography (HPLC), or a combination of these techniques. The limit of detection needs to be established given the dilution of the dosage form in the medium. Fiber optic detection in situ for direct measurement of the dissolved drug in the dissolution vessel is often used in human health drug product testing but may be less applicable in veterinary medicine due to possible spectral interferences from the complex excipients often found in the dosage forms [43]. If HPLC is used, the method should be demonstrated to specific for the analyte of interest and validated to current regulatory standards. To complete the method development, parameters such as automated sampling, appropriate tubing, and sample filtration should be characterized to determine if there is an incompatibility with the material or if drug retention is an issue. Stability of the drug substance throughout the in vitro test should also be demonstrated.

During development, the discriminating ability of the in vitro release method for its intended purpose should be demonstrated. Quality control release methods to confirm batch-to-batch quality, consistency, and stability should monitor critical

attributes of the formulation or manufacturing process including, for example, particle size distribution, release rate, polymorphic form, and compression force [44, 45]. A thorough understanding of the discriminating ability of the method is essential during development. The data generated by a discriminating in vitro drug release method could be used to support SUPAC. Although it is preferable to discriminate between batches with respect to critical attributes that can impact bioavailability, it may not always be feasible [46]. If an in vitro method is used for bioequivalence, discriminating power should be established between a bioequivalent and non-bioequivalent batch based on in vivo results.

Results should not be highly variable, and any unexpected results or trends in the data should be explored. Visual observation of the test can often provide insight and direction when investigating variable results. Variable dissolution results may be a result of minor differences in the formulation or manufacturing process, or contributing factors from the method itself such as coning (the tendency of solids to aggregate forming a cone at the bottom of the vessel), dosage forms sticking or floating, the need for deaeration of the medium, apparatus type, and speed [23].

Sampling time points are dependent on the intended use of the method and the type of dosage form. Early in development, dissolution profiles (i.e., many sampling time points) are often used to understand in vitro performance as a function of time for comparison to in vivo results to provide insight into the applicability of the method. Infinity time points are useful to ensure full release of the active ingredient and demonstrate content uniformity during formulation development [14]. The duration of the test for most immediate release dosage forms is 15–60 min as opposed to hours for extended release products. Drug release considerations for some fast-dissolving technologies, such as orally disintegrating tablets (ODT) may be even faster. ODTs are often measured by disintegration alone or in conjunction with dissolution. ODTs generally must disintegrate in 30 s or less [47].

9.5.2 Method Validation

After development and characterization, the method of quantitation should be validated for specificity, linearity, accuracy, precision, and robustness. Specificity should be demonstrated with respect to the excipients, other APIs, or impurities/degradants present. This is most often achieved by analyzing a placebo containing all formulation ingredients other than the drug substance(s). Linearity and range are established using solutions of the drug substance over a range of concentrations that bracket the intended concentration range of the drug product. Accuracy and recovery are demonstrated by preparing samples in the presence of all dosage form excipients in dissolution medium over a range of concentrations that bracket the intended concentration range of the drug product. Precision is established through repeatability of measurement (replicate measurements of standard and/or sample solutions) and intermediate precision to determine the impact of random influences in the laboratory (analysis performed by a second analyst, often using different

instrumentation on a different day). Robustness evaluates the impact of minor, deliberate changes in the method. Robustness should be explored for both the dissolution method and the method of quantitation. For the dissolution method, buffer strength, pH, surfactant levels, speed of the apparatus, and temperature should be altered and the impact on the results obtained explored [14, 23]. The design and execution of robustness for the method of quantitation is dependent on which analytical technique is being used, i.e., HPLC, UV–VIS, or electrochemical detection. Appropriate key parameters should be identified during development and the impact of minor modifications should be demonstrated.

9.5.3 Specification Setting

In vitro release test specifications that can differentiate between acceptable and unacceptable batches of drug product are essential for ensuring patient safety and therapeutic benefit. Specifications are established based on acceptable clinical, pivotal bioavailability and/or bioequivalence batches, in addition to historical data from test batches generated during drug product development [45]. When IVIVC has been established, all batches that meet the defined dissolution specifications are considered bioequivalent. If IVIVC is not possible, then specifications are justified based on the manufacturing process capability and batch data from pivotal clinical and stability studies, but may lack any relevance to the in vivo performance of the drug product. As a quality control release method, the manufacturing process changes that have the most potential to influence the release profile of the drug substance should be explored during development, and specifications developed to discriminate between a batch manufactured properly versus a batch manufactured with deviations from the process. Regardless of the relationship between the in vitro release method and its intended use, the specification should be realistic, scientifically sound, and specific to the use of the test.

For immediate release drug products, three distinct categories exist for specifications: single-point, two-point, and dissolution profile comparison [13]. Single-point specifications are suitable as a routine quality control test for highly soluble and rapidly dissolving APIs (BCS Class 1 or 3). A single-point specification of not less than 85% ($Q = 80\%$) in 60 min or less is typical. Two-point specifications for slowly dissolving or poorly water soluble APIs (BCS Class 2), one at 15 min and the other at a later time point with not less than 85% released (60 min or less), are used for characterizing drug product quality and routine testing. For BCS Class 4 compounds, multiple time points or a dissolution profile may be necessary to ensure in vivo performance and quality control. Dissolution profile specifications are preferred for SUPAC-related changes and bioequivalence of immediate release dosage forms. Comparisons are made between profiles generated for the drug product before and after a change has taken place [13].

For modified release dosage forms, dissolution profiles are often utilized with sufficient points to demonstrate consistency in the profiles from batch to batch.

A minimum of three time points are included in the specification: the first at approximately 20–30% drug release to ensure that premature release, i.e., dose-dumping, is not a concern; the second at approximately 50% drug release; and a third at approximately 80–85% release. Alternate or additional time points may be used with accompanying scientific justification [48].

For unconventional dosage forms, including some modified release dosage forms and many of the dosage forms common to veterinary medicine as described earlier, the in vitro release test can provide information regarding the release characteristics of the drug product but often does not correlate with in vivo behavior. Therefore, the method is most useful for monitoring stability of the drug product and ensuring manufacturing control. For these types of methods, emphasis should be placed on the influence of critical quality parameters and the impact of process changes on the release characteristic of the drug product [1, 48]. Irrespective of the dosage form and mechanism of release, the drug product is expected to meet the dissolution specifications for the duration of its shelf life. When IVIVC has not been established but stability studies reveal changes in the dosage form as a function of condition and storage time, then there may be a need to evaluate the significance of the changes in vivo. When changes in the dosage form occur as a result of the stability storage condition, the specifications may be modified to reflect the change, assuming bioequivalence of the aged dosage form has been demonstrated [13, 48].

9.6 In Vivo/In Vitro Correlation

In vitro/in vivo correlation (IVIVC) models are developed to demonstrate the biological relationship between the in vitro release profile and in vivo performance of a dosage form, most often modified release drug products. To establish IVIVC, pharmacokinetic parameters such as C_{max} or AUC after administration of the dosage form are compared to the release profile obtained during in vitro testing of the dosage form. This correlation is then expressed quantitatively using deconvolution techniques and statistical moment calculations [1, 49, 50]. The ability to achieve IVIVC is dependent on the properties of the formulation, in vitro release method, and the physicochemical properties of the drug substance. Its usefulness extends to formulation screening, dissolution method development, specification setting, and bioequivalence justification [1, 49]. In addition, IVIVC can be utilized to support biowaivers and bioequivalence without the need for additional in vivo studies, a huge cost and time savings [51].

There are three correlation levels described in the USP, depending on the relationship between the plasma drug concentration–time curve and the in vitro release profile. Level A correlations are the highest that can be achieved. They describe a point-to-point relationship between in vivo absorption and in vitro release. In this case, the in vitro data can completely replace in vivo data in the event of manufacturing changes, formulation modifications, or product strength adjustments. Level B correlations utilize statistical moment analysis, such that the mean in vitro dissolution

time is compared to the mean in vivo dissolution time. A point-to-point correlation is not achieved. Level C correlations relate one dissolution time point to one pharmacokinetic parameter, resulting in a single point correlation. Level B and level C correlations cannot predict in vivo performance. Therefore, in vitro data cannot be substituted for in vivo data for manufacturing or drug product changes [1].

Many sources describe methodologies utilized to establish IVIVC with respect to human health medicine [1, 49, 52]. However, very few exist for veterinary medicine. When embarking on the development of an in vitro release testing method for a veterinary dosage form, the desire is to maintain a biological relevance. Therefore, for reasons discussed throughout this chapter, the novel dosage forms often utilized in veterinary medicine, the difficulty in achieving physiological conditions within the confines of the recognized apparatus and test conditions, and the associated cost and time required for in vivo clinical studies make IVIVC difficult to attain for veterinary medicine.

9.7 A Non-compendial Release Test Case Study: The CIDR Intravaginal Insert

An example of a veterinary product whose in vitro release test development was challenging and met much of the challenges described in this chapter was the CIDR intravaginal insert. This was due to many contributing factors including the unique shape, size and high drug load of a highly water insoluble drug (progesterone). The CIDR intravaginal insert comprises a homogenous dispersion of progesterone throughout a silicone skin that is injected molded and cured at high temperature over an inert "T"-shaped nylon spine as shown in Fig. 9.1 [53–58]. The nylon spine

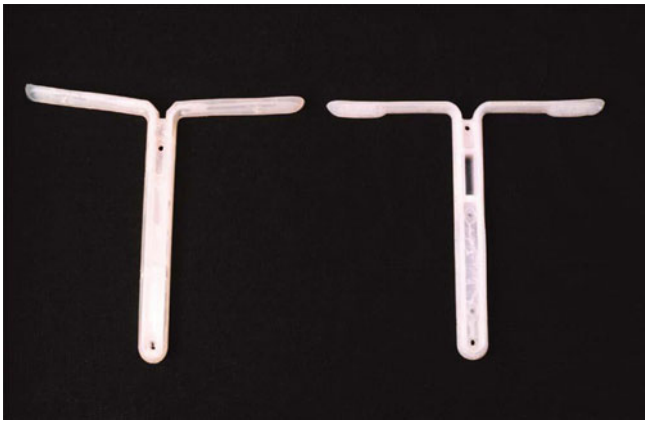

Fig. 9.1 CIDR intravaginal inserts for cattle. (*Left*) CIDR-B or CIDR 1900 Cattle Insert; (*right*) CIDR 1380 Cattle Insert

Table 9.2 Physical and chemical characteristics of the CIDR intravaginal insert

Parameter	Dimensions	Issues in relation to drug release test development
Shape	"T" shaped	Unfamiliar geometry for direct insertion into a dissolution vessel of nominal volume 1,000 mL
Physical strength	Rigid with flexible wings that flex at the hinge region	Difficult to maintain insert in its "T" shape inside a dissolution vessel of nominal volume 1,000 mL
		Insert tends toward opening to its natural "T" shape after flexing the wings closed
Size	Tip-to-Tip distance = 14 cm	Tip-to-Tip distance and body length prohibit free rotation of a blade stirrer
	Body length = 15 cm	Body distance results in body exposing some of the insert above the dissolution media if 1,000 mL of media is added to the dissolution vessel
Progesterone load (%w/w)	CIDR-B = 10% w/w CIDR Cattle Insert = 10% w/w	High drug load of a very water insoluble drug
Progesterone load (g)	CIDR-B = 1.9 g CIDR Cattle Insert = 1.38 g	Solubility issues in the receptor medium Maintenance of sink conditions

provided form and shape to the insert. The physical and chemical characteristics of the delivery system are given in Table 9.2.

Compendial apparatus and conditions were a starting point for method development; however, modifications were needed to achieve the desired in vitro release test for this dosage form. The observations given in Table 9.2 (i.e., it was too physically large and the water insoluble drug in too high an amount in the insert) provided the justification for modifying the compendial apparatus specified in the USP. In the absence of guidance provided by the veterinary medicine regulators such as CVM or EMA and the limited number of publications available to describe the testing procedures of novel veterinary dosage forms, steps were taken to develop an in vitro drug release test for this product based on the scientific principles inherent within the compendial monograph for drug release as described in the USP. Some of the resolutions to the problems encountered are described below.

9.7.1 Locating and Maintaining the Insert in the Dissolution Flask

Although the size and the geometry of the insert was large, by folding the wings back toward the body of the insert, it could be fitted inside a dissolution flask of nominal volume 1,000 mL. However, following insertion it prohibited a blade stirrer to be introduced into the dissolution flask, and therefore there was no means of stirring the dissolution media. In addition, the natural resistance to the flexed position tended to push

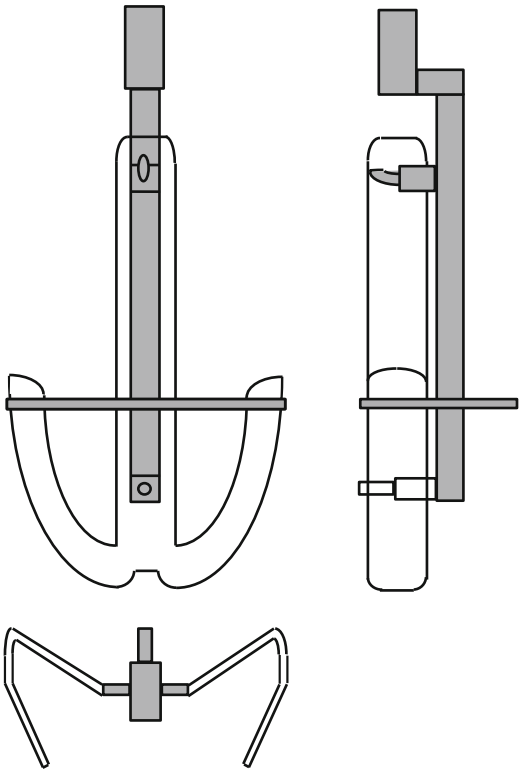

Fig. 9.2 Specially designed holder was accurately manufactured and qualified that exactly positioned, rotated, and maintained the CIDR intravaginal insert in a precise position within the dissolution vessel. Front elevation (*top left*), side elevation (*top right*), and plan view (*bottom left*). From ref. [61]

the insert out of the dissolution vessel. This problem was solved by manufacturing a specially designed holder (Fig. 9.2) that located the insert in a fixed position and immobilized the wings. It was designed to allow the insert to rotate on its own axis and was of a size that permitted the insert to be located within the dissolution vessel. Rotation of the holder caused the insert wings to act as stirrer blades. By stringent design, exacting manufacture, post-manufacture dimensional qualification, and location of the insert 5 mm from the bottom of the flask, the arrangement could produce reproducible hydrodynamics at any given stirring speed.

9.7.2 Volume of Dissolution Media

Once located on the specially designed holder, and even though the insert was accurately positioned only 5 mm from the bottom of the flask, the long body length of the CIDR intravaginal insert resulted in some of the body to be exposed above the dissolution media when 1,000 mL of media was added to the dissolution vessel and stirred. This necessitated and justified the need to use 1,100 mL of dissolution media. Fortuitously 1,100 mL of dissolution media could be held within a dissolution vessel of nominal volume 1,000 mL and did not spill over the sides even when subjected to elevated stirring speeds of 150 rpm.

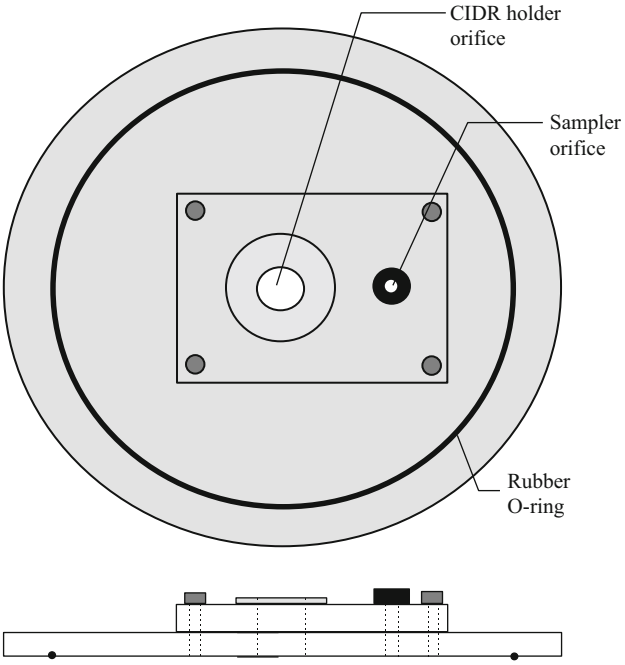

Fig. 9.3 Specially designed lid was manufactured to ensure evaporation was kept to a minimum over the test duration. (*Top*) Plan view; (*bottom*) side view. From ref. [61]

9.7.3 Evaporation

The final drug release test that was developed was conducted over a 24 h period (to assure >80% release at $t=\infty$). When a conventional lid was used to cover the dissolution vessel, this extended period of time caused excessive amounts of evaporation of the dissolution media to occur. Since it is not acceptable for evaporation to occur during a dissolution test [59, 60], a specially designed lid was manufactured that incorporated flanges and an O-ring to ensure that evaporation was kept to a minimum over the test duration (Fig. 9.3).

9.7.4 Maintenance of Sink Conditions

Although mimicking in vivo conditions is preferred for an in vitro drug release test, the poor water solubility of progesterone precluded the use of physiologically realistic media. The use of surface active agents did not sufficiently increase progesterone solubility in water to allow sink conditions to occur. Repetitive and frequent replacement of such large volumes of water during the release test was considered inappropriate. Therefore the development process looked toward identifying a solvent that,

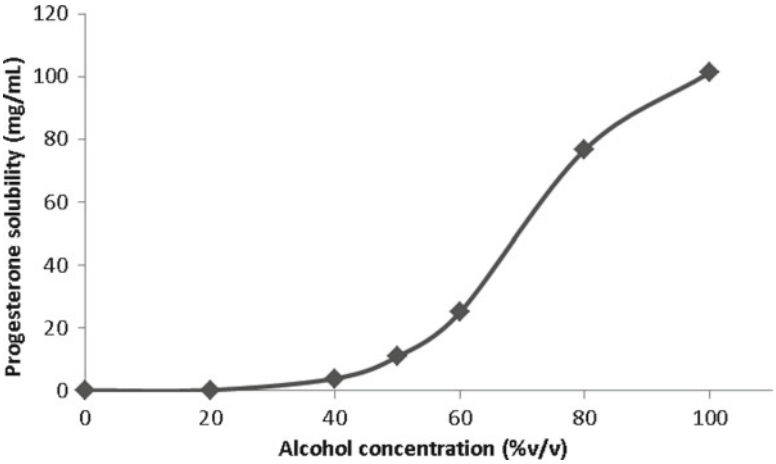

Fig. 9.4 Effect of alcohol concentration on the solubility of progesterone in water:alcohol mixtures. Data from ref. [61]

when mixed with at least some water, would result in sink conditions prevailing over the time course of drug release. The solvent identified was ethanol (Fig. 9.4].

Progesterone exhibited sufficiently high solubility in ethanol (alcohol) to allow a 66.6:33.3 %v/v (2:1 parts alcohol:water) mixture to permit sink conditions to prevail during the release of progesterone from the CIDR B (1.9 g progesterone) or CIDR 1380 Cattle Insert (1.38 g progresterone). This value was chosen based on knowledge of the volume of dissolution media used in the dissolution vessel (1,100 mL), the potential total amount of drug that could be released from the insert at time infinity (1.9 g) and progesterone's solubility at different water:alcohol mixtures.

9.7.5 IVIVC

An IVIVC could not be established due to the observation that the mechanism of release in vitro was different to that observed in vivo [61].

9.7.6 Final Method

The final method is shown in Fig. 9.5 and the parameters for the test are given in Table 9.3. This methodology was described in regulatory documents submitted for successful New Zealand, Australian, and USA registration. The same methodology was used for the PCL intravaginal insert [62]. The developed method was demonstrated to be discriminatory with respect to the critical quality attributes that could impact in vivo performance of the intravaginal insert [63].

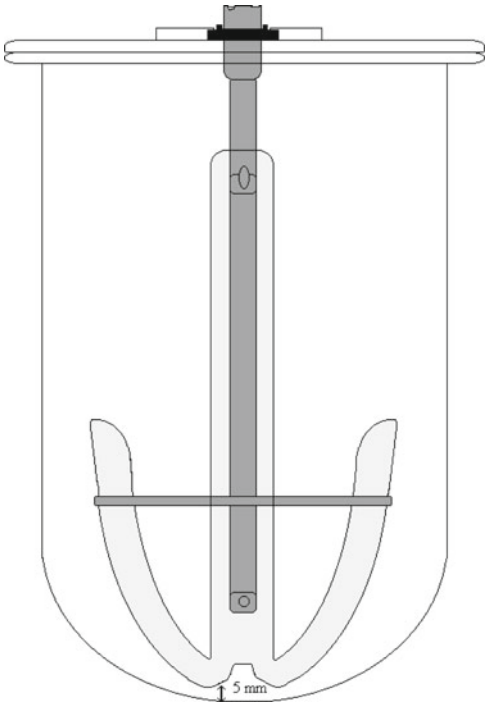

Fig. 9.5 Final drug release test method for QC testing of the CIDR-B and CIDR 1380 intravaginal inserts. From ref. [61]

Table 9.3 Final drug release test method for QC testing of the CIDR-B and CIDR 1380 intravaginal inserts [61]

Parameter[1]	Value
Volume of dissolution media	1,100 mL
Rotation speed	100 rpm
Temperature	39°C
Holder	Specially designed (Fig. 9.2)
Lid	Specially designed (Fig. 9.3)
Position of holder from bottom of dissolution flask	5 mm
Composition of dissolution media	66.6:33.3 %v/v alcohol:water
pH and ionic strength	No adjustment
Time points	2 min, 2, 4, 8, 12, 24 h
QC parameter	Release rate ($\mu g/cm^2/h^{1/2}$)
Analytical method	UV
Approximate batch range variation in release rate under final test conditions	1,300 ± 10%

In addition the method was demonstrated to be specific, robust, precise and discriminatory with respect to the critical key attributes that differentiate an acceptable versus unacceptable batch, both at release and on stability. The limitation of the test was that, because an IVIVC could not be established, the method could only be

used as a quality control tool to pass or fail manufactured batches. It could not be used as a surrogate for bioequivalence, biowaivers, or SUPAC. Specifications were developed based on the rate of drug release. They were considered to be realistic (based on a pivotal pilot batch and multiple manufacturing batches), specific to the intended use of the test (QC tool to assess the performance of manufacturing batches), and scientifically justified (with supporting data collected from in vivo studies).

9.8 Conclusions

The development of an in vitro release test for veterinary medicine can be challenging due to many contributing factors including the unique physiology of the target animal, species differentiation, and the novel dosage forms utilized. Of the above, the novel dosage forms are the most difficult because of their unique size, varied geometric shape, high drug load, immediate or modified release mechanism, and the physicochemical properties of the API and drug product. Compendial in vitro test apparatus and conditions as described in the USP or Ph. Eur. were developed with the intent of mimicking in vivo conditions in humans dosed with conventional dosage forms. Although the compendial apparatus and conditions are a starting point for method development, modifications are often needed to achieve the desired in vitro release test for a veterinary dosage form. Justification to support the use of non-compendial conditions must be demonstrated and provided to the regulatory authorities. Alternate analytical techniques may be used in conjunction with or in replace of in vitro release testing when demonstrated to be discriminatory with respect to the critical quality attributes that can impact in vivo performance of the dosage form. Irrespective of the final in vitro test conditions, the development of an in vitro release test should be comprehensive and the method should be demonstrated to be specific, robust, precise, and discriminatory with respect to the critical key attributes that differentiate an acceptable versus unacceptable batch, both at release and on stability. Specifications should be realistic, specific to the intended use of the test, and scientifically justified with supporting data collected throughout the development of the drug product.

In human health medicine, emphasis during in vitro method development is placed on achieving IVIVC. A biorelevant in vitro release test that has the ability to predict in vivo behavior can aid with formulation development, bioequivalence, biowaivers, and SUPAC. Collectively, a biorelevant in vitro release test can lead to a tremendous cost and time savings as a result of the ability to minimize the number of clinical studies required. However, achieving IVIVC or even a biorelevant in vitro release test is a challenge for veterinary medicine. The lack of biorelevant test conditions that accurately mimic the physiological conditions of the target animal species is the biggest hurdle. Through development, modifications to the test conditions from those described in USP or Ph. Eur. to better match the physiological conditions are possible but must be scientifically justified. For example, increasing the pH of the dissolution media to mimic the conditions in a ruminant gut would be within

scientific reason. In the case of modified release novel dosage forms with release rates that can extend for months, alternate analytical approaches may be better suited at mimicking in vivo behavior than the compendial apparatus.

Unfortunately, scientists tasked with the challenge of developing an in vitro release test for a novel veterinary dosage form have a limited number of resources available. Little guidance is provided by the veterinary medicine regulators such as CVM or EMA. A limited number of publications describe the novel veterinary dosage forms and the in vitro release tests that are used to characterize them. Therefore, during development, open dialogue with the regulators is highly encouraged to collect their opinions on the analytical approach being taken. Irrespective of the dosage form, intended use, or test conditions, sound scientific rationale and conclusive supportive data should be demonstrated throughout the development of an in vitro release test for use with veterinary pharmaceuticals.

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Chapter 10
Long Acting Rumen Drug Delivery Systems

Thierry F. Vandamme and Michael J. Rathbone

Abstract Because of the unique anatomy of the digestive system of ruminant animals, drug delivery technologies can be designed to be retained in the rumen for prolonged periods permitting drug release for durations of up to 12 months (or even longer). Since the beginning of the 1980s different technologies have been developed to prolong the release of antibiotics, anthelmintics, trace elements, growth promoting agents, and minerals to the rumen of cattle and sheep. Successful development of intraruminal devices must take into account the anatomy and physiology at the site of drug release from the technology and the disease state the drug is treating. The formulation scientist should have a sound knowledge of physical pharmacy and an appreciation of plastic product design. This chapter describes historical and recently developed long acting veterinary rumen technologies, defines the relevance of the anatomy and physiology of the rumen to the development of a ruminal drug delivery technology, provides an insight into the disease state of nematode infestation as an example of a clinical condition for which long acting drug products have commercial value, and describes a case study of a delivery system that was developed specifically to deliver anthelmintic compounds for the treatment of nematode infestations.

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10.1 Introduction

Many challenges face the formulation scientist when developing a long acting veterinary drug delivery technology. Whether one is developing a drug product for a small animal or a livestock animal, obstacles include the anatomy and physiology of the animals which varies greatly between species (and within the same species); different sizes and weights of animals; the need to devise some method of administering the product to the animal; the need to devise some method for retaining the product in the animal for the duration of release; and, for livestock animals, the fact that the meat, milk, or eggs of these animals will be consumed and therefore the protection of the health of the consumer must be considered.

In the past 40 years, many long acting veterinary drug delivery technologies have been developed specifically for use in animals. These advancements have led to the improvement of veterinary practice, animal health and welfare, livestock animal management practices, and the improvement in the treatment of key pathologies [1]. For small animals these technological advances include insecticidal collars which were produced by extrusion (the first of which contained diazion against the fleas and the ticks); antibiotics protected in a premixing technology; shampoos using spherulites in order to obtain a prolonged action; formulations containing interferon for cats; patented nutritional supplements intended to manage the anxiety and the phobias of the dogs and cats; a spray containing corticoid with action targeted for the dermatoses without systemic effects (due to the properties of the molecule); an implant containing deslorelin for the chemical castration of the male dogs; and more recently the first vaccine against the canine leishmaniose recorded in Europe. For livestock animals one can cite implants for cattle containing norgestomet (Syncromate-B) and estradiol valerate (Crestar®); intravaginal inserts containing progesterone in inert silicone such as the PRID® and CIDR®; intravaginal polyurethane sponges containing progestins; intraruminal bolus containing minerals or trace nutrients, antibiotics, growth promoting agents, and anthelmintics using products such as Captec device, Paratect Flex, Ivomec bolus, and TimeCapsule.

A long acting veterinary drug delivery system is of particular value in the livestock industry. This value has arisen because of the intensive methods of rearing ruminant animals such as cattle and sheep. This has resulted in the need for reducing the number of administrations of drugs to livestock animals since such animals are difficult to handle, time consuming to herd and treat, and get stressed when handled (which leads to a reduction in growth or production). Fortunately for the formulation scientist, ruminant animals such as cattle and sheep have a digestive tract which possesses anatomical characteristics (specifically the rumen) that provides the opportunity for the retention of appropriately designed long acting drug delivery technologies. This book chapter introduces the reader to the area of intraruminal drug delivery by describing some historical examples of long acting rumen technologies to provide the reader with an insight into the novelty and "out-of-the-box" thinking needed in this area and to present some working examples of technologies from which mechanisms to administer and retain a long acting product in the rumen

in the animal can be deduced. The chapter also describes some recently developed long acting veterinary rumen technologies and defines the relevance of the anatomy and physiology of the rumen to the development of a ruminal drug delivery technology. The disease state of nematode infestation is described to provide an example of a clinical condition for which long acting drug products have provided commercial value to the livestock industry. In the final section of the book, a case study describes a delivery system that was developed specifically to deliver anthelmintic compounds for the treatment of nematode infestations.

10.2 Digestive Anatomy in Ruminants

This topic has been described in detail in Chap. 3 of this book. The pertinent aspects for long acting veterinary drug delivery include:

The stomach of ruminants has four compartments: the rumen, reticulum, omasum, and abomasum. The rumen is the largest of the four compartments and has anatomical characteristics that are ideal for drug delivery.

The rumen mucosa comprises stratified squamous epithelium. This is not considered an absorptive type of epithelium. The rumen is not a good site for absorption, therefore its function to the formulation scientist is a chamber in which to locate and retain a long acting drug delivery technology.

The rumen is a large fermentation chamber (±125 L), providing an anaerobic environment, constant temperature and pH, and good mixing. Well-masticated substrates are delivered through the esophagus on a regular schedule. The rumen is large enough to accommodate a delivery technology, indeed, it can simultaneously contain multiple delivery technologies. It is a dynamic environment providing good mixing; it will produce forces on a delivery technology that are strong enough to cause erosion or abrasion (which can be used to advantage by the formulation scientist). The factor that limits the size of the delivery technology is its ability to be administered via the mouth and esophagus.

Ruminants evolved to consume and subsist on roughage—grasses and shrubs built predominantly of cellulose. Common cellulosic pharmaceutical excipients (e.g., hydroxypropylmethyl cellulose) cannot be used to delay the rate of release of a drug as the material will be rapidly and easily digested.

Ruminants produce prodigious quantities of saliva. Published estimates for adult cows are in the range of 100–150 L of saliva per day. The rumen contains ample water to facilitate the release process.

The rate of flow of solid material through the rumen is quite slow and dependent on its size and density. The rumen is a stable and constant environment for drug delivery.

Ruminants are well known for “chewing the cud.” Rumination is regurgitation of ingesta from the reticulum, followed by remastication and reswallowing. It provides for effective mechanical breakdown of roughage and thereby increases substrate surface area to fermentative microbes. Methods must be devised in order to prevent

the delivery technology from being regurgitated and expelled from the rumen. A change in geometric shape before and after administration and density are two common methods. Particulate dosage forms will be subjected to the process of chewing the cud; therefore, they must be designed to resist destruction of their physical properties by mastication.

Fermentation in the rumen generates enormous quantities of gas: about 30–50 L/h in adult cattle and about 5 L/h in the sheep or goat. Polymers allow diffusion of gases. This could affect the performance of the device. Or the gases found in the rumen of animals could be used to develop a novel rumen delivery technology.

10.3 An Example of a Clinical Condition Whose Treatment Can Benefit from a Long Acting Drug Delivery Technology

10.3.1 Gastrointestinal and Pulmonary Helminths

Ruminal infestations due to gastrointestinal nematodes are very frequent in the temperate zone. Particularly during the first year grazing season, calves and lambs are particularly sensitive to this kind of infestation. The clinical symptoms of the disease express themselves as loss of appetite, loss of weight, and/or diarrhea. Consequently, the control of such infestations is of great importance to the livestock industry and for this reason, numerous studies have been initiated in the fields of epidemiology and controlled release drug delivery in order to eradicate these infestations.

To control such infestations different antiparasitological formulations having anthelmintic properties have been developed. The anthelmintic formulations, intended for cattle and sheep, comprise either transcutaneous liquid dosage forms intended to be applied to the back of the ruminants or formulations that are mixed with the animals' food or drink.

Nematodes that infest the digestive tract are, together with the liver flukes and the dictyocaulus, the most important agents of parasitological diseases of livestock (Fig. 10.1). Infestation can go unnoticed since the unequivocal clinical symptoms are often absent. The disease may only be apparent following death of the animal. However, infestation causes high losses in productivity which are significant from an economic point of view. In cattle infestations are mainly caused by the family of the trichostrongylidae such as Trichostrongylus, Ostertagia, Dictyocaulus, Haemonchus, and Nematodirus. These nematodes, except for Dictyocaulus which is the agent responsible of the verminous bronchitis, live as a parasite on all the digestive tract of the ruminants. They can be located at different places of the gastrointestinal tract: rumen, reticulum, omasum, abomasum.

The parasitological phase of the cycle of the trichostrongylidae is shown in Fig. 10.2. It is a direct cycle during which any intermediate host is not brought in.

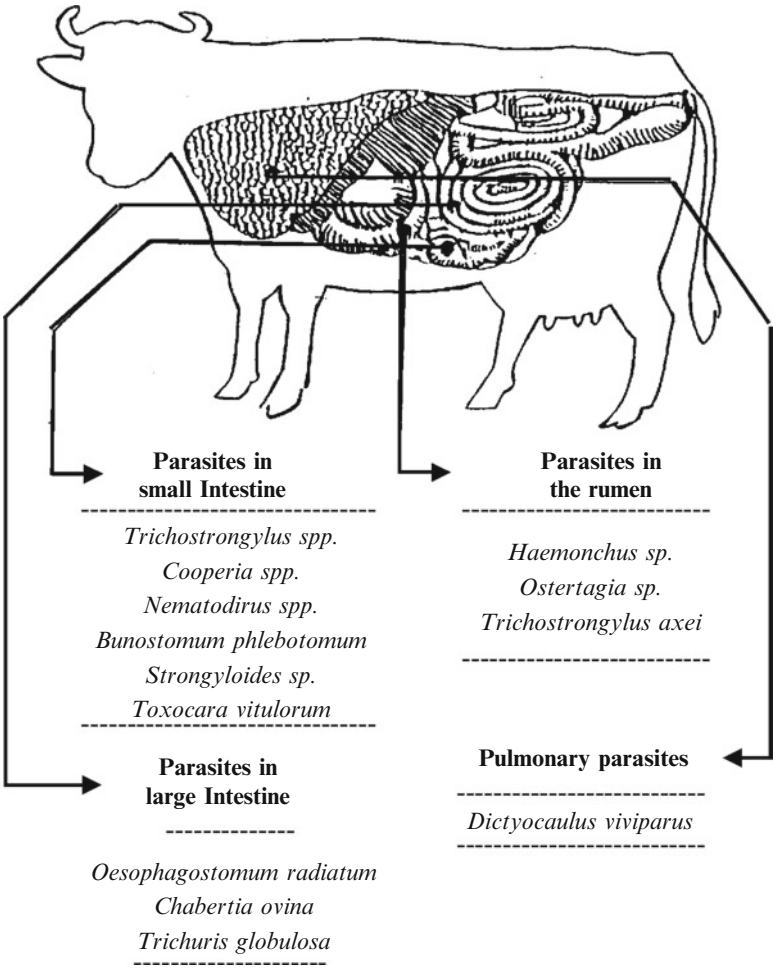

Fig. 10.1 Localization of the different gastrointestinal and pulmonary nematodes in cattle

In the rumen, under the effect of the high content of carbon dioxide (CO_2), the larva loses her cuticle and a sloughing phase (L3 to L4; Fig. 10.2) takes place, following the species, into the mucous membrane of the abomasums or of the small intestine.

With respect to the infestation rate, if the ruminants are not treated by any anthelmintic drug, firstly, the disease naturally decreases rapidly as the temperature increases in the spring (Fig. 10.3 for the Northern Hemisphere). However, the infestation rate then increases quickly from June to a maximum in August. The disease, also called verminosis, is particularly prevalent during July and August when the infestation rate is at its highest.

The economic losses generated by the repeated handlings of the animals during a treatment are difficult to be quantified but can be considerable. The rationale for

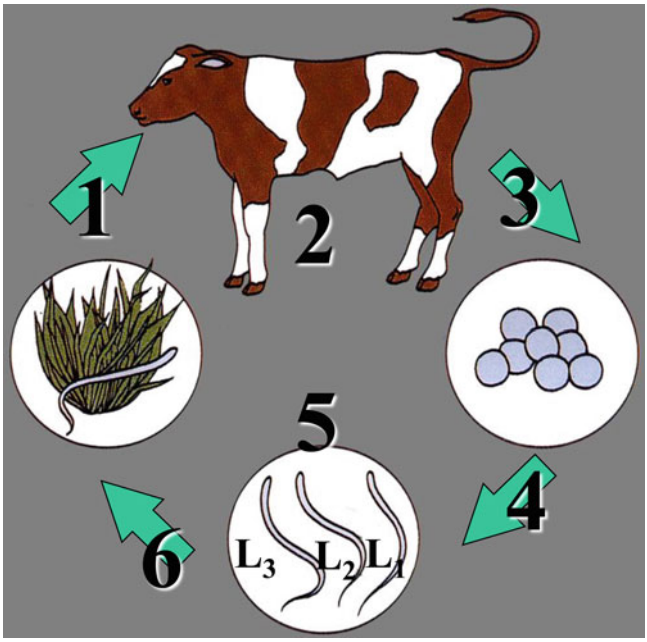

Fig. 10.2 Different parasitological phases during the cycle of gastrointestinal nematodes by ruminants

developing a long acting drug delivery technology for this disease is that a reduction of the number of handlings would allow the cattle breeders to realize some economic benefits. A well-designed delivery system that effectively delivers the drug for the right duration at the right time, in the right manner (pulsed or continuous delivery), to the right place will also provide benefit to the farmer by resulting in an increase in stock weight and a lower herd death rate at the end of the pasture season.

10.3.2 Gastrointestinal Nematodes and Immune System

10.3.2.1 Immunity to Gastrointestinal Nematodes of Cattle

Most of the common parasites found in cattle are able to stimulate an effective level of protective immunity in most of the herd population after the grazing animals have been on the pasture for several months. In such cases, reinfection with these parasites results in a significant reduction in the number of worms that become established in the grazing animals. Parasites such as *Dictyocaulus viviparous* and *Oesophagostomum radiatum* are extremely effective in eliciting strong protective immune responses [2]. A primary exposure of previously naive cattle to infection or

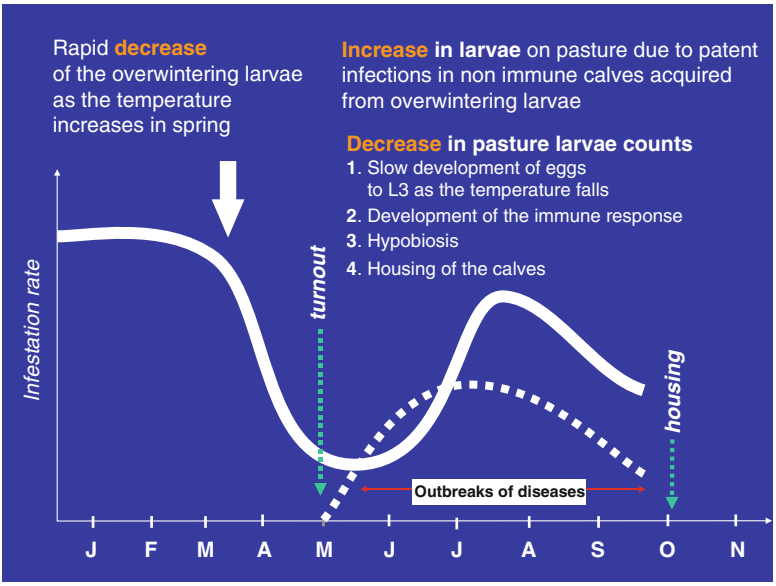

Fig. 10.3 Evolution of the larvae amounts on pastures without anthelmintic treatment

even to parasites antigens results in a very significant reduction in the number of parasites that can become established after a subsequent infection [3–5]. The parasites largely remain a problem for only the youngest ruminants in the herd. Other parasites such as *Cooperia* sp. and *Haemonchus placei* require a longer period of exposure before this level of protective immunity is observed, but even with these parasites, calves at the end of their first grazing season will exhibit a significant reduction in the number of incoming larvae that can successfully become established [2]. On the contrary, cattle remain susceptible to infection by *Ostertagia* for many months, and immunity that actually reduces the development of newly acquired larvae is usually not evident until the animals are more than 2 years old. It is this prolonged susceptibility to reinfection that this parasite remains the most economically important gastrointestinal nematode in temperate regions of the world.

Although ruminants may remain susceptible to reinfection for a prolonged period of time, the immunity to infection with gastrointestinal nematodes in cattle can be manifested in a number of ways. It was observed [6–8] that for *Ostertagia* infection immune responses, the number of parasites developing after subsequent infection was reduced. The overall result of all these manifestations of the immune response was a reduction in parasite transmission within the cattle herd. Furthermore, Gasbarre et al. [2] showed that the immunity to *Ostertagia ostertagi* and *Cooperia concophora* was not the same within individuals in the herd and that different immune mechanisms in different herd members may be an important contributor to resistance to different parasite species.

10.3.2.2 Interaction of Gastrointestinal Nematodes and the Immune System

Many assumptions concerning the immune mechanisms responsible for the different types of functional immunity have been advanced [9]. All infections tend to preferentially stimulate one of two types of mutually antagonistic immune responses [10, 11]. These responses referred to as Th1- or Th2-like arise as the result of the stimulation of different subsets of T-helper lymphocytes [2]. The result of stimulation of either of these subsets is the elaboration and secretion of a wide range of cellular communicators termed cytokines. Each of these cytokines exhibits very specific effects on all cell types bearing a surface receptor for the given cytokine. Cytokines can exhibit a variety of effects on different cell types, ranging from stimulation to inhibition. The cytokine network is highly regulated and a number of different cytokines can have the same effect on the same cell type, and many even share the same receptor.

Gastrointestinal nematode infections in mammals elicit a very strong Th2-like response [12], which is characterized by high levels of the cytokine Interleukin 4 (IL4), high levels of IgG1 and IgE antibodies, and large numbers of mast cells. At the present time, it seems evident that the protective responses are complex, and there are no single dominant effector mechanisms [13].

In cattle, with regards to host immune responses, the most extensively studied gastrointestinal nematode is *Ostertagia ostertagi*. It was observed that within 3–4 weeks of experimental infection [14, 15], and 2 months of exposure to infected pastures [16–18] previously naive calves show significant rises in anti-*Ostertagia* antibodies in the peripheral circulation. From these studies, these authors showed that the antibody responses were detectable using a wide range of parasite-derived antigens and involved all major immunoglobulin isotypes. These authors also reported that at the same time, extensive changes were being seen in the local tissues of the abomasum. Following several studies [19, 20], in the first 3–4 days after infection, there was a significant increase in the size of the regional lymph nodes draining the abomasum, and by 4–5 weeks after infection, the weight of these nodes reached 20–30 times that of the same lymph nodes taken from uninfected age and size-matched calves. From these studies, Gasbarre [19] concluded that this increase in size was a result in an increase in number of both parasite-specific lymphocytes, and that lymphocytes did not recognize the parasite antigen. Additional studies [20, 21] showed that the percentages of B lymphocytes in the nodes were higher indicating a preferential expansion of these cells and that there was a corresponding decrease in the percentage of T cells. An increased percentage of B lymphocytes has also been observed [22] in the draining lymph nodes. Also a concomitant decrease in the percentage of T cells in both naturally [23] and experimentally [24] infected animals has been observed. Furthermore, there is a high level of expression of IL4 in both the draining lymph nodes and in lymphocytes isolated from the mucosa [20, 24]. At the present time, the cross-regulation by IL4 and IFN-γ is considered to be one of the major factors driving immune responses to the Th1 and Th2 phenotypes. Gasbarre et al. [2] found that in the case of *Ostertagia*, elicited immune responses are not simply the stereotypic Th2 response as seen in other gastrointestinal nematode infections and in terms of the effector populations found in the tissues surrounding the

parasites, *Ostertagia* does not appear to be typical. Naturally, but not experimentally infected cattle were shown to exhibit increased numbers of mast cells and eosinophils in the mucosal tissues [22]. Generally, nematode infections induce dramatic changes in the tissues surrounding the parasite such as mucosal mast cell hyperplasia, generation of globular leucocytes, eosinophilia, increased mucus secretion, and increases in the mass and activity of smooth muscle in the gut [25]. The fact that *Ostertagia* infections appear to be very efficient stimulators of a number of lymphocyte subpopulations, but very poor inducers of effector cell populations, indicates that *Ostertagia* has evolved a means to suppress or evade protective immune mechanisms. A number of potential suppressive mechanisms have been proposed including: the generation of suppressor cells [26], polyclonal activation of the immune system [19], and the elaboration of parasite products that regulate cell growth [27].

10.4 Intervention by Long Acting Drug Delivery Technologies in the Gastrointestinal Nematode Lifecycle

The field of pharmaceutical technology offers clinicians the opportunity to enhance treatment through the optimal design of new dosage forms through the application of the principles of controlled release science and technology. Recent knowledge in the field of epidemiology and pharmacology can define the optimal delivery profiles that a long acting drug release technology should strive to provide in either a pulsed or continuous fashion over extended time periods (Fig. 10.4). Such delivery systems offer the clinician interested in the treatment of gastrointestinal nematodes the opportunity to deliver the drug into the blood system of the animal in a continuous fashion (as symbolized by the blue line in Fig. 10.4) or in a pulsed fashion,

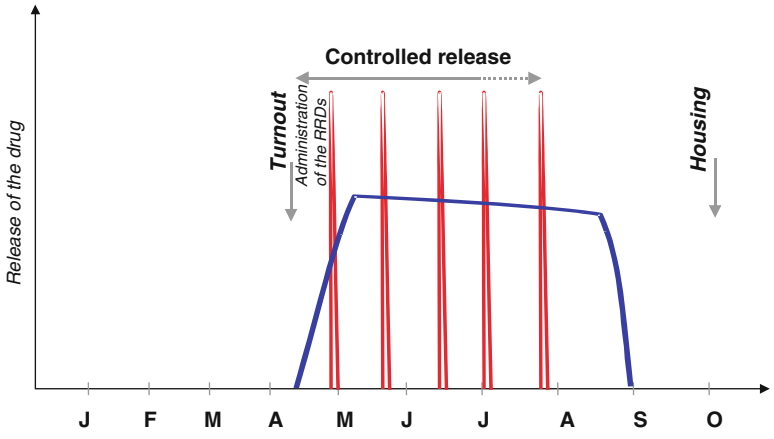

Fig. 10.4 Scheme of the pulsed or slow release of an anthelmintic drug from a ruminal drug delivery device

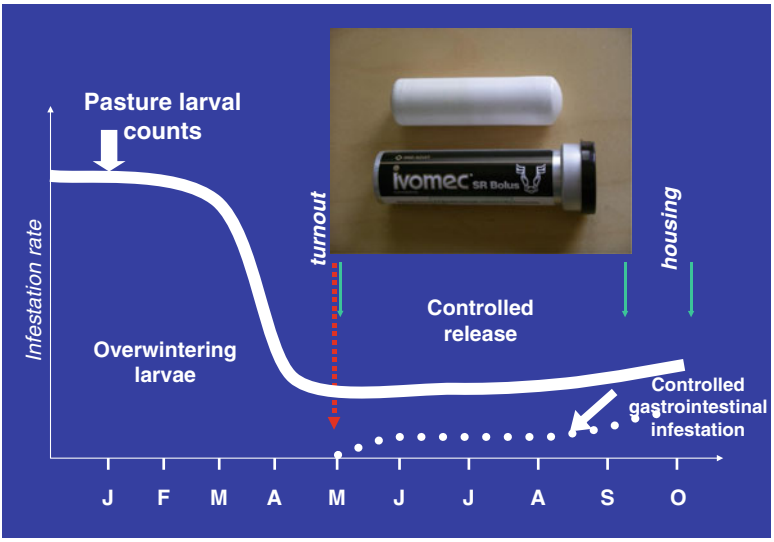

Fig. 10.5 Evolution of the larvae amounts on pastures after the release of an anthelmintic agent when the amount of the larvae is at a low rate of infestation

e.g., 23 days (as symbolized by the red lines in Fig. 10.4). So, which profile would best suit the delivery of an anthelmintic agent for the eradication of gastrointestinal nematode in an infected animal?

The level and the duration of exposure to gastrointestinal nematode infections are of crucial importance for the development of acquired immunity in the first season grazing calves. An excessive reduction of host–parasite contact by chemoprophylaxis, pasture management, or both causes a diminished level of acquired immunity. Moreover, the level of acquired resistance is negatively related to the degree of suppression of host–parasite contact [28]. Whether or not a reduced resistance against establishment and development of gastrointestinal nematode infections has a negative effect on weight gain in the second grazing season depends both on the intensity of the prophylaxis used and on the level of the challenge infection. From cross-sectional serological surveys, it was shown that pharmaceutical parasitic control in the first grazing season in calves tends to be overprotective. Possible consequences of over-treatment, besides higher treatment costs and more drug residues in animal products and in the environment are a reduced level of acquired immunity and increased selection for anthelmintic resistance. Correct pharmaceutical intervention is therefore of great relevance.

From these considerations, it appears (Fig. 10.5) that, although the intraruminal devices afford some advantages and allow the control of the appearance of gastrointestinal infestations, they suffer from several disadvantages, namely:

(1) Release of the anthelmintic agent when there is a low rate of infestation
(2) In the case of the intraruminal devices that provide a constant release of drug, the absence of release during the period of July–August while the infestations are the most important

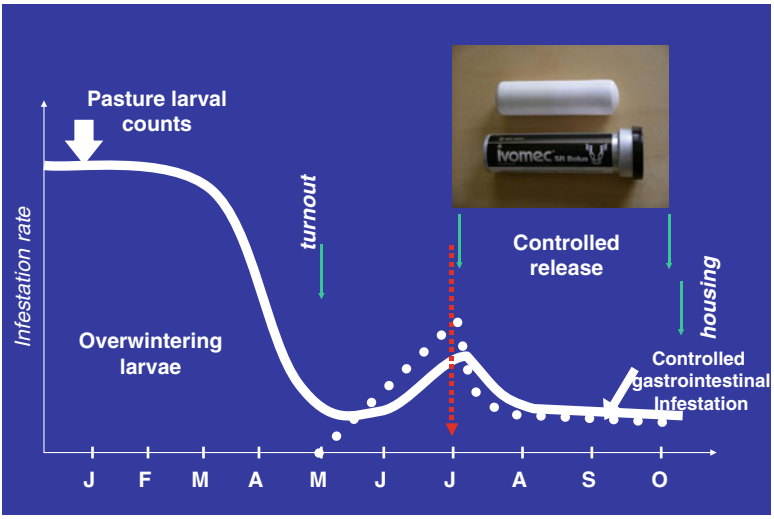

Fig. 10.6 Evolution of the larvae amounts on pastures after the release of an anthelmintic agent when the amount of the larvae is at a high rate of infestation

(3) No increase of the drug delivered to cattle during their optimal period of weight increase during the grazing season
(4) Absence of contact between the animal and the parasites at the beginning of the grazing season due to the low amount of larvae which are present on the grass at this time

Therefore, it follows that the optimal profile for a drug to be delivered to treat gastrointestinal nematodes. The drug delivery system should be devised to (Fig. 10.6):

(1) Avoid a useless overprotection for the ruminants
(2) Ensure an optimal release of the anthelmintic drug during the larval infestations, namely, during the period going from July to August
(3) Ensure a deferred release time of the anthelmintic agent in order to allow the animal to increase its immunity in order to begin the second grazing year in good conditions
(4) Preferably ensure a pulsed release with an increasing amount of the drug released proportionally to the increasing of the weights of the animals rather than a continuous release
(5) Avoid the release of the drug at an inopportune time (namely from April to June in the Northern Hemisphere) and therefore allow an increase in the immunity by encouraging a contact between the animals and the parasites when the level of the parasites is low, and finally
(6) To foresee the possibility of a concomitant administration of other drugs (such as some irradiated larvae during the beginning of the grazing season) or a fasciolide drug (at the end of the grazing season or at the housing)

From the previous figures, it is easy to understand that to construct such an ideal rumen device the following aims must be taken into account:

(1) Release of the anthelmintic agent during larval infestations (namely July–August)
(2) To avoid a release of the anthelmintic agent at an inopportune time (April–June)
(3) The possibility to increase the innate immunity by delaying release in order to allow a contact between the parasites and the grazing animals
(4) To foresee the possibility of a concomitant administration of other drugs (irradiated larvae or a fasciolide drug)

10.5 Ruminal Drug Delivery Systems

In order to provide the reader with an insight into the complexity associated with the design of ruminal drug delivery systems, in this section we provide examples of ruminal drug delivery systems and describe two more recent systems to highlight the advances that have taken place in this area of drug delivery.

Ruminal drug delivery systems are designed to be retained in the rumen of cattle, sheep, and goats by either a change in geometry or by increasing its density (see Chap. 3). A nondegradable ruminal drug delivery system will generally remain in the rumen for the life of the animal. The delivery system is administered to the animal using a balling gun.

10.5.1 Compressed Bolus

In its simplest form, an intraruminal drug delivery system consists of a large bullet-shaped mass resulting from compression of powders or metals. Examples include bolus (tablet) formulations containing oxytetracycline, sulphadimidine, or various trace elements. Drug release occurs in the rumen via erosion or diffusion of the compressed bolus for periods ranging from several days to 120 days or more. In many countries, slow-release compressed boluses have been used to successfully supplement cattle and sheep with selenium, copper, iodine, and cobalt. An Australian team [29] developed an intraruminal device for slow release of iodine in sheep. Each device contained 1,000 mg iodine and released 0.5–1.1 mg iodine per day over a period of 3 years.

10.5.2 Extruded Bolus

Extrusion can be used to manufacture rumen bolus. One example of this approach is the TimeCapsule (Fig. 10.7). Invented in New Zealand this delivery system

Fig. 10.7 The TimeCapsule for facial eczema. The white inner core comprises zinc oxide and extrudable excipients which are covered by a *green waxy* material.

slowly administers zinc oxide for up to 6 weeks for the treatment of facial eczema. It comprises primarily of zinc oxide together with sufficient extrudable agents that enable the formulation to be extruded under high pressure into a rod. Following extrusion the rod is cut to length and then one end is shaped into a semicircle. The zinc oxide core is then dipped into, and covered by, a waxy material. Drug release occurs via erosion from the exposed end of zinc oxide core. Zero order release is observed due to the presence of the waxy coating which prevents the zinc oxide core from eroding from the sides. When some of the zinc oxide core has eroded away, the waxy coating is no longer supported by the core and chips away thereby ensuring a constant surface area of the core is exposed to the rumen environment

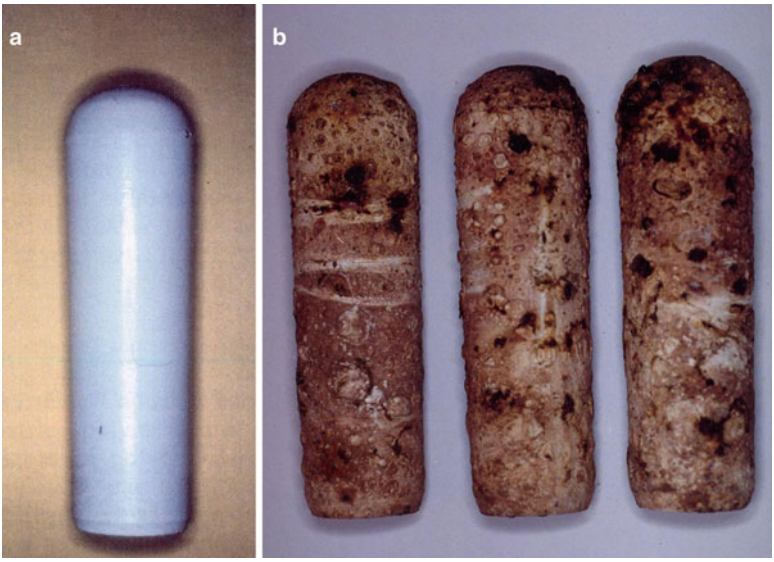

Fig. 10.8 Picture of the Chronomintic® bolus before (**a**) and after (**b**) administration showing that its polyurethane coating remains intact throughout the administration period thereby preventing erosion of the inner matrix occurring from the outside to the inside of the matrix

10.5.3 Paratect Flex®

The Paratect Flex bolus comprises a flat trilaminate sheet manufactured using the polymer ethylene vinyl acetate containing morantel tartrate in the middle laminate layer. The flat sheet is rolled into a cylindrical shape prior to administration and retained in this configuration using a water soluble film. This allows the device to be administered via the back of the throat; however, following administration the film dissolves causing the flat sheet to unroll and be of a shape and dimension that prevents regurgitation by the animal. For economic reasons, the Paratect Flex® Bolus was withdrawn from the market.

10.5.4 Chronomintic®

Chronomintic® is a matrix device with the capacity to slowly release the anthelmintic drug levamisole (Fig. 10.8a). The matrix core is made up of particles of iron and levamisole hydrochloride. The core is bored in its center, the dimensions of which control the release of the drug. The external surface of the matrix core is covered with an impermeable polyurethane coating designed to prevent erosion of the core from the external surface. The levamisole, isomer laevogyrous of the

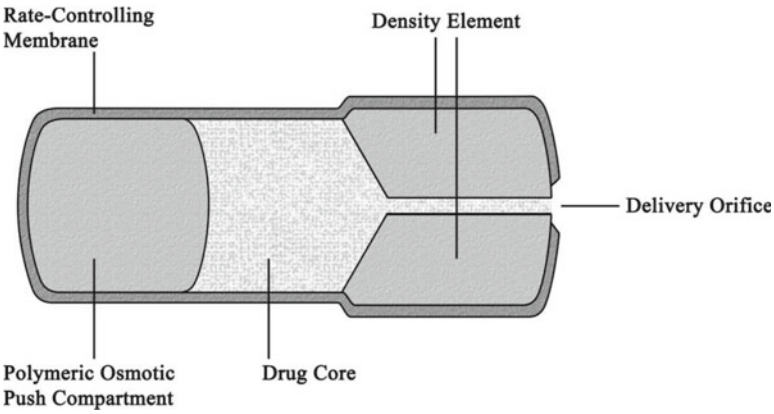

Fig. 10.9 Cross-section of the Ivomec® SR bolus

tetramisole, belongs to the group of the imidazothiazoles. It is particularly active against the larval and adult forms of the dictyocaulus. It is also effective against digestive worms. After administration the bolus remains in the rumen due to the presence of the iron particles in the matrix which increases the density of the delivery system to >2.5. During the grazing season the matrix core slowly degrades, but the polyurethane coating remains intact (Fig. 10.8b). An initial amount of 2.5 g of levamisole is released during the first 24 h; the anthelmintic drug is then released gradually over a 90-day period.

10.5.5 Ivomec® SR Bolus

The Ivomec® SR Bolus was introduced in 1996 by Merck & Co. as a means of long lasting control of internal parasites in cattle. Due to the presence of ivermectin, the bolus also controls several tick species on cattle. Therefore, the Ivomec® SR Bolus found applications for the control of both endo- and ectoparasites in cattle weighing 125–300 kg. The bolus consisted of an osmotic pump (Fig. 10.9). It provided a sustained release of ivermectin in the animal at a uniform rate of approximately 12 mg/day for about 135 days.

10.5.6 Repidose® 750 or 1250 with Systamex®

Repidose® 750 or 1250 with Systamex® (Fig. 10.10) is a bolus programmed to eradicate the gastrointestinal worms (adult and larvae) in beef animal. The delivery system can also eradicate verminous bronchitis, when the release of the drug coincides

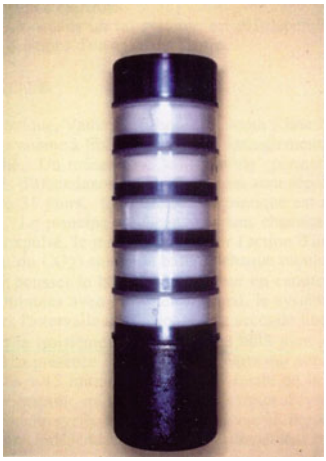

Fig. 10.10 Repidose® 750 or 1250 with Systamex® bolus

with an infection by dictyocaulus. However, in a strongly infested pasture, Repidose® 750 or 1250 with Systamex® does not replace vaccination against verminous bronchitis. The oxfendazole, the anthelmintic drug contained in this delivery system, also exerts an ovicidal effect. The bolus contains 5 therapeutic doses which are pulse released at regular times up to 130 days after administration. The first tablet is released approximately 21 days after administration.

10.5.7 Captec Device

The Captec device ® (Fig. 10.11) was the first intraruminal drug delivery technology to use a change in geometry to retain itself in the rumen of livestock animals. Its design comprised a hollow tube (capsule) that was open at one end and which contained specially designed wings at the opposing closed end. The wings were optimized for flexibility and, prior to administration, were held back along the capsule body using water soluble tape. Following administration the water soluble tape dissolved allowing the wings to spring open and form a shape that could not be regurgitated by the animal. The plastic capsule was filled with tablets containing a complex formulation of drug and excipients. Before loading the tablets into the Captec device a long metal spring was placed in the closed end of the capsule. Once loaded the open end of the capsule was sealed with a plastic seal that contained a hole that acted as a delivery orifice of fixed diameter. Drug release occurred following softening of the tablet formulation with rumen fluid. The softened formulation was then extruded out of the delivery orifice under pressure from the spring. Erosion of the softened extruded formulation that exposed itself beyond the delivery orifice

Fig. 10.11 Captec device

assured continuous release in a zero order fashion. The device was used to deliver various anthelmintic compounds to sheep. It was also used for several other active compounds.

10.5.8 Housed Tablet Technology

Recently, Wunderlich et al. [30] and Rathbone et al. [31] described a modification of the Captec device (Fig. 10.12). The plastic design has demonstrated over time to be safe to the animal and permit easy administration. Its vacant inner allows for the ready loading of drugs formulated into tablets. Rathbone and his coworkers reformulated the tablet and eliminated the need for the spring, while increasing the capsules versatility in delivery duration, rate, and drug type that could be delivered from such a technology (Fig. 10.13a). The workers demonstrated that the new formulation could deliver drugs exhibiting a range of physicochemical properties from the water soluble, lipid soluble to completely insoluble into the rumen of cattle. An alteration to the formulation excipient rations, drug load (up to 70%w/w), or a change to the diameter of the delivery orifice (located in the side of the plastic capsule), or the number of delivery orifices could tailor in vivo release rates and/or delivery periods from a few days to up to 9 months (Fig. 10.13b). The device contained fewer components than the original Captec device, utilized a three component tablet formulation, eliminated the need for a metal spring in the device, and exhibited the ability to deliver both water soluble and lipid soluble drugs over periods ranging from a few days to 9 months.

Fig. 10.12 Components of the housed tablet technology

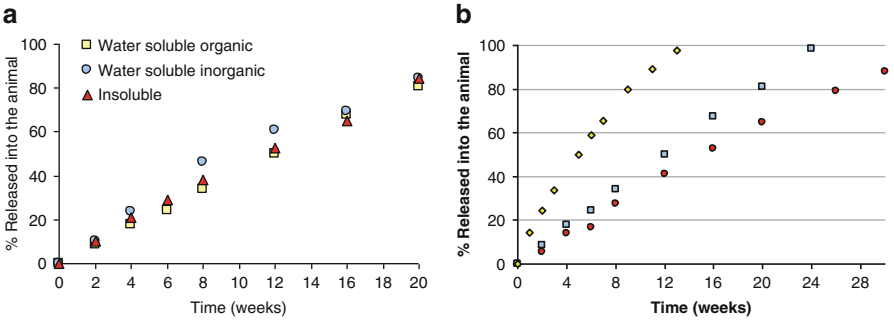

Fig. 10.13 In vivo drug release from the housed tablet technology showing versatility of the technology for drug release: (**a**) delivery of compounds exhibiting a range of physicochemical properties from water soluble to lipid soluble to completely insoluble at the same rate and duration and (**b**) alteration to the formulation excipient rations, drug load (up to 70%w/w), or a change to the diameter of the delivery orifice (located in the side of the plastic capsule) allowing tailoring of the in vivo drug release rate and/or delivery periods from a few months to up to 9 months

10.6 Vandamme Rumino-Reticulum Technology: A Technology to Rationally Treat Gastrointestinal Nematodes

The clinical condition and ideal characteristics of an intraruminal drug delivery system that is designed to treat gastrointestinal nematodes is provided in Sects. 10.3 and 10.4. Thus far in Sect. 10.5 of this chapter, we have provided an overview of rumen drug delivery technologies that predominantly afford only continuous release or offer pulsed release at fixed time intervals. In this final section, we describe a drug delivery technology that has been designed to be sufficiently versatile to meet the demands for delivering a drug in a fashion that provides the ideal delivery profile (Sect. 10.4) for the treatment of gastrointestinal nematodes.

All the studies that evaluated the new Rumino-Reticulum Device (RRD) were achieved using fistulated cattle in order to be able to enter and remove the RRD directly into the intended site of release; thereby exposing the delivery system to the actual in-use conditions that the final product will find itself in (Fig. 10.14).

To optimize drug release, the RRD were designed and constructed by assembling different elements containing the drug symbolized in Fig. 10.15 by the green color and separated one another by a degradable monofilament [32]. The first element is covered by a cap pierced by some holes in order to maintain the device (having a density higher than 2.5) in the bottom of the rumen of the cattle.

The release of the drug is achieved by the following mechanism: firstly, the ruminal liquid dissolves the anthelmintic agent; secondly, this liquid goes inside the cavity pierced on one side by a hole and maintained on another by a degradable monofilament (Fig. 10.15). At each extremity of the monofilament, a seal prevents the ruminal liquid to pass into another compartment before biodegradation of the first has occurred. When the monofilament has degraded, the two parts of the compartments separate from one another and allow the ruminal liquid to reach the next compartment. The crossing of the ruminal liquid from one compartment to another one is governed by the chemical nature of the monofilament.

By examining different kinds of biodegradable monofilaments, for example a monofilament of Monocryl® and a monofilament of polydioxanone, it was found that it was possible to have a breaking time ranging from 22 to 86 days, respectively (Table 10.1).

By choosing the appropriate polymer for the monofilaments to construct the RDD, namely, by choosing for the first compartment a polydioxanone monofilament, the ruminal liquid was able to progress to the second compartment only after 86 days. Conversely, by choosing the polymer Vicryl® (2/0) to make the monofilament, the time will be 23 days. These new RDD provide the opportunity to fulfill the requirements for delivering anthelmintics as described in Sect. 10.4.

Following these research studies, the device was manufactured and tested using 30 first season grazing calves aged from approximately 5 months which were divided into three groups. One group was designated as untreated controls (group 1) and salvage treatment was to be administered only when clinical signs of gastroenteritis appeared; the two remaining groups were assigned to different treatments as

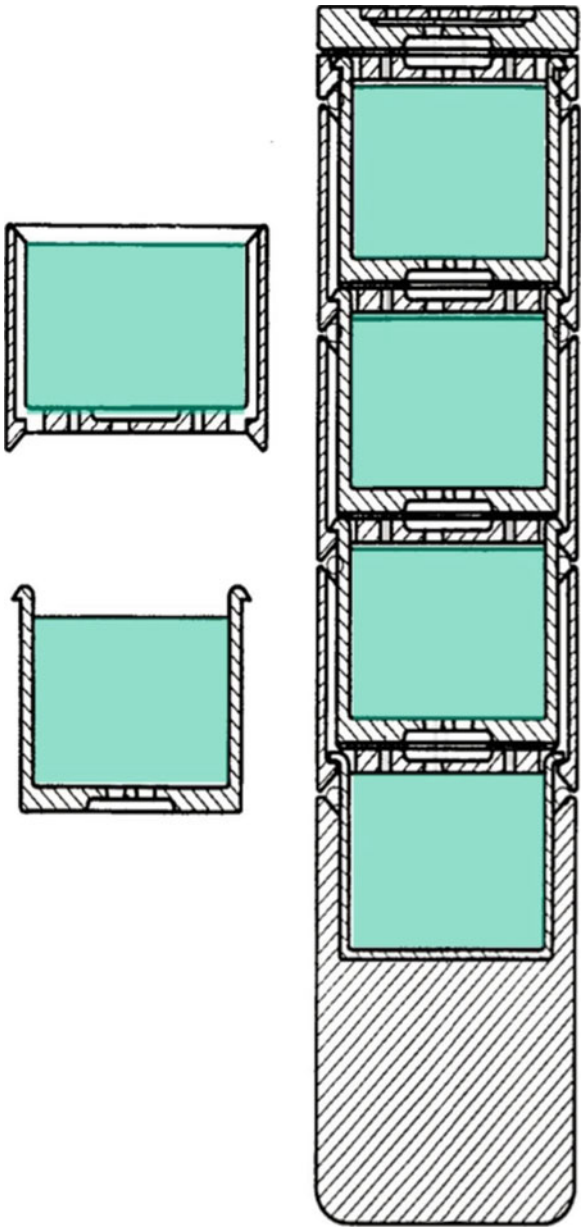

Fig. 10.14 New concept of a pulsed ruminal delivery device

follows: group 2 received early season suppression with an intraruminal slow release Ivomec® SR Bolus administered immediately prior to turnout and group 3 were treated with the new RDD constituted of five compartments, each one containing 12 mg of ivermectin.

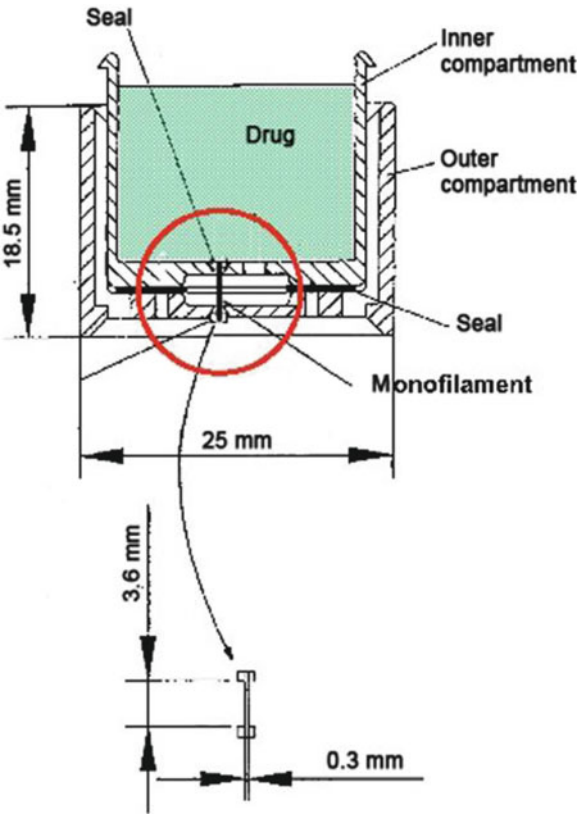

Fig. 10.15 Detail of one compartment of the new concept of a pulsed ruminal delivery device

Table 10.1 In vitro and in vivo breaking times of the biodegradable monofilaments ($n = 10$)

		Breaking time	
Trade name	Chemical composition	In vitro	In vivo
Vicryl® (2/0)	PLGA 10:90	22 ± 0.4 days	23 ± 1.0 days
Monocryl® (2/0)	PGCL 75:25	21 ± 0.3 days	22 ± 1.0 days
PDSII® (2/0)	Polydioxanone	85 ± 0.8 days	86 ± 1.0 days
Dexon® (2/0)	Polyglycolic acid	21 ± 0.6 days	20 ± 1.0 days
Maxon® 0 n°3	Polyglyconate	55 ± 0.2 days	53 ± 0.5 days

PLGA polylactic glycolic acid; *PGCL* polylactic caprolactone

Within each groups, several means of evaluating the performance of each group were undertaken. The first one was the evaluation of the serum pepsinogen concentration. This was determined and expressed in milliunits of tyrosine (mU Tyr). Ostertagia and Cooperia IgG antibodies against crude L3, L4, and adult antigens (4 mg/mL) were determined in an enzyme-linked immunosorbent Elisa assay.

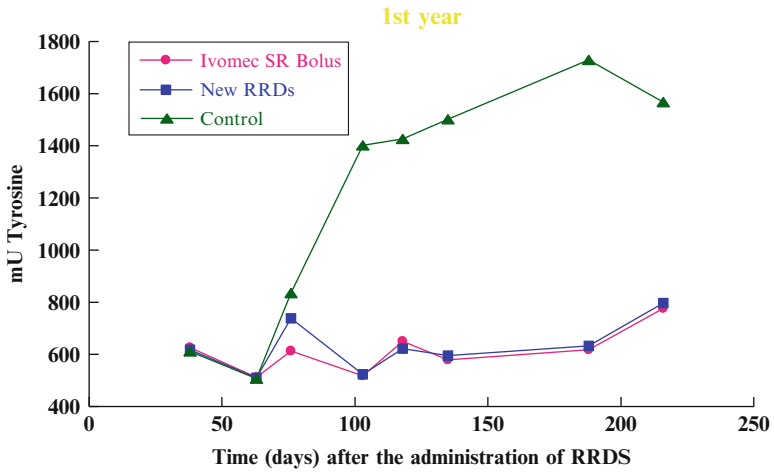

Fig. 10.16 Mean serum tyrosine concentrations of untreated calves (*filled triangle*), of calves treated with ivermectin sustained-release devices (Ivomec® SR Bolus) at turnout for first season grazing (*filled circle*), of cattle treated with new RRDs at turnout for first season grazing (*filled square*)

Figure 10.16 shows that the values of the milliunits of tyrosine were much more important for the control and for the group receiving the new the RDD meaning that exposure was much more important for the two first groups.

The second evaluation concerned the fecal egg counts which were carried out using the Mac Master technique. Briefly, the geometric group means of eggs per gram (EPG) were plotted. For larval determination, third stage larvae were collected after 10 days incubation at 25° from a pooled feces culture of each group. From the results (Fig. 10.17) we can observe that fecal egg counts were lower for the group receiving the RDD than the two other treatments.

Concerning the third, and the most important evaluation (Fig. 10.18), during the first grazing season we observed no significant differences in the mean weight of the cattle between the two groups receiving an anthelmintic treatment either by the osmotic pump Ivomec® SR Bolus or a new RDD, and a significant improvement in mean weight gain compared to the untreated group.

After housing, the cattle were weighted regularly and during this period of time no significant difference was observed between the two groups treated with anthelmintic agents during the first grazing season (Fig. 10.19). When the cattle were turned out at the second grazing season (symbolized by the red arrow on the graph) the cattle did not receive any anthelmintic treatment. From the results of the mean weights of the cattle, it was observed that the weight of the cattle which received the new RDD during the first grazing season was significantly higher compared to the second group. This was due to the development of a best immunity during the first grazing season for the group which received the new RDD. At the end of the second grazing season, a difference of a mean of 33 kg per cow was observed between the two treatment groups in favor of the RDD.

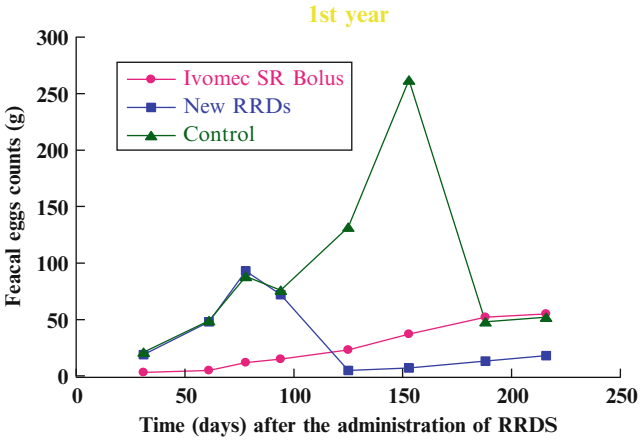

Fig. 10.17 Geometric mean number of eggs per gram of fresh feces (EPG) for untreated calves (*filled triangle*), calves treated with ivermectine sustained-release devices (Ivomec® SR Bolus) at turnout for first season grazing (*filled circle*), cattle treated with new RRDs at turnout for first season grazing (*filled square*)

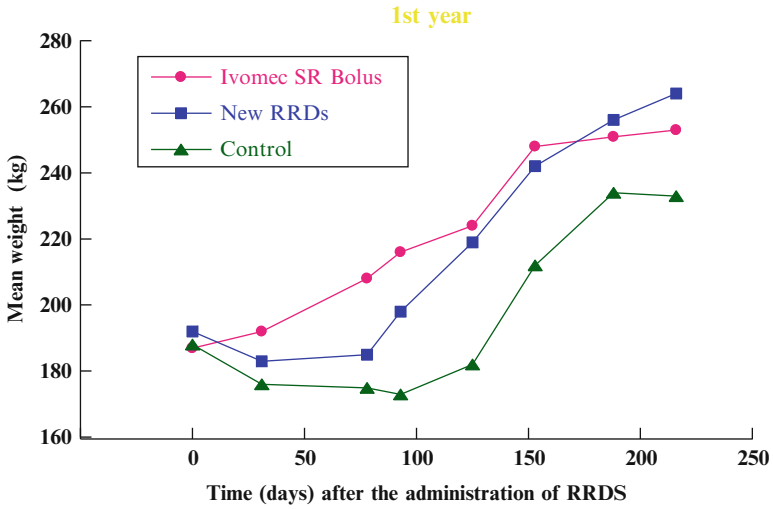

Fig. 10.18 Mean cumulative weight gain (kg) of untreated calves (*filled triangle*), calves treated with ivermectine sustained-release devices (Ivomec® SR Bolus) at turnout for first season grazing (*filled circle*), cattle treated with new RRDs at turnout for first season grazing (*filled square*)

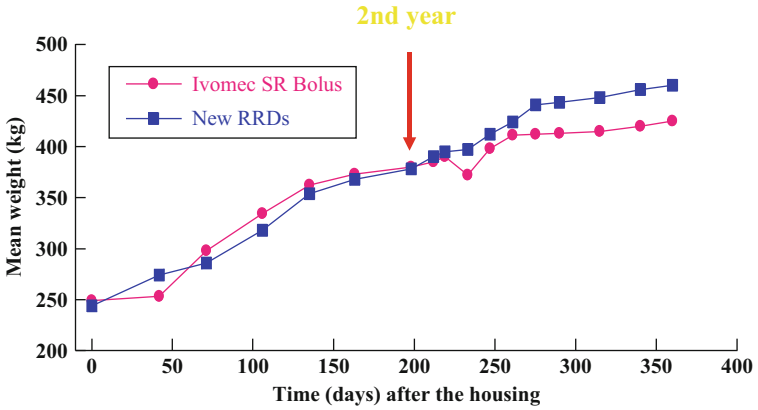

Fig. 10.19 Mean cumulative weight gain (kg) of calves during the second grazing season, treated with ivermectine sustained-release devices (Ivomec® SR Bolus) at turnout for first season grazing (*filled circle*) ($n = 10$), cattle treated with new RRDs at turnout for first season grazing (*filled square*) ($n = 10$)

10.7 Concluding Remarks

The development of an intraruminal drug delivery technology is complex and requires consideration of numerous factors including the anatomy and physiology of the site of release, the disease state that the drug is treating, a sound knowledge of physical pharmacy, and an appreciation of plastic product design. A number of examples have been given in this chapter of intraruminal drug delivery systems that highlight the innovative nature of the process. In addition, a new intraruminal device has been described (RDD) which addresses the unique needs of a given clinical condition (treatment of gastrointestinal nematodes) leading to the ability to (1) a delayed release (variable following the chemical nature and the thickness of the monofilament which is used); (2) a sequential and a pulsed release of the drug; (3) the possibility to increase the amount of the dosing released during the grazing season; (4) the possibility to change the anthelmintic agent during the grazing season and therefore to avoid the phenomenon of resistance; and finally to release the drugs during all the grazing season.

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Chapter 11
Controlled Release Intravaginal Veterinary Drug Delivery

Michael J. Rathbone and Christopher R. Burke

Abstract This chapter describes the current scope of intravaginal drug delivery in livestock. Pre-formulation, manufacturing, pharmacokinetic, and technological aspects of product design are considered. Historical developments are briefly reviewed, while product developments that have occurred over the past decade are described in detail. The chapter concludes with a look into the future and suggests some future needs that, if filled, could extend the use of vagina of livestock as a route of administration and make available a wider range and scope of products for use in the industry.

11.1 Introduction

The vagina of farmed animals has been successfully utilized as a portal for the systemic delivery of a number of pharmacologic compounds. Several conceptual and commercially available intravaginal veterinary drug delivery systems have been developed for this purpose [1–12]. The vagina of livestock animals such as cattle and sheep is an attractive site for drug delivery due to the ease with which drug delivery systems can be administered and removed, its biological properties which

This chapter is dedicated to my friend and colleague Patrick J. Burns who passed away last year. A leader in the field of estrous control, he will forever be admired by me for his imagination, tenacity, knowledge and friendship.

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are conducive to drug delivery, its ability to retain drug delivery systems for prolonged periods (weeks) and, although this route for drug delivery is sex specific, the uneven distribution of females within the farmed animal population means that delivery systems developed for this route will serve the majority of the population owned by the farmer [9]. For drugs that are susceptible to gastrointestinal or hepatic metabolism, vaginal delivery may provide an alternative to that of the oral route of administration [9].

History shows that steroids have been administered via this route to control the estrous cycle of farmed animals [1–12]. The management of estrous control of livestock through pharmacologic intervention using intravaginal inserts containing progestins represents a valuable farm management tool to enhance production, enrich genetic stock characteristics and assure that animals are pregnant to coincide with management systems associated with seasonal drying off and milking activities [9]. Ease of use, excellent retention rates, ease of removal and normal fertility rates have contributed to the success of such systems [8, 9].

The first long-acting estrous control product became available to farmers in the mid-1960s (polyurethane sponge for use in sheep) [1–3]. This was followed some years later with the development of a progesterone impregnated silicone spiral shaped device (PRID for use in cattle) [1–3]. Since then, several progesterone impregnated silicone inserts have appeared on the market [1–12]. In the past decade or so, a number of progesterone intravaginal inserts have been described (CueMate, TRIU-B, PRID Delta, PCL Intravaginal Insert, electronic inserts and CueMare). These systems have provided the producer with effective hormonal control treatments and provided animal scientists with tools to examine and extend our knowledge on the estrous cycle of farmed animals [11]. Indeed, the commercial success of these products can in a large part be attributed to the efforts of the animal scientists whose research has provided estrous synchrony programs that comprise combinations of slow release progesterone intravaginal inserts together with exogenously administered hormones (e.g., estradiol, GnRH and prostaglandin-F2α) that regulate follicular and luteal development to more tightly synchronize the timing of insemination and maximize fertility to the synchronized inseminations [11]. The resultant programs offer producers and veterinarians an important means of effective control over the estrous cycle of cattle, sheep and goats, and in some cases alleviate the serious reproductive challenges of high-yielding systems.

This chapter describes the range of intravaginal drug delivery technologies used in livestock for estrous control of cattle, sheep, goats, pigs and horses. Preformulation, manufacturing, pharmacokinetic and technological aspects of product design are considered. An overview of the historical developments in this area is provided. One main focus of the chapter is to describe product developments that have occurred over the past decade or so. The chapter closes with a look into the future and suggests the needs in the area that, if filled, could extend the use of the vagina of livestock as a route of drug administration and make available a wider range of products that span a larger range of drugs which treat a greater number of clinical conditions for the benefit of veterinarians and producers for the purpose of animal health, production and reproduction.

11.2 Vaginal Anatomy, Histology, Pathophysiology, and Physiology

The anatomical and physiological features of the vagina of farmed animals will affect drug absorption and delivery system design. They are, therefore, important considerations in the development and optimization of an intravaginal veterinary drug delivery system.

11.2.1 Anatomy

The vagina of farmed animals is an important region of the reproductive tract. It is open to the exterior via the vulva, and has specialized secretory activity and functions. Its specialized functions include the ensheathment of the penis during intercourse, it is the lowermost part of the birth canal and therefore has an important role during parturition and it acts as an excretory duct for discharge. Its secretions of mucus serve to nurture spermatazoa during the period of ovulation during the estrous cycle. In addition, the cervical mucus acts to block the entry of pathogens into the uterus. The vagina must perform these functions at all times even during the continuous physiological changes in the local environment associated with the estrous cycle and with pregnancy. These functions have implications on the design of delivery systems for this route. Thus, the delivery system must be designed for ease of application to reduce or prevent the likelihood of the introduction of pathogens into the vagina; it must possess some form of retention mechanism that acts independent of the cyclic changes in physiology and which allows it to remain in place for the duration of delivery. In addition, release of drug from the delivery system must be relatively insensitive to the physiological changes which are taking place during its entire insertion period to ensure appropriate and reproducible plasma levels over the administration period. Also, the delivery system must have minimal effect on the vagina and its environment, e.g., it must not promote local reaction, must not produce excessive stimulation of mucus or cause infection.

The female reproductive organ of mammals can be broadly divided into external genitalia, comprising the vulva and vestible; and internal genitalia being the vagina, cervix, uterus, fallopian tubes and the ovaries [13–15]. The vagina is a fibromuscular tube situated in the pelvic cavity under the rectum and over the urinary bladder. It connects the uterus to the external genitalia, thereby serving both as a copulative organ, and a passage canal for the neonate during the birthing process [14–16]. The foremost (cranial) boundary of the vagina is the cervix, while the rearmost (caudal) boundary is the point immediately cranial to the external urethral orifice where the hymen is located [15]. The hymenal fold is an extension of the mucous membrane [17, 18] and most prominant in prepubertal states. The hymen is prominent in horses and pigs, but rudimentary in adult cattle. The length of the vagina in mature females varies from as short as 7.5 cm in sheep, goats and pigs to as long as 30 cm in cattle

[13–15, 18]. It rests in a collapsed state in animals such as cattle and horses [13–15], and the surface of the vestibule and vagina of cattle contains prominent folds, which are longitudinally disposed [19].

The vagina is serviced by a rich venous plexus including the vaginal vein which drains into the internal iliac vein (internal pudendal vein in mares [15]) and then into the vena cava for a direct route back to the heart. In contrast to other species, the cow has an accessory vaginal vein with no corresponding artery. The cow has a more prominent venous network in the vestibular region, and is the only animal to have a vestibular vein arising from the internal pudendal vein. Blood vascularization and flow rate dynamics are dependent on ovarian steroids, particularly estrogen [20, 21].

11.2.2 Histology

11.2.2.1 Vaginal Wall

The wall of the vagina is composed of three distinctive layers [18]. From deepest to the most superficial to the vaginal lumen are (1) adventitia, (2) muscular coat, and (3) mucous membrane layers.

11.2.2.2 Mucous Membrane

The mucus membrane (or mucosa) consists of a *lamina propria* of dense connective tissue covered by an epithelium [18].

The connective tissue of the lamina propria in humans is less dense towards the muscular middle layer of the vaginal wall and may be referred to as the submucosa. Papillae of the lamina propria extend into the epithelium, more so in the posterior regions of the vagina (sheep [22, 23], humans [18], and cattle [19, 24].

In the bovine vestible, these projections were measured at about 200 μm in length and 50–100 μm at the base, becoming narrower and concentrated caudally [24], and absent in the anterior regions [18].

A dense network of fine elastic fibers is present in the outermost region of the lamina propria in humans [18], immediately under the epithelium. These fibers are also disposed inward through the lamina propria and become condensed in the walls of blood vessels within the muscular middle layer. Blood vessels are located in the depths of the lamina propria [18], and decrease in diameter towards the superficial region of the stroma underlying the epithelium [19].

11.2.2.3 Epithelium

The epithelium of the vagina is generally described as being a stratified (multiple layer of cells) squamous (irregular, flattened cells) tissue type. Similar types of epithelium are found in the oral cavity, skin and eyes [25].

Vaginal epithelium is responsive to ovarian steroids, particularly estrogen produced by maturing follicles [18, 26]. Rapid growth and thickening of the epithelium are observed around the time of estrus (cattle, [27], goat [28], sheep [23], and pig [29]).

11.2.2.4 Endocrine Dependency

Epithelial developments are dependent on steroid stimulation, particularly estrogen from active ovarian follicles [26].

Luteal secretion of progesterone from the ovaries is also considered to stimulate cellular activity in the vaginal mucosa [28].

Various studies have attempted to correlate endocrine status during the estrous cycle to exfoliate cytological descriptions [14]. These events are considered to be closely associated in some species (i.e., sheep, goats, rodents, cats, and dogs), but not in others (i.e., cattle, horses, and pigs).

11.2.3 Physiology

11.2.3.1 Secretions

Mucus is secreted from the epithelial cells and glands of the cervix and anterior vagina [30], particularly during the estrogenic phase of the cycle [31, 32]. This secretion is considered essential for sperm transport [33, 34]. Cow has been the subject of much study concerning these mucins [35], the major reason being that mucins of marked physical differences are secreted throughout the estrous cycle and during pregnancy [35]. The amounts of mucus and its moisture content are reduced during the diestrous period. The weight of mucus in the vagina of cows and heifers soon after being slaughtered was reported to vary widely from 0.2 to 7.26 g [33, 34]. It was noted that some of the fluids may have been lost between slaughter and recovery of the organs while the animal was in a recumbent position. Moisture content in vaginal mucus was found to range from 90 to 98%, comparatively higher than that of cervical mucus (80–90%). The weight and solidity of cervical mucus increase from 0.7 g in a nonpregnant cow to 3 g and 14 g in early and mid-gestation of pregnancy [33, 34].

11.2.3.2 Physiochemical Properties of Mucus

A chemical analysis of mucins has shown them to be of the "blood-group substance" type, being composed of fucose, galactose, glucosamine, galactosamine, sialic acid, acetyl, ash, nitrogen, and 13 different amino acids [35]. Compositional differences between the cow estrous mucin and pregnancy mucoids have been characterized [35]. Sialic acid is an integral part of the mucoid molecule, and the content varies between the mucoids [36]. Estrous mucin contains only 1–1.5% dry matter, compared to either diestrous mucin (2–3%) or pregnancy mucin (4–5%) [28, 29].

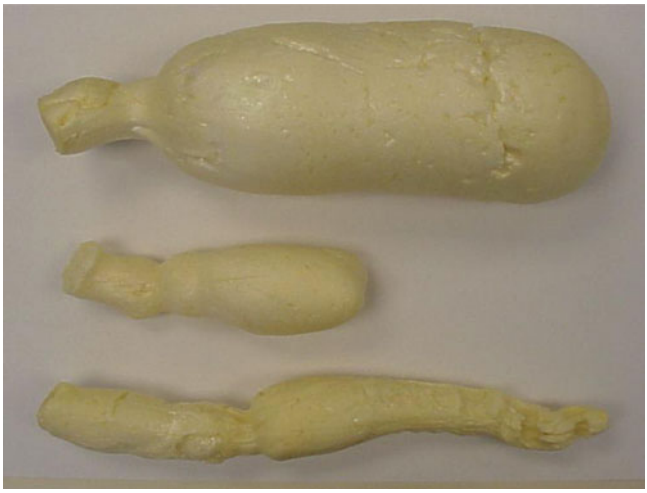

Fig. 11.1 Typical casts of livestock vagina (from *top to bottom*: cow, sheep and pig)

These mucins are considered to exist in a random coil form [34] with molecular weights of around 4×10^{-6} M [36]. The estrous mucin is twice as viscous and probably exists in a much more expanded form [36]. Viscosity of cervical mucus is minimal during estrus, probably to support sperm transport, and maximal during pregnancy, forming a cervical plug to enhance protection of the fetus developing in the uterus [37, 38].

Vaginal surface pH can be measured with a glass pH electrode and is highly correlated with the pH of vaginal mucus at various sites within the vagina of women [30, 37]. The pH in women ranged from 4 to 6, considerably more acidic than in cattle which is reportedly neutral to alkaline (pH 7.0–8.9 [34, 38–40]) or only slightly acidic (pH 6.6 [41]). The flow of more acidic mucus from the cervix (pH 6.8) into the vagina during estrus in the cow had the effect of lowering vaginal pH when measured during the metestrous period [38]. Acidity was considered to contribute to infertility in problem breeder cows and the vagina was routinely douched with a mild sodium bicarbonate solution just before breeding [41]. This practice probably disappeared when vaginal pH between groups of cows with normal or impaired fertility was found to be equivalent [41].

Very little bacteria is seen to grow on mucins [30, 42], probably due to the lysosome-like activity within mucin [43].

To rationally develop an intravaginal veterinary drug delivery system, the physical characteristics of the vaginal environment need to be understood. In a recent study, Rathbone et al. [44] developed a method capable of characterizing the physical dimensions of the vaginal cavity of three farmed animals (cow, pig, and goat). The method involved the administration of expanding foam into the space of the object being characterized. The method was fully validated with respect to its ability not to over-expand the cavity being measured. Typical casts are shown in Fig. 11.1.

11.3 Drugs Administered Via the Vagina of Livestock

To date, intravaginal veterinary drug delivery systems have only been developed for the administration of synthetic and natural hormones such as progesterone, methyl acetoxy progesterone, fluorogestone acetate, and estradiol benzoate [1–12]. However, since progestins are among the most widely used reproductive drugs in veterinary medicine [45], the introduction of controlled release hormone intravaginal inserts for the delivery of progestins has been welcomed by veterinarians and producers. The reason for this is that to control the estrous cycle of livestock, most progestin treatment protocols require 1–2 weeks (or longer). Because of the short biological half-life of progestins, conventional injections (or oral feed preparations) need to be administered daily to maintain efficacious blood levels [45]. Such daily treatments are inconvenient, time consuming, and prone to missed dosing [45]. The introduction of long-acting intravaginal progestin delivery systems overcame these problems. Ease of use, excellent retention rates, ease of removal, and normal fertility rates have contributed to the success of such systems [1–3, 11].

11.4 Design Challenges

11.4.1 Administration

Livestock do not have the ability to self-administer intravaginal delivery systems. Veterinarians and producers therefore need some means by which to easily (and without causing harm, pain, or damage to the animal) administer intravaginal inserts. Insertion is achieved using applicators: plastic instruments designed to allow loading of the intravaginal insert into it at one end and easy release of the insert into the vaginal cavity through the use of some release mechanism that is operated by the person administering the insert (Fig. 11.2). Rathbone et al. [8] have discussed in length the design constraints associated with both the applicator and the need to design the intravaginal delivery system around the vaginal applicator. Single-handed applicators are preferred [8].

11.4.2 Retention

A device will only be effective if it remains in the vagina for the duration of the treatment period [8, 9]. Poor retention rates would reduce the overall efficacy of a treatment. Rathbone [8] defined a rule of thumb for intravaginal insert retention rates: if an intravaginal veterinary drug delivery system is to be of practical use, it must possess a retention rate of at least 95%.

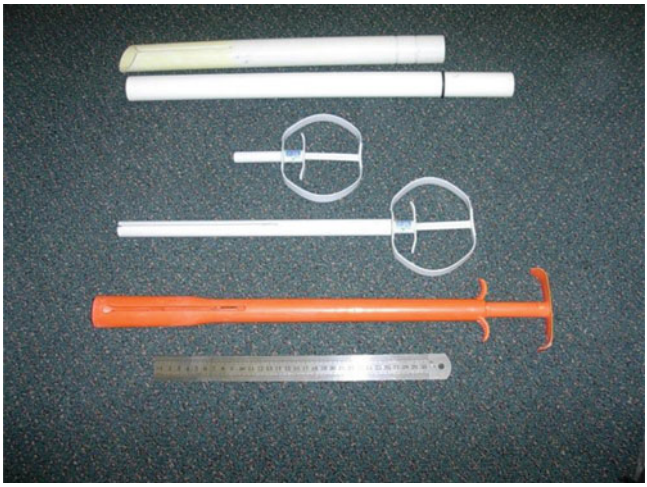

Fig. 11.2 Plastic applicators used to administer intravaginal products to livestock. *Top to bottom*: PRID Insert applicator; the CIDR Sheep and Goat Insert applicator; the CIDR 1380 Cattle Insert applicator; and Cue-Mate applicator

Intravaginal veterinary drug delivery systems utilize an "expansion mechanism" to retain the device in the vagina for the duration of treatment [1–3]. Following insertion, either the entire device expands outwards (e.g., sponges or PRID) or only a part of the device expands outwards (e.g., the "wings" of the CIDR-B or CIDR-G). In both cases, the expanded device exerts gentle pressure on the vaginal wall resulting in intravaginal retention.

11.4.3 Removal

The majority of delivery systems administered intravaginally to animals are not biodegradable and therefore require some means of removal from the vagina to end the treatment. Removal must be achieved quickly, safely, and without damage to the vaginal mucosa. Typically, removal of devices is aided by the addition of a "tail" into the design of the device (Fig. 11.3). The tail should not interfere with any administration process, be long enough to protrude out of the vulva after the insert has been administered, and extrude sufficiently so that an end user can grasp it firmly enough to pull the device out of the vagina without slippage. The tail is usually made of a plastic, blue in color, and curved so that it hugs the contour of the rear end of the animal. These features prevent investigation by herd mates. The CIDR Pig Insert [3, 9, 46] does not have a tail since the treated pigs are penned with very inquisitive herd mates who were observed to pull on a protruding tail causing it to be removed before the duration period was achieved. This necessitated the design of a removal tool for the CIDR Pig Insert (Fig. 11.4).

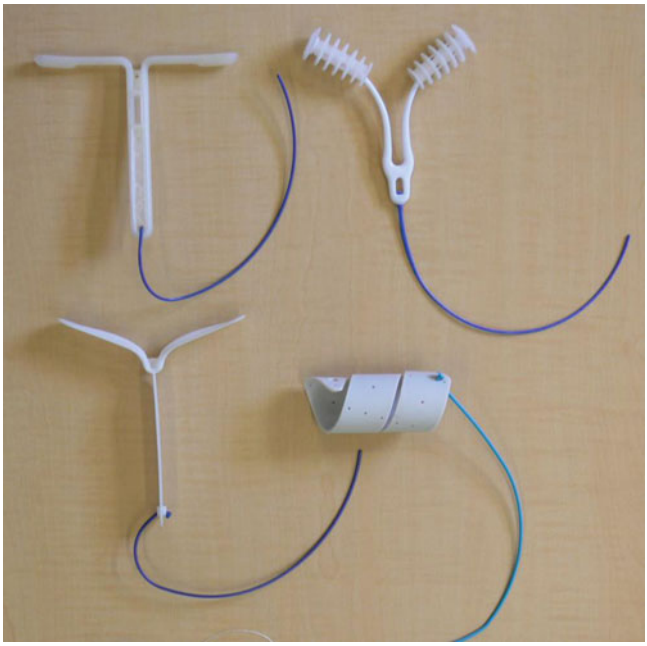

Fig. 11.3 Various intravaginal inserts showing plastic tails attached to the *lower* end of the insert body that protrudes out of the vagina during treatment and which is grasped and held firmly while pulling in order to remove the insert from the vagina of the animal to terminate the treatment period. *Top left*: CIDR 1380 Cattle Insert; *Top right*: CueMate; *Bottom left*: PCL Intravaginal Insert; *Bottom right*: PRID

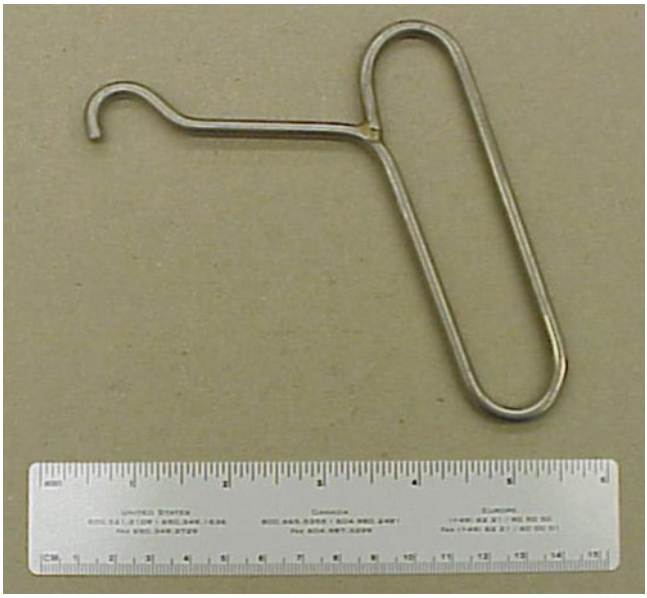

Fig. 11.4 Removal tool for the CIDR Pig Insert

11.4.4 Size and Shape

The size and shape of an intravaginal drug delivery system are complex and dictated by the need for the insert to be flexible, change shape, expand, or contract, depending upon the designers' intended administration and retention mechanism [8]. The key considerations in the final design revolve around it being safe to use, not cause damage to the animal upon insertion, while in place and during removal. The dimensions of the delivery system (length during and after insertion, diameter, and width during and after insertion) are dictated not only by the anatomical constraints and resilience of the vagina but also by the applicator design. This interrelationship between vaginal anatomy and applicator design has been discussed by Rathbone et al. [8] and should be undertaken in joint consideration with delivery system requirements relating to release rate.

11.4.5 Drug Loading

A characteristic of progesterone containing intravaginal inserts is their high drug load and poor dose utilization which leads to a high residual content after removal. Rathbone et al. [47–49] optimized the original CIDR-B for drug content and residual drug load resulting in a reduction in drug load from 1.9 to 1.38 g and a residual content lowering from 1.4 to 0.6 g. This was achieved by thinning the skin. Theoretical calculations suggested that a skin thickness of 0.8 mm with a corresponding initial drug load of approximately 1 g could provide the same release rate and amount of drug delivered as the original CIDR-B; however, the final skin thickness over the nylon spine of the commercially available was 1 mm. Skin thicknesses of less than 1 mm were limited by reliable production of a uniform thickness over the entire nylon spine due to manufacturing reproducibility.

11.4.6 Manufacturing

11.4.6.1 Equipment

Intravaginal veterinary inserts are manufactured by the process of injection molding using commercial sized injection molding machines [8]. Generally, spines are manufactured separately and are drug free. The silicone skin is injection molded around the spine and heat is used to accelerate curing of the thermoplastic polymer silicone. Exceptions to this are the PCL Intravaginal Insert, which does not require a spine and is molded one-piece.

Prior to injection molding, silicone and progesterone must be homogenously mixed. This is usually conducted in a planar-type mixer. The process of mixing will introduce unwanted air bubbles into the mix. Unwanted air bubbles affect product quality attributes such as weight of the insert and drug contentand are removed by vacuum in a separate part of the manufacturing process.

11.4.6.2 Molds

The silicone skin is cured in a mold usually made of stainless steel which has cut into it four or more mold cavities. The molds are precision cut and usually polished. Great care is undertaken to maintain the molds in premium condition. Heating elements heat the molds and air holes allow air to be released during manufacture.

11.4.6.3 Curing Times

As mentioned above, silicone is a thermoplastic, and therefore the higher the temperature, the faster the curing time. This can be used to advantage during manufacture of silicone inserts and temperatures approaching 200°C are used for the CIDR products [1–3]. This results in short cycle times and increases the cost effectiveness of the process. This can be tolerated by the very heat stable drug progesterone.

11.4.6.4 Packaging

Because intravaginal insert for livestock are of a large size, packaging can be problematic. Upwards of ten inserts are generally contained in each package. On the positive side, progesterone is a very stable compound and retains its chemical stability for many years when formulated and manufactured into intravaginal veterinary inserts. Thus, the packaging only needs to be adequate to afford some physical protection to the inserts during storage and transport. There is no requirement to invest in more expensive packaging that affords protection against light, humidity, or gasses such as oxygen.

11.5 Product Design Principles

Rathbone et al. [8, 9] have described the general considerations for developing a controlled release drug delivery system for the control of the estrous cycle of domesticated livestock. These are listed in Table 11.1. The reader is referred to these publications for further details of the processes involved when designing an intravaginal veterinary drug delivery technology for livestock.

Table 11.1 Formulation development issues when designing an intravaginal veterinary drug delivery system

Intellectual property issues relating to actives, use and process patents
Physiochemical properties of the drug
Interactions between the drug and the formulation ingredients
Duration of release
Formulation processing conditions and facilities with respect to both initial development and scale-up
Sterility issues
Tissue residue issues in food producing animals
Ability to fit within farm management practices
Packaging requirements
Method of administration
Dose utilization
Cost of raw materials
Size of the delivery system
Successful formulations must offer improvements in control of the estrous cycle
Cost effectiveness
Safety to the end-user
In vivo stability
Retention of the delivery system

From ref. [8]

11.6 Historical Product Developments

Rathbone et al. [1–4, 9] have documented the historical developments in this area up to 2001. This period featured intravaginal veterinary drug delivery systems including polyurethane sponges containing synthetic progetins, silicone-based inserts containing the naturally occurring hormone progesterone including the PRID, CIDR-B, CIDR 1380 Cattle, CIDR Pig Inserts; electronically controlled inserts (Intelligent Breeding Device and EMIDD) capable of delivering multiple drugs at a predefined time, either pulsed or continuous fashion; and a biodegradable insert called the PCL Intravaginal Insert. The reader is referred to these comprehensive references on this topic.

11.7 Significant Technological Developments Over the Past Decade

11.7.1 CIDR 1380 Cattle Insert

The CIDR 1380 Cattle Insert is the optimized version of the CIDR-B (Fig. 11.5) which was originally developed in the 1980s. It comprises a nylon spine over which

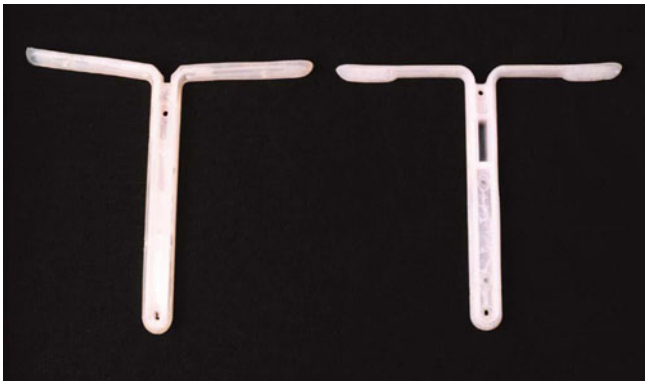

Fig. 11.5 The original CIDR-B (*left*) and the optimized version named the CIDR 1380 Cattle Insert (*right*)

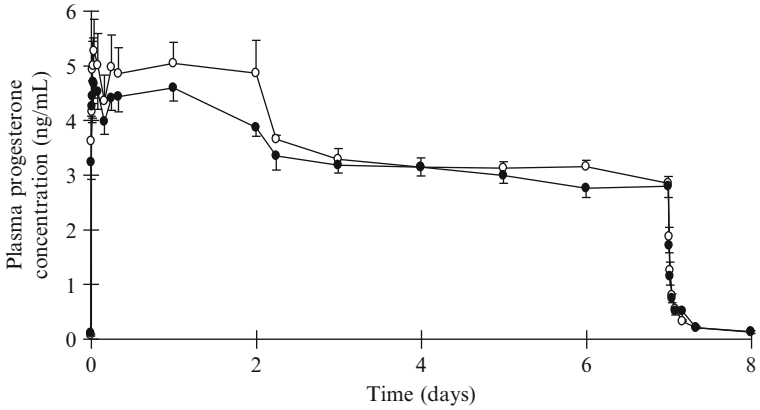

Fig. 11.6 Blood plasma progesterone profile for CIDR-B Intravaginal Insert versus redeveloped CIDR 1380 Intravaginal Insert (*open circle* CIDR-B insert, *filled circle* CIDR 1380 Intravaginal Insert, $n = 8$, error bars = SEM) [47]

a 1-mm-thick skin of silicone containing a homogenous dispersion of 10% w/w progesterone is injection molded [1–3]. Molding temperatures approaching 200°C are used to rapid cure the silicone and consequently reduce cycle times, thereby increasing the efficiency of the production process.

After successful completion of extensive animal trials, the CIDR 1380 Cattle Insert received FDA approval to be marketed on the US market in 2002 for use in beef heifers and cows.

The CIDR 1380 Cattle Insert was re-registered on existing CIDR-B markets based on it being bioequivalent to the CIDR-B product [47] (Fig. 11.6).

Fig. 11.7 CIDR 1380 Cattle Insert and PCL Intravaginal Insert. Both inserts contain 1,380 mg of progesterone homogenously distributed throughout silicone (CIDR 1380 Cattle Insert) or Polycaprolactone (PCL Intravaginal Insert)

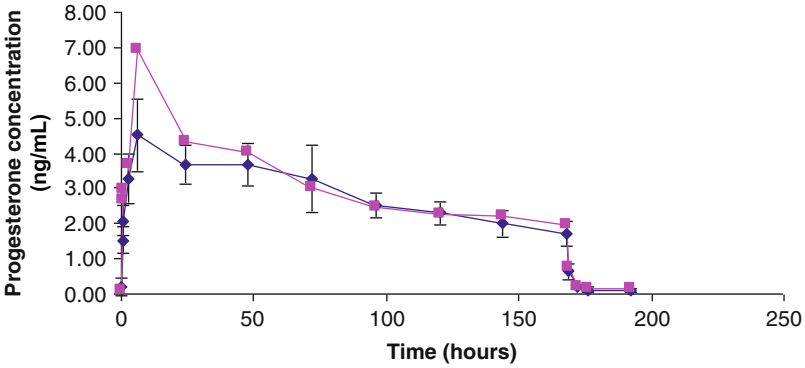

Fig. 11.8 Bioequivalence study comparing the PCL Intravaginal Insert (*filled diamond*) with the CIDR 1380 Cattle Insert (*filled square*) [50]

11.7.2 PCL Intravaginal Insert

The PCL Intravaginal Insert (Fig. 11.7) received registration approval on both the New Zealand and Australian markets based on it being a generic equivalent product to the CIDR 1380 Cattle Insert [50–52].

Although the blood profiles did not show equivalency in C_{max} (Fig. 11.8 and Table 11.2), both these agencies accepted the argument that C_{max} played no role in the efficacy of the insert and that its lower peak height compared to the CIDR 1380 Cattle Insert did not impact on the safety of the insert. On both these markets, approval was sought and awarded based on the product being a generic equivalent, therefore avoiding the need to submit expensive and time-consuming clinical trials to the agencies.

Table 11.2 Optimized PCL intravaginal insert

Parameter	Ratio (%)	Lower 90% limit (%)	Upper 90% limit (%)
AUC	93.0	85.5	101.2
C_{max}	87.9	79.4	97.2

To be considered bioequivalent, FDA Guidance for Industry #35 states limits for AUC and C_{max} as 80–120 for the lower and upper 90% limit, respectively. As can be seen in Table11.2, the lower 90% limit failed to meet these specification limits for the PCL Intravaginal Insert. The insert was therefore unable to undergo registration through the generic equivalent pathway.

11.7.3 Intelligent Breeding Device

The Intelligent Breeding Device has been described previously [1]. In addition to using electronics to control the delivery of progesterone from an intravaginal insert, and to being the first intravaginal insert to be able to pulse dose compounds other than progesterone during insertion, the Intelligent Breeding Device possesses another unique feature. It is the first intravaginal insert to be inserted using a disposable applicator. Figure 11.9 shows the device packaged within its applicator and subsequent photographs show how the insert was administered. The application was a one-piece plastic molding that had foldable wings at one end. These wings could be unfolded and then the head of the device inserted into the vagina of the cow until the open wings rested on the vulva. By pressing against the device, it could be pushed into the vaginal cavity and the applicator removed and disposed.

11.7.4 Smart1

The Intelligent Breeding Device (Fig. 11.9) underwent redevelopment and was reintroduced on the New Zealand market as the Smart1 (Fig. 11.10). The Smart1 contained improved electronics, better sealing to prevent moisture ingress during insertion, a novel retention mechanism and incorporated the use of a concertina bladder that contained an improved progesterone formulation (Fig. 11.10).

11.7.5 Cue-Mate

The Cue-Mate (Fig. 11.3) is the only commercially available intravaginal insert to offer the opportunity to reuse the supporting wishbone-shaped spine upon which removable silicone fluted pods are fixed (Fig. 11.11).

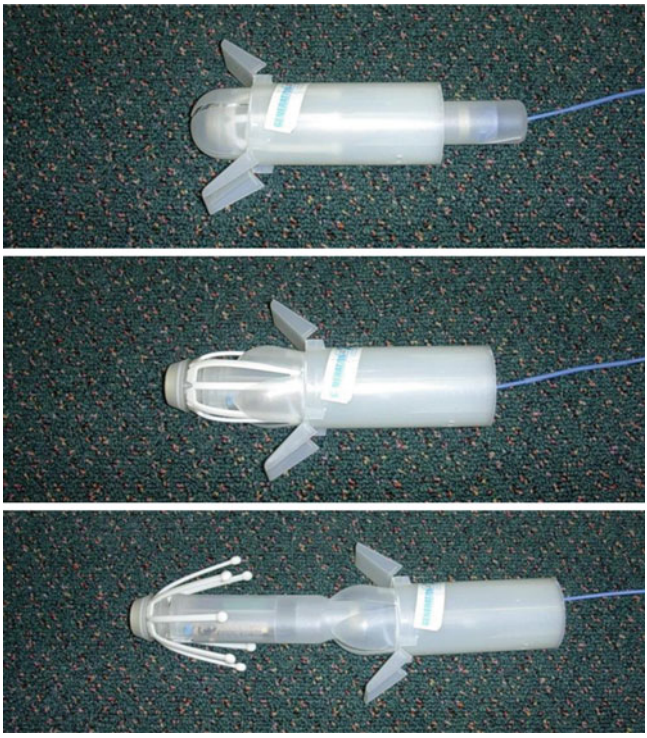

Fig. 11.9 Intelligent Breeding Device showing unique disposable applicator

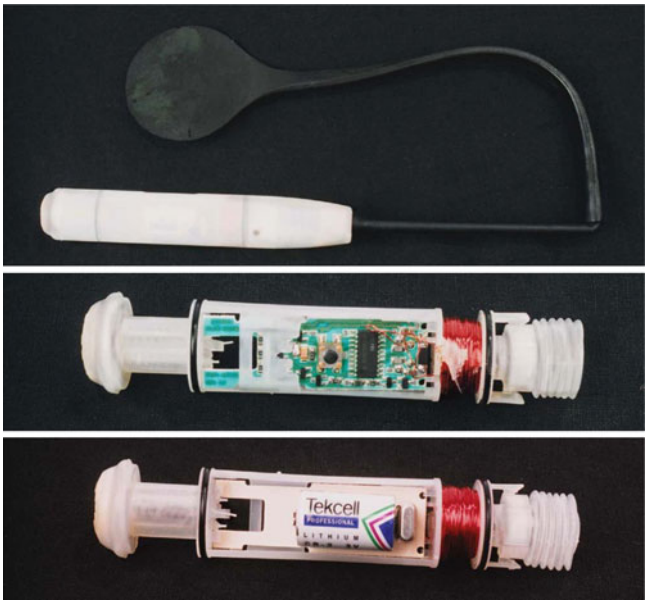

Fig. 11.10 Smart1 electronically controlled multi drug releasing intravaginal insert for cattle

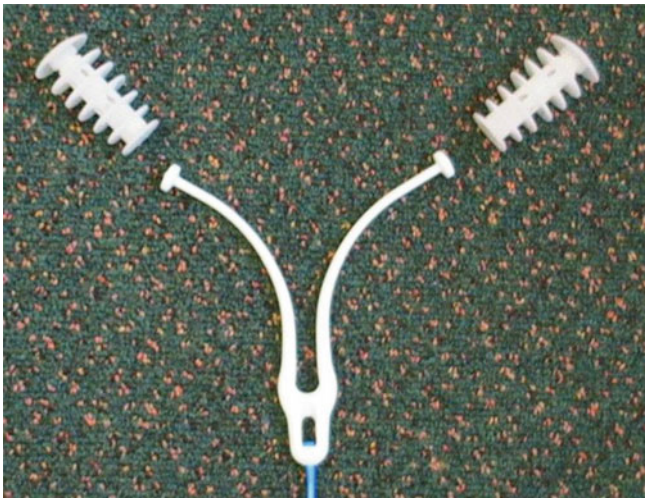

Fig. 11.11 Cue-Mate showing removable pods and reusable spine

11.7.6 Cue-Mare

The Cue-Mare is an intravaginal insert formulated for horses. It has been shown to be useful in transitional mares that have a history of estrus behavior of longer than 10 days and follicles of less than 25 mm [53]. The insert has been reported to produce an 87% ovulation response rate within 7 days of insert removal and a 52.9% conception rate for mares bred after insert removal. The insert has been shown to maintain an initial blood level of >2 ng/mL for 5 days and terminal blood levels above 1 ng/mL for 10 days [53].

11.7.7 TRIU-B

The TRIU-B is an intravaginal insert marketed for sale in South America (Fig. 11.12).

It is impregnated with 1 g of progesterone. The TRIU-B has a unique cross-shaped structure with anatomic non-sliding bands, and its inner structure with a new poliamidic formulation allows for better anchorage, while minimizing device losses and local inflammatory reactions [54].

11.7.8 DIB-V Intravaginal Insert

The DIB-V Intravaginal Insert has a V-shape design and comprises a pliable silicone elastomer which contains1.0 g of progesterone [55]. It is used for the regulation of the estrus cycle, treatment of postpartum, and to decrease the inter-calving interval in heifers and cows (Fig. 11.13).

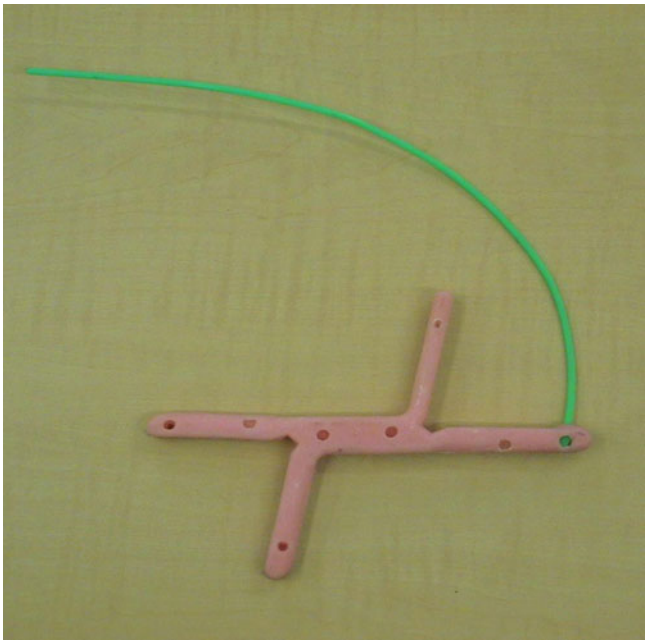

Fig. 11.12 TRUI-B showing alternating arms to assist retention in cattle

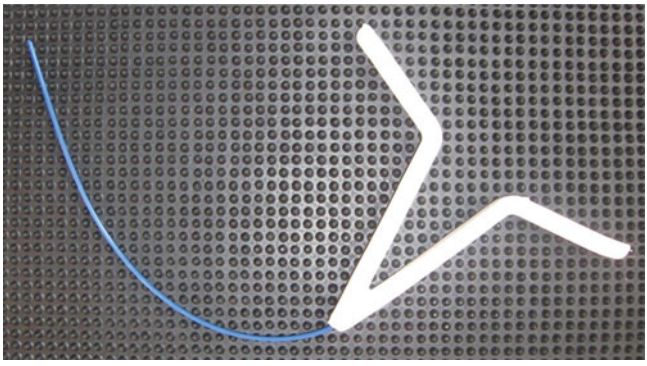

Fig. 11.13 DIB-V showing unique V shape and plastic tail

11.7.9 PRID Delta

The PRID Delta is a triangle-shaped intravaginal insert containing progesterone homogenously distributed throughout the silicone that is manufactured by a bi-injection process (Fig. 11.14).

Fig. 11.14 PRID Delta showing triangular shape and filament tail

11.8 Future Prospects

There is very little point of differentiation between the existing cattle inserts currently marketed. All provide the same (or very similar) blood profiles, result in the same efficacy, and exhibit very similar environmental, human, and animal safety profiles. Price is determined by manufacturing costs, but these are similar for all existing products. All contain similar amounts of progesterone and silicone, and are therefore similarly priced. Because of the high residual progesterone remaining in the inserts upon removal, an opportunity to reduce drug load and therefore cost of manufacture exists. However, Rathbone and coworkers [47–49, 56–58] investigated a range of physical parameters that affected progesterone release from silicone matrix. These authors demonstrated that a reduction in drug load resulted in a reduction in release rate and a corresponding lowering of plasma levels below 2 ng/mL [47–49, 57]: the level required to be sustained to ensure efficacy of the treatment [2]. Interestingly, raising the drug load above 10% w/w did not increase blood plasma levels [47–49, 57]. Surface area had an effect on plasma progesterone concentrations [47–49, 57]; however, the surface area of the insert is limited by the anatomy of the animal vagina. Silicone skin thickness is limited by manufacturing constraints. Rathbone concluded that there was little scope for optimizing a silicone insert any more than the physical and chemical properties associated with the CIDR 1380 Cattle Insert. However, a recent publication demonstrated that it is possible to reduce the drug load of silicone inserts resulting in a lower residual while still

successfully delivering the amount of progesterone required (about 0.6 g) to maintain plasma progesterone levels above 2 ng/mL [59]. Silicone polymers are expensive to purchase, and there is an inherent need to incorporate a high amount of progesterone into a silicone polymer-based intravaginal insert. Therefore, there exists the potential for an estrous control product for sheep and/or cattle whose cost of manufacture is cheaper compared to current products. If such a product could be designed and developed, even though it would offer no advantage over existing products in terms of efficacy and safety, it could compete with existing products based on a price differential and would find uptake by the end user.

The potential exists for a much wider use of vaginal drug delivery systems than that which currently exists within the livestock industry. The short list of suitable drug candidates is a limitation for this route of veterinary drug delivery. Drugs must exhibit certain inherent physicochemical characteristics in order to be useful for this route. These include rapid absorption characteristics, be effective in relatively small doses, have a low minimum effective concentration, exhibit some degree of solubility in the vaginal fluids, be sufficiently stable under the conditions of manufacturing and upon storage, have a wide margin of safety, be nontoxic to the end user, be nonirritant to the vaginal mucosa, and be compatible with the polymer selected for the delivery system.

The cost effectiveness and availability of new materials and the development of innovative delivery systems may lead the way toward extending the drug candidate list to drug delivery in the vagina of animals. A commercially available estrous control product for use in pigs would service a market need. Incorporation of other drugs, in addition to progestins, into vaginal drug delivery platforms, and delivery of such compounds in desired amounts for required durations offers future opportunities.

As is common with other mucosal routes for drug delivery, the bioavailability of many compounds is relatively low via the vagina, but the ability to retain delivery systems in the vagina of farmed animals provides an unexploited opportunity to deliver a range of pharmacologically active compounds, which may be extended further by the co-administration of permeation enhancers [60].

Electronically modulated drug delivery of the type illustrated by the Intelligent Breeding device and Smart1 holds promise for application in cases where multiple drugs are needed and which are administered at different times in the treatment program. Each intervention costs money in terms of drug products and veterinarian time, travel and expertise. The development of a device that combines several drugs can slow release or pulse on demand, has the capability to be manufactured at low cost per treatment, and offers opportunity for potential exploitation. Such a device is described by Bunt et al. [61] who also describe some of the challenges during its development [61–65]. Conceptually, such a device has endless possibilities such as estrus detection and automated estrus control using telemetry [66].

11.9 Concluding Remarks

This chapter has described the current status and highlighted the technology needs in the area of intravaginal veterinary drug delivery that, if filled, could extend the use of the vagina of livestock as a route of administration and make available a wider range of products that addresses a wider spectrum of clinical conditions to those working in the animal health, production and reproduction industries. Novel intravaginal drug delivery technologies will need to be further developed and commercially supported to meet existing and future clinical needs.

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Chapter 12
Veterinary Long-Acting Injections and Implants

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Abstract The animal health industry has a strong interest in controlled release parenteral formulations and has been very receptive to innovations in drug delivery technology for both production and companion animals. These dosage systems minimize the need for repeated injections while giving therapeutic benefit for extended periods of time making commercial development of these products worthwhile. This review chapter highlights the variety of long-acting injectable and implant technologies that have been investigated and commercialized for veterinary applications.

12.1 Introduction

Controlled release parenteral dosage forms have been of interest to the animal health industry for many years and have been discussed in several reviews [1–8]. Many different technologies have been evaluated for long-acting injectables and implants, some of which have led to commercial successes.

The long-acting dosage forms offer many advantages: reduced stress levels in the animals associated with less frequent dosing, improved patient and owner compliance, reduced or more manageable side effects on the patient, as well as patent protection for the product innovator.

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This review will discuss the technologies that have been investigated and highlight some of the commercial products in the global animal health marketplace. From the authors' viewpoint, the marketed long-acting injectable and implant formulations can be classified into the following categories: (1) aqueous dispersions or solutions; (2) oily injections; (3) in situ depots; (4) microspheres; and (5) implants. This review will go through each formulation technology with a listing of currently marketed products followed by a description of selected products reported in the literature. The majority of information for these tables was captured from product labels and websites of regulatory agencies [9–14].

12.2 Excipient Choices

Long-acting injectables and implants used in animal health typically utilize biocompatible and safe excipients in the formulations. Novel and proprietary excipients are evaluated for safety and biocompatibility either by themselves or in the finished product testing [15]. Formulators usually choose excipients from the GRAS (generally recognized as safe) listing [16]. To assist in excipient selection, there are literature reviews that describe solvent systems and other excipients used in parenteral products [17, 18] and books describing pharmaceutical ingredients [19–21]. The FDA Inactive Ingredients Database is another useful tool [22]. The 2005 FDA guidance from the centers associated with human health describes expected preclinical testing required for these novel excipients [23].

12.3 Aqueous Dispersions and Solutions Dependent on Drug Properties

There are several animal health long-acting injectable aqueous formulations available in the market. Two examples of these are listed in Table 12.1. The long-acting aspects of these drug products are not enabled by the formulation but rather by the drug properties themselves.

The PROGRAM® 6 Month Injectable for cats is a prepackaged ready-to-use syringe containing a sterile suspension of 10% lufenuron, whereas CONVENIA® is a reconstitutable lyophilized product containing the cephalosporin cefovecin sodium for use in cats and dogs. The CONVENIA formulation contains methyl and propyl parabens as preservatives, sodium citrate and citric acid as buffers, as well as sodium hydroxide or hydrochloric acid for pH adjustment. The diluent (10 mL) contains 13 mg/mL of benzyl alcohol, as a preservative, and water. The antimicrobial activity of the drug product lasts for up to 14 days [24]. Cefovecin has an exceptionally long half-life in dogs and cats because the drug binds to plasma proteins which acts as a reservoir for the drug [25].

Table 12.1 Aqueous-based marketed animal health long-acting injectables

Tradename (API)	Company	Indication	Species	Government references	US Patent references	Duration[a]
PROGRAM® 6 Month Injectable (Lufenuron)	Novartis AH	Flea control	Cats	NADA # 141–105 APVMA 49630 APVMA 49631	4,798,837 5,416,102 5,240,163	6 M
CONVENIA® (Cefovecin sodium)	Pfizer	Antibiotic	Dogs; Cats	NADA# 141-285, EMEA/V/C/000098	6,020,329 7,378,408	14 D

Note: This is not an exhaustive list of products or information for each product
[a]*D* day; *M* month

Other aqueous formulations include PACCAL® Vet which is a water-soluble formulation of paclitaxel. This veterinary oncological product is based on Oasmia's micellar XR-17 platform which does not require premedication and abolishes CREMOPHOR® EL related side effects. This product candidate is currently in trials for the mastocytoma indication in dogs [26]. Recently, the FDA granted PACCAL Vet with the Minor Use Minor Species (MUMS) designation (see Sect. 12.8) for treatment of resectable and non-resectable squamous cell carcinoma in dogs [27]. In addition, a formulation of doxorubicin (DOXOPHOS® Vet), also based on Oasmia's micellar platform, has entered clinical trials.

12.4 Oily Based Injectables

A suspension or solution of active ingredient in oil and/or nonaqueous solvent system is another formulation approach to long-acting injectables. Several chapters and reviews discuss this topic in detail [28–30]. There are many oils and nonaqueous solvents to choose from, including fixed oils (e.g., olive oil, sesame oil, castor oil), isopropyl myristate, ethyl oleate, benzyl benzoate, polyethylene oleic triglycerides, thin vegetable oil (fractionated coconut oil), and liquid polyethylene glycols (PEGs). For detailed properties, see Murdan and Florence's chapter or the review by Spiegel and Noseworthy [17, 30]. Drugs are formulated in oily vehicles for several reasons including low solubility in aqueous media, irritation of aqueous formulations, drug instability in aqueous media, or the desire to attain sustained release. The intramuscular (IM) route of administration is suggested because subcutaneous (SC) injection is sometimes associated with pain on injection or irritation [30].

Upon subcutaneous injection, the majority of the water-immiscible oil is confined to the injection site. A depot is formed, usually in the shape of a flattened oblate or prolate spheroid similar to a lentil or football, respectively. The shape determines the surface area which is thought to be a critical factor in drug absorption. The drug usually absorbs faster than the oil. The rate of drug absorption from various oily solvents is controlled by the distribution coefficient (K) of a compound between an oily solvent and saline [29]. Although a slow clearance rate of oil is beneficial, the oil should be totally absorbed from the site in order to decrease accumulation and side effects [30].

When developing an oil-based system, the ideal oil is stable and nonreactive to the drug, biocompatible, a good solvent or dispersing agent for the drug, and inert with respect to the primary packaging [30].

Selected products based upon lipophilic or nonaqueous solvent systems are detailed in Table 12.2.

Some simple approaches were evaluated during the development of long-acting oxytetracycline (OTC) formulations [32]. The approaches were essentially a variation on the theme used for procaine or benzathine penicillin where prodrugs are prepared that are less soluble or insoluble at the injection site and are slowly dissolved and absorbed from the injection site.

Table 12.2 Lipophilic/nonaqueous-based marketed animal health long-acting injectables

Tradename (API)	Company	Indication	Species	Government reference	US Patent References	Known excipients	Duration
EXCEDE® (Ceftiofur crystalline free acid)	Pfizer (Pharmacia & Upjohn Co)	Antibiotic	Cattle, swine	NADA # 141-209, 141-235	5,721,359; 7,829,100		7 D
LIQUAMYCIN® LA-200® (Oxytetracycline)	Pfizer	Antibiotic	Cattle, swine	NADA # 113-232	4,018,889	2-pyrrolidone [4]	
OXY-TET® 200 and BIO-MYCIN® 200 Injectable Solutions (Oxytetracycline)	Vetmedica (Boehringer Ingelheim)	Antibiotic	Cattle, swine	ANADA # 200-008	–	PEG 400, Sodium formaldehyde sulfoxylate, magnesium oxide, water, monoethanolamine	
PEN BP-48® (Penicillin)	Teva Animal Health	Antibiotic	Cattle	NADA # 065-498	–	–	
COBACTAN™ LA (Cefquinome sulfate)	Merck (Legacy Intervet SPAH)	Antibiotic	Cattle, swine				2 D
POSILAC® 1 Step (Sometribove Zinc, bovine somatotropin)	Elanco (Legacy Monsanto)	Milk enhancer	Dairy cattle	NADA # 140-872	5,739,108		14 D
IVOMEC® Gold (Ivermectin)	Merial Limited (A Sanofi Company)	Parasiticide	Cattle	CRMV-SP 3085; SAGARPA Q-3596-12	–	distilled acetylated monoglycerides/triacetin/ hydrogenated castor oil 55/40/1	42–140 D
CYDECTIN® LA 10% Cattle (Moxidectin)	Pfizer (Legacy Fort Dodge)		Cattle	APVMA 60116	–		
CYDECTIN 2% Sheep (Moxidectin)	Pfizer (Legacy Fort Dodge)		Sheep	APVMA 58532	–		
DECTOMAX® Injectable Solution (Doramectin)	Pfizer	Parasiticide	Cattle, swine	NADA # 141–061	5,089,480; 6,001,822		
MICOTIL (Tilmicosin phosphate)	Elanco	Antibiotic	Cattle, sheep	NADA # 140–929	4,820,695; 5,574,020	PG, water [31]	3 D
NUFLOR® (Florfenicol)	Merck (Legacy Intervet SPAH)	Antibiotic	Cattle	NADA # 141–063	4,235,892; 5,082,863	NMP,PG/PEG solution [4]	
NUFLOR GOLD® (Florfenicol)	Merck (Legacy Intervet SPAH)	Antibiotic	Beef and non-lactating dairy cattle	NADA # 141–265		2-pyrrolidone/ triacetin solution	
ONSIOR® (Robenacoxib)	Novartis	Anti-inflammatory		EMEA/V/C/000127		PEG400/ethanol with poloxamer 188	

Note: This is not an exhaustive list of products or information for each product

Table 12.3 Drug chemistry, formulation, and administration route effect on testosterone esters

Ester	Formulation	Route of administration	Duration of effect
Testosterone propionate	Oil solution	IM	3–4 days
Testosterone *n*-valerate	Oil solution	IM	9 days
Testosterone propionate	Emulsion	IM	3–4 days
Testosterone propionate	Small crystals	IM	8 days
Testosterone propionate	Large crystals	IM	12 days
Testosterone propionate	Pellet	SC	4–5 days

Early work also evaluated different testosterone esters (for example, propionate or *n*-valerate) and showed how the duration of release of steroid esters can be varied via either different prodrugs or different formulations of the same prodrugs as shown in Table 12.3 [33, 34].

A 7.5% suspension of cefquinome was developed for injection in cattle for the treatment of bovine respiratory disease associated with *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* sensitive to cefquinome. The product is registered in the EU as COBACTAN™ LA 7.5% and is formulated in medium chain triglycerides thickened with aluminum stearate. Cefquinome sulfate is an antibacterial of the cephalosporin group which acts by inhibition of cell wall synthesis. It is characterized by its broad therapeutic spectrum of activity. As a fourth-generation cephalosporin, it combines high cellular penetration with high stability against beta-lactamases which predict a lower probability for selection [35].

NUFLOR GOLD® is an injectable solution of the synthetic antibiotic florfenicol in 2-pyrrolidone and triacetin [36]. It is used in beef and non-lactating dairy cattle for the treatment of bovine respiratory disease associated with *Mannheimia haemolytica, Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* [37].

EXCEDE® (ceftiofur crystalline free acid) for swine and horses is a single dose injectable long-acting antibiotic treatment against respiratory disease clinically proven to deliver at least 7 days of therapeutic plasma levels [38, 39]. The formulation is based on ceftiofur in a crystalline form in a ready-to-use sterile suspension in oil [40].

EXCENEL® RTU for beef and lactating dairy cattle and swine is a ready-to-use formulation of the hydrochloride salt of ceftiofur, a broad-spectrum cephalosporin antibiotic. Each milliliter of the suspension contains ceftiofur hydrochloride equivalent to 50 mg ceftiofur, 0.5 mg phospholipon, 1.5 mg sorbitan monooleate, 2.5 mg sterile water for injection, and cottonseed oil [41].

Significant work on the controlled release delivery of bovine and porcine growth hormone (somatotropin) was undertaken in the animal health industry in the 1990s. Several reviews summarized the many types of long-acting delivery systems that were investigated [42–44]. One commercial product in the marketplace is POSILAC® (sometribove zinc suspension for injection), a sterile, prolonged-release subcutaneously injectable, oil-based formulation in single-dose syringes, each containing 500 mg sometribove zinc [45]. The composition is shown in Table 12.4. The active ingredient, recombinant bovine somatotropin (rbST), is biologically equivalent to the natural pituitary-derived somatotropin, and is used as a supplement that increases milk production in healthy lactating cows [47].

Table 12.4 Composition of POSILAC® [46]

Component	Content (%)
Sometribove Zinc	37.4–39.4
Aluminum monostearate	3.03–3.13
Oil, food grade	57.57–59.47

A range of oily LA injectable antiparasitic formulations for ruminants has been brought to the market in recent years, spanning the major types of macrocyclic lactone compounds.

The first generation of long-acting ivermectin formulations (1% w/v) was an oil-based preparation (propylene glycol/glycerol formal 60:40) which provided a slow absorption from the subcutaneous space and an extended persistence of concentration in the bloodstream and tissues of parasite location.[48] A concentrated (3.15%) ivermectin long-acting injectable solution (IVOMEC® Gold) [49] has been introduced into several veterinary pharmaceutical markets. The product is administered to cattle at 630 μg/kg. IVOMEC Gold formulation exhibits a unique thixotropic effect, where solution agitation causes a decrease in formulation viscosity, thus facilitating application of the product, indirectly helping to slow absorption and provide the optimal plasma profile. Because of its pharmacodynamic characteristics of absorption and slow excretion, IVOMEC Gold reaches maximum effectiveness against ticks from 14 to 17 days after application, with control of this parasite persisting for up to 75 days [50].

Another example of an oily injectable formulation is the CYDECTIN® LA Injectable family of products containing moxidectin as antiparasitic agent. The long-acting effect of moxidectin is due to its lipophilic nature and its ability to be absorbed by body fat and be released over time into the bloodstream. The CYDECTIN 10% Long Acting Injection for Cattle is an oil-based, clear, yellow, slightly viscous, sterile solution containing 100 mg/ml moxidectin and 70 mg/ml benzyl alcohol, formulated as a subcutaneous injection to be administered in the dorsal surface of the ear of cattle [51–53]. CYDECTIN 2% LA for Sheep contains 20 mg/ml of moxidectin and is injected in the base of the ear of the sheep [54].

DECTOMAX® Injectable Solution [55] is a ready-to-use, sterile solution containing 1% w/v doramectin (10 mg/mL) in an oily base of ethyl oleate (<25%) and sesame oil (>70%).[56] DECTOMAX Injectable Solution is indicated for the treatment and control of gastrointestinal roundworms, lungworms, eyeworms, grubs, sucking lice, and mange mites. In cattle, DECTOMAX is formulated to deliver the recommended dosage (200 μg/kg of body weight) when given by SC or IM injection at the rate of 1 mL/110 lb of body weight. This product also has gained approval for use in swine for IM injection [57].

12.5 In Situ Depots

In situ forming depots are delivery systems that can easily be injected into both production and companion animals. They are relatively low-viscosity liquids prior to injection which undergo a rapid change in physical form upon injection

subcutaneously in the animals. Several reviews [58, 59] have described the basic principles of these systems. Over the years, many types of in situ forming systems have been evaluated and reported for veterinary applications, including, for example, those based on biodegradable polymer precipitation (e.g., ATRIGEL® [60]), sucrose acetate isobutyrate (SAIB) depot formation [61], and lipid-based liquid crystalline phase transitions [62]. Several of these technologies have been used for commercial products, as described in Table 12.5.

Merial recently received regulatory approval for a 5% eprinomectin-based LAI cattle product from the FDA [63]. LONGRANGE™ is based on the biodegradable polymer precipitation technology. It has durations of persistent effectiveness ranging from 100 to 150 days for lungworm and roundworms [64]. According to the Freedom of Information Summary, LONGRANGE contains 5%w/v eprinomectin, 5% poly(lactide-co-glycolide) (PLGA), and N-methyl-2-pyrrolidone (NMP) [65].

Another product utilizing in situ forming depots with biodegradable polymer precipitation technology is made by SR Veterinary Technologies, LLC. SR Veterinary Technologies LLC, a subsidiary of Wildlife Pharmaceuticals, has a pipeline of sustained release products for veterinary applications. The SR Veterinary delivery system is a biodegradable liquid polymer, a nonaqueous diluent, with the drug in solution or suspension within the polymer. The drug slowly releases over time as it diffuses from the polymer matrix and the polymer degrades [66]. Buprenorphine SR is already available for compounding through the custom pharmacy division of Wildlife Pharmaceuticals, ZooPharm. Buprenorphine SR releases over 72 h [67]. The biodegradable polymer delivery system is a 50:50 (w/w) solution of a polymer dissolved in NMP [68, 69]. The biodegradable polymer is a copolymer with a 50:50 molar ratio of DL-lactide to ε-caprolactone. The copolymer has an average molecular weight of approximately 5,500 Da [68].

Thorn BioScience, LLC focuses research on controlled release animal pharmaceuticals through two delivery systems developed with Southern Research Institute (SRI) and Southern BioSystems (SBS). “The delivery system utilizes sucrose acetate isobutyrate (SAIB), a fully esterified sucrose molecule which is a highly viscous and highly hydrophobic, resin-like material in pure form. The addition of small amounts of organic solvents to SAIB greatly reduces its viscosity to an injectable consistency for drug delivery. Once injected, the organic solvent dissipates into the tissues leaving behind a SAIB/drug delivery matrix which releases the drug by diffusion. SAIB has been used to deliver actives for periods of up to 1 month. It is degraded by enzymatic hydrolysis into sucrose and its aliphatic acids. In addition, SAIB has bio-adhesive qualities which have been utilized to deliver therapeutics topically and to deliver antigens on mucosal surfaces” [70]. A patent by Tipton et al [71] discloses formulation approaches with SAIB. A simple approach is mixing the SAIB with a low-viscosity solvent. SAIB is a direct food additive approved in over 40 countries [72].

An example of a commercial product based on the in situ depot of SAIB in the animal is SUCROMATE®. SUCROMATE Equine, 1.8 mg/mL of deslorelin acetate, is an intramuscular injectable sustained release gonadotropin releasing hormone (GnRH) analog for use in horses. It is prepared as a suspension in SAIB

Table 12.5 In situ depot-based marketed animal health long-acting injectables

Tradename (API)	Company	Indication	Species	Government reference	US Patent reference	Duration
LONGRANGE™ (Eprinomectin)	Merial Limited (A Sanofi Company)	Parasiticide	Cattle	NADA # 141–327	6,733,767	100–150 D
SUCROMATE® Equine (Deslorelin acetate)	Thorn Bioscience	Ovulation	Horses (mares)	NADA # 141–319	6,051,558	2 D
BUPRENORPHINE SR™ (Buprenorphine hydrochloride)	SR Veterinary (A Wildlife Pharma Company)	Pain management	Cats, dogs, exotic species, lab animals	N/A (compounded)	–	3 D

Note: This is not an exhaustive list of products or information for each product

and propylene carbonate in a 70:30 w/w ratio. The drug product is indicated for inducing ovulation within 48 h of treatment in cyclic estrous mares with an ovarian follicle between 30 and 40 mm in diameter. This product is intended to optimize breeding management [73, 74].

12.6 Microspheres

Over the years, substantial effort was put into developing and understanding microsphere-based drug delivery systems [75]. This field has gained significant interest in the animal health industry with some commercialized products. Selected products are described in Table 12.6.

For dogs noncompliant with the monthly heartworm preventative oral treatments and for pet owners with preference for less frequent administration, the injectable sustained release dosage product PROHEART®6 and PROHEART 12 SR are available heartworm preventatives. PROHEART 6 is a sustained release (SR) formulation of moxidectin-impregnated microspheres injected subcutaneously, providing single dose continuous protection against heartworm for 6 months. The product consists of two separate vials: Vial 1 containing 10% moxidectin sterile microspheres is constituted with Vial 2, a sterile vehicle (see Table 12.7 for suspension composition). The subcutaneous dose is 0.05 mL of constituted suspension/kg body weight [76]. PROHEART 6 remains the only 6-month injectable heartworm preventive approved in the United States.

It must be noted that PROHEART 6 has been a controversial product since its US approval in 2001. PROHEART 6 was voluntarily recalled by the manufacturer in 2004 based on FDA's concerns regarding reports of serious side effects and reintroduced to the US market in June 2008 under a risk minimization and restricted distribution program (RiskMAP) [77] with post-approval label and Client Information Sheet revisions. The decision was based on the results of toxicologic studies coupled with the lower adverse event frequency in international markets. PROHEART 6 is the first veterinary drug marketed under a RiskMAP that allows veterinarians and the owners to weigh the risk with the benefits of a 6-month injection that maximizes protection. Veterinarians enrolled in this program completed in-depth training in order to participate. A similar product, PROHEART SR-12 Injection Once-A-Year Heartworm Preventative for Dogs, which contains approximately three times the dose of moxidectin as PROHEART 6, is registered in Australia and marketed there since October 2000 [78].

An article written by PR Pharmaceuticals, Inc. was published in 2004 regarding ivermectin loaded PLGA microparticles in dogs. PLGA with a lactide:glycolide molar ratio of 85:15 and a molecular weight of 136 kDa was utilized. Ivermectin was loaded at levels of 15, 25, 35, and 50 wt% within the PLGA matrix and dissolved in ethyl acetate. This solution was mixed with 1 % polyvinyl alcohol to form an oil-in-water emulsion. The resulting microspheres were injected in a 2.5 wt% carboxymethylcellulose in water with methylparaben. Dogs were dosed at 0.5 mg/kg ivermectin

Table 12.6 Microsphere-based marketed animal health long-acting injectables

Tradename (API)	Company	Indication	Species	Government reference	Patent reference	Known excipients	Duration
PROHEART® 6 (Moxidectin)	Pfizer (Legacy Fort Dodge, American Cyanamid)	Heartworm	Dogs	NADA # 141-189	6,340,671	Fat based microspheres	6 M
PROHEART 12 SR (Moxidectin)	Pfizer (Legacy Fort Dodge)	Heartworm	Dogs	APVMA 51805	–	Fat based microspheres	12 M
SMARTSHOT™ Family (Vitamin B12 and Selenium)	Novartis and AgResearch Limited	Growth	Sheep	APVMA 51158	NZ329447; AUS711760	Arachis (peanut) oil	8 M
Celerin™ (Estradiol benzoate)	PR Pharmaceuticals	Growth	Cattle	NADA # 141-040; 141-041	5,288,496; 5,401, 507; 5,427,796	–	12 M

Note: This is not an exhaustive list of products or information for each product

Table 12.7 Composition of the PROHEART® 6 reconstituted drug product (per mL) [76]

Component	Content (per mL)
Moxidectin	3.4 mg
Glyceryl tristearate	3.1%
Hydroxypropyl methylcellulose	2.4%
Sodium chloride	0.87%
Methylparaben	0.17%
Propylparaben	0.02%
Butylated hydroxytoluene	0.001%

administered subcutaneously. Drug released for at least 287 days, and heartworms were not found in any ivermectin microsphere treated group [79].

The SMARTSHOT™ family of injections has Vitamin B12 encapsulated within PLGA microspheres by solvent evaporation. The PLGA referenced in the patent is in a molar ratio of 95:5 with an inherent viscosity of 0.70 dl/g at 30°C in chloroform. These microspheres are suspended in peanut oil with the help of a resuspending agent [80].

One research project utilizes encapsulation in a nonconventional way. Eden Research PLC announced in early 2012 that Teva Animal Health had formally decided to enter into a licensing agreement for natural encapsulation [81]. Eden has developed an encapsulation technology using yeast cells to deliver a slow release of terpenes for agricultural and nonagricultural uses. The technology is the encapsulation of terpenes in either hollow β-glucan particles or cell wall particles. The press release discusses the use of yeast cells, which are composed of biopolymers β-glucans, mannan, and chitin. The terpene payload is released on contact with moisture. Pores close as the particle dries, trapping the remaining terpenes [82, 83]. According to Eden, the sustained release of the terpenes overcome their volatility, phytotoxicity, and poor solubility [81].

12.7 Implants

Implants have long been used in animal health, mainly for production enhancement in cattle. Since the 1950s, compressed tablet implants containing estrogenic anabolic steroids have been administered for 2–3-month intervals to improve feed conversion and the rate of weight gain in beef cattle. The economic benefits of growth enhancers have been well documented. Commercially available implants used in cattle production are inserted under the skin of the back side of the ear where they slowly release the drugs into the animal's systems. The number and size of the implants vary among commercial products, as do the commercial devices for implanting. Current products contain similar types of ingredients—an estrogen or estrogen-like drug that stimulates growth hormones in the animal's pituitary gland. The technical product use literature stresses the importance of proper implanting technique as the key to obtaining consistent productivity results. Examples of some commercially available implant products are shown in Table 12.8.

Table 12.8 Marketed animal health long-acting injectable implants

Tradename (API)	Company	Indication	Species	Government reference	US Patent reference	Duration
RALGRO® family (Zeranol)	Merck AH (Legacy Intervet)	Growth	cattle, sheep	NADA # 038-233, 141-192	–	
REVALOR® family (Trenbolone acetate / Estradiol)	Merck AH (Legacy Roussel UCLAF)	Growth	cattle	NADA # 140-897, 140-992	4,192,870	
REVALOR® XS (Trenbolone acetate / Estradiol)	Merck AH (Legacy Intervet, Roussel)	Growth	cattle	NADA # 141-269	6,498,153	200 D
FINAPLIX® family (Trenbolone acetate)	Merck AH (Legacy Intervet)	Growth	cattle	NADA # 138-612	3,939,265	
COMPUDOSE® (Estradiol / Oxytetracycline)	Elanco (Legacy Ivy Animal Health)	Growth	cattle	NADA # 118-123	4,191,741	
SYNOVEX® (Trenbolone acetate / Estradiol)	Pfizer (Legacy Wyeth/Fort Dodge)	Growth	cattle	NADA # 009-576, 011-427, 141-043, 200-367	–	
SMART GUARD™ (Ivermectin)	Virbac	Heartworm	dog	*SAGARPA Q-0042-357	–	12 M
SUPRELORIN® family (Deslorelin acetate)	Virbac (Peptech Limited)	Ovulation	dog	EMEA/V/C/000109	–	6 or 12 M
OVUPLANT™ (deslorelin acetate)	Peptech Limited	Ovulation	Horses (mares)	Dechra NADA # 141-044	5,545,408	

The mechanism of API release from the compressed tablet drug product (manufacturing technology used for FINAPLIX®, SYNOVEX®, RALGRO®, and REVALOR® implants [84]) is slow erosion. COMPUDOSE® implants are made in a continuous process by coating a nonmedical silicone core with a thin layer of silicon containing micronized crystalline estradiol-17β [85]. The strand is heat-cured and then cut into 3 cm lengths. The estradiol-17β is released from the matrix by diffusion. Because of the problems with implant retention in the ear, the implants were coated with the antibiotic oxytetracycline (OTC) to prevent infection at the injection site. The OTC addition significantly improved the overall implant retention [86].

REVALOR XS is a 200-day implant developed for steers fed in confinement. Each pellet contains 20 mg of trenbolone acetate and 4 mg of estradiol for a total dose of 200 mg of trenbolone acetate and 40 mg of estradiol. Each dose contains four uncoated pellets (for the first 70–80 days of release) and six pellets coated with biodegradable polymer coating which provides the prolonged release through the remainder of the 200-day duration [87]. Quality control of the coating process is a critical parameter during the manufacturing process [88]. The REVALOR website [89] indicates that Near-Infrared (NIR) Spectroscopy is used in the manufacturing process for real-time in-process assessment of the polymer coating layer thickness. This in-process analytical technology tool is utilized in the manufacturing process. The NIR probe is located inside the coater which allows for continuous monitoring of the coating thickness during the coating manufacturing process.

These implant products are labeled for the US market as being "manufactured by a non-sterilizing process." In 2000, the Center for Veterinary Medicine issued a policy letter revising their Policy and Procedures Manual Guide 1240.4122 describing the sterility and pyrogen requirements for veterinary injectable drug products. The revision includes additional information referencing the USP and the need for validated processes that assure sterility and pyrogen levels that are within established limits [90]. The policy states, "It is the policy of the Center for Veterinary Medicine that (1) all injectable drug products (including intra-mammary products) be sterile except euthanasia products and the ear implants for bovine and ovine species, and that (2) pyrogen levels in sterile veterinary drugs should not exceed established limits. Approved products (including intra-mammary) which fail to meet the requirements for sterility and pyrogens must be labeled as being 'manufactured by a non-sterilizing process.'"

The use of implants for delivery of parasiticides has been reported in the literature [91]. Ivermectin released from a crosslinked poly(ortho ester) matrix over a 6–12-month duration has been shown to be effective in canine heartworm prophylaxis.

A long-acting ivermectin implant was developed by Smart Drug Systems [92–94] based on a matrix-type cylindrical rod having an inner core of ivermectin with excipients and silicone which was covered in silicone. Linear ivermectin release was demonstrated from ivermectin implants in rats. Since ivermectin is a hydrophobic drug, the researchers used additives in the inner core to increase the solubility of

the ivermectin as the infiltrating fluids penetrated the implant. The duration of release was prolonged with the addition of additives (e.g., polyethylene glycol 4000 to 3 months or desoxycholate sodium to over a year). This served as the basic technology behind the SMART GUARD™ ivermectin implant.

SUPRELORIN®, a long-acting cylindrical implant containing deslorelin acetate, a synthetic gonadotrophin-releasing hormone analogue in a matrix consisting of hydrogenated palm oil and lecithin, is used in male dogs to make them temporarily infertile [95]. The manufacturing process involves compression, slugging, dry granulation, low-temperature extrusion, and cutting into the proper implant length with a rotary cutter. The implants are loaded into the implanters, individually sealed into laminate foil pouches, and sterilized by electron beam irradiation.

Another type of growth promoting implant was fabricated by either direct compression or by extrusion from castorwax, ethylcellulose, and cimaterol [96]. Implants releasing an average of 0.72 mg cimaterol/day for 6 weeks were implanted into lambs. Feed efficacy and carcass quality were compared with animals given controls (placebo implants) or medicated feed. The 6-week study showed the SC implants were 10–20-fold more efficacious than the oral administration in promoting growth and reducing fat deposition in the lambs.

Mechanical type devices have been used as prototype injectable delivery systems in animals as well. These usually have been fabricated with a nondegradable biocompatible outer material. One example consisted of an impervious polystyrene casing over a multi-dose capsule containing a stack of active ingredient and excipient spacer pellets. Moxidectin was compressed into pellets and placed into a hydrogel-based delivery device which expanded on contact with the body fluid and served as the driving mechanism for the moxidectin drug release [97]. In vivo release was monitored in sheep indirectly using fecal egg counts, and the results demonstrated that the sustained release device could control nematode diseases in livestock throughout an entire season with a single administration.

A variety of fatty acid salts of a synthetic growth hormone releasing hexapeptide were formulated into implants using direct compression and coated with different biocompatible polymers leaving the short ends exposed. The daily release rate in vitro was shown to be dependent on the molecular weight of the fatty acid, the higher the molecular weight, the less soluble the derivative, and the slower the daily release rate. The absolute rate of hexapeptide released was shown to be dependent on the ratio of the total hexapeptide to implant surface area which was modified using a variety of biodegradable or nondegradable polymers to give zero-order release. Radiolabeled implants were implanted into cattle. The in vivo implants released an average of 3.2 mg of hexapeptide daily giving good correlation with the 3.1 mg released in vitro over the 43-day study [98].

A variety of uncoated and coated implants were reported in the literature for the delivery of porcine somatotropin (PST) [43]. For example, Clark et al prepared complexes of aromatic aldehydes and PST and compressed them into implants which were injected SC behind the ear in swine. An increase in terms of both

average daily weight gain and feed-to-gain (F/G) at the end of the 21-day study was seen over the sham control [99]. Steber prepared poly(ethylacrylate methylmethacrylate) partially coated layered PST implants which gave 27-day sustained release of PST when implanted in pigs [100].

Recently a number of nontraditional implants were moved into a late-stage development or have been already introduced into the market.

Royer Animal Health, LLC, a subsidiary of Royer Biomedical, Inc., developed implantable MIII Cisplatin Beads (INAD 10-829), indicated for equine sarcoids [101]. The product, resorbable cisplatin beads (~3 mm), utilizing Royer Biomedical's Matrix III and R-Gel controlled release drug matrix, has been shown to be safe and efficacious in clinical use. An application for MUMS approval is pending.

TR-CLARIFEYE™ (by Healionics Corporation and marketed by TR BioSurgical, LLC) [102, 103] is a unique veterinary glaucoma implant intended for treatment of primary glaucoma and is made from the proprietary STAR—Sphere Templated Angiogenic Regeneration—biomaterial scaffold. STAR is designed to enhance biointegration that in this application restores aqueous outflow, reduces fibrosis, and improves tissue integration. TR BioSurgical also announced encouraging results using a bioscaffold in canine patients with advanced osteoarthritis. The device is implanted in or near injured tissue and provides a structural matrix for existing repair cells, such as stem cells or fibroblasts, to infiltrate and heal tissue. The new bioscaffold implant contains no drugs, cells or growth factors and is eventually resorbed by the infiltrating cells. The implant is made from a proprietary, copolymerized collagen and is sterile, cost effective, and non-immunogenic [104].

BioVeteria Life Sciences, LLC has developed a denatured collagen implant resembling early-stage collagen (similar to embryonic-like collagen). The tissue repair matrix (TR Matrix) bioscaffold implant provides an advanced cellular matrix for infiltrating cells to repair damaged tissue [105].

The area of veterinary surgical resorbable implants provides an example of successful synergy with human pharmaceutics. On the human side, the Recombinant Human Bone Morphogenetic Protein-2/Absorbable Collagen Sponge (rhBMP-2/ACS) was developed for use in spinal surgery, treatment of acute tibial fractures, and in oral surgery. In animal health, rhBMP-2/ACS implant for dogs was designed to support the treatment of diaphyseal fractures, currently under regulatory review under MUMS designation. The European Medicines Agency's scientific advisory committee for veterinary medicines, the CVMP, has recently granted a marketing authorization under the trademark TRUSCIENT® [106].

12.8 Minor Use Minor Species Product Candidates

Interestingly, many implantable and injectable product candidates under development have taken advantage of the Minor Use and Minor Species Animal Health Act of 2004 (the "MUMS Act") [27] that provides the animal health industry incentives

intended to make more drugs legally available to veterinarians and animal owners for the treatment of minor animal species and uncommon diseases in major animal species. In addition to the eligibility for waivers from user fees, the sponsors are eligible to apply for grants to support safety and effectiveness testing. Seven years of marketing exclusivity is granted upon approval or conditional approval. The "conditional approval" "allows the sponsor to make the drug available before collecting all necessary effectiveness data, but after proving the drug is safe in accordance with the full FDA approval standard and that there is a reasonable expectation of effectiveness. The drug sponsor can keep the product on the market for up to 5 years, through annual renewals, while collecting the remaining required effectiveness data" [27]. A comprehensive list of veterinary implantable and injectable products, approved or under consideration for the MUMS designation, is presented in Table 12.9 and includes oncological products, hormonal implants, injections, resorbable implants, and others [27].

12.9 Regulatory Considerations

Implants and long-acting injectables are regulated drug products. These finished products are usually pyrogen free and sterile. The formulator needs to design the formulation and the formulation processing to take this into account. Will the product be manufactured under aseptic processing conditions or will it be terminally sterilized? In addition to traditional testing for potency, degradation products, sterility, and endotoxins, testing for drug release is usually needed for production batch release. A rugged in vitro drug release rate test that can be used to show intervals for initial release (lack of dose dumping), an extended release period, and the end of delivery portion (typically a cumulative percent release of at least 80%) is usually developed as an accelerated release test for QC purposes. This topic has been discussed at several workshops [107, 108].

Adequate shelf life stability is needed for implants and long-acting injectable products. Ideally, the finished product will have 2 years of shelf life (preferably at room temperature) to support marketing of the product. In addition, drug product that is fresh, as well as at the end of its expiry dating, needs to be safe and efficacious in vivo.

The importance of the delivery device needs to be considered and developed in the pharmaceutical development program. If the device is part of the primary package for the product, additional data may be needed for the regulatory filing.

Another regulatory aspect that needs to be considered is residual solvents. As in human pharmaceuticals, residual solvents need to be controlled [109]. In certain cases in animal health, a chemical listed on the guidance will be used as an excipient. In these cases, the excipient is not considered a residual solvent, but an integral part of the drug product. Additional work may be needed to ensure there is no adverse effect on safety.

Table 12.9 List of veterinary implantable and injectable products under the MUMS designation

Established trade name & species	Dosage form	Intended use	Sponsor
sGnRHa & domperidone OVAPRIM®, (Ornamental fish)	Injection	For the induction of spawning in ornamental fish	Syndel Laboratories, Ltd.
Salmon Gonadotropin Releasing Hormone analog OVAPLANT®	Intra- muscular implant	For the induction of spawning in mature salmonids	Aquatic Life Sciences c/o Western Chemical
Deep Scleral Lamellar Cyclosporine Implant (Horses)[a]	Implant	For the management of severe equine recurrent uveitis (ERU) that is refractive to standard treatment and in the absence of blindness, cataract, glaucoma, or active inflammation in the affected eye or eyes	Acrivet, Inc.
Paclitaxel – PACCAL® VET (Dogs)	Injection	For treatment of non-resectable Grade II and III mast cell tumors in dogs that have not received previous therapy except corticosteroids	Oasmia Pharmaceutical AB
Cisplatin, MIII Cisplatin Beads (Horses)	Implantation	For the local treatment of equine sarcoids up to 3 cm in diameter, excluding lesions that are flat, or those that are located within 1.5 cm of a vital anatomical structure, such as the eye, urethra, or rectum	Royer Biomedical Inc.
Recombinant Human Bone Morphogenetic Protein-2/ Absorbable Collagen Sponge (Dogs)	Implantation	For the treatment of diaphyseal fractures as an adjunct to standard surgical care using open fracture reduction	Fort Dodge Animal Health (currently Pfizer Animal Health)
Tulathromycin DRAXXIN® Injectable Solution (Non-lactating goats)	Injection	For the treatment of respiratory disease associated with *Mannheimia haemolytica* in non-lactating goats	Pfizer Animal Health
Tulathromycin DRAXXIN Injectable Solution (Sheep)	Injection	For the treatment of respiratory disease associated with *Mannheimia haemolytica* in sheep	Pfizer Animal Health
Replication competent amphotropic murine leukemia virus with a cytosine deaminase gene (TOCA 511) and oral flucytosine (5-FC) (Dogs)	Intracranial injection (TOCA 511) followed by oral flucytosine 3 weeks later	For the treatment of primary malignant brain tumors in dogs	Tocagen, Inc.
Paclitaxel – PACCAL® VET (Dogs)	Injection	For the treatment of resectable and non-resectable squamous cell carcinoma	Oasmia Pharmaceutical AB
Alpha-neurotoxoid – PANAVIRA® (Dog)	Injection	For the treatment of local disease in stage II and III oral malignant melanoma	Nuovo Biologics LLC
Luteinizing Hormone-Releasing Hormone analog (LHRHa) (Channel catfish)	Injection	For use as a spawning aid for female channel catfish (*Ictalurus punctatus*)	Eagle Aquaculture, Inc.
Luteinizing Hormone-Releasing Hormone analog (LHRHa) (Channel catfish)	Implant	For use as a spawning aid for female channel catfish (*Ictalurus punctatus*)	Eagle Aquaculture, Inc.

[a]Terminated at sponsor's request

12.10 Conclusions

Many different long-acting parenteral drug delivery technologies have been investigated in the veterinary field, resulting in commercial products in all the major technology categories. Innovation will continue to be needed as companies strive to develop products that advance animal healthcare and improve animal lives.

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Chapter 13
Intramammary Delivery Technologies for Cattle Mastitis Treatment

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Abstract Mastitis is an infection of the udder caused by bacterial pathogens entering the mammary gland via the teat canal. It is the most prevalent infectious disease in adult dairy cattle. This chapter provides an overview covering the classification of mastitis, anatomy and physiology of the bovine udder, economic impact of mastitis, internal features and histology of the mammary gland, and therapeutic strategies with emphasis on the role of controlled drug release technologies in cattle mastitis prevention and control.

13.1 Introduction

Mastitis is an infection of the udder caused by one of the several bacterial pathogens (streptococcus, staphylococcus, coliforms) entering the mammary gland via the teat canal when the teat sphincter is relaxed following milking or suckling. It is the most prevalent infectious disease in adult dairy cattle. Mastitis causes a huge economic loss to dairy industries all over the world [1, 2]. Many drugs of various classes, including anti-inflammatory drugs, vitamins, and vaccines have been used for the treatment of mastitis [3–5]. Mastitis is the most frequent reason for antibiotic use in dairy farms [6] and contributes to a substantial portion of the total drug and veterinary costs incurred by the dairy industry [7].

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The mammary gland of the dairy cow requires a non-lactating period when approaching parturition to optimize milk production in the subsequent lactation period. This period is called "the dry period." Generally, 40–60 days are recommended. After the dry period there is a transition from the non-lactating to the lactating state, called the prepartum period. The lactation period that follows the prepartum period lasts about 300–310 days.

Prevention of new infections during drying-off period prior to the cessation of lactation is a major challenge for dairy farmers. The non-lactating udder is prone to bacterial infection, with new infection rates being highest in the early dry period and approaching parturition [8]. After drying-off, the closure of the teat canal by the formation of a keratin plug greatly influences the incidence of intramammary infections (IMIs) during the dry period. At present, antibiotic therapy at the end of lactation is the most effective means of eliminating existing infections and preventing new ones.

However, despite the long history of the use of antibiotics to treat IMI in dairy cows, the prevention of new infection appears to offer greater long-term benefits. The success rate from using antibiotics is very low and there are a number of problems associated with their use in the treatment of IMI. One limitation of the present therapy approach for the dry period is that it provides little or no protective effect against new infections during the prepartum period. Most available dry cow products do not persist late into the dry period and, consequently, the udder is left susceptible to new IMI during the prepartum period. Efforts to control mastitis with management practices have not been shown to reduce new dry-period infections.

In addition, the presence of antibiotic residues in milk for human consumption is a major concern in today's dairy industry [7]. Further, the multiple usages of antibiotics for either therapeutic or prophylactic purposes in the dry period may lead to the emergence of antibiotic resistance [3, 9]. Antibiotic use in the animals is often cited as a potential cause for antibiotic resistance in humans, since antibiotic-resistant bacteria may be transferred to humans via food from animals [9, 10]. The possibility that the use of antibiotics in food-producing animals may be restricted or banned in the future is of concern to the dairy industry today [10, 11].

The economic effects of antibiotic use also deserve mention since discarded milk production during antibiotic treatment, together with the decrease in yield, accounts for the largest economic loss associated with mastitis. Reducing the use of antibiotics to prevent mastitis would greatly benefit the dairy industry.

Recently, non-antibiotic prophylactic agents that act as physical barriers to seal the teat internally during the drying-off period have been developed as an alternative method of controlling IMI. Success in controlling new IMIs during the dry period by sealing the teat opening using a non-antibiotic product containing bismuth subnitrate has been reported in the literature [8, 12–18]. However, there has been no conclusive pharmacokinetic or residue data available in target animals [19]. Besides, the possibility of a toxic effect from bismuth appearing either directly in the milk or indirectly in the environment needs to be considered.

There are also some reports on external seals being used to protect the teats from the outside as a barrier membrane. These have had only limited success in reducing

IMIs during the dry period, largely due to poor persistence on the teat [20]. For these reasons there is a need for a more appropriate dosage form to be developed for improved mastitis prevention and a subsequent reduction in antibiotic use.

The development of new delivery systems capable of controlling the release of drugs is of great interest, and a challenging task, in the veterinary field. Early development of controlled-release drug delivery systems was more aimed at human pharmaceuticals with the veterinary field following suit in the mid-1970s [21]. In the past few years there has been strong interest in the development and use of long-term drug delivery modalities for both companion and farm animals, with controlled-release drug delivery systems offering numerous benefits in the animal health field. Advances in pharmaceutics have led to the development of novel drug delivery systems and medical devices. These technologies offer promising options to the dairy industry for use against IMI.

IM infusion products currently used to treat mastitis are oily suspensions or solutions. However, the development of injectable sustained-release delivery systems has become an increasingly attractive area of research in recent years for both human and veterinary application. These technologies offer advantages such as ease of application, prolonged site retention, and improved efficacy and bioavailability [22].

13.2 Classification of Mastitis

Mastitis is the most prevalent infectious disease of adult dairy cattle [23]. It is an infection of the cow's udder that causes physical, chemical, and microbiological changes [24]. It is caused by bacteria and is characterized by pathological changes in the mammary tissue and abnormal secretions from the mammary gland. Several species of bacteria (streptococcus, staphylococcus, coliforms) and fungi (mycoplasma) have been identified as causes of mastitis [23]. A total of 137 microbial species have been isolated from the bovine mammary gland [25]. *Escherichia Coli* (*E. coli*) and *Streptococcus uberis* (*S. uberis*) are the most common causes of environmental mastitis and are increasingly implicated in low somatic cell count (SCC) herds [26]. Mastitis occurs when the udder becomes inflamed due to the release of leukocytes into the mammary gland in response to an invasion of the teat canal, usually by bacteria. These bacteria multiply and produce toxins that cause injury to milk-secreting tissue and various ducts throughout the mammary gland. Elevated leukocytes, or somatic cells (SC), cause a reduction in milk production and alter milk composition. These changes in turn adversely affect the quality and quantity of dairy products.

The normal SCC of milk from a normal cow may be less than 200,000/ml and can be even lower than 100,000 during first lactation [27]. The SCC of milk from an individual cow is used as an indicator of udder health.

Udder infections may develop during the lactating period or the dry period. Infection rates are highest in the early dry period, although these infections often do not persist or develop into clinical mastitis until the next lactation [23].

The signs of mastitis vary according to host factors and invading pathogens. Based on the severity of the disease, mastitis can be classified into subclinical mastitis and clinical mastitis, and based on the pathogen source, mastitis can be divided into environmental mastitis and contagious mastitis.

13.2.1 Subclinical Mastitis

There are no visible disease signs in the mammary gland with subclinical mastitis and the milk from cows with subclinical mastitis appears normal. However, inflammatory cells are present in high numbers, and can only be detected with an appropriate SCC test [3]. Elevated SCC often results in a reduction in milk production which decreases further with increasing SCC.

13.2.2 Clinical Mastitis

High SCC is associated with clinical mastitis, which is visually detectable in the production of abnormal milk, characterized by clots and clumps and water secretion in the milk, and visible changes of the udder such as redness and swelling. In severe cases, there may be systemic signs such as elevated temperature, anorexia, and septicemia, which may cause animal death [28]. Both forms of mastitis produce significant economic losses due to rejected milk, deteriorated milk quality, early culling of the cow, the cost of antibiotics, and increased labor cost for farmers. Mastitis pathogens have been categorized according to the epidemiology of the infection-inducing pathogens as contagious or environmental, more specifically based on their distinct characteristics of distribution and interaction of pathogens with the teat and teat duct [29].

13.2.3 Contagious Mastitis

Contagious mastitis is caused by contagious pathogens. The main contagious pathogens are *Staphylococcus aureus* (*S. aureus*), *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, and *Mycoplasma* species. With contagious pathogens, the organism adapts to survive within the host, in particular within the mammary gland, and is capable of causing subclinical infections. These are typically evident in the elevation SCC of milk from the affected quarter. These organisms reside in the teat skin, colonize in the teat ducts, and cause injury to the mammary gland. *S. aureus* is the most common cause of IMIs [16]. The principal reservoirs of *S. aureus* are the udder and teat skin and the milk of infected glands [30]. The infected mammary gland is the main source of these organisms in a dairy herd and they are normally spread from cow-to-cow at the time of milking [31]. The organisms have the capacity to penetrate

tissues producing deep-seated foci; hence, intramammary antibiotic therapy is not completely successful in eradicating staphylococcal mastitis [3].

13.2.4 Environmental Mastitis

Environmental mastitis is caused by pathogens present in the surroundings where the cows live. Environmental pathogens are not adapted to survive within the mammary gland of the host. Environmental pathogens include a heterogeneous group of bacteria. The most commonly isolated environmental pathogens are Enterobacteriaceae particularly *E. coli* and *S. uberis* (*S. uberis*) [32]. These organisms exist in various sites within the cow's body including the gut, genital tract, and tonsils and can multiply in cattle litter or water. At least one-third of all cases of clinical mastitis are caused by environmental streptococci, with the proportion varying widely between herds [13].

13.3 Economic Impact of Mastitis

Bovine mastitis is one of the most important diseases affecting the world's dairy industry; it causes a heavy economic loss to milk producers all over the world [33–36]. Losses caused by lowered milk production, treatment, and replacement costs are enormous. Milk dumped after antibiotic usage is the major cost associated with clinical mastitis. Discarded milk and decreased production account for about 85% of the cost of clinical mastitis. The prevalence of subclinical mastitis may be 15–20% and the incidence rate for clinical mastitis around 32–71 cases per 100 cows per year [4, 37]. Mastitis has been estimated to cost dairy producers from $180/cow/year, with 70–80% of this arising from subclinical mastitis. The US market size for intramammary antibiotics is in the order of US $68 million compared to the $17.7 million for the New Zealand market. Worldwide, annual losses due to mastitis have been estimated to be approximately US $35 billion. In the USA, the annual costs of mastitis have been estimated to be US $1.5–2.0 billion, while losses from milk production due to subclinical mastitis and higher cow replacements costs associated with high SCC were estimated at US $960 million [38].

13.4 Anatomy and Physiology of the Bovine Udder

The bovine udder is a skin gland. It consists of four separate mammary glands clearly divided into right and left halves by a longitudinal furrow called the intramammary groove (Fig. 13.1] each drained by a teat. The four secretory glands are structurally separate and function independently. Lactating udders often weigh up to 60 kg [39].

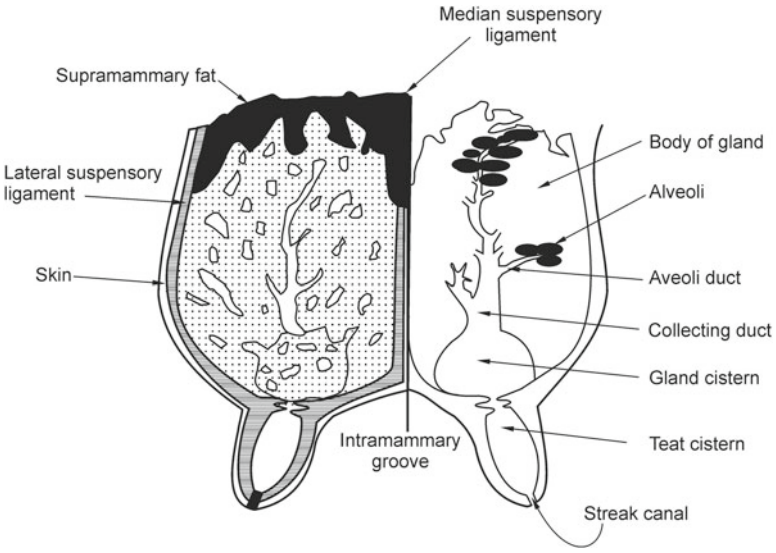

Fig. 13.1 Cross section of the bovine mammary gland

The surface of the skin or epidermis of the udder is composed of stratified squamous epithelium and is covered with fine hair [23]. The skin, while providing only limited support to the udder, provides protection from abrasions and from invasion by microorganisms. Fine connective tissues connect the skin to the udder and coarser connective tissue attaches the front quarters to the abdominal wall. The teats are hairless and have a rich supply of blood vessels. Teat skin is thin and devoid of the sebaceous gland. The teats vary in shape from cylindrical to conical [23].

The udder is supported by the skin, and lateral and median suspensory ligaments. The lateral suspensory ligaments are one of the main supporting structures of the mammary gland. The lateral suspensory ligament is a fibrous, nonelastic structure arising from the sub-pelvic tendons (tendons attaching organs to bone). These tendons are above and posterior to the rear udder. The lateral suspensory ligaments extend along both sides of the udder and also into the interior of the udder.

The median suspensory ligament of the udder is composed of elastic tissue arising from the midline of the abdominal wall and extending between the right and left halves of the udder to join with the deep lateral suspensory ligaments. The median suspensory ligament forms the center of gravity of the udder and carries the majority of the weight of the udder. The ligament provides a distinct internal separation between the left and right halves of the udder.

13.5 Internal Feature and Histology of the Mammary Gland

Histologically, mammary glands are greatly modified and enlarged sweat glands with dilated alveoli that store milk. The interior of each quarter is composed of the teat cistern, gland cistern, milk duct, and glandular tissue. The glandular tissue

or secretory portion contains millions of microscopic sacs called alveoli. Each alveolus is lined with milk-producing epithelial cells and is surrounded by muscle cells that contract and squeeze milk from the alveolus during milking. Blood vessels bring nutrients to each alveolus whereas epithelial cells convert them into milk. The teat orifice, called the teat canal or streak canal, is the terminus of an extensive alveolar and tubular system which synthesizes and collects milk. The teat canal is an important component of the teat. It is a thick muscular tissue that is lined with antibacterial substances and closes the teat when milk is not being extracted. Each teat has a single narrow teat canal, which dorsally opens into a wider teat cistern lined with bilayered epithelium. The average length of the teat canal in the living cow is 10.8 mm and the distal and proximal diameters are 0.4 mm and 0.77 mm, respectively [23, 40]. The teat canal is the primary physical and chemical barrier to the invasion of mastitis pathogens into the udder. The lining of the teat canal like the skin of the teat consists of a stratified squamous epithelium, and its surface continually undergoes keratinization to form sebum-like material which fills the lumen of the canal [40]. Teat canal keratin acts as a barrier to the microorganisms that cause mastitis [40, 41]. This material is rich in long-chain fatty acids having a bacteriostatic effect on certain bacteria [42]. The teat canal is surrounded by a true sphincter of smooth muscle fibers, which function in maintaining a tight closure of the canal (Fig. 13.1), thereby inhibiting entry of microorganisms and also preventing leakage of milk from the mammary gland [43]. Quarters having apparent teat canals (lack of tight closure) have a greater incidence of infection [44].

The teat wall comprises five distinct tissue layers: the superficial epidermis, the dermis, an intermediate layer, fibrous lamina propria, and, internally, the epithelium of the teat canal. Just above the teat canal are series of folds known as Furstenberg's Rosette. These folds radiate in all directions and flatten out when milk accumulates within the mammary gland to aid in the retention of milk [40].

The teat cistern (sinus papillaris), located above the Furstenberg's Rosette, is a cavity within the teat capable of holding 30–60 ml of milk (Fig. 13.2). The teat cistern is lined with numerous longitudinal and circular folds of the mucosa, which tend to overlap and serve as a storage place for bacteria during incidents of mastitis. The gland cistern (sinus lactiferous) is located just above the teat cistern and is capable of storing 100–400 ml of milk. The gland cistern is connected to the teat cistern, which opens into the narrow teat canal [40, 45].

The gland and teat cisterns are continuous, but in most cows a definite circular constriction of dense connective tissue called the annular (cricoid) fold is seen between the two cisterns.

The gland cistern serves as a collection point of major ducts (10–20) which branch repeatedly to eventually drain the alveoli, the milk-secreting tissue. The alveoli are composed of a single layer of epithelial cells. The epithelial lining is surrounded by contractile epithelial cells known as myoepithelial cells. These cells contract in response to the hormone oxytocin, resulting in milk being squeezed out of the alveolar lumen and into the ductules. The alveolus is surrounded by a connective tissue basement membrane outside the myoepithelial cell.

The epithelial cells are capable of extracting nutrients from blood, synthesizing milk components (fat, protein and lactose) and secreting milk into the lumen of the alveoli. The basal region of the alveolar cells is attached to the myoepithelial cells

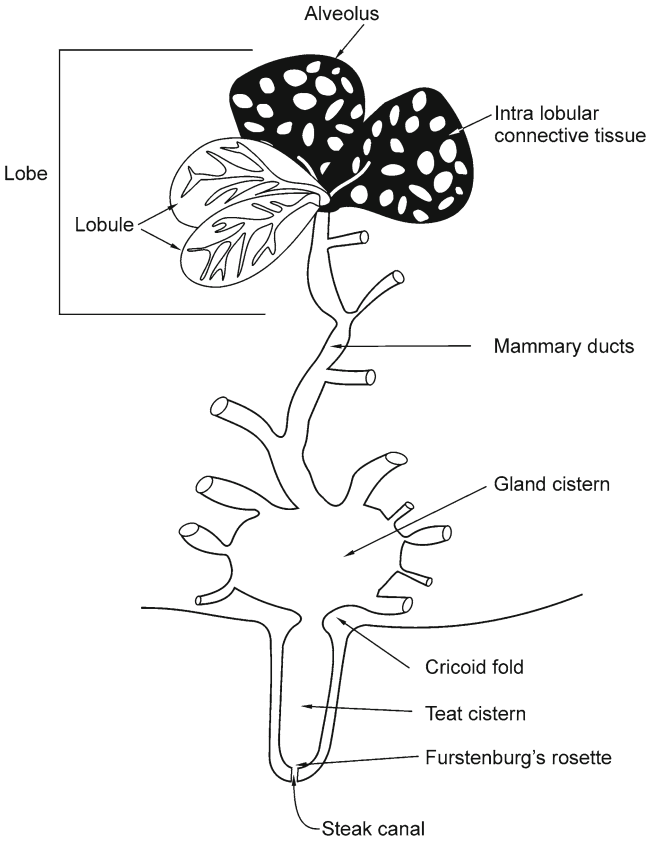

Fig. 13.2 Mammary duct and lobule alveolar system [46]

or the basement membrane. A lactating secretory cell is the basic unit of milk synthesis. Milk precursors are taken from the blood into the cell through the basal and the lateral membrane and milk is discharged into the lumen through the apical membrane. Individual cells are joined to their neighbor cells by tight junctional complex structures located around the apical portion that forms a tight barrier. This prevents the passage of materials between cells under normal conditions. Secretory cells are probably also bound to adjacent cells through gap junction, which allow low molecular weight materials to pass from one cell to another. The intracellular exchange may help a given alveolus to synchronize milk discharge from various secretory cells into the lumen.

The shape of the alveolar cells depends on the status of distension of the alveolus. The cells are firmly joined to one another by junction complexes at the intercellular boundary below the luminal surfaces although the junctional complexes are not always well defined.

From the luminal surfaces, numerous microvilli arise. The epithelial cell, the major component of the alveoli, is composed of many intracellular elements.

The udder is supplied with very large amounts of blood enabling milk production (500 liters of blood per kg of milk). In addition to transporting the basic components of milk to the glandular parenchyma, the blood flow also supplies immunoglobulins (IgG1 and IgG2) and complement proteins. The immunoglobulins are a heterologous group of glycoprotein synthesized by cells of the lymphocytes. Blood flow also acts as a carrier in transporting to the udder any parenterally administered drugs that can diffuse through the parenchyma.

13.6 Therapeutic Strategies

Many drugs of various classes including antimicrobial and anti-inflammatory drugs as well as vitamins, and vaccines have been used for the treatment of mastitis [3–5]. Antibiotics have been the drugs of choice most often advocated in clinical practice and research as a therapy for mastitis by either the intramammary route or, less commonly, the systemic route. There are two different approaches of treatment based on the treatment period: treatment during the lactating period and treatment during the non-lactating (drying-off) period.

Mastitis is present in every herd; however, rates of infection and types of bacteria causing mastitis vary greatly between herds. Control of bovine mastitis is very difficult using antibiotics because of the diversity of pathogens capable of causing the disease. Therefore the control of environmental and contagious pathogens, with improved preventative methods that help reduce the use of antibiotics would be more appropriate.

13.6.1 Treatment of Mastitis with Antibiotics

For over 50 years, intramammary infusions of antibiotics have been used to treat clinical mastitis. The drug can be administered intracisternally and/or through the systemic route. The intramammary route is the most common route for the administration of drugs for the treatment of mastitis [3]. The efficacy of an antibiotic agent depends on a number of factors including the susceptibility of the etiological agent involved, the type of lesion produced, and the pharmacokinetics of the administered drug. The primary strategy in antibiotic therapy is to eliminate the microorganism by delivering an optimal amount of drug to the site of invasion and maintain an effective concentration level for an adequate time. This could be achieved by the development of an appropriate formulation with an increased residence time at the site of infection. Failures in the treatment of acute mastitis with intramammary antibiotic therapy are due to poor or uneven distribution of the drug through the intensely swollen udder parenchyma and blockage of the milk duct system by inflammatory products [3]. Furthermore, *S. aureus* can survive in the intracellular space of the tissue; therefore intracisternally administered drugs may not gain access to these

pathogens. Thus bacteriological failures may occur even when the organisms are sensitive to the antibiotic used.

From the therapeutic perspective, the mammary gland is a compartment isolated from the rest of the organs in a cow's body. Consequently, the success of the systemic treatment necessitates passage of the drug from the blood to the milk, through the lipid membranes of the mammary gland. Antibiotic passage across this barrier occurs through passive diffusion, i.e., it is dependent upon the concentration gradient of the drug across the barrier with the limitation that only lipophilic substances are able to cross the barrier. A systemic administration requires the use of high doses of antibiotics to maintain the minimum inhibitory level of drugs in the udder compared to those from intramammary administration. Therefore, the systemic route is recommended only for acute cases of IMI when the parenchyma is intensely swollen, which means that the milk duct is blocked by inflammatory secretions which prevent the distribution of the drug throughout the udder.

For mastitis treatment the intramammary route is primarily used for the delivery of the drug into the udder through the teat canal. The main advantage of this route is that high concentrations of drugs are achieved in the target tissue. However, in acute mastitis poor distribution of the drug may occur due to the swollen udder parenchyma and blocked milk ducts due to the inflammatory products. Furthermore, staphylococci can survive inside the tissue; therefore, the intracisternally administered drug may not gain access to these microorganisms. Systemic treatment in addition to intramammary treatment is recommended to overcome these problems [47]. The choice of a particular route of administration depends on the severity of disease, type of organism, and physicochemical properties of the drug.

Cows are treated for IMIs during the lactating period. In the drying-off period drugs are administered to treat mastitis as well as to prevent new IMIs that may occur during the early dry period.

13.6.2 Dry Cow Therapy

In dry cow therapy, the drug is usually injected directly into the mammary gland after the last milking. The use of long-acting intramammary treatment at the time of drying-off is the recommended mastitis control therapy. Dry cow treatment produces a higher cure rate for existing infection than therapy during lactation and can prevent new IMIs and clinical mastitis [13]. The advantages of a dry cow therapy are:

1. Uniform levels of drug can be maintained in the udder in the short term as the gland is not being milked.
2. Less economic loss though not having to discard drug contaminated milk.
3. Much higher doses of drugs can be administered in the dry period than during the lactation period.

13.6.3 Therapy During Lactation

Clinical mastitis during lactation is usually treated by an intramammary infusion of antibiotics [48]. Antibiotic therapy of lactating cows increases the risk of antibiotics residues in meat or milk; therefore long-acting formulations cannot be used for lactating cows. As a result the treatment of mastitis in lactating cows is problematic due to low drug efficacy and antibiotic residue in the meat and milk.

13.6.4 Antibiotic Efficacy

Due to the large variation in response not only among herds but also among cows in the same herd, the efficacy of antibiotics to treat mastitis is very difficult to evaluate. Variations may occur in the type of organism involved, the location of the infected site, the degree of udder indurations, herd management, and the criteria used in assessing clinical and the bacteriological response [1]. The clinical efficacies of different antibiotics reviewed are shown in Table 13.1.

In view of the diverse range of cure rates, it is not possible to draw firm conclusions about the relative effectiveness of different products. However, data consistently show a greater bacteriological cure rate in the treatment of *Streptococcus agalactiae* infections compared to *Staphylococcus aureus* [1].

The main issues in the treatment of mastitis are therapeutic failure caused by inadequate prevention during the prepartum period (poor persistence of the therapeutic agent in the udder for the entirety of the dry period), re-occurrence of infections due to the intracellular localization of bacteria, continued bacterial colonization that begins during drying-off but persists throughout the dry period to induce clinical mastitis at the beginning of the next lactation, and the effectiveness of single-dose therapies during lactation [49]. The choice of antibiotics seems to have very little effect in treating mastitis caused by *Staphylococcus* or *E. coli* [50].

Current control methods are not equally effective against all mastitis pathogens and have little effect on pathogens originating from the environment. Moreover, existing treatments are not very successful and cure rates are poor. Mastitis caused by *S. aureus* is variable and cure rates are only about 25–26% [1]. The most deducible reasons for this may be the poor distribution of the drug in the inflamed udder and the emergence of antibiotic-resistant staphylococci.

Conventional intramammary products are mainly oil-based suspensions. These are poorly formulated and have inherent disadvantages with respect to ease of administration, removal, and performance. Most available dry cow products do not persist late into the dry period and leave the udder vulnerable against new IMIs during the prepartum period [1]. In addition, these formulations suffer from inherent physical stability problems such that the dispersibility of conventional IM suspensions is often difficult to achieve in the field. Dose uniformity can be an issue with these oil-based products [51]. Two important factors which are considered vital to

Table 13.1 Efficacy of mastitis treatment with different antibiotics [1]

Causative organism	*Staphylococcus aureus*			*Streptococcus agalactiae*		
Antibiotics	Mean% cure	Range	No. of reports	Mean% cure	Range	No. of reports
Penicillin	32	0–87	12	84	50–100	11
Cloxacillin	41	21–84	14	92	40–100	8
Neomycin	27	25–36	2	27	27	1
Tetracycline	54	17–96	8			
Chloramphenicol	33	20–48	7	75	61–84	2
Erythromycin	63	51–76	2			
Spiramycin	70	45–82	2			
Rifampicin-SV	66	65–66	2	75	75	1
Penicillin + Streptomycin	39	21–78	5	91	91	1

determine the effectiveness of pharmaceutical suspensions are homogeneity of the suspension and the dose measured for administration [52]. It is unlikely that conventional oil-based IM dosages packed in syringes remain homogeneous at the time of administration.

Another problem associated with lipophilic dry cow products is poor retention in the udder. Ziv and Saran have reported that highly lipophilic dry cow products disappeared within hours of administration [53, 54]. Their value as a drying-off product is therefore questionable. In addition, antibiotics which are highly bound to the serum protein will have low absorption and therefore are less effective. The difference in pH levels of mastitis milk (6.8–7.2) and normal milk (6–6.5) also affects the absorption of the drug since the absorption potential of the drug from the site of administration depends on the ionization of the drug in the milk. Since ionization (due to pH changes) relies on the severity of the disease, the drug absorption potential in the udder may be highly compromised in an infected udder [54, 55]. Further, the distribution and binding of antibiotic to serum and milk protein is also affected by pathological changes such as the blockage of milk ducts by inflammatory products or by cell debris and the swelling of udder parenchyma in diseased quarters [47]. Oil-based IM formulations may be poorly retained within the aqueous environment of the bovine udder.

The inadequacies of current methods of mastitis control have led to the search for new measures, particularly for a more appropriate dosage form to be developed. The current limitations in IMI control/treatment signal the need for a formulation that is physically stable, is easy to administer, and remains at the site of administration for a reasonable period of time.

While antibiotics have traditionally been used on a curative basis, problems associated with the control of mastitis during the drying-off period have also not been sufficiently dealt with. One of the most recent approaches that offer potential solutions for the control of new IMI occurring during the drying-off period is a non-antibiotic teat seal formulation. An important aim of this thesis is to develop a new teat seal technology for controlling IM infections occurring during dry period. The following section reviews current teat seal products in the market.

13.7 Review of Teat Seal's Role in Prevention of IMI

Although mastitis has been treated using antibiotics for more than 50 years, it continues to be a major problem for the dairy industry. In particular, non-lactating mammary gland at early stages of drying-off and close to parturition is susceptible to bacterial infection arising from environmental bacteria. Prevention of new infections in dairy cows during the drying-off period is a major challenge for dairy farmers. The closure of the teat canal after drying-off by the formation of a keratin plug prevents the entrance of bacteria. A keratin plug is typically formed naturally within 16 days of drying-off; however, it can take up to 60 days to form a plug and the teat to close.

Dry cow therapy with antibiotics is an important strategy for controlling IMIs during the drying-off period. However and despite the use of antibiotics, control of mastitis is not completely successful due to the diversity of IMI causing bacteria, reinfection due to incomplete elimination of microorganisms, uneven distribution of antibiotics, and development of antibiotic resistance bacteria. Limitations of current methods of mastitis control and concerns over antibiotic residue in milk and meat highlight the need to consider alternative approaches for prevention and control of pathogens causing IMIs. Internal and external teat seals provide a physical barrier against IMIs during dry period. They offer a viable alternative to antibiotic dry cow therapy when administered to teats of uninfected udders at drying-off. However, they have had only limited success largely due to poor persistence during the dry period [20, 56]. The need to address removal after use deserves further consideration also.

The non-lactating udder is prone to bacterial infection with new infection rates being highest in the early dry period and later approaching parturition [8]. More than 50% of clinical mastitis cases originate during the dry period [32, 56]. The closure of the teat canal at drying-off by the formation of a keratin plug (derived from epithelial cells lining the teat canal) is a key factor influencing the incidence of new IMI during the dry period. Some components of the keratin plug have known microbicidal activity [42, 57]; however the major role of such a plug is to serve as a physical barrier preventing microbial access to the teat sinus [41]. The longer the teat canal remains open the greater the chance of infection. Cows can form a keratin plug in the teat canal after drying-off within 16 days [58], but in some cows it can take up to 60 days for a plug to form and the teat to close completely. Approximately 50% and 5% of teats remain open after 7 and 50 days of the dry period, respectively. It is highly likely that environmental bacteria can invade the teat cistern via the sphincter during this period [8]. The development of teat seals is an alternative strategy to prevent new IMIs occurring before a keratin plug has formed. A number of internal and external seals for sealing the teat as an artificial skin seal have been studied over the last 25 years [17]. Teat seals can be classified into the following categories; Internal Teat Seals, External Teat Seals, Teat Plugs, and Inserts.

13.7.1 Internal Teat Seals

Conventional internal teat seals have shown some promising results in preventing new cases of mastitis during the dry period when infused into the teats of uninfected cows at drying-off. They contain a heavy inorganic bismuth (65% bismuth subnitrate) and aluminum monostearate in liquid paraffin base and are infused from a plastic syringe, of a similar type to those used for the infusion of intramammary antibiotic [18, 59]. Studies have shown that this bismuth-based intramammary teat seal is as effective as dry cow antibiotic therapy for the prevention of new dry-period infections [12, 14, 16, 18].

It is reported that cows that formed a keratin plug soon after drying-off had a lower incidence of infection during the dry period [60]. A study has also shown that internal teat seals behave in a similar way to the natural keratin plug-in forming a barrier to environmental pathogens. Huxley et al. studied the efficacy of a teat seal when compared with long-acting antibiotic preparations containing cephalonium [8]. Results showed that the quarters that received the teat seal acquired significantly fewer new IMI compared with quarters that received antibiotics in mastitis cases caused by *E. coli*, and all major pathogens. Furthermore, Berry and Hillerton [12] reported that a commercial teat seal product (Orbeseal) in combination with an intramammary product containing cloxacillin resulted in no incidents of clinical mastitis during the dry period. Woolford and coworkers reported on the clinical efficacy of teat seal (Teat seal) compared with a lactating cow preparation containing cloxacillin benzathine, a dry cow preparation containing 250 mg cephalonium (positive control) and negative control (no treatment) [17]. A total of 528 cows were included in this study, with each quarter within a cow randomly assigned to one of the four treatments. All quarters were bacteriologically negative at the time of infusion. During the dry period, the use of teat seal resulted in an 18-fold lower clinical mastitis than the negative control and was similar in effect to the positive control [17].

Despite the success of teat seal products in reducing new IMIs in clinical studies, there are some issues which need to be addressed. Notz indicated that there are some problems related to the application of the teat seal (Orbeseal®) in practice. In addition to inadequate and poor retention, subsequent failure to seal the teat was reported [61]. Conventional teat seal products contain 65% bismuth subnitrate. Some reports indicate that bismuth can induce neurotoxic effect in both human and animals [62]. Some of the side effects in human therapy with bismuth are encephalopathy, nephropathy, stomatitis, and colitis [63]. The half-life of bismuth in humans is reported to be between minutes and several years [63]. The implications of these observations have not been addressed for the use of inorganic bismuth as a component of teat seal preparations.

13.7.2 External Teat Seals

The external teat seal which is used to cover the teat end like a second skin has been studied for the prevention of new IMI during the dry period. Effect of an external teat seal (StrongholdTM, DryFlexTM) application on the occurrence of clinical mastitis was studied by Corbellini et al. [64]. Results showed a 50% reduction in the incidence of clinical mastitis. In 50% of cases the teat was blocked for five days during the dry period. In another study, the adherence of StrongholdTM, DryFlexTM was assessed in mid dry period [65]. The results showed that the adherence was slightly higher in the mid dry period compared to drying-off and pre-calving [65].

The external teat seal only persists for about five to eight days. Because of this insufficient persistence at the teat end, external teat seals cannot always be implemented successfully [20, 65]. These studies indicated that the application of external teat seal for the prevention of IMI while providing benefits has had only limited success in reducing IMIs during dry periods.

13.7.2.1 Teat Plugs and Inserts

In the past, teat plugs and inserts have been used to keep the teat open after injury [66]. Huston and Heald [67] studied the effect of a polyethylene coil that was fitted intramammarily on cows five days after their first parturition. Cytological evaluation of tissues of the gland cistern epithelium after three weeks indicated significantly fewer bacterial isolations and a lower occurrence of clinical mastitis in treated quarters compared to control quarters [67]. Another study performed by Nickerson and coworkers investigated an intramammary device in the form of a rod made of fluorinated ethylene propylene plastic of different weights, and its effect on incidences of mastitis [68]. The device failed to provide protection. The teat canal is usually kept tightly closed by the teat sphincter muscles; this limits bacterial entrance to the teat orifice. Insertion of teat plugs and inserts may endanger the antibacterial properties of the teat canal by causing damage to the tissues of the teat canal, which ultimately increases the cow's risk of mastitis infections.

13.7.3 Prospective of New Formulations Development

As discussed earlier, delivery of drugs to the mammary gland in cattle is challenging. This is mostly due to the anatomical and physiological constraints of the mammary gland. Moreover, many of the commercially available intramammary delivery systems are poorly formulated and suffer from inherent physical stability problems. Currently, treating a lactating cow for mastitis involves infusing antibiotics into the teat of the cow daily using a single syringe for each of the four teats for each treatment. This results in huge numbers of syringes being required and major handling, storage, and disposal issues.

The use of natural polymers as well as synthetic polymers in human and veterinary drug delivery continues to be an area of active research. Despite the fact that various synthetic as well as natural polymers have been investigated for human and veterinary drug delivery application, very little work has been done along the lines of using hydrophilic polymers to develop intramammary delivery systems. Such systems as intramammary teat seals or sustained release of a therapeutic agent intended for IM delivery may have advantages over traditional oil-based formulations. Such polymer-based systems could serve as a matrix to control the release of a therapeutic agent intended for IM delivery; they may also form a physical barrier to shield the IM gland from invading pathogens. The following section describes polymeric controlled-release systems with emphasis on their promising role for treatment and prevention of IMIs.

13.8 Controlled-Release Delivery Systems

When drugs are administered in the form of immediate-release dosage form fluctuations in blood drug concentration may consequently lead to undesirable toxicity and reduced efficacy. Factors such as frequent dosing and unpredictable absorption have led to the use of controlled-release drug delivery systems. Maintaining a constant therapeutic drug concentration for an extended period of time is challenging. For a safe and efficient therapy, drug concentration should lie within the therapeutic window over the desired period of action. A dosage form that releases one or more drugs in a predetermined pattern for a fixed period of time either systematically or to a specified target organ is called a controlled drug delivery system [69]. The primary objectives of controlled drug delivery are to ensure patient compliance and safety. This is achieved through a better control of plasma drug levels and less frequent dosing.

Controlled-release drug delivery systems offer one or more of the following benefits or advantages [70]:

1. Controlled administration of therapeutic doses at a desirable delivery rate
2. Maintenance of drug concentration within an optimal therapeutic range for a prolonged duration of action
3. Maximization of treatment efficacy
4. Reduction of adverse side effects
5. Minimization of frequent dosage administration
6. Enhancement of patient compliance
7. Protection from degradation of some drugs with in vivo short half-life

13.9 Types of Controlled-Release Drug Delivery Systems

Controlled-release drug delivery systems are broadly categorized as polymeric and lipid-based carriers. Whilst the use of lipid-based systems in the area of intramammary drug delivery is well established, polymeric systems are yet to be fully exploited. The following sections will focus on the various polymeric controlled-release systems that are currently of clinical significance for human and animal health.

13.9.1 Diffusion-Controlled Systems

In diffusion-controlled systems the release rate of a drug is determined by its ability to diffuse from an inert membrane barrier, usually made of an insoluble polymer [71]. Diffusion-controlled systems can be classified into two categories: reservoir and matrix systems.

13.9.1.1 Reservoir (membrane systems)

In these systems the drug is encapsulated in a polymeric membrane. Drug diffusion through the membrane is the rate-limiting step and controls the overall drug release rate [72, 73]. A saturated concentration of drug inside the reservoir is essential to maintain a constant concentration gradient across the membrane. The release of the drug from a reservoir system is governed by Fick's first law of diffusion, which describes the transport of molecules by a concentration gradient given as [71]:

$$J = -D\frac{dc}{dx}, \tag{13.1}$$

where J is the flux of drug across a membrane (in amount/area-time), D is the diffusion coefficient of the drug in the membrane (area/time), and dc/dx is the change in concentration of the drug in the membrane over a distance of x.

The disadvantage of such a system is the danger of dose-dumping if the membrane is ruptured. Another disadvantage is in the ability of large molecules such as proteins and peptides to diffuse through the polymer membrane.

13.9.1.2 Matrix (monolithic systems)

In matrix systems the drug is uniformly dissolved or dispersed in a polymer matrix. Diffusion occurs when the drug passes from the polymer matrix to the environment [72, 73]. The diffusion rate decreases as the release of the drug continues. Since the drug has a gradually longer distance to travel a longer diffusion time is required.

The main disadvantage of this system is that it cannot provide a zero-order (concentration independent) release. The removal of the remaining matrix after the drug has been released from such systems also poses a problem.

13.9.2 Chemically Controlled Systems

Chemically controlled systems can be broadly classified into bioerodible/biodegradable systems and pendent chain systems.

13.9.2.1 Bioerodible and biodegradable systems

The polymer matrix in these systems erodes due to the presence of hydrolytically or enzymatically labile bonds [74]. As the polymer erodes, the drug is released to the surrounding medium [75, 76].

13.9.2.2 Pendent chain systems

In these systems the drug molecule is chemically linked to a polymer backbone and the drug is released by a hydrolysis or enzymatic cleavage. The rate of drug release is controlled by the rate of hydrolysis [77]. This approach provides an opportunity to target the drug to a particular cell type or tissue. Natural polymers such as polysaccharides as well as synthetic polymers such as polylysine, copolymers of 2-hydroxypropylamide, and others have been used as drug carriers in such systems.

13.9.3 Solvent Activated System

Solvent activated systems can be classified into osmotic controlled and swelling controlled systems.

13.9.3.1 Osmotic controlled systems

In these systems osmotic pressure is used as the driving force to generate a controlled release of the drug. In an osmotic controlled system, either an osmotically active drug or an osmotically inactive drug along with an electrolyte is surrounded by a semipermeable membrane with a small orifice [71, 78]. The membrane allows permeation of water but not the drug. When the tablet is exposed to water, water will flow into the tablet due to the osmotic pressure difference. The rate of water influx controls the overall rate of drug release. The release rate remains constant as long as the drug concentration across the membrane is constant [79].

13.9.3.2 Swelling controlled-release systems

When a polymer comes in contact with an aqueous environment, it begins to absorb water. This water uptake can lead to a considerable swelling of the polymer. Because of the swelling, the drug, dispersed in the polymer, begins to diffuse out. Therefore, drug release depends upon two simultaneous rate processes: water diffusion into the polymer and polymer chain relaxation [80–82]. The continued swelling of the matrix causes the drug to diffuse out at a faster rate. The overall drug release rate is controlled by the rate of swelling of the polymer network.

13.9.4 Modulated Release Systems

In these systems, drug release is controlled by external stimuli such as pH [83–85], ionic strength [84], temperature [71, 85, 86], solvent exchange, magnetism [87],

and ultrasound [71, 88]. Hydrogels, which would respond to these external stimuli, can be used as controlled-release devices. Hydrogels by definition are network of hydrophilic polymers that can swell in water and hold a large amount of water without dissolution [86, 89]. Hydrogels have significant structural advantages that can provide them with properties similar to biological tissues. They also manage to maintain mechanical integrity. Formulations of hydrogels exhibit a range of physical, chemical, and biological properties that can provide controlled, pulsed, and triggered drug release characteristics. Drug transport mechanism depends on drug size and aqueous solubility, water polymer partition, coefficient polymer composition, and degree of hydration. The property of the polymer swelling that often results from the glassy rubbery transition of the dry form is used as a drug-delivery control mechanism.

Whilst the aforementioned controlled-release mechanisms have been extensively exploited for developing safe and more efficient veterinary drug delivery systems for ruminal, vaginal, parenteral, and topical applications; they remain underutilized in the intramammary field.

13.9.5 In Situ Gelling Systems

Amongst the potential delivery systems that can be infused into the mammary gland, in situ gelling systems can serve either as a vehicle for controlled delivery or as a physical barrier. Formulations that gel upon administration of liquid polymeric systems when injected/infused into the body have attracted increasing interest [90–92]. These systems are injectable low viscosity fluids prior to injection and undergo a rapid chemical/physical change induced by the surrounding physiological environments when introduced into the body. Thus an in situ formed depot forms a reservoir to control the release of a drug within the desired tissue, organ, or body cavity [93]. Moreover, such systems can transform into a semisolid or solid mass and presume the shape of the surrounding cavity, serving as a barrier membrane in addition to serving as a reservoir for extended drug release [94]. Such polymer formulations can gel in vivo in response to various triggers such as solvent exchange, temperature, pH, and ionic strength [94–96]. This concept has been investigated for numerous delivery applications such as parenteral delivery [91, 92, 97], intratumoral delivery [98], tissue engineering [99], gene delivery [100], ophthalmic delivery [101–103, and oral delivery [104–109].

The type, molecular weight, concentration of the polymer, and drug load can control the release rate of drugs from the depot and the system can be tailored to meet the desired therapeutic effect. Natural polymers such as chitosan [110, 111] have been studied for use in oral and ophthalmic drug delivery [104, 106, 112]. Similarly xyloglucan [102, 103, 108, 113] and gellan gum [103] have been studied for ophthalmic drug delivery. Cellulose derivatives such as ethyl cellulose and HPMC have been studied for ophthalmic drug delivery [114–116] and rectal drug delivery [117].

Synthetic polymers such as poloxamers have been studied as in situ gelling vehicles for intratumoral [98], intraperitoneal [118], ophthalmic [119], and parenteral drug delivery [120–122]. Polyacrylic acid has been studied as an in situ gelling delivery vehicle for gene delivery [100] and ophthalmic delivery [101, 115], and polyacrylic acid/chitosan interpolymer complex have investigated as vehicles for oral delivery [123, 124]. Non-polymeric materials such as glycerol monooleate [22, 125] and sucrose acetate isobutyrate (SAIB) [126] have also been studied for the controlled release of bioactive materials in in situ depot-forming delivery systems for delivering a variety of drugs in humans and in the veterinary field.

In addition to drug delivery applications, in situ gelling systems have also been studied as tissue sealants and adhesion barriers in medicines in the surgical area [127] and for wound dressing purposes [128–132]. Following hydration of the wound dressing containing carboxymethylcellulose sodium (CMC), the formation of a cohesive gel has been effective in encapsulating large populations of potentially pathogenic bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* under the gelled surface, as well as being immobilized within the swollen fibers [129]. The ability of some wound dressings to sequester and retain microorganisms for controlling infection was studied by Bowler et al. [133].The study has demonstrated that a hydrofiber dressing containing alginate effectively sequesters and retains microorganisms upon exposure to simulated wound fluid, and may therefore provide a passive mechanism for reducing the microbial load in wounds and in the surrounding environment [133].

The unique characteristics of these gels, including their softness and tissue compatibility, are advantageous as they may reduce mechanical and frictional irritation to the tissues. Moreover, formulations can be tailored to allow rapid solidification at the site of administration.

As mentioned earlier, there are several possible mechanisms that can lead to the formation of a gel or a semisolid depot in situ. Based on the mechanism of gel formation these systems can be categorized as thermosensitive gels, pH-sensitive gels, ion cross-linked gels, SABER delivery systems, and systems based on polymer precipitation.

13.9.5.1 Thermosensitive Gels

Some polymers show a phase transition when the temperature is raised above its lower critical solution temperature to form a gel. Aqueous solutions of poly(ethylene oxide-b-propylene oxide-b-ethylene oxide) (PEO-PPO-PEO) block copolymer also known as poloxamers have been shown to undergo in situ gelling at physiological temperature [134]. Application of this system was studied for an antitumor drug by Amjii and coworkers [98]. Following intratumoral administration of paclitaxel–poloxamer 407 formulations, improved antitumor efficacy was observed. Application of Poloxamer 407 has been also studied for the release of human a growth hormone [135] and interlukin-2 [118].

Aqueous solutions of triblock copolymers of polyethylene glycol and poly(lactic/glycolic acid or PEG-PLA-PEG) were developed by Jeong et al. [136, 137]. It is a free-flowing solution which exists in a liquid state at room temperature and changes into gel at body temperature and has shown potential to control the release of a drug. The results in the above study showed that a model hydrophilic drug, ketoprofen, was released over two weeks with a first-order release profile, whereas a model hydrophobic drug spironolactone was released over 2 months [97].

13.9.5.2 pH-sensitive Gels

Charged, water-soluble polymers may form reversible gels in response to changes in pH. Chitosan solutions exhibit a liquid–gel transition around pH 7, when pH changes from slightly acidic to neutral. Carbopol (Polyacrylic acid, PAA) in combination with HPMC was used for the ophthalmic delivery system of an antibacterial ofloxacin, based on the concept of pH-triggered in situ gelation [115]. The formulation provided release of the drug over an eight-hour period. Gel formed in situ following oral administration of 1% (w/v) aqueous solution of gellan gum (Gelrite or Kegel), an anionic deacetylated polysaccharide, to rats and rabbits were evaluated by Miyazaki et al. [107]. The formulation contained calcium ions in complex form, the release of which in the acidic environment of the stomach caused the gelation of the gellan gum. The bioavailability of theophylline from gellan gel formed by in situ gelation in the stomach was increased by four to five times in the rats and threefold in the rabbits, compared with that from commercial oral formulations [107]. A water insoluble interpolymeric complex (IPC) containing poly(methacrylic acid) (PMA) and poly(ethylene glycol) (PEG), in a biocompatible co-solvent system that transforms into a gel at physiological pH, has been demonstrated to control the release of macromolecular drugs such as proteins and oligonucleotides [138].

13.9.5.3 Ion Cross-linked Gels

Water-soluble charged polymers may also form gels when reacted with di- or trivalent counter ions. The aqueous solution of alginate which is a biodegradable polysaccharide with mannuronic and guluronic acid repeating units and gellan gum (Gelrite) is cation-sensitive in situ gelling polysaccharides [103, 139] which form gels when mixed with divalent cations such as calcium [104, 106]. Oral administration of aqueous solutions of either gellan gum (1.0%, w/v) or sodium alginate (1.5%, w/v) containing calcium ions in complex form resulted in the formation of gel depots in rabbit and rat stomachs as a consequence of the release of the calcium ions in the acidic environment [107]. In vitro studies demonstrated diffusion-controlled release of paracetamol from the gels over a period of 6 hours [104]. Gels formed in situ following oral administration of aqueous solutions of sodium alginate (1.0–2.0% w/v) and theophylline in rats were evaluated by Miyazaki et al. [106]. They resulted in an increase of theophylline bioavailability of 1.3–2 in rats [106].

13.9.5.4 SABER systems

SABER is controlled-release platform which can be used to deliver a wide range of therapeutics over a period of weeks to 3 months. SABER technology is an in situ gelling system based on a non-polymeric scaffold of sucrose esters, such as SAIB [126, 140, 141]. In this technology, a highly viscous product forms on contact with water. Addition of co-solvents such as ethanol and *n*-methyl-2-pyrrolidone dilutes the SAIB and reduces its viscosity. As soon as the formulation is injected, the solvent will diffuse out of the formulation, which immediately regains its high viscosity and solidifies thus entrapping the drug, which will be released for an extended period of time. An injectable gel formulation of rhGH using the SABER system sustained-release depot formulation was recently studied by Okumu et al. [126]. In this study the sustained release of intact rhGH in vivo for at least 7 days was achieved [126].

13.9.5.5 Polymer precipitation

Another mechanism that has been explored in drug delivery is the polymer precipitation method based on the polyester class of polymers. An in situ polymer depot is formed in this method due to polymer precipitation induced by solvent exchange because of the insolubility of the polymer in water [95]. If a drug is incorporated it will be entrapped in the polymer depot, forming a reservoir for release of the drug slowly over time. The recently developed Atrigel system (Atrix Laboratories, Fort Collins Colo, USA) is based on a polylactide and polyglycolide/NMP system. A luteinizing hormone-releasing hormone (LHRH) agonist leuprolide acetate product (Eligard) has been approved for the treatment of prostate cancer [142]. Another product Atridox® [8.5% doxycycline) is used for periodontal treatment and localized sub-gingival delivery of doxycycline [142, 143]. ATRISORB®, a membrane barrier for the periodontal pocket based on the ATRIGEL system, is also in the market [95, 143].

Examples of some in situ gelling products that are already in the market and in the development stage are shown in Table 13.2.

13.9.5.6 Application of In Situ Gelling Systems in the Veterinary Field

Very little research has been performed on in situ gel-forming systems in the veterinary area. The product DOXIROBE, an in situ gelling product based on the ATRIGEL (PLGA-PLG/NMP) system, is marketed by Pfizer for the treatment and control of periodontal diseases in dogs. Another product ATRISORB, a barrier membrane for tissue regeneration also based on the ATRIGEL system, was studied in the premolar and molar teeth of beagle dogs and resulted in new periodontal supporting tissues becoming reconstituted on root and furcation surfaces [156].

Table 13.2 Marketed and in advanced clinical development in situ forming gel/depot systems, modified from [94]

Product	Therapeutic category	Delivery system	Development stage	References
OncoGel®	Oncology	ReGel®	Phase II trial	[97, 136, 144–146]
ATRISORB®	Dental gel	ATRIGEL®	Marketed	[95, 143, 147–150]
ATRIDOX®	Dental gel	ATRIGEL®	Marketed	[94, 95, 147, 148]
Eligard®	Oncology	ATRIGEL®	Marketed	[95, 151, 152]
Postoperative pain depot	Pain management	SABER®	Phase II	[126, 140, 141, 153]
BST-CarGel®	Cartilage repair	BST gel®	Under clinical development	[110, 154]
Elyzol®	Dental gel	Glycerol monooleate	Marketed	[125]
Doxirobe®	Dental gel for dogs	ATRIGEL®	FDA Approved	www.pfizerah.com
Timoptic-XE®	Ophthalmic	Gelrite®	Marketed	[103, 155]

ReGel—PLGA-PEO-PLGA
ATRIGEL—Polyester/organic water miscible solvent
SABER®—Sucrose acetate isobutyrate/ethanol
Gelrite®—Gellan gum

Furthermore, Poloxamer 407 has been investigated for the delivery of ceftiofur, intended to treat foot infection in cattle by Zhang et al. [157]. In vitro release study indicated a zero-order release of the drug from poloxamer gel.

SABER delivery system has been studied in delivering progesterone and estradiol for veterinary application [158]. SABER system was also studied for the veterinary application of GnRH analogue deslorelin [159, 160]. SABER Mate E (Equine), a short-acting controlled-release dosage form containing deslorelin acetate in SABER, has been studied in gilts and mares [159–161].

The use of one of these systems can prolong the release of drugs from a few hours to a few months. This technology offers advantages over traditional dosage form as it reduces animal handling frequency. These studies clearly show that there is scope for more research to be done in the area of in situ gelling delivery systems for application in the veterinary field, specifically for the application and treatment of IMIs. However, the development of such a system must address the drawbacks shown by the aforementioned in situ methods.

Most thermosensitive polymers are of limited use for parenteral administration, mainly because they are not biodegradable. The PEG and poly(lactic acid) block copolymer systems were liquid at temperatures around 45° C and gelled upon cooling to body temperature [137]. However, despite the biocompatibility of the copolymers and their biodegradability, the need to heat the solution to incorporate the drug and administer the system makes this approach impractical. With the precipitation method, incomplete gel formation can result in a high initial release and cause local or systemic toxicity [94].

Hydrophilic polymers activated by water enable the formation of in situ gels based on the interaction between water and polymer. These polymers do not respond to the presence of triggering stimuli such as pH, ionic strength and temperature but respond to the presence of water or biological fluids, which change the polymer structure enabling the drug to be released at a controlled rate from the dosage form.

13.10 Concluding Remarks

Mastitis, an infection of the bovine mammary gland, is caused by a number of pathogens, most frequently bacteria. Given the anatomical and physiological constraints that render drug delivery to the mammary gland problematic, and the diversity of bacteria that cause mastitis, mastitis has been difficult to control. The use of antibiotics has, for many decades, been the standard mode of treating mastitis. Despite extensive antibiotic usage, mastitis remains the most prevalent disease in dairy cows and causes a considerable economic loss to the dairy industry. Although antibiotics are integral in the battle against mastitis, there is an increased concern over emerging antibacterial resistance due to the overuse of antibiotics in cattle animals. Therefore, there is a need to search for non-antibiotic alternatives to control this prevalent disease.

The formation of a natural keratin plug occurs in cows during the dry period to close off the teat canal. This can take from one week to sixty days. While the keratin plug is an important natural form of resistance against mastitis during this period the delay in its formation can leave the teat canal vulnerable to infection. Awareness of the economic impact of mastitis is driving the development for control programs. Such programs aim at early detection of mastitis via the identification of the causative organism(s) and prevention of their transmission by removing the source of the infectious agent (infected cows, vectors, fomites, etc.). Understanding the mammary defense system, anatomy and physiology, microbes, their habitats and virulence, milking cycle and dynamics, as well as the mode of action of various therapeutics agents including antibiotics is critical in achieving effective mastitis control.

The issues arising from widespread use of antibiotics leading to resistant bacterial strains, poor cure rates, and reoccurrence of infections after antibiotic therapy, all highlight the need for a new technology that can control such infections more effectively.

Due to the aforementioned limitations and concerns regarding the treatment and control of mastitis in food-producing animals, it is important to reflect on existing technologies for the prevention and control of mastitis. Critical appraisal of existing technologies and their modes of action will indeed improve and overcome some of the inherent formulation and stability problems. Nevertheless, there will always be a need for innovative intramammary drug delivery technologies that can ideally serve multiple purposes, such as prevention, symptom alleviation, and cure of cattle mastitis.

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Chapter 14
Veterinary Vaccines

Martin J. Elhay

Abstract The technology of controlled release has always been attractive to veterinary vaccine developers as it has potential to bring a level of convenience, efficacy, and compliance to the use of vaccines. The ability to have in a single therapy or device all that it is required to sensitize and protect an animal may deliver access to new markets, provide differentiation to existing products, and solve hitherto unmet needs in veterinary immunology. However, few controlled release vaccine formulations are available at this time despite the sophistication of the materials and devices used for controlled release of medical and veterinary therapeutics. Recent advances in our understanding of how the immune system is sensitized is beginning to allow us to apply new and old technology of controlled release to vaccines across a number of livestock and companion animal species. The technology allows us to control how antigen and adjuvant are presented to the host with increasing fidelity resulting in appropriate levels and duration of immunity. The discovery that many adjuvants work by stimulating the innate immune system has led to the development of molecular adjuvants, which are well suited to devices and controlled release formulations. As controlled release vaccine formulations near late development there are regulatory concerns that need to be addressed as familiar and unfamiliar vaccine components are presented to regulators in a persistent form.

14.1 Introduction

The case of controlled release of veterinary vaccines in general is an interesting one in the realm of veterinary medicine. Unlike other preventative or therapeutic drugs, which may be directed toward a single biological target, the aim is to present to the

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immune system a package consisting of an immunogen and immunostimulatory molecules in a particular context that will afford relevant and, usually, sustained immunity. This does not necessarily mean that there needs to be continuous high levels of active ingredient but, rather, judicious presentation of antigen to the immune system in the context of appropriate immune signals. This is achieved normally by repeated immunization with vaccine formulations that characteristically are a mixture of antigen and adjuvant. An adjuvant is required to provide the host with a signal [1, 2] that indicates that the antigen should be treated by the body as foreign or a danger to the host and consequently neutralized by one or a number of immune mechanisms. The mechanisms of adjuvant action are beyond the scope of this chapter; however, it needs to be noted that adjuvants have a complex role in both activation of the immune system and presentation of antigens through release or retention of antigen. It is clear that existing veterinary vaccines have an innate release profile of antigen and immune stimulatory signals; however, in many applications this release is not adequately controlled, requires revaccination, or does not provide adequate, relevant, or sustained immunity. It is for these reasons that the technology of controlled release is being applied to veterinary vaccines to provide convenience and efficacy and to open up potential markets that conventional vaccines cannot satisfy.

14.2 Evolution of the Immune System and Vaccination

The immune system of mammals is designed to protect the young in the first days, months, and years, while their immune defenses are not complete, from attack by pathogens [3]. As such it has evolved to respond to pathogens and pathogen associated molecules presented to the immune system either neutralized (e.g., by maternal antibody) or in some otherwise nonlethal form (e.g., low numbers or amount). Furthermore, the hard wiring of the innate immune system to recognize pathogen associated molecular patterns [4] is evidence that the immune system has developed to recognize pathogens and respond appropriately. Many of today's successful vaccines are modified live versions of the pathogen and are often viruses. These vaccines take advantage of the fact that the vaccine form of the pathogen will only have limited replicative or infective capacity in the host before the host overcomes the virus. For many vaccine applications, however, this is not a strategy that can be followed, and killed, or subunit, versions of the pathogen are the only options available for obtaining relevant antigen. Killed and subunit antigens, however, are notoriously less efficacious than living attenuated organisms. They need, therefore, to be given in several doses usually at quite high levels to achieve some effective level of immunity [5]. Some antigens such as tetanus and botulinum toxoids give very long-term immunity and may represent special cases of antigens that naturally elicit excellent long-lasting immune responses with botulinum toxoid being efficacious as a single dose vaccine without any particular need for a special formulation [6].

14.3 Controlled Release as the Key to Making Better Vaccines

Traditionally vaccines have been given a number of times so that the host can develop adequate immunity [7]. The first dose introduces antigen in the context of some sort of danger signal, usually in the form of an adjuvant, and results in a rapid expansion of antigen-specific B-cells [8]. Without the persistence of antigen in the right context, it is likely that this first response will diminish. Reintroduction of the target antigen and associated co-stimulation will usually result in the completion of the immunizing process with expansion B cells and somatic hypermutation resulting in higher affinity antibodies to the antigen [9]. Sometimes another immunization is required for adequate immunity to be achieved. Even so in many veterinary situations, such as ruminant clostridial antitoxin immunity, antibody levels are only maintained at protective levels for a number of months [10]. It is in situations such as these that a method that either simulates current vaccine protocols or otherwise produces long-lasting effective immunity is desired. There is then a case for using controlled release technology developed for human pharmaceutical application or vaccines in a veterinary context. As for the human vaccine application of these technologies, the underlying immunologic mechanisms of sensitization need to be considered.

14.4 Continuous or Pulsatile Release

Controlled release of antigen and adjuvant that mimics vaccination protocols may be sufficient for some antigens such as tetanus toxoid where immunity is relatively long lived. For others a release profile that constantly boosts for example every 3, 6, or 12 months might be required. The question arises then if a phased or continuous release is required. Some have argued that continuous release may lead to tolerance of the antigen [11] and indeed, experimentally, soluble antigen can render antigen-specific B cells anergic [12]. It is possible that this phenomenon may be due to the lack of a concomitant danger signal that can be provided by adjuvant [13]. The danger signal, provided by adjuvant, would ensure that antigen is always seen by the immune system in the context of co-stimulation. An advantage of manipulating the kinetics of vaccine release through controlled delivery is that an effective immunizing therapy can be delivered, potentially, with a single administration of vehicle, device or implant. As mentioned earlier, vaccination schedules characterized by two or three doses can be mimicked with systems that deliver in a pulsatile manner [14–16]. However our understanding of how the immune system develops a response to infecting pathogens informs us that, under appropriate circumstances, continuous delivery of antigen in the right context may result in superior immunity [17, 18]. Whether a pulsatile or continuous release profile is decided upon depends as much on the technology available as the mechanism of developing immunity. Thus to achieve pulsatile or stepped release of vaccine there are several methods available including: administration of coated and uncoated vaccine with immediate and delayed release kinetics [19], mixed phase systems such as microparticles

suspended in an aqueous adjuvant [15], poly(lactic-co-glycolic) (PLGA) microspheres with an oil core [20], exogenous adjuvant incorporated into microparticles [14], or a mixture of particles with different release times (Gander et al. 1993 cited in [14]). Needless to say many of these approaches are complex and potentially costly. Dispensing with the need for a stepped delivery of vaccine can increase the flexibility for controlled release if continuous release can afford the immunity required. In these situations attention must be paid to the magnitude and rate of delivery to ensure that immunologically relevant quantities of antigen are presented with appropriate co-stimulatory signals [13, 21]. Satisfaction of these criteria can result in good immunity from a continuous release system without the need for emulating current vaccination protocols in a single dose therapy.

14.5 Role of Adjuvants in Controlled Release

As mentioned above, key to the success of controlled delivery of vaccines, especially continuous delivery, is the co-delivery of the appropriate co-stimulatory signals [22–24]. The primary signal comes from the interaction of the antigen in the context of major histocompatibility complex molecules with its cellular cognate receptor. The second signal comes from engagement of cellular co-receptors ensuring the close and specific interaction of antigen presenting cells with sensitizing B and T cells. The third signal comes from soluble mediators released by the antigen presenting cell that have tremendous influence on the type and quality of the subsequent immune response [9]. Adjuvants have a role in mediating or stimulating all three signals directly or indirectly. As a vehicle for delivering the vaccine the very physical presence of the adjuvant (or controlled release device) can cause damage on injection and thus send danger signals to the innate immune system. The chemical nature of the adjuvant may cause toxicity resulting in further damage including cell necrosis, which will lead to inflammation. Some adjuvants directly engage pathogen associated molecular pattern receptors such as Toll-like receptors (TLRs) and thus signal the innate system directly [25]. Thus by stimulating directly or attracting antigen presenting cells, adjuvants initiate the immune sensitizing process and influence its outcome depending on the physical and chemical properties of the adjuvant. When combined with a controlled release delivery mechanism there is further fidelity in the control of that sensitization process. Until recently co-stimulation has been in the form of traditional adjuvants such as aluminum salts and oil emulsions. Now with the increase in our understanding of how adjuvants work through particular stimulatory pathways, a molecular approach can be applied. We now understand for example that responses to Monophosphoryl lipid A (MPL), a derivative of lipopolysaccharide (LPS), are mediated through TLR 4, Polyinosine-polycytidylic acid (Poly I:C), an analogue of double stranded RNA, works through TLR 3, and CpG oligodeoxynucleotides (unmethylated cytidine guanidine bacterial DNA motifs; [26]) exert their effect through TLR 9, to name just a few (reviewed in [25]). These molecular adjuvants are well suited to controlled release systems and are finding their place already in experimental systems [27, 28]. The advantage

of molecular adjuvants may be realized as decreased reactivity, compared to conventional chemical adjuvants, and perhaps decreased volume allowing for increased payload or lower overall delivery volume.

14.6 Kinetics of Antigen Delivery to Lymphoid Organs and the Importance of Antigen Persistence

Delivery of the correct antigenic and immune stimulatory signals needs to be timely and geographically appropriate [17, 29]. That is antigen and immunostimulatory signals need to be delivered with the site of injection (e.g., intradermal or subcutaneous), site draining lymph nodes, and changes in cell populations in mind. Although some measure of controlled release and antigen delivery to antigen presenting cells can be afforded by conventional adjuvants [30], controlled release systems have an enormous potential over conventional vaccines in providing this capability. Furthermore, depending on the controlled release device or system, it is possible to target the same lymph node with antigen and immune signals as discussed above. Since the persistence of antigen on follicular dendritic cells (FDC) is a feature of long-lived effective immune responses, the amount of antigen released by a device or system over time may be of critical importance for the development of sustained effective responses. Continual or repeated release of antigen has the potential to resupply or replace the need for lymph node FDC. Secondary lymphoid tissues are adapted to maximize the chances of antigen interacting with the immune system and subsequent generation of an effective antibody response [31]. Subsequently, it takes a number of days to develop a primary response and ideally persistence of antigen would continue until specific antibody has formed to allow immune complexes to form and establish a presence on FDC in secondary lymphoid tissue. However many injectable vaccines deliver a bolus of antigen that may or may not be available in a form that results in antigen persistence. Even aluminum gel-based vaccines deliver much of their antigen within a few hours and the rest in a few days [30, 32]. For some antigens this may not be effective (e.g., antigens such as *Clostridium perfringens* D toxoid) and so a constant supply of antigen, draining to the local lymph node, would be highly effective. In some cases the presence of large amounts of antigen in a particular site can lead to the formation, *de novo*, of tertiary or ectopic lymphoid tissue, which may be involved in the immune response to persistent antigen including the presence of FDC [33, 34].

14.7 Applications Requiring Controlled Release in Veterinary Medicine

In human health there is a clear need for single dose vaccines that was recognized by the World Health Organization in the late 1980s [35]. The greatest impact of vaccines is often seen in countries that can least afford them. Furthermore logistical

reasons often means that compliance is difficult to achieve for vaccines that need one or more follow-up doses to be effective [16]. In animal health the motivating factors are somewhat different though overlaid on the same immunological basis. A major cost to livestock farmers is morbidity and mortality caused by diseases that can be prevented or ameliorated by vaccination with vaccines that are currently available. These vaccines need to be given at least twice and, depending on the duration of immunity required, booster doses need to be given at intervals as frequently as every 3 months though more usually 12 months. The high cost of labor, diesel, and time, therefore, means there is a problem with adherence to recommended protocols for vaccination. An extreme example of this situation is that of extensively ranged cattle in the north of Australia that are mustered infrequently due to harsh climatic conditions and terrain. These cattle would benefit greatly from immunization with a single dose vaccine for a variety of diseases prior to turning out before the wet season. To obtain that single dose efficacy some sort of controlled release vehicle or device is required. It is obvious that single dose vaccination would give the farmer a cost benefit for vaccines that need to be given more than once. In addition to compliance there are other reasons for using single dose vaccines. For companies, the availability of such vaccines can open up markets such as the aforementioned north of Australia, that currently do not use vaccines available in temperate regions where farming practices allow more frequent animal handling. Another potential use of controlled release vaccines could be in overcoming maternally derived antibody [36], which is a problem for several livestock and companion animal species. In this situation sensitization can continue in the face of maternal antibody. When maternal antibody finally wanes antigen can continue to be presented in the context of a vaccine [37], in this case one delivered in a controlled manner.

14.8 Examples of Controlled Release Vaccine Application in Target Species

The commercial and practical application of controlled release for drugs in livestock and companion animals is well established. In contrast most of the work in developing systems for controlled release of vaccines has been limited to laboratory species in the main. There are few examples of veterinary application of controlled release systems for vaccines (Table 14.1). Much of the work has been carried out in mice and rats for antigens/disease targets of veterinary importance. Tetanus toxoid is an important vaccine antigen for humans, but is of course a very important target for livestock and horses especially. Controlled release tetanus toxoid vaccines have been examined in rodent models [38–41] and in sheep [42–44]. Other important antigens for veterinary species include *Dichelobacter nodosus* [19] and *Pasteurella haemolytica* [45]. The human pathogen *Bordetella pertussis* was examined using the VacciMax™ liposome in oil single dose delivery system [46]. *B. pertussis* is closely related to *B. bronchiseptica* that is involved in infectious tracheobronchitis in dogs and atrophic rhinitis in pigs. Viral antigens bovine herpesvirus 1gpD [47, 48] and avian metapneumonia virus [49] have also been examined. An interesting non-disease target for controlled release application in veterinary medicine is immunocontraception.

Table 14.1 Examples of controlled release technologies used in animal species or against pathogens of veterinary importance

Technology	Dose profile	Target disease/antigen	Species	Result	References
Microspheres					
PLGA and PLA	Continuous erosion	Tetanus toxoid	Mice	Negative effects of erosion on antigen stability	[38]
Poly-lactide microspheres	Continuous erosion	Tetanus toxoid	Rats	No toxicity	[39]
Spermine-Alginate microspheres	Intramuscular	Bovine Herpesvirus 1 glycoprotein D	Mice	Dose sparing	[47]
Alginate microspheres	Oral delivery	Ovalbumin model antigen	Cattle	Mucosal immunity in the lung	[71]
Alginate microspheres	Oral delivery	*Pasteurella haemolytica* outer membrane protein	Mouse	Improved oral and subcutaneous efficacy following encapsulation	[45]
Poly-lactide co-glycolide (PLGA) and chitosan microspheres	Continuous erosion	Tetanus toxoid	Guinea pigs	Trehalose stabilized protein. Antacid to balance pH changes	[41]
PLGA	Continuous erosion	GnRH-I immunocontraception	Sheep	Indicated requirement for co-adjuvant	[54]
PLGA	Oculonasal delivery of encapsulated DNA or protein	Metapneumovirus F-protein	Turkeys	Prime with DNA and boost with protein	[49]
Implant					
Osmotic swelling pump device (silicon rod, porous polyethylene cap)	Pulsatile	Tetanus toxoid powder	Sheep	Equivalent to aluminum adjuvanted tetanus toxoid	[42]
Cholesterol lecithin biodegradable implants Quil A adjuvant	Continuous and prime-boost	Recombinant *Dichelobacter nodosus* pili (footrot)	Sheep	Safer and equivalent immunity to non-implanted 2 dose vaccines	[19]
Collagen minipellet	Continuous release	Tetanus toxoid and diphtheria toxoid	Mice	Better antibody levels than aluminum hydroxide gel	[40]
Collagen minipellets	Continuous erosion	Model antigens clostridial toxoids	Sheep	Equal or better immune to aluminum hydroxide gel	[43]
Silicone devices	Continuous erosion or release	Model antigens clostridial toxoids	Sheep	Equal or better immune to aluminum hydroxide gel	[44]
Injectable silicone implant including rovInterleukin 1β	Continuous	Avidin (model)	Sheep	Recombinant IL-1 required for effective immunity	[13]
PLGA	Extrusion, evaporation and molding	Porcine Zona Pellucida	Wild horse others	Reduced fertility	[51]
Liquid phase					
VacciMax™	Liposome in oil	*Bordetella pertussis*	Mice	Higher antibody levels than conventional adjuvant	[46]
Agarose hydrogel	Continuous release	Bovine Herpesvirus 1 glycoprote in D DNA	Cattle	Equivalent immunity to intradermal immunization	[48]

For blocking fertility in animals while maintaining sexual activity (and herd integrity), horses can be vaccinated against porcine zona pellucida glycoprotein [50]. However to more effectively vaccinate wild animals a single dose controlled release form is preferable [51]. Another contraceptive vaccine target is gonadotrophin releasing hormone (GnRH, see also Chap. 16 by McDowell). It is notoriously difficult to develop long-lasting immunity to GnRH and so repeated injections are required to obtain even year-long duration of immunity. This situation is acceptable for control of boar taint in male pigs [52] and estrous-related behavior control in mares [53] where short duration of effect is satisfactory. For long-term or permanent sterilization and behavior modification, however, a controlled release mechanism is required. For livestock this has been attempted with some success in sheep with a PLGA microspheres containing GnRH [54]. Taken together a number of studies have looked at controlled release as a delivery mechanism for killed vaccines for veterinary applications. No consistent approach has emerged perhaps reflecting the diversity of applications and multiplicity of technological approaches available.

14.9 Technologies for Controlled Release of Veterinary Vaccines

14.9.1 PLGA and PELA

By far the most examined material for use as a controlled release vehicle is PLGA [55]. This is the same material found in resorbable sutures used in surgery and so has a long history of clinical use and safety [33, 56]. Using different ratios of the lactic and glycolic acid components different release kinetics can be achieved. Injectable microspheres containing the antigen can be made in such a way that there can be continuous or sometimes pulse-phased release of antigen [20]. To formulate as a vaccine delivery vehicle usually involves solvents that may be detrimental to the intended payload [16]. As PLGA breaks down there is a change in pH leading to an acidic environment that may affect vaccine antigens detrimentally. A solution to this problem is to include protein stabilizers and antacids [41]. Furthermore there are difficulties in sterilizing microspheres due to their size and gamma irradiation may not be suitable for the PLGA nor the antigen's integrity. Related to PLGA is the block copolymer poly-lactide poly(ethylene glycol) PELA (reviewed in [57]), which may have some advantages over PLGA for vaccine delivery due to its more hydrophilic nature.

Nevertheless interest continues in PLGA as controlled release vehicle for veterinary vaccines. Recently a prime-boost strategy has been described for vaccination of turkeys against metapneumovirus [49] where turkeys were immunized with microparticles enclosing the antigen and microparticles with adsorbed plasmid for the same antigen in a prime-boost strategy. Importantly the microparticles were delivered via the oculonasal route, which is route suitable for vaccination large numbers of chicks.

14.9.2 Alginate and Naturally Derived Controlled Release Materials

A number of naturally derived compounds, such as collagen [40, 43], chitosan [41, 58], cholesterol/lecithin/Quil-A [19], hyaluronic acid [59], carageenan [60], and alginate [61] have been used as slow release carriers/adjuvants (see Table 14.1]. Many of these have been developed in the hope of generating a controlled release matrix that can be formulated with antigen under mild conditions. Furthermore, their biocompatibility and generally safe chemical nature make them ideal for immunizing via mucosal routes so that relevant local immunity can be generated. Alginate microparticles are created by cross-linking unbranched glycouronans from brown seaweed species with divalent cations [62]. Alginate especially has been used for pathogens of veterinary importance and for inducing mucosal immunity in livestock. Alginate microspheres have shown promise delivering important vaccines for respiratory disease in cattle including bovine herpesvirus [47], *Pasteurella haemolytica* [45], and *P. multocida* [62]. Although alginate has been attributed as having immune modulating properties [62] the relatively benign nature of alginate and other hydrogels means that it may be necessary to deliver these vaccine delivery systems with additional adjuvant to ensure the desired type of immunity and sufficient duration of immunity.

14.9.3 Oils

Oil-based adjuvants are widely used in veterinary applications because of their efficacy and relatively low cost. The mechanism of action is thought to be due to the irritating capacity of the oil and the fact that they can form a depot for antigen release [62, 63]. Oil adjuvants are not normally regarded as controlled release per se; however their hydrophobicity lends them to delay the release of hydrophilic or aqueous emulsified components. In certain forms they have proven to be very effective delivery mechanisms providing single dose efficacy. The success of oil adjuvants as single dose vaccines appears to depend on antigen and perhaps the species of animal to be vaccinated. So for example, for cattle, botulinum toxoids C and D can be formulated as a very successful single dose water-in-oil-in-water vaccine [6], though the antigen may have important single dose capabilities on its own as a Quil-A aluminum phosphate gel also gives single dose efficacy for these antigens [6]. Water-in-oil adjuvants are routinely used for immunization of fish [64] and these are generally regarded as effective although the fish immune system is notoriously slow to react immunologically [65]. For fin fish vaccination there is an absolute requirement for single dose efficacy and it is perhaps fortuitous that oil based adjuvants tend to form free floating depots when injected intraperitoneally [66]. An adaptation of the conventional water-in-oil presentation involves replacing the water phase with liposomes containing commercial or commercial ready vaccine.

This system (VacciMax™) affords true single dose efficacy for *Bordetella pertussis* [46] and recombinant hepatitis B surface antigen [67] regardless of antigen or species target. A further variation of this approach is to freeze dry the liposome component (DepoVax™) and dissolving them in the oil [68]. One can speculate that this water-free oil vaccine may have advantages by stabilizing antigen and co-adjuvant as well as contributing further to the slow release profile of this vehicle.

14.9.4 Implants

Implants have an inherent attractiveness as a mechanism for delivery of vaccines as they can provide flexibility and the ability to deliver single dose efficacy. Many variations have been tried (Table 14.1] and reflect the different approaches attempted in different species and with different antigens. They have their own drawbacks including limitations in injection location and potential for residue or retention of device materials. Continuous [13] and pulsatile release [69] implants have been examined for veterinary and non-veterinary applications. Implants have an advantage over liquid or suspension vehicles in that they can provide added physical structure that conveys their functionality. PLGA implants, for example, by virtue of their smaller surface area to volume ratio will degrade and release antigen slower than a suspension of microspheres [51]. The extended time of release will give the immune system time to fully develop a response to the vaccine antigen [44]. Another method for achieving the same result is to make use of enclosed, expanding hydrogels that can drive the release of components from a blind ended rod and thus give the profile of antigen release required [42]. The delivery of an implant rather than a suspension/liquid may confer other advantages such as the ability to be removed following accidental user administration or even to facilitate delivery at a distance [70]. The latter example of ballistic delivery can open up the use of vaccines for wild or feral species such as wild horses where re-vaccination is next to impossible and single dose delivery of the complete vaccinating program is a necessity.

14.10 Impediments to the Adoption of Controlled Release Solutions

It seems that there are a number of technologies, therefore, that are available and that have the right physical characteristics to allow controlled release of veterinary vaccines. Why, therefore, are there not more on the market or in late term development? Firstly, many of these technologies are not cost effective for widespread use in livestock. It is in livestock, both intensive and extensive, that a need for controlled release vaccine solutions is most required. Most vaccines for livestock are cheap and those for poultry and fish are extremely inexpensive. For some cattle, sheep, fish etc. rehandling is a costly if not impossible operation. In these circumstances,

therefore, the cost of an implant needs to have adequate return on investment and be comparable in cost to current vaccination protocols or therapies. In addition any device providing long term efficacy must not adversely affect carcass quality and be easily removed if it is not in fact degraded or is degradable. For companion animals, although compliance is an ever present problem, repeated administration is easily managed by a revisit to the veterinarian. This brings us to the second impediment to the adoption controlled release solutions to vaccines namely the requirements of modern regulatory agencies. In food producing animals, residue analysis is routine so that human safety can be ensured. In the case of the use of controlled release devices or formulations there is probably going to be a persistence of the device, components of the device and or the antigen and other excipients including adjuvant. We know from studies of conventional vaccines that the components will persist for days to months. Therefore as regulatory agencies increase scrutiny of veterinary vaccines we need to be mindful that the persistence of components due to formulation in a controlled release form may have implications for animal and human safety. Persistence of immunological components may also have effects beyond their chemical nature including the formation of ectopic lymphoid tissue [35; DeVeer, Elhay and Meeusen, unpublished observation) or other injection site reactions that may have consequences for hide and carcass quality in livestock species and, for companion animals, unwanted esthetic consequences. Related to the residue issue is that of non-biodegradability of the device or vehicle providing controlled release. This is perhaps unique to livestock and resource animals in that nonbiodegradable implants may interfere with mechanical processing of tissues or hides. One solution for livestock is to administer the device in the ear; however, this site of delivery is becoming increasingly "crowded" by the plethora of implants and tags used to treat and identify livestock. The final reason that may explain why there are not more veterinary vaccines available in controlled release form is related to expectation of the broad utility of these technologies. Many of the technologies claim to be the solution for all vaccine requirements. There are two problems with this. The technologies are often at an early proof-of-concept stage, perhaps with data obtained in laboratory animals. Seldom are the technologies presented to potential partners for commercialization having been tested in target species. These controlled release technologies need to be in a much more developed state before they can be considered for commercialization. This creates a conundrum for inventors who are often short of funds and expertise required to get target animal data easily. Another problem for those purveying new technologies including controlled release methods is that each application is unique and no technology can satisfy the different demands of the many different real and potential products in the market. For example, it can be that once in a controlled release form, vaccine effects can have kinetics that may change their efficacy pattern and thus may not fit the product profile. In respect to this, many inventors, who often are very familiar with the market, wish to see their technology being used and are surprised and disappointed when the harsh realities of technical development costs and true market potential are made evident during licensing or co-development with a commercial partner.

14.11 Conclusion

A multitude of technologies have been, and are being, examined for controlled release of veterinary vaccines and some of these may have great potential. However it has been recent advances in our understanding of how the innate immune system senses danger through pattern recognition receptors, the development of molecular adjuvants as ligands for these, and an understanding of the geographical and timing issues surrounding development of effective immunity that provide the greatest promise for controlled release of veterinary vaccines and vaccines in general. Finally, close cooperation between those developing new controlled release technologies and those that are destined to market them is essential if we are to see practical, effective and cost conscious solutions to application of controlled release veterinary vaccines.

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Chapter 15
Delivery Systems for Wildlife

Arlene McDowell

Abstract Delivery remains a key challenge that hinders the successful remote administration of compounds utilized in the management of free-ranging wildlife. Pest wildlife occurs worldwide and management of this group of animals is shifting from lethal control methods to administration of agents that reduce the fertility of the pest species. Oral delivery of biocontrol agents is the favored route of administration; however, significant hurdles need to be overcome to achieve therapeutic in vivo effects. Regulation requirements for products to control fertility in wildlife are evolving as novel products are being developed. This chapter will outline current strategies for delivery of a number of bioactive compounds to wild animals and includes contemporary research in the field.

15.1 Introduction

Wildlife species encompass a broad range of animals living in similarly diverse habitats. In the broadest sense, wildlife can be defined as non-domesticated, free-ranging animals. This chapter will focus on terrestrial, vertebrate wildlife, although it is acknowledged that the need to deliver bioactives to species in aquatic environments is also an area of active research. A group of wild animals that are becoming increasingly important are wildlife pests [1] and this chapter will outline delivery of control agents to pest wildlife.

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Wildlife pests can cause environmental damage and have economic impacts when they impinge on production animals or agricultural and forestry practices. Wildlife can also transmit disease to other animals as well as humans e.g., the common brushtail possum is a reservoir for bovine tuberculosis in New Zealand [2] and a range of wild animals including racoons, foxes, and coyotes carry the rabies virus in the United States [3]. It has also been estimated that 60% of emerging infectious human diseases are caused by zoonoses and that 72% of these originate in wildlife [4]. The pandemics of influenza A H1N1 (swine flu) and avian influenza A H5N1 (bird flu) in recent years have brought to the forefront the importance of managing the health of animal populations.

The compounds to be delivered to wildlife parallel those used in veterinary medicine for companion animals and livestock and include vaccines, antibiotics, ectoparasiticides, anesthetics, and analgesics as well as fertility control agents. Whilst there is a considerable body of research focused on refining the delivery of therapeutic molecules to humans, livestock, and companion animals, the application of these delivery strategies to a wildlife situation is less advanced. This provides the opportunity for innovation in design of delivery systems given the diverse range of species and environments encompassed in wildlife management. Controlled release technology is prudent for applications with animals because of the reduced dosing frequencies and sustained effects and will be particularly suitable for a wildlife setting.

15.2 Management of Wildlife

15.2.1 Kept Wildlife

There is a great diversity of wild animal species that are kept in managed conditions in zoos, wildlife parks, rangelands, and animal sanctuaries. Administration of anesthesia is the most common requirement for drug delivery and an essential task to facilitate the handling of wild animals in these captive settings to prevent injury to both the animal and the handlers. The aggressive or timid nature of many wild animals means that administration of anesthetic agents must be done remotely, typically using darts. Excellent texts are available on the use of anesthetics and analgesics in non-domesticated animals and so will not be covered further here. The reader is referred to West et al. [5] and Kreeger [6] for further information on this topic.

As part of the management of kept wildlife, fertility control agents are sometimes administered. Porcine zona pellucida (ZPZ) was the first immune-based contraceptive used for wild species [7] and has since been delivered as an immunocontraceptive agent in a range of captive zoo species around the world including zebra (*Equus grevyi*), giraffe (*Giraffa camelopardalis*), and American black bears (*Ursus americanus*) [8]. ZPZ has also been investigated as an antifertility agent for the introduced European red fox (*Vulpes vulpes*) in Australia; however, there was minimal antibody response when ZPZ was administered [9].

Table 15.1 Considerations for the use of remote delivery systems to administer contraceptives to individual, unrestrained wildlife [10]

Advantages	Disadvantages
1. Specific animals in a population can be targeted	1. Target animal must be within range of the projectile device
2. Body weight can be estimated for dosing accuracy	2. Animal should be >15 kg to enable a sufficient target
3. Cost per animal lower than capturing then administering contraceptive	3. When treating few animals, costs are high
4. Flexibility in the formulation type possible (liquid, solid, etc.)	4. The devices can fail due to mechanical problems
5. A range of volumes can be delivered	5. The noise generated by the device may scare nearby target animals
6. Possible to incorporate dyes with the delivery system to mark treated animals	6. Personnel need to be trained to use the devices effectively

The most common delivery system used to administer contraceptives to individual, free-ranging animals is again dart systems projected from devices such as blowpipes or guns. Kreeger [10] has outlined the advantages and disadvantages of remote delivery systems for unrestrained animals (Table 15.1), with access to the animal being a key feature that dictates the feasibility of projectile dart systems.

15.2.2 Pest Wildlife

Globally a variety of wild animals are considered pest species (Fig. 15.1). An animal can be considered a pest because (a) it has been introduced into a foreign ecosystem and are often referred to as invasive species or (b) a native species has become over-abundant. Pest wildlife cause zoonotic diseases, damage the environment, compete with native animals, consume native flora, threaten endangered animals and are involved in wildlife–human conflicts (e.g., aircraft strikes and damage to property). The goals of managing wildlife pests are to reduce the size of the pest population to mitigate the deleterious impacts of the overabundant species [11]. A method commonly utilized to control pest wildlife is culling; however, this is controversial and does raise questions about humanness [12].

Biological control is a favored option and relies upon interrupting one or more key biological processes in the target species to cause either death or infertility. Immunocontraception has received a great deal of attention in the literature as a method of fertility control. With this technique, animals are immunized with key proteins involved with reproduction, such as egg coat proteins or sperm proteins. The body then recognizes these self-proteins as foreign and mounts an immune response against them with the result being infertility. That is, they act as a vaccine. Whilst this strategy is plausible, there are a number of limitations with immunocontraception for wildlife [13]. The first of these is administering the agent to animals

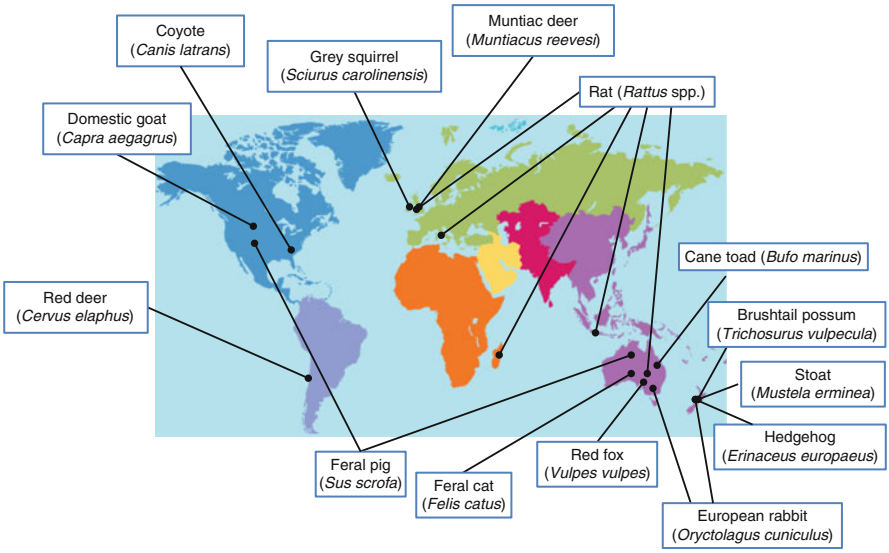

Fig. 15.1 Wildlife pests—a global problem

in the field. Studies to date have all been based on delivery via an injection, commonly followed by a booster [14, 15]. A system that relies upon injecting the animal is not feasible for the majority of wild, free-ranging animals. Individual animals also respond variably to an immune challenge; therefore a proportion of the population will not be affected by the immunization [16]. An important outcome of this is that only non-responding animals would then produce offspring. As there is a major genetic component to the immune system, these animals and their offspring in turn are likely to also be non-responders. Thus, the proportion of the population that failed to respond to the immunocontraceptive would rapidly increase over time [14]. In contrast, chemical sterilitants, or hormonal contraceptives, are alternative control agents that would act directly on target cells or tissues and not rely upon an immune response. Research in this area has been largely focused around luteinizing hormone-releasing hormone (LHRH) due to its critical role in the regulation of reproductive function.

Whether the agent is an immunocontraceptive or a chemical sterilitant, the challenge is to safely deliver these biological control agents to free-ranging, feral animals with a widespread distribution across diverse terrain and often in remote and inaccessible areas. Cost will always be an important consideration in the design of a delivery system, particularly when the target population is large and occurs over a large land area that necessitates remote delivery and is not economically valuable. When administering bioactives to free-ranging animals in a natural ecosystem where many different species coexist, the issue of target specificity is very important. Broad-scale distribution of an oral delivery system containing an active compound has the potential to affect nontarget species [17]; thus lures or attractants for

Table 15.2 Examples of products used to deliver a range of agents to wild animal species

Delivery system	Bioactive delivered	Target animal species	Reference(s)
Devices			
Boar-Operated-System (BOS™) feeder	Fertility control agents, vaccines or toxins	Feral pigs	[51]
Bio-bullet	Brucella abortus vaccine	Bison	[52]
Paxarms Syringe darts	Various liquid drugs	Wild dogs, whales, bears, monkeys	[53]
Oral baits			
PIGOUT®	1,080 poison	Feral pigs	[54]
DiazaCon™	Avian contraceptive (20,25-diazacholesterol dihydrochloride)	Pigeons and other feral birds	[18]
OvoControl®-G	Nicarbazin	Canada geese	[48]
OvoControl®-P	Nicarbazin	Rock pigeons	[47]
ONRAB®	Rabies vaccine, live adenovirus vector (AdRG1.3),	Red fox (*Vulpes vulpes*), Striped skunks (Mephitis mephitis)	[55]
Raboral V-RG®	Rabies *Vaccinia* vaccine	Racoons (*Procyon lotor*), Coyotes (*Canis latrans*)	[56]
Oral lipid matrix			
Liporale™	BCG Vaccine	Common brushtail possum (*Trichosurus vulpecula*), Badger (*Meles meles*)	[3]
INJECTIONS			
GonaCon™	LHRH	White-tailed and black-tailed deer, California ground squirrels, domestic cats, feral swine, wild horses, bison	[47, 48]
Particulate delivery systems			
Immune Stimulating Complexes (ISCOMs)	Phocid Distemper Virus 1	Seals	[34]
Equity™	Gonadotropin-releasing factor vaccine	Horses	[34, 36]

the target species are a key component for a broadcast delivery system. For example, the PIGOUT® product (Table 15.2) for feral pigs (*Sus scrofa*) in Australia is a fish-flavored cereal matrix to attract pigs and is dyed a dark green color to minimize uptake by nontarget species, in particular birds. The product FeralMone® (Animal Control Technologies Pty Ltd, Victoria, Australia) is an aerosol spray attractant of fermented egg odor that improves bait uptake by foxes and feral dogs.

Concomitant to the issue of attractants is that of palatability of the dosage form. Ideally, a single bait should contain a sufficient dose of the bioactive that, if consumed, would be able to achieve the desired biological effect in the target species. Thus, there should be consideration given to the taste of the dosage form (and bioactive, if relevant) to ensure that the product is consumed in sufficient quantities by the target species. For example, the active ingredient in DiazaCon™ (Table 15.2) is

bitter and so masking agents are necessary to increase palatability of this oral bait [18]. OvoControl®-G for geese (Table 15.2) has been produced as a bait that is shaped like corn and dyed yellow so that it resembles a natural food source of the geese and will be acceptable to them [19]. Pre-feeding of baits that do not contain the active ingredient is also a strategy often employed in the field to achieve acceptable uptake of baits [20].

A specialized aspect of delivery systems for wildlife applications is the stability of formulations under extremes of environmental conditions, as would be encountered in a field situation following broadcast distribution [1]. It can be expected that formulations would be exposed to a range of temperatures including wide day/night fluctuations, extremes of humidity, freeze/thaw cycles, ultraviolet light and rainfall, and fungal as well as microbial contamination. Delivery systems must therefore incorporate features that will protect the bioactive compound without compromising efficacy.

15.3 Delivery Systems for Wildlife

Delivery systems that are feasible for use to free-ranging wildlife can be broadly classified as disseminating or non-disseminating based on their mode of transmission.

15.3.1 Disseminating Delivery Systems

Disseminating (or transmissible) delivery systems are attractive because they are able to spread the bioactive unaided through the target population by means of a vector. An example of a disseminating system that has been investigated for the common brushtail possum, a pest species in New Zealand, is the parasitic gut nematode (*Parastrongyloides trichosuri*). The nematode can rapidly infect large numbers of animals, and when introduced into a parasite-naïve population of brushtail possums, the nematode had spread across 400 ha of Kahurangi National Park in 52 weeks and was present at a high prevalence [21].

Other disseminating delivery systems currently under investigation are viruses, potentially engineered as a vector to carry genes for immunocontraceptives or as pathogens. For example, the macropod herpes virus (MaHV) causes fatal infections in marsupials such as wallabies and kangaroos in Australia [22]. Possum adenovirus (PoAdV-1) has been selected as the most promising candidate as a vector because the genomes are easy to manipulate and the viruses are readily transmitted between individuals. A single genotype of PoAdV-1 has been isolated and sequenced from individuals from different localities in New Zealand [23]; however, it has not been possible to replicate this virus in cell culture, thus limiting further development.

With all genetically modified organisms (GMOs) and disseminating systems, there is international concern about the inadvertent transfer of these organisms, and the genes they carry, to countries where they would affect native animals. This remains as a significant hurdle to the implementation of delivery systems incorporating GMOs.

15.3.2 Non-disseminating Delivery Systems

Non-disseminating delivery systems offer the advantage that the risks of uncontrolled spread are removed. The strategy of employing oral baits (Table 15.2) is a fundamental technique to remotely administer agents to wildlife. There is a range of particulate delivery systems that are currently in the laboratory development or field trial stage and these will be outlined below. These particulate systems may ultimately be incorporated into an oral bait capitalizing on the existing knowledge of bait formulation, palatability, field stability, and optimal density placement in the field, etc.

A novel non-disseminating system that has recently been developed for use in veterinary medicine is bacterial ghosts. Bacterial ghosts are empty envelopes of intact cell membrane from lysed Gram-negative bacterial cells from a number of genera (e.g., *Escherichia coli*, *Vibrio cholerae*, and *Salmonella typhimurium*) [24, 25]. Recombinant proteins can be loaded into various compartments within the bacterial ghosts (inner or outer cell membrane, periplasmic or cytoplasmic space) and expressed once within the host after the ghosts have been taken up by the dendritic cells and processed [24]. Bacterial ghosts can enhance T-cell activation and systemic and mucosal immunity and have been investigated as delivery systems in the prophylactic vaccines for animal pathogens including *Actinobacillus pleuropneumoniae* in pigs (see [24]). An advantage with bacterial ghosts is that they retain their immunostimulatory membrane structure [25]; however, the immune response after oral administration of ghosts containing ZP antigen to the common brushtail possum was low [26].

Virus-like particles (VLPs) are another non-disseminating delivery system that also relies on the immune system to elicit a biological response. VLPs are composed of structural proteins that self-assemble to form an organized capsid structure [27]. Once formed, the particles can then be expressed in a range of yeast or bacterial host cells. Therapeutic agents can be entrapped within the shell of the VLP and used to deliver the payload following oral delivery. With an antigen epitope bound to them, VLPs mimic the immunostimulatory portion(s) of native viruses to have their biological effect [28]. The potential of VLPs as delivery systems has not yet been fully realized due to the problems with an inconsistent product when production is scaled up [27]. Further, acquired immunity can result from the use of VLPs, although to some degree this can be overcome by creating chimeric forms of the VLP structure [27]. VLP vaccines against the human papillomavirus (HPV) and hepatitis B are available on the market for human and animal patients [29]; however, their use in a wildlife setting is untested.

Incorporating the bioactive into a polymeric nanocarrier is an attractive formulation option for protecting the bioactive from enzymatic degradation and facilitating subsequent uptake at the target site. Colloidal delivery technology is well developed in the human field and to a smaller extent with farm and companion animals; however, its application to a wildlife management situation has been limited.

Colloidal systems, such as liposomes, are a versatile delivery system as their size and composition can be manipulated and they have both lipophilic and hydrophilic domains that can be utilized to carry drugs and vaccines [30, 31]. They have potential to be used for the oral delivery of peptides and proteins, although in vivo results are variable [32] as liposomes are unstable at low pHs in the gastrointestinal tract [33]. Immunostimulatory complexes (ISCOMs) are colloidal structures based on liposomes with the addition of the adjuvant Quil A to the phospholipid and cholesterol liposome structure. Antigen is incorporated within the ISCOM structure. Morein et al. [34] provide an excellent review on ISCOMs. More recently, ISCOMATRIX has been developed that is an adjuvant comprised of the same components as ISCOMs, but does not include antigen [35], giving the user the flexibility to add their antigen of choice. For example, the ISCOMATRIX adjuvant has been used in the Equity™ product (Table 15.2) as a contraception for horses [36].

A vaccine formulation for bovine tuberculosis (Tb) has been developed for the common brushtail possum in New Zealand [37]. The Bacillus Calmette-Guerin (BCG) vaccine is administered to prevent transmission of the disease from this wildlife reservoir and vector to economically important farmed cattle and deer. Cross et al. [38] have developed a novel oral formulation for this Tb vaccine to immunize the brushtail possum that combines a vaccine delivery vehicle within a bait. The formulation is a pharmaceutical-grade, edible lipid matrix (Lipid-PK) that can deliver live BCG bacilli. These authors have also incorporated attractants (10% chocolate powder and 0.67% anise oil) into the matrix to enhance the palatability and so uptake of the oral baits [38]. A similar Lipid-PK formulation is being investigated by Clark et al. [39] as an oral bait to vaccinate the Eurasian badger (*Meles meles*) against Tb in the United Kingdom.

Entrapment of the bioactive within polymeric nanoparticles carriers provides protection of the active compound to be delivered. Nanoparticles are in the size range of 10–1,000 nm and particulates in this sub-micron size range have been shown to be absorbed across the gut epithelium and enter the systemic circulation [40]. The small size of nanoparticles is an advantage to enable particulate uptake by epithelial cells [41]. The polymer wall formed during the synthesis of nanoparticles reduces contact with degrading gastrointestinal enzymes once ingested by the animal and thus protects the payload, an important feature in the delivery of protein and peptide bioactives [42, 43]. Further advantages are their stability in biological fluids and the controlled drug release profile that can be manipulated by the choice of polymer [44]. Work in our research group has demonstrated that following intra-cecal administration of poly(ethyl cyanoacrylate) (PECA) nanoparticles containing D-Lys^6-GnRH (Gonadotropin-releasing hormone) to the brushtail possum, it was possible to elicit a biological response of a reduction in plasma luteinizing hormone (LH) concentration. This provides evidence that intact D-Lys^6-GnRH peptide is able to cross the gut epithelia, enter systemic circulation and reach the anterior pituitary [45].

15.4 Regulatory Aspects of Delivery of Bioactives to Wildlife

There are very few therapeutic agents that are approved for use in wildlife species. Use of these compounds such as antibiotics and anesthetics is often on the basis of previous experience in similar animal species and trial and error. Wildlife veterinarians often use medications registered for livestock or companion animals, where the formulations are not designed for wild animal species. Differences in anatomy and physiology between species mean that pharmacokinetic and pharmacodynamic parameters are not necessarily transferable. The reader is directed to Martinez et al. [46] for an excellent review on the differences in gastrointestinal physiology between animal species. Consequently, there is uncertainty about how animals will respond to the administration of a given compound.

The FDA's Office of Minor Use and Minor Species Animal Drug Development within the Centre for Veterinary Medicine is charged with facilitating the approval of drugs for use in minor species, including wildlife species. Animals that are classified as "minor species" are all species except dogs, cats, cattle, horses, pigs, chickens, and turkeys. The Minor Use Minor Species (MUMS) Act of 2004 gives incentives for pharmaceutical companies to approve a drug for use in minor species and aims to make more drugs available for use in minor species.

As discussed above, reducing fertility is the favored option to manage pest wildlife. Registration of fertility control products for administration to animals has similar requirements in Europe, America, and Australasia [47]. Data required by the registering authorities include; chemistry of the active, efficacy, metabolism and kinetics, and toxicology [47]. Of importance for fertility control agents that will be spread by broadcast distribution, fate in the environment, hazards to nontarget species and tissue residues must also be demonstrated [47, 48]. The specific data requirements for a new product depend on whether the agent is classified by the regulators as a pesticide or a veterinary medicine [47].

In America, prior to 2006 wildlife contraceptives were regulated by the Food and Drug Administration's Centre for Veterinary Medicine (CVM) through the completion of Investigational New Animal Drug files [48]. However, because wildlife vaccines were used in a natural outdoor environment (forests, grasslands, bush, etc.), the Environmental Protection Agency (EPA) became responsible for their regulation [13]. OvoControl®-G (Table 15.2) was the first contraceptive to be registered by the EPA following the new agreement with the FDA. The FDA's CVM continues to regulate the use of vaccines in companion animals, livestock, and zoo species [48].

In the European Union, the Animal Health Strategy is based on the standards and guidelines of the World Organisation for Animal Health (Office international des Epizooties—OIE). The Veterinary Medicines Directorate is responsible for the safe and effective use of veterinary medicinal products in the United Kingdom. In the United Kingdom and Europe, regulation of fertility control products is complex and again depends on how each country defines the class of compound (e.g., as a pesticide or vaccine) [47].

In New Zealand, the New Zealand Food Safety Authority's (NZFSA) Agricultural Compounds and Veterinary Medicines (ACVM) Group is responsible for approving veterinary medicines under the Agricultural Compounds and Veterinary Medicines Act 1997. For fertility control products, the Environmental Risk Management Agency (ERMA) is responsible for approval and registration in New Zealand. In Australia, registration of all agricultural and veterinary products is centralized to the Australian Pesticides and Veterinary Medicines Authority (http://www.apvma.gov.au) and includes fertility products or use with wildlife [47].

Whilst there may be advantages to disseminating delivery systems for biocontrol agents, their regulation will be fraught since by definition, when released into the environment, these agents are able to spread through animal populations [49].

As with traditional veterinary medicines, global harmonization of agents for fertility control of wild animals is also desirable to facilitate the development of novel products [50]. Currently the requirements for the registration of veterinary medicines are coordinated through the VICH (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products). The VICH is a trilateral program that involves the cooperation of the United States, Japan, and the European Union with Australia/New Zealand and Canada as observers. Currently there are no specific Guidelines within the VICH for products used for wild animals; however, the Topic "Ecotoxicity" would be relevant to this area (http://www.vichsec.org/en/topics.htm#3).

15.5 Conclusion

More sophisticated delivery systems are being developed for wildlife that enables the effective delivery of therapeutic compounds to this challenging and diverse group of animal patients. Pharmaceutics and pharmaceutical technology have application in areas other than human research and are applicable to the management of pest wildlife. Formulation strategies used in traditional pharmaceutics can be applied to enhance oral delivery of bioactives to wildlife [1]. It is hoped that continuing collaboration between wildlife managers, biologists and pharmaceutical scientists can solve the interesting challenges in delivering bioactive agents to wild animals.

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Chapter 16
Human: Veterinary Technology Cross Over

Alan W. Baird, Michael J. Rathbone, and David J. Brayden

Abstract Emerging drug discovery technologies are helping us to break from the traditional simplistic cycle of animal experimentation for human applications with "spin off" benefits for veterinary medicine. A more coordinated effort can develop synergies. In this chapter we attempt to profile how those technologies harnessed independently for human or veterinary medicine have related features. We discuss shared approaches and requirements that are reaping benefits for both human and veterinary patients.

16.1 Introduction

"*Every advance made in human medicine affects the progress of veterinary science*…." [Encyclopaedia Britannica Eleventh Edition (1910–1911)] and perhaps the converse is equally true. From traditional beginnings, therapeutic and diagnostic practices have benefitted from developments in basic sciences through physics, chemistry, biology, mathematics, and increasingly through computer power. One hundred years after the statement was published, we are fortunate enough to be in a period when the revolutions in molecular technology and mathematical modeling are colliding in an exciting manner. Researchers today have access to techniques beyond the imagination of their counterparts 10 years ago. In this chapter we explore how human and veterinary science as well as clinical practice are sharing technological progress.

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The previous chapters of this book explore comparative aspects of drug delivery related to animal health. Although fundamental principles of anatomy, physiology, and biochemistry govern pharmacokinetic (PK) profiles of all species, complexity in how certain drugs are orally absorbed, protein bound, metabolized, and eliminated via transporters in different species means that reliance on allometric scaling to predict dosing requirements between species is usually erroneous. Optimized clinical trial design to profile PK even in the same species has yet to be achieved as data is not normally required by regulatory authorities in the pediatric, geriatric or the sick patient, even though clearance may differ in each. Still, the concept that man represents just one other species is justifiably promoted throughout this collection. Science is in the early stages of understanding how genetic differences within, as well as between, species can affect the way drugs work. The genomes of rodents are turning out to be remarkably similar to humans. In many cases, even genetic linkages have been conserved. As such new information and understanding accumulates, maintained by the relatively new discipline of bio-informatics, genetically defined animal models become increasingly valuable in gaining an understanding of human disease. Pharmacogenomics offers as many opportunities in rational drug discovery in veterinary medicine as it does in human medicine in order to tailor molecules to have improved safety and efficacy in stratified populations within and between species. Recent technical developments including sequencing of the genomes of a range of species and the development of molecular techniques are sometimes referred to as "omics." Such powerful approaches have encouraged developments in data management, computation and analysis, leading to the "new" science of Systems Biology.

16.2 Biomarkers

The term biomarker is one that is used differently in different settings. Some important accepted biomarkers are based on sensitive chemical detection of specific analytes in biological fluids [1]. Such technology is applicable in both human and animal domains. For example, chemical detection of xenobiotics [2] ranges from biosurveillance of pollutants in the environment, through forensic applications, including doping of athletes (humans, greyhounds, or horses) to therapeutic drug monitoring for agents with a low therapeutic index. Military applications of biomarkers have also been described [3] as well as their applications in space medicine [4]. Other examples include biomarkers to identify illegal growth promoters in beef cattle [5] and to detect contaminants in food [6]. Diagnostic parameters (heart rate, blood pressure, growth rate, blood biochemistry) are biomarkers that are homeostatically maintained with normal ranges, and are therefore useful indicators of health or of pathological changes. Similarly, biomarkers may be used to indicate whether xenobiotics administered to an animal may affect functional parameters and, in the case of therapeutics, can indicate whether normal function is being restored.

It is now possible to detect low-abundance receptor targets including specific sequences of nucleic acids and proteins as well as aspects of post-translational modification in complex mixtures using microarrays: capture, reverse-phase, tissue, lectin, and cell-free expression [7]. These technologies, many of which are commercially available and used in clinical diagnoses, also have research value in proteomics studies including future biomarker discovery, protein interaction studies, enzyme-substrate profiling, immunological profiling, and vaccine development. The need to detect extremely low-abundance proteins in complex mixtures has provided motivation for the development of sensitive, real-time, and multiplexed detection platforms. Recently, biomarkers for some human diseases have emerged, including prostate specific antigen (PSA) for prostate cancer [8], C-reactive protein (CRP) for heart disease [2], and an inverse correlation between plasma glycosylated hemoglobin (HbA (1c)) and Type II diabetes [9]. Using biomarkers from blood or urine offers surrogate measures of pathology affecting poorly accessible tissues or organs or even cells in solid tumors [10]. Biomarkers are also essential in developing toxicology methodology by enhancing the specificity and sensitivity of prediction assays. One consequence such an approach has had is to reduce the numbers of animals used in toxicity testing [11].

Sophistication in biomarker discovery has been extended through "omics" techniques [12–15] to identify relevant markers rapidly. Biomarker discovery tools include transcriptomics (the study of RNA transcript expression), metabolomics (the study of metabolite expression), and proteomics (the study of protein expression patterns) [16, 17]. Currently, a validated biomarker may be used in high throughput screening in human or in veterinary pathology, drug discovery, and in safety assessments [18]. Identification of clinically important protein and genetic biomarkers of phenotype and of specific biological function is an expanding area of research that will extend diagnostic capabilities [19]. Biomarkers also provide useful information related to vaccine design and delivery [19]. With respect to preventive vaccines, biomarkers, for example in responses to BCG challenge [20], have potential to predict outcome and/or to predict effectiveness. In the case of therapeutic vaccines, biomarkers may be used to predict subject immune response and safety, reflected by genetic features of the host that relate to quality and type of immunity generated.

Biomarkers have traditionally provided information regarding diagnosis, prognosis, and treatment of genetic disorders, as well as for indications of therapeutic efficacy in treating infectious and non-infectious diseases across species. Bioinformatics methods have empowered this approach greatly by profiling biomarker patterns in clinical metabolomics, using chemical fingerprints that biological processes produce. Data mining tools are used to interrogate data gathered from complicated studies informing future clinical applications [21].

In addition to sequence information (of DNA, RNA, and proteins), epigenetics is also beginning to provide methods with which to identify biomarkers of health and disease. For example, histone modification [22, 23] or altered DNA methylation patterns showing up in genome-wide association studies (GWASs) may have future applications in identifying loci associated with common diseases [24]. Perhaps the

presence of hand-held PCR machines [25] in every clinic, office, or even farm is not too far off.

Before concluding this section on biomarkers, it is worth remembering that animals, or populations of animals, have been used throughout history as *de facto* surrogate predictive biomarkers for human toxicity. Examples range from canaries used in coal-mines as indicators of methane gas contamination to environmental sentinels of environmental hazards (e.g., trout sensitivity to cadmium toxicity in fresh water) [26, 27].

16.3 Drugs for Human Use Developed in Animals

Traditional drug discovery has employed whole animals and animal tissues for in vivo and in vitro drug research [28, 29]. Thus, animals have always contributed to research leading to market authorization of new medicines for man. Animal models of human diseases [30–32] have been useful in basic and applied research, although their relevance at the level of accurate pathology and toxicology as well as for predictive therapeutic efficacy in man tends to be disease-dependent [33, 34]. One historic example, often cited, is the discovery of insulin's role in human Type 1 diabetes using dogs [35], which unveiled the eventual role for porcine insulin in replacement therapy. For many decades, animal sources of insulin were used in humans, with drawbacks, including immunological sensitization as well as the possibility of contaminated sources, many of which have been overcome following the development of recombinant humanized insulin [36, 37]. Ironically, therapeutic benefits of new drug entities which were originally developed for human use using animal models of disease and toxicology may return to a veterinary setting when an established marketed human drug is leveraged for veterinary applications. An example is Clomicalm® (clomipramine hydrochloride) (Novartis Animal Health, Basel, Switzerland), a reformulated human tricyclic antidepressant, now approved to treat separation anxiety in dogs [38]. For many human conditions or diseases including cancers, stroke, and Alzheimer's disease, there are no really useful animal models [39–43] and this has delayed therapeutic advances. Less known is that other animal models have been highly successful and have led to predictive therapeutic outcomes in man, an example being rodent experimental autoimmune encephalomyelitis (EAE), which was instrumental in development of the first antibody therapy to α4 integrin for multiple sclerosis patients [44]. There is now considerable interest from human clinicians in using canine clinical cases that display *spontaneous* cancers for testing investigational therapies as an alternative to rodent-induced cancers, which usually have limited relevance to man [45].

With developments in genetic engineering, specific traits can be introduced into animals. So-called "knock-out" or "knock-in" animals can be engineered either to generate an animal with a genetic deficiency or alternatively to express a specific gene in a form which provides insight into human disease. Although most transgenic studies use inbred mice, rabbits [46], sheep [47, 48], pigs [49, 50], chickens [51], and non-human primates [52, 53] have been described.

Genetic engineering is well established, for example, to make pest-resistant crops and to produce pharmaceuticals from bacteria. With respect to animals, the advantages of genetic engineering over conventional breeding methods include speed and specificity. This has led to "precision" breeding [54] with applications ranging from animals engineered as sources of "products" for medical applications, to pedigree companion animals. Current transgenic studies have developed, for example, mice with "human" cells or organs. Sentinel chimeric animals with enhanced sensitivity to pathogens or to environmental chemicals may even be created as "early warning" systems for human infectious diseases [55].

Transgenic animals (chimera or hybrids) may carry a human gene inserted into their genome using techniques of recombinant engineering. These include using stem cells (embryonic or adult) growing in tissue culture with the desired DNA or alternatively injecting the desired (human) gene into the pro-nucleus of a fertilized egg [56]. Either of these methods may be employed to generate animals which express human genes. Animals containing viable human tissues, cells, or genetic information have arisen through research which is aimed at generating animal models of human disease, as well as to develop and produce new therapeutic entities. Not least due to ethical concerns, a controversy is under way [57, 58]. Applications of transgene technologies are vast in potential and range. For example, transgenic probiotica have been considered as a vector for targeted drug delivery [59] and various approaches have been investigated for gene transfer [60, 61], an approach which when it matures may provide benefits for human and animal patients alike. Similarly, use of siRNA with the potential to silence any targeted gene of interest [62], in certain pathologies caused by excessive or inappropriate gene activation, might equally benefit animal and human populations [63].

Yet another technique in which non-human animals may be engineered to produce a human pharmaceutical has resulted in generation of a human protein, antithrombin, which was the first "biological" made in a transgenic animal to receive regulatory approval for human therapy (ATryn® GTC Biotherapeutics, MA, USA). Specifically, this approach harnessed another physiological advantage since the anti-thrombin is secreted in the milk of transgenic goats. This is further proof of principle that efficient methods may be designed to produce biologically active human recombinant proteins in secreted animal fluids.

16.4 Drugs for Animal Use Developed in Humans

One of the reasons for a relative reduction in numbers of animals in drug discovery has been increasing reliance on (human) cell-based assays. Cell-based approaches [64], including high throughput screens [65, 66], which have greatly accelerated the number of candidate drugs that can be evaluated for efficacy and also for safety [67, 68]. Most pharmaceuticals currently used in animals were originally developed for human use, a major exception being the avermectins. In contrast, the microsomal triglyceride transfer protein inhibitor, Slentrol ® (Dirlotapide, Pfizer Animal Health, Groton, USA), is a drug which is restricted to use only in obese dogs [69].

Recent product approvals of new chemical entities follow this trend of specific design for veterinary species. An example is the tyrosine kinase inhibitor, Palladia® (Toceranib, Pfizer Animal Health, Groton, USA), which was approved in 2009 for treating cutaneous mast cell tumors in dogs [70]. A recent development in the US is that a consortium made up of 19 veterinary schools is inviting owners to allow the use of investigational human cancer therapies in dogs expressing cancers before they have been approved for man and this is providing additional safety and efficacy animal data as well as evidence of remission in some cases [71]

However, formulations and dosages must be tailored according to specific species needs and applications. Some aspects of drug use in animals therefore differ from their use in humans. For example, antibiotics have been employed as growth promoters in food animals, for which there are legitimate concerns over resistance generation in man from drug residues. Regulations therefore mandate withdrawal periods between use of drug and slaughter for human consumption. For some drugs there are species-related patterns of sensitivity. Notwithstanding the general similarities between vertebrate species, drug absorption varies within as well as between species. Considering the anatomical differences between monogastrics, hindgut fermenters, foregut fermenters, and ruminants, the potential for differences in PK profiles is marked and this has led to different drug administration technologies in which, for example, device engineering advances have been dominant for intraruminal and intravaginal delivery in production animals [72, 73]. However, since the same computational algorithms to assess PKs related to cell targeting (rates of diffusion, binding, internalization, and systemic clearance) there are shared unifying concepts for all species. For a single drug entity, PK equivalence (i.e., bioequivalence) which is determined by rate and extent of absorption of the active substance will differ between species. Bioequivalence studies are often part of the application to regulatory authorities for generic veterinary medicinal products to address issues of safety and of efficacy when compared with the reference "parent" medicinal product [74].

16.5 Drug Delivery

Modern aspects of drug delivery to veterinary species have been extensively reviewed [75–77]. The pattern of drug discovery using animal models leading to applications in humans which then re-emerge as improvements in animal health care is generally not followed in delivery technologies where there is a history of device-led species-specific research. Different target groups include humans, companion animals, food-producing, performance, zoo, laboratory, and wild animals. Varying experimental, applied, commercial, and ethical issues pertain to drug delivery to these different populations.

Terminology which underpins the science of drug delivery includes the terms bioavailability and bioequivalence. The number of molecules of a biologically active molecule at its receptor for an appropriate amount of time (recognizing that this may not be constant) provides a host of challenges. For some cases controlled release of drugs is governed by varying need (i.e., when plasma or tissue concentrations of

drugs require (a) to be maintained around a constant desired level, (b) to be delivered in a tissue- or organ-specific manner, (c) to be "ligand targeted", or (d) to be delivered in a pulsatile fashion).

Traditional drugs (the original "magic bullets" [78]) were typically low molecular weight moderately aqueous-soluble orally delivered molecules, which can dissolve in lipid bilayers. The oral bioavailability of small molecules is much greater than that of large molecular weight, hydrophilic, and metabolically unstable biotech peptides and proteins. Therefore, insulin's therapeutic use demanded injected systemic administration and over 10 other injected proteins for man have had their half-lives improved using PEGylation [79], whereas attempts at non-injected peptide and protein delivery have been relatively unsuccessful to date. Recently however, Mannkind (CA, USA) has been permitted to proceed with two human Phase III inhaled insulin trials for mealtime administration [80], so it seems that dismissal of the possibility of a pulmonary insulin product after the fall of Pfizer's Exubera® may have been premature.

Standard (and nonstandard) routes of drug administration are often classified by their site(s) of administration regardless of whether local or systemic effects are desired. These encompass:

Topical (also referred to as local; including epicutaneous, enema, eye drops, eardrops).

Mucosal (also referred to as enteral; including (oral, rectal gastrointestinal sublingual buccal, naso-pharyngeal, airway, uro-genitary tract, ocular, and integument).

Systemic (also referred to as parenteral; including intravenous, intraarterial, intraosseous, intramuscular, intradermal, subcutaneous, intracardiac, intrathecal, intraperitoneal, intravesical, intravitreal, intracavernous, epidural, intracerebral, and intracerebroventricular).

Thus, drug delivery is limited by anatomical, biochemical, and physiological features of the target species, accounting for the preponderance in the pharmacopeia of small water-soluble drugs over larger, polar, or non-soluble agents [81]. The desire to deliver such compounds in the right amount at the right time at the right place has produced the science of drug delivery and controlled release. Advances in this area have been rapid and enormous but there is a long road to travel. Prediction of PK profiles has become integrated into design, optimization, and selection of drugs. Much attention has been paid to the delivery of oral formulations [82–84]. Preclinical screening protocols include in silico, in vitro, in situ, ex vivo, and in vivo approaches. Comparisons and relative benefits of each of these approaches have been recently reviewed [85].

16.6 Pharmacogenomics, Transcriptomics, Proteomics, Metabolomics, Glycomics, and Lipidomics

Veterinary medicine is in the early stages of understanding how genetic differences in animals can affect the way drugs work. The field of pharmacogenomics offers promise in veterinary medicine, as it does in human medicine. Studies in pharmacogenomics

may lead to genetic tests which can be used to determine drug safety in specific breeds of dogs. The US Food and Drug Administration's Critical Path Initiative is designed to help move appropriate medical innovations that are safe and effective out of the laboratories to where they can help human and animal patients [86].

Not without a little irony, the remarkable advances up to and after the Human Genome Project delivered its map, human and veterinary consequences of research have begun to conjoin. The irony is that as we increasingly recognize the importance of personalized medicine, we continue to rely on restricted strains of laboratory animals with virtual genetic identity. Furthermore, breeding programs for companion and livestock animals have resulted in pedigrees of inbreeding [54]. The potential of rapid and inexpensive sequencing technology for genetic risk and clinical utility brings a new way of approaching clinical interventions in humans [87]. Whether the revolution has arrived or not [88], health professionals in all areas will be involved in applications of genomics.

With respect to domestic animals, breed specific differences in response to drugs have been reported [89]. Notwithstanding, discovery programs and regulatory authorities persist in selecting inbred strains of animals for their work. However, transgenic animals are likely to influence future activities [56]. Problems with using inbred strains include failure to acknowledge that many interventions will be actually applied to outbred (i.e., genetically mixed) populations. For example, over 400 breeds of dog are recognized worldwide, with some breed-related medical issues. These include specific metabolic diseases which are genetically determined. Consequently genomic insights have significance for dogs as patients and also for dogs as animal models of disease, especially for spontaneous cancers [86]. More broadly, breeding interest groups have begun to use genomics to reduce the incidence of inherited diseases in their breeds. With respect to gene transfer, early work performed in mice to provide proof of principle is being extended to large animals which tend to be outbred, and perhaps less different to humans than are mice [90, 91].Therapeutic gene transfer to companion animals is also becoming established [92].

16.7 Biologicals

To complement Chap. 15 of this book (Veterinary Vaccines), we review how advances in human and veterinary science may be translated into applications in clinical practice with specific examples. The first vaccines were derived from a bovine corollary of a human disease (cowpox and smallpox [93]). Biologicals derived from animals extend to Type II diabetes, an example being exenatide [94–96] a polypeptide glucagon-1-peptide analogue derived from a reptile (*Gila monster*). Other examples of animal-derived complex materials which have use as human therapies include hormones for replacement therapy (bovine and porcine insulin as described above) as well as serum proteins such as animal immunoglobulins or specific fractions as antitoxins and antivenoms [97, 98]. Interestingly, the recognition of the merit of the antitoxin against diphtheria was not evident at the

time its discoverer, Emil von Behring, received the first Nobel Prize in Medicine or Physiology [99].

The definition of a biological product has developed with time, and regulatory authorities take responsibility for regulation of both biological and small molecule pharmaceuticals. There is a growing range of recombinant therapeutic proteins and monoclonal antibodies available for treatment of human diseases. These materials are produced by biological processes, rather than chemical synthesis. With respect to noninjected delivery, biologicals are challenging with respect to their deployment as clinically useful drugs, as discussed above.

Regarding current "unmet needs," vaccines and other weapons related to surveillance, prevention and treatment of infectious diseases are likely to be the areas of most success in the near future. However, in an analytical comparison of two viral diseases of animals (Foot and Mouth Disease and rinderpest [100]) superficially similar problems have different outcomes based on efficacy of the vaccines, transmission rate of viruses, potential cross-reactivity between different strains of each virus, implementation of regional and international campaigns, and cost/benefit drivers in husbandry and commercial sectors. Whereas rinderpest has been eradicated [101], it is likely that Foot and Mouth Disease and its direct effects on animal health and commerce will be around for a long time to come.

Transfusion of whole blood, or blood components, including antibodies applicable in passive immunization as described above, has been described for hundreds of years. One of the first reports of blood transfusion was in the seventeenth century from a sheep to a human [102] and the associated technology was very simple. Since that time transfusion in human medicine has become commonplace although there are attendant risks of immunological side effects and of contamination. Therapeutic transfusions in non-human animals display species-specific and varying levels of compatibility. In contrast to human blood transfusion, although domestic animals do have different blood types cross matching is not generally required before (the first) transfusion, as antibodies against non-self-cell surface antigens are not expressed constitutively. However, careful management is required [103] and future developments in artificial blood elements, including erythrocytes, may have benefits for human and animal patients alike [104].

Tissue or organ transplantation has also developed over time. The first heart transplanted into a human patient was that of a chimpanzee [105]. The failure of this early procedure was, interestingly, not due to immunological rejection [106]. Xenotransplantation has come a long way and currently the science of regenerative medicine is forging ahead. Mice, including genetically engineered animals, have been at the forefront of research. "Humanized" cells and tissues are increasingly used to study disease processes [107] and to generate useful insights for translational biology [108]. The process of replacing tissues or cells, including stem cells, is beyond the scope of this chapter. Gene-based treatments, cell-based treatments, cloning, and use of tissues and tissue products will continue to be developed. Specifically with respect to xenotransplantation, including transplantation of non-human cells, tissues, or organs into humans, the importance of ethics and regulation on research, development, and commercialization of regenerative medicine cannot be overemphasized [109, 110].

16.8 Medical Devices

A medical device is a product which is used for medical purposes in patients, in diagnosis, therapy, or surgery and differs from a medicinal product (or pharmaceuticals), which achieves its principal action by pharmacological, metabolic, or immunological means. "Medical device" is a legal term and has been defined by regulatory agencies across the globe. For example, in the United States, the Federal Food, Drug, and Cosmetic Act defines medical devices as "*an instrument, apparatus, implement, machine, contrivance, implant, in-vitro reagent, or other similar or related article, including any component, part, or accessory thereof, which is intended for use in the diagnosis of disease or other conditions; in the cure, mitigation, treatment, or prevention of disease in man or other animals; or which is intended to affect the structure or any function of the body of man or other animals*."

Medical devices range from the simple to complex. In addition, medical devices include in vitro diagnostic products, such as general purpose lab equipment, reagents, and test kits, which may include monoclonal antibody technology. Certain electronic radiation emitting products with medical application and claims meet the definition of medical device. Examples include diagnostic ultrasound products, X-ray machines, and medical lasers. In vitro diagnostics are tests that can detect diseases, conditions, or infections. Some tests are primarily used in the diagnostic laboratory, other health professional settings, on the farm (for animals of commercial importance), or for individuals to use at home.

Not all technological advances which support human or animal health care are "esoteric." Innovations in practical medical devices include mobile applications which are software programs that run on smart phones and other mobile communication devices. Development of mobile medical applications is opening new and innovative ways for technology to exchange information [111, 112] and also to improve health and health care [113]. These specific technological developments may come to match that of the telephone itself, with equivalent impact on clinical practice [114].

Specifically with respect to drug delivery, medical devices have benefitted from technology developments. The challenge of administration of volatile general anesthetics to non-human species generated different sized delivery masks designed for the target animal. As delivery methods progressed anesthesia machines, ventilators, and related equipment have been improved [115]. Other aspects of drug delivery to humans and non-human species have also evolved in parallel. For example, aerosols are widely used to deliver payloads to airway mucosa for local delivery [116]. In this case, metered dosing and patient compliance are significant issues. However, treatment of systemic diseases by airway administration of drugs remains largely ineffective [117]. Use of fentanyl patches in companion animals is a good example of human to animal transition [118, 119].

Either synthetic or natural, biomaterials are widely used in human and animal health care provision. For example, biomaterials have been developed for joint

replacements, bone and cartilage replacements, tissue adhesives, orthodontic implants, cardiac valves, skin repair devices (artificial tissue), and prostheses. Novel approaches to biomaterials in tissue engineering [120] and in drug delivery [121] have generated much excitement, patents, and a burgeoning literature. In spite of this, the number of biomaterials, devices, or systems that have been successfully developed for the application remains small [122]. With respect to "payload" delivery (of genes, DNA, pharmaceuticals) the focus is beginning to move towards novel biomaterials, tissue engineering, stem cell, and non-live gene delivery systems. Consequently, it is increasingly useful to assess specific interactions between the cell/host and particular biomaterials of interest [123, 124].

16.9 Conclusions and Perspectives

Human and veterinary health care rely on the same fundamental principles and, often at different rates, support each other through common research, applications of technology, and sharing of experience. The possibility of students at medical or veterinary schools being instilled with the concept that each cell, tissue, or organ system is an independent unit is still real. However, we are recognizing that the principles for regulation and control of biochemical and physiological principles are surprisingly conserved and that there are no logical boundaries between molecular biology, physiology, pharmacology, pathology, therapeutics, patient care, and herd health. It is encouraging that a number of initiatives related to "One Health" with respect to animal and human science are undergoing a renaissance. Zoonotic diseases must have been recognized in pre-history [125]. During the twentieth century, scientists and many (but not all) clinical practitioners became so specialized that we could often "not see the wood for the trees." However, these pioneers generated the jigsaw pieces of knowledge which, as they are assembled, produce a clearer picture for integrated medical thinking [126, 127].

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