Saurabh Bhatia

Natural Polymer Drug Delivery Systems

Nanoparticles, Plants, and Algae

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Chapter 1
Nanotechnology and Its Drug Delivery Applications

Abstract Drug delivery is attractive approach for medicine field, as more potent and specific drugs are being developed. With the integration of nanotechnology, so-called smart drug-delivery systems integrate biosensing functionalities which sustain independently in vivo reaction control that resulting in part unique features of the term Nanomedicine. Nanotechnology is one of the very frontiers of science today. Current polymeric research is dominantly participating in the advancement of nanotechnology by offering the controlled release of therapeutic agents in constant doses over prolong periods, cyclic dosage, and tunable release of both hydrophilic and hydrophobic drugs. Many biomaterials can be used to this end, offering extensive chemical diversity and the potential for further modification using nanoparticles. Conventional methods of drug delivery present several disadvantages, mainly due to off-target effects that may originate severe side and toxic effect to healthy tissues. New drug delivery systems based on nanoscale devices showing new and improved properties and developed as promising solutions for achieving desirable therapeutic efficacy. Here, we provide a broad overview of novel nanoparticle based drug delivery systems, covering its innovations, applications and commercialization systems using both natural and synthetic polymers.

Keywords Drug delivery • Cancer therapy • Nanoparticles • Toxicology • Pharmaceuticals

1.1 Introduction

Various researchers are currently working on nanotechnology especially in medicine and further more specifically in drug delivery. Wide ranges of materials are under examination investigation for drug delivery and more specifically for cancer therapy. Potential sectors in biomedical field has started using nanoparticles to minimize the toxicity and side effects of drugs and up to recently did not realize that carrier systems themselves may impose risks to the patient. The kind of hazards that are introduced by using nanoparticles for drug delivery are beyond that posed by conventional hazards imposed by chemicals in classical delivery

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matrices. For nanoparticles the knowledge on particle toxicity as obtained in inhalation toxicity shows the way how to investigate the potential hazards of nanoparticles. The toxicology of particulate matter differs from toxicology of substances as the composing chemical(s) may or may not be soluble in biological matrices, thus influencing greatly the potential exposure of various internal organs. This may vary from a rather high local exposure in the lungs and a low or neglectable exposure for other organ systems after inhalation. However, absorbed species may also influence the potential toxicity of the inhaled particles. For nanoparticles the situation is different as their size opens the potential for crossing the various biological barriers within the body. From a positive viewpoint, especially the potential to cross the blood brain barrier may open new ways for drug delivery into the brain. In addition, the nanosize also allows for access into the cell and various cellular compartments including the nucleus. A multitude of substances are currently under investigation for the preparation of nanoparticles for drug delivery, varying from biological substances like albumin, gelatin and phospholipids for liposomes, and more substances of a chemical nature like various polymers and solid metal containing nanoparticles. It is obvious that the potential interaction with tissues and cells, and the potential toxicity, greatly depends on the actual composition of the nanoparticle formulation. This paper provides an overview on some of the currently used systems for drug delivery. Besides the potential beneficial use also attention is drawn to the questions how we should proceed with the safety evaluation of the nanoparticle formulations for drug delivery. For such testing the lessons learned from particle toxicity as applied in inhalation toxicology may be of use. Although for pharmaceutical use the current requirements seem to be adequate to detect most of the adverse effects of nanoparticle formulations, it cannot be expected that all aspects of nanoparticle toxicology will be detected. So, probably additional more specific testing would be needed.

1.2 Historical Prospects of Nanotechnology

Though researchers have been learning nanoscience facts for many years, scientific growth in the second half of the twentieth century offers precious tools that allows the scientist to study and develop materials in the nanoscale size range and facilitated to formalize nanotechnology as a scientific field [1]. Historical representation of development phases encompasses various concepts and research work related with nanotechnology has started in the 1980s was caused by the convergence of several experimental work e.g. the discovery of the scanning tunneling microscope in 1981 and the discovery of fullerenes in 1985 [1]. Based on the explanation and wide acceptance of concepts of nanotechnology a book called Engines of Creation publicized in 1986 [2]. Nanotechnology was first described by the Feyman (1959), Professor, Californian institute of technology during his lecture in American physical society. During this lecture he has used

the statements such as "*There's Plenty of Room at the Bottom*" which clearly means the prospect to create nanosize objects with the use of atoms as structural particles was considered [3]. Presently this speech is considered as beginning of nano-technological paradigm [3]. Later on in 1974, Taniguchi uses term "*nano-technology*" at the international conference in order to explain the super thin dispensation of objects with nanometer precision and nanosized mechanism [4]. During 1985 Harold Kroto, Sean O'Brien, Robert Curl, and Richard Smalley (Rice University researchers) discovered the Buckminsterfullerene (C_{60}), more commonly known as the buckyball, which is a molecule similar to a soccer ball in shape and composed entirely of carbon, as are graphite and diamond [5]. In the similar year of 1985, Bell Labs's Louis Brus revealed colloidal semiconductor nanocrystals (quantum dots), for which he shared the 2008 Kavli Prize in Nanotechnology [6]. In 1988 the first university course of "Nanotechnology and Exploratory Engineering" was taught by FI's president Eric Drexler at Stanford [7, 8]. Carbon nanotubes discovered in 1991 by Sumio Iijima bear a resemblance to rolled up graphite, while they cannot really be made that way [9]. During the same period Vice President for Science and Technology, J.A. Armstrong, spoke at the Symposium on the 100th Anniversary of the Birth of Vannevar Bush. "I believe that nanoscience and nanotechnology will be central to the next epoch of the information age, and will be as revolutionary as science and technology, at the micron scale have been since the early 70's." In 1992 the first book published on Nanosystems: Molecular Machinery, Manufacturing, and Computation by K. Eric Drexler [8]. Rest events are mentioned Table 1.1.

1.3 Promising Role in Drug Delivery

Recent researches have evidenced extraordinary development of research and applications in the nanoscience and nanotechnology. There is a growing interest in the confidence that nanoscience when functionalized in therapeutic field will certainly pass on the significant advances in the diagnosis and treatment of disease. The most expected functions of nanotechnology in medicine include both in vitro and in vivo diagnostics, drug delivery, nutraceuticals and production of improved biocompatible materials [39–43]. Additionally nanotechnology has also proven for their significant applications in medical devices and other instruments utilized in medical field. Several nanoforms especially nanoparticles with advance designs and structures act as an essential device to comprehend these applications. To comply with medical applications considering the safe utilization of nanoparticles in medical field the Royal Society and Royal Academy of Engineering (2004), established size range (size $\leq$100 nm) for the particles that can easily comply with guidelines and regulations and can safely used in medical field [44]. Nevertheless, such range does not essentially influence their role in medical applications. Owing to their unique features such as their surface to mass ratio (much larger than that of other particles), their quantum properties and their capability to adsorb and carry other therapeutic

Table 1.1 List of historical events reported in nanotechnological science

Year	Event and researchers involved	Even description	References
1992	C.T. Kresge and colleagues at Mobil Oil	Nanostructured catalytic materials MCM-41 and MCM-48	[10]
1993	Moungi Bawendi of MIT	Discovered a method for controlled production of nanocrystals (quantum dots)	[11]
1993	First Feynman Prize in Nanotechnology awarded	For modeling a hydrogen abstraction technique significant in nanotechnology	[12]
1994	Nanosystems textbook used in first university course	Book was based on the book Nanosystems: Molecular Machinery, Manufacturing, and Computation	[13]
1995	Foresight Institute awarded the 1995 Feynman Prize in Nanotechnology to Nadrian C. Seeman (Ph.D., chemistry professor at New York University)	In appreciation of revolutionary work to synthesize complex three-dimensional structures with DNA molecules	[14]
1996	NASA's Ames Research Center is begins work in computational molecular nanotechnology	NASA's Ames Research Center is becoming a significant force in computational molecular nanotechnology	[15]
1996	First nanobio conference	Based on "Biological approaches and novel applications for molecular nanotechnology" was held December 9–10, in San Diego, CA	[16]
1997	Commercialization started by Zyvex	The first nanotechnology development company, started business	[17]
1998	First DNA-based nanomechanical device pioneered by Dr. Seeman and his co-workers at New York University	This invention was based on the creation of devices from branched DNA molecules This scientific work was reported in January 1999 issue of Nature	[18, 19]
1999	Ho and Lee investigated facts of chemical bonding by structuring a molecule [iron carbonyl $Fe(CO)_2$] from constituent components [iron (Fe) and carbon monoxide (CO)]	This work was performed with the help of tunneling microscope	[20]
1999	Mirkin, discovered dip-pen nanolithography® (DPN®)	For cell biology research, nanoencryption, and other applications	[21]
1999	First Nanomedicine book published	Nanomedicine, Volume I: Basic Capabilities 1st Edition by Robert A. Freitas, CRC Press	[22]

(continued)

Table 1.1 (continued)

Year	Event and researchers involved	Even description	References
1999	First safety guidelines drafted by Neil Jacobstein	For Responsible Nanotechnology Development	[23]
2000	Commercialization making use of nanotechnology began appearing in the marketplace	Various consumer products based on nanotechnology appeared. During the same time President Clinton announces U.S. National Nanotechnology Initiative	[24]
2001	Feynman Prize in Nanotechnology awarded	For speculation of nanometer-scale electronic devices and for production and description of carbon nanotubes and nanowires	[25]
2002	Feynman Prize in Nanotechnology awarded	For employing DNA to facilitate the self-assembly of new structures and for advancing our skill to represent molecular machine systems	[26]
2003	Feynman Prize in Nanotechnology awarded	For representing the molecular and electronic structures of novel materials and for incorporating single molecule biological motors with nano-scale silicon devices	[26]
2003:	Halas, West, Drezek, and Pasqualin at Rice University developed gold nanoshells	These nanoshells when converted in nano size range to absorb near-infrared light, provide a platform for the innovation, diagnosis, and management of breast cancer without persistent surgery, biopsies, or systemically destructive radiation or chemotherapy	[27]
2003	Emergence of National Nanotechnology Initiative or the science, engineering, and technology research and development for nanoscale projects President George W. Bush increased funding for nanotechnology	On December 3, 2003 Bush signed into law the 21st Century Nanotechnology Research and Development Act	[28]
2004	Britain's Royal Society and the Royal Academy of Engineering published Nanoscience and Nanotechnologies: Opportunities and Uncertainties	This document supports issues that address environmental, potential health, ethical, social and regulatory issues associated with nanotechnology	[29, 30]
2004	Feynman Prize in Nanotechnology awarded	For manipulating stable protein structures and for synthesizing a novel enzyme with an modified function	[31]

(continued)

Table 1.1 (continued)

Year	Event and researchers involved	Even description	References
2005:	Winfree and Rothemund at California Institute of Technology developed theories for DNA-based computation and "algorithmic self-assembly"	In these theories computations are set in the process of nanocrystal growth	[32]
2005	Feynman Prize in Nanotechnology awarded	For synthesizing various single molecular functional nanomachines and for producing macromolecules of intermediate sizes with desirable shapes and functions	[33–37]
2006:	Tour and colleagues at Rice University built a nanoscale carmade	These nanoscale carmade was synthesized with oligo(phenylene ethynylene), alkynyl axles and four spherical C60 fullerene (buckyball) wheels	[33–37]
2006	Feynman Prize in Nanotechnology awarded	For his invention in molecular computation and algorithmic self-assembly, and for producing complex two-dimensional arrays of DNA nanostructures	[33–37]
2007	Feynman Prize in Nanotechnology awarded	For building molecular machine systems that function in the area of Brownian motion, and molecular machines based upon two-state mechanically interlocked compounds	[33–37]
2008	Feynman Prize in Nanotechnology awarded	For his achievement in molecular electronics and the production of molecular motors and nanocars, and for theoretical contributions to nanofabrication and sensing	[33–37]
2009–2010	Seeman and colleagues at New York University	Created several DNA-like robotic nanoscale assembly devices	[33–38]
2010	Andre Geim and Konstantin Novoselov have just won the 2010 Nobel Prize in Physics	For groundbreaking experiments regarding the two-dimensional material graphene, and their work has opened a broad frontier in nanotechnology	[33–37, 39]
2011	First programmable nanowire circuits for nanoprocessors		[33–37]
2012	The National Nanotechnology Initiative launched two more Nanotechnology Signature Initiatives	Nanosensors and the Nanotechnology Knowledge Infrastructure-bringing the total to five NSIs	[33–37]

(continued)

Table 1.1 (continued)

Year	Event and researchers involved	Even description	References
2014	The National Nanotechnology Initiative releases the updated 2014 Strategic Plan. The National Nanotechnology Initiative releases the 2014	Progress Review on the Coordinated Implementation of the NNI 2011 Environmental, Health, and Safety Research Strategy	[33–37]

substances make these nanoparticles (NPs) as an attractive tool for medical purposes. One of the most important features of nanoparticles that encourage their utilization in drug delivery is relatively large surface which is functional and can potentially bind to adsorb and carry other compounds such as drugs, probes and proteins. Nanotechnology yet to face more challenges or establishing the better perceptive of the patho-physiological basis of disease, explore more advance diagnostic opportunities, and yield better treatments. Nanoparticles range comply dimensions below 0.1 μm or 100 nm, however size >100 nm is required for loading adequate quantity of drug onto the particles. Positive attributes of engineered nanoparticles is that besides of their potential usage in drug delivery, drug itself can be formulated in nano range and function as its own "carrier" [45–47]. Nanoparticles are advantageous since they can be engineered according to the mode of delivery and target sites. Therefore different types of engineered materials can be used for the composition of the engineered nanoparticles such as biomaterials (phospholipids, lipids, lactic acid, dextran, chitosan) can be used safely or materials those are either synthesized or having chemical nature e.g. carbon, silica, various polymers, and metals can be used for this purpose. Nanoparticles can be engineered according to the type of interaction required with cells since biomaterials acts differently than non biological components mentioned above. As a result nano engineering may offer different nanoparticles with different chemical composition and possibility different action. In most of events solid NPs may be used for drug targeting which have to release the drug after reaching the intended diseased site in the body. So to anticipate the effective drug delivery system biodegradable nanoparticle formulations are required to transport and release the drug in order at specific site. Nevertheless non-degradable particles are usually experimented in designed model studies to determine the behavior of nanoparticles. Previous nanoparticles based toxicity reports discovered the extreme relative toxicities obtained from inhaled nanoparticles as part of the accidental release of ultrafine or nanoparticles by combustion derived processes such as diesel exhaust particles [48–52]. It has been realized that these combustion derived ultrafine particles/nanoparticles are associated with serious health problems [53] such as blood coagulation and pulmonary immune adjuvant effects, inflammation [54] and cardiovascular effects [52]. Since ultrafine and nanoparticles falls under nanorange (100 nm) so both terms are equivalent, therefore nanoparticles may be supposed of having similar toxicities as associated with engineered nanoparticles (environmental pollutant).

1.3.1 Nanoparticles and Drug Delivery

Current nanomedicine science constitutes nanometer scale complex systems (10–1000 nm), having two essential components. Among these two one is a active pharmaceutical ingredient [43], whereas nanoparticle formulations of the drug itself can also be possible [45–47]. Either of these two system results in special function related to preventing, treating, or diagnosing diseases. Such potential system sometimes called smart-drugs or theragnostics [55]. Nano-bio-technologies aided theragnostics play a major role in drug delivery to achieve more specific drug targeting and delivery, minimize toxicity profile while maintaining therapeutic effects, better safety and biocompatibility, and faster development of new safe medicines. Selection of suitable carrier system requires the study of following basic factors that are involved in drug delivery and designing:

- Good understanding of biodistribution and targeting
- Good information of biocompatibility and functionality
- Knowledge of formulation stability and shelf life
- Information related with drug incorporation and release

During the drug delivery when carrier is used exclusively the possible adverse effects of residual material should be considered. In this respect biodegradable nanoparticles with a limited life span as long as therapeutically needed would be optimal. There are different types of chemical structures available (natural or synthetic) for the preparation of nanoscale [56]. However none of the carrier system complies with the parameters discussed above to the full extent. Therefore more development is required in polymeric chemistry and its related nano-therapeutical applications which can provide more interesting basis to deal with this issue in a promising way. For medicinal or diagnostic purpose nanoparticles are entrapped and this entrapment results in enhanced drug delivery or uptake by, target cells and/or to minimize toxicity of the free drug to non-target organs. During both events increase of therapeutic index is reported which will certainly results in enhanced therapeutic efficacy (e.g. tumor cell death) with toxicity to other organ systems. Thus for these purposes long-lived and target-specific nanoparticles are created. In most of the cases substances are biodegradable resulting in drug release after complete degradation. Most critical problem encountered by the particulate drug carriers is their entrapment in the mononuclear phagocytic systems that are present in the liver and spleen [57–59]. Nevertheless, targeting of organ may be favorable as in the case of liver targeting of nanoparticles in treating liver ailments like tumor metastasis or hepatitis. Nanoparticles can be surface modified with polyethylene glycol which results in its delayed retention time in the blood stream by hindering its recognition and phagocytosis by the mononuclear phagocytic system [60–62]. Moreover PEG modification can also affect the distribution and minimize in vitro toxicity when gold nanorods were modified using PEG. Nanoparticle coating is required to avoid agglomeration. Different types of coating can be employed to avoid

agglomeration. This can be achieved by and keeping the particles in colloidal suspension including various polymers like poly(vinylpyrrolidone) (PVP), polyethylene glycol (PEG), etc, natural polymers like chitosan, pullulan, dextran, etc., and surfactants like sodium dodecylamine, oleate, etc. [63]. Seki et al. demonstrated that size of particle affects the NP distribution as reported for liposomes for which a lower liver uptake was found for the smaller vesicles (200/300 nm versus 25/50 nm) [64].

Slight variation in size affects the actual distribution and thus bioavailability [65–69]. In liposomal delivery size greater than 100 nm enhances the clearance rate by the mononuclear phagocytic system whereas charge factor is more considerable for those particles that are having size less than 100 [70], however this is not true for all particles since composition will be important as well. In addition to degradation certain more strategies can be used to trigger the release of the drug e.g. light and heating may be used to enhance the therapeutic effect (cell death) or for local drug release, respectively. Thus selective release of the content after specific localization can be encouraged by using thermosensitive nanoparticles e.g. at 42 °C doxorubicin showed enhanced cytotoxicity then at 37 °C using copolymers of poly-L-lactide (PLLA) and polyethylene glycol [71]. Furthermore the discharge of photosensitizers from nanoparticles can be encouraged by using photodynamic therapy. Such light dependent release can potentially induce cytotoxicity as demonstrated for PLGA nanoparticles containing zinc(II) phthalocyanine [72] and indocyanine green [73].

1.3.2 Use of NPs Formulation in Drug Delivery

Different physiological barriers, solubility of drug under in vivo conditions (different biological fluids), permeation rate, and environment of targeted site decides the fate of the drug or carrier or whole formulation. Primary objective of drug delivery is to deliver the drug at desirable place in the body thereby preventing the potential side effects to non diseased organs. Such type of specific cellular or tissue or organ mediated nanoparticulate target system is especially required in cancer treatment where the tumor may be localized as distinct metastases in various organs. Undesirable cell toxicities associated with therapeutics restrict the utilization of chemotherapeutics. Targeted drug delivery system especially local drug delivery or drug targeting lead to increase in drug concentrations and provides strategies for more compartment specific therapy. Owing to the unique futures of nanoparticles such as binding and stabilization of proteins, small size which allows penetration of cell membranes and lysosomal escape after endocytosis, it can be used as potential tool in drug delivery to encourage more specific targeting of organelle, cell, tissue or organ or any compartment specific in the body. Chemotherapeutics entrapment in form of nanoparticles has been reported in various researches [74–76].

Significant features of liposomes(nanosized phospholipid "fatty" structures) such as small, flexible and biocompatible are explored in several reports. Such features allow them to bypass smallest arterioles and endothelial fenestrations without causing clotting. Currently potential materials such as (co-)polymers and dendrimers at the nanosize range are being utilized to improve the distribution of encapsulated or attached drugs.

Among the anticancer chemotherapeutic agents, paclitaxel (taxol) is currently under more intensive study due to its potential cytotoxicity against tumor cells. Under in vitro conditions paclitaxel based nanoparticle formulation reported for its enhanced cytotoxicity for tumor cells. In addition it has been discovered therapeutic efficacy was enhanced at the same time under in vivo animal model. Furthermore encapsulation of paclitaxel in vitamin E TPGS-emulsified poly (D,L-lactic-co-glycolic acid) nanoparticles resulted in higher and prolonged effect then the effective concentration in *vivo*.

In addition to the size, surface characteristics play an important role in particle uptake, distribution and effects. Schins et al. and Albrecht et al. studied acute and chronic models of surface modified micro quartzes with their toxicity [77, 78]. They have observed the uptake of PVNO-polymer coated quartz by macrophages without toxicity and showed no genotoxicity in epithelial cells or acute and chronic inflammation whereas naïve quartz caused these effects to a great extent. Polymer particles mediated altered body distribution for two different types of particles was reported [79]. He has reported that after intraperitoneal administration PMMA particles having size range of about 1.4 μm and about 6.4 μm can be easily recovered in contrast with PS particles having size of about 1.2, 5.2 and 12.5 μm from the spleen. So far this situation hasn't applied over nano range, however surface modification of polystyrene particles suggest various effects on cellular oxidative [80], mitochondrial ROS formation and burst blood coagulation [81]. Moreover polyethylene glycol coated nanoparticles enhance the time in circulation for the nanoparticles [60–62]. Primary objective of entrapment of drug is to reduce toxicity of the free drug to non-target organs and ultimately to enhance delivery to, or uptake by, target cells. To achieve these set of goals establishment of long-lived and target-specific nanoparticles is required. Entrapment of nanoparticles in the mononuclear phagocytic system as present in the liver and spleen is a major setback during nanodrug delivery system [57, 59, 82, 83]. Nevertheless liver targeting is encouraged during the treatment of disease like tumor metastasis or hepatitis. Certain drugs are supposed to be transported to liver such as migration of oligonucleotides for modification of gene expression when bound to biodegradable polyalkylcyanoacrylate nanoparticles [84]. As suggested earlier PEG mediated surface modification of nanoparticles reduces recognition and phagocytosis by the mononuclear phagocytic system [59–62].

Decline in therapeutic efficacy and inference with liver function was reported after the entrapment of drug in form of nanoparticles. Fernandez-Urrusuno et al. reported the transient liver alterations after acute and chronic intravenous adminis-

tration of cyanoacrylate and polystyrene nanoparticles [57]. He has also reported the liver associated inflammation after secretion of acute phase protein α1-acid glycoprotein by hepatocytes. Noticeably owing to the local release of oxidative species antioxidant defenses of hepatocytes were depleted. In contrast a study based on the comparative evaluation between insulin-chitosan nanoparticles to chitosan solution and chitosan powder formulations shown that insulin-chitosan nanoparticles were less effective in terms of bioavailability and lowering blood glucose level in both a rat and sheep mode [82]. This study has proven the potential of nanoformulation in enhancing drug delivery without loss of drug activity.

1.3.3 Cellular and Intracellular Targets

In addition role of organ or cellular targeting, the fate of the nanoparticles within the cells also decides the effectiveness for drug delivery system. Nanoparticles usually suffer from intracellular degradation in endosomes or lysosomes. Therefore for exhibiting the potential activity the drug should be designed/ encapsulated to encourage their release into the cytosol. In contrast Edetsberger et al. demonstrated that the particle size of about 20 nm escape from cellular uptake without involvement of endocytic mechanisms [83]. Since it has been reported that chemical features (e.g. surface charge) also determine the fate of nanoparticles in cells which may results in efficient internalization in endosomes and cytosol, and localization in the nuclear region [84]. This was found in surface functionalization of gold nanoparticles with PEG resulted in effective internalization. Certain nanoparticles such as poly(DL-lactide-co-glycolide) nanoparticles couldn't be able to escape from endosomes hence ingested by cells by endocytosis [85]. Therefore change in surface charge is required to escape nanoparticle from these endosomes into the cellular cytoplasm. This concept was implemented over PLGA nanoparticles to allow their escape from endosomes and resulting in cytoplasmic delivery of the incorporated drugs. Theory of easy escape of positive surface charge nanoparticles from the endosomes was supported by Panyam et al. [86]. In contrast with positively charged he has suggested that negatively charged polystyrene nanoparticles did not reach the cytosol but remained in the endosomal compartment of the smooth muscle cells.

Bourges et al. suggested that there is a possibility of specific targeting to retinal pigment epithelium cells [87]. Åkerman et al. developed the small quantum dots having size less than (<10 nm), for specific targeting of peptide coated dots to the vasculature of lungs and tumors [88]. Additionally conjugation of polymer shells with quantum dots was later studied by Ballou et al. [89]. In this report he has described the role of quantum dots cores coated with hydrophilic polyethylene glycol to trigger the half life time. Nevertheless drug passage in to by lymph nodes was demonstrated by Ballou et al. [89]. In this study translocated quantum dots could be examined up to 4 months after administration. Therefore greater chances

of accumulation were observed. It has been reported that polymeric coating especially with PEG abolishes the uptake of drug by the reticuloendothelial system of liver and spleen whereas 40–50 nm magnetic PEG coated nanoparticles were well taken up by endocytosis [90].

Weissenböck et al. adopted the strategy to increase cellular binding by carbohydrate binding ligands on the surface of biodegradable and biocompatible poly(D,L-lactic-co-glycolide) acid (PLGA) nanospheres [91]. Such enhancement in cellular binding may results in enhanced activity of the drug presented as or incorporated in nanoparticles. Moreover it has been reported that conjugation of specific protein e.g. with antibodies to the nanoparticle surface may allow a more specific targeting of the particles [92, 93]. Ultimately it has been concluded that surface modifications of nanoparticles offer enormous opportunities for medical applications like uptake, intracellular transport and drug targeting in terms of cellular binding.

1.3.4 The Brain—The Ultimate Target for Drug Delivery

From several perspectives the brain is a challenging organ since incidence of degenerative diseases in the brain will increase with the aging population and secondly blood brain barrier (BBB) found to act as rate limiting factor in drug delivery to brain in fighting against various the central nervous system (CNS) disorders. The most critical issue in brain drug delivery is that the blood brain barrier fence interior from the exogenous substances [94]. Blood brain barrier is equipped with some limiting physiological factors such as reticuloendothelial system and protein opsonization, which play major role in decreasing the transport of drug. Usually almost all therapeutic drugs including small molecules do not cross the blood brain barrier. From brain physiology point of view endothelial barrier is having tight junctions at the interface with the brain astrocytes. Some of the alternative mechanisms can be adopted to facilitate the transport in normal conditions such as using endogenous BBB transporters resulting in active efflux transport, receptor mediated transport and carrier mediated transport. Nevertheless utilization of drug in form of nanoparticles facilitate transport of drug by compromising barrier properties intentionally or unintentionally [95–97]. Several approaches employed to improve the drug delivery across the BBB. Owing to its size (ranges from 1 to 1000 nm), functionalization characteristics and ability to reduce the need for invasive procedures can be utilized as career for drug delivery and found to play a significant advantage over the other methods of available drug delivery systems to deliver the drug across the BBB. Various mechanisms and strategies involved in this process. These strategies are exclusively based on the on the type of nanomaterials used and its combination with therapeutic agents (such materials include non-viral vectors of nano-sizes for CNS gene therapy, polymeric nanoparticles and liposomes, etc.). Olivier et al. suggested that transport of the drug across blood brain barrier can be possible by the toxic effect of nanoparticles (about 200 nm) on cerebral endothelial cells, while

Kreuter et al. contradicted this study by utilizing similar nanoparticles with size range about 300 nm [95, 96]. Moreover Lockman et al. reported that effect was not common for a different type of nanoparticles [97]. Due to the reported ill effects of nanoparticles some devices such as implanted catheters and reservoirs are encouraged in effective drug delivery to the CNS. Currently several researchers are working on different nanomaterials to improve the safety and efficacy level of drug delivery devices in brain targeting. It's advisable to determine the physical and chemical association between drug, carrier, excipients and desired organelle, parasite, cell, tissue and receptor, for drug delivery into the brain. Transport of nanoengineered device at cellular levels can be achieved by -fluidic channels [96]. Various drug delivery systems such as nanoparticles, nanogels, liposomes, microspheres, and nanobiocapsules have been employed to improve the bioavailability of the drug in the brain. However, so far, biodegradable polymeric nanoparticulate careers and microchips are found to be more effective therapeutically in treating brain tumor. Distinct strategies based on physiological concepts can also be employed to stimulate the transcytosis ability of specific receptors expressed across the BBB. According to reports low density lipoproteins related protein with engineered peptide compound created an area for introducing Angiopep peptide as a new potential therapeutics.

Previous studies have determined that nanoparticles having different surface characteristics such as neutral nanoparticles and particles having low concentrations of anionic nanoparticles were found to have no effect on BBB integrity. In contrast NPs with high concentrations of anionic nanoparticles and cationic nanoparticles were toxic for the BBB. It was later discovered that neutral or cationic nanoformulations showed lesser uptake in brain in contrast with anionic nanoparticles at low concentrations exhibit higher uptake in brain. It has been concluded that surface charge of nanoparticle plays an important role in determining toxicity and brain distribution profiles [97]. Surfactant assisted transport of nanoparticle across the blood brain barrier was recently reported with the polysorbate (Tween) surfactants. Coating of nanoparticle with polysorbate (Tween) surfactants stimulates endocytosis mechanism via the Low Density Lipoprotein receptor of the endothelial cells after adsorption of lipoproteins form blood plasma to the nanoparticles [98]. Moreover it has been further revealed the function of apolipoprotein-E for carry drugs across the BBB. However failure in transportation of drug across the BBB occurs when apolipoprotein-E variants that did not recognized lipoprotein receptors [99]. Later on it was realized that recognition and interaction with lipoprotein receptors on brain capillary endothelial cells plays an important role in brain uptake of the drug. Transport of several drugs can also be attained by masking their features or limiting their binding efficiency/sites to cellular efflux systems e.g. p-glycoprotein. Binding of drug with p-glycoprotein limits its cellular transportation and encourage the drug removal from cells. P-glycoprotein belongs to ATP dependent efflux transporters that plays have a major role in preventing delivery into the brain [100]. P-glycoprotein role become more critical in drug resistant tumor cells where there expression becomes high and act as multidrug resistance protein to limit the entry

of drug inside the cell. It was observed that entrapment of certain cytotoxic drug such as in surfactant coated nanoparticles increases the brain drug uptake and its toxicity towards p-glycoprotein expressing tumor cells. Alyautdin et al. reported that surfactant coated poly(butyl) cyanoacrylate nanoparticles have been used to deliver drugs to the CNS [101]. Similarly Koziara et al. determined the effect of entrapment of a cytotoxic drug paclitaxel in cetyl alcohol/polysorbate nanoparticles and further he hypothesized that paclitaxel nanoparticles restrict paclitaxel conjugation to p-glycoprotein and successive efflux from the cells [102]. This hypothesis led down conclusion which would consequently trigger the brain and tumor cell drug levels. Many other additional routes to deliver the drug in brain which could circumvent the BBB such as via migration along the olfactory or trigeminal nerve endings could only possible after the deposition on the olfactory mucosa in the nasal region [103]. This procedure was experimented by translocation of ultrafine ^{13}C particles in the brain olfactory bulb after inhalation exposure. Based on amount of manganese present in different parts of the brain, Elder et al. demonstrated the role of solid NP like manganese oxide to translocate to the brain by the olfactory route [104]. In subsequent report nanoparticles have been functionalized by conjugation with bioactive ligands-lectins to the surface of poly (ethylene glycol)-poly (lactic acid) nanoparticles to trigger specific uptake via the inhalation route nanoparticles. Similarly Gao et al. utilized wheat germ agglutinin to enhance the brain uptake of such functionalized NP by encouraging their binding with N-acetyl-D-glucosamine and sialic acid. Nevertheless transport of drug across BBB and the olfactory route only report for up to 2 % nanoparticles uptake in contrast with the drug delivery that desires to make substantial increments prior to use [105].

Latest concepts and techniques should be applied to design and develop the drug delivery careers for efficiently delivering the drug across the BBB at a safe and effective manner. Among all current advance delivery systems nanoparticles are found to be the most effective careers in delivery of conventional drugs, vaccines, recombinant proteins, as well as nucleotides. Drug features such as pharmacokinetic properties (such as biodistribution, bioavailability and drug release characteristics) can be improved by nanoparticulate drug delivery systems. Nanoparticulate drug delivery systems allow the controlled and effective release of the drug with site specific drug delivery targeting to tissue or cell with reduced side effects. Hence application of nanotechnology in pharmaceutical biotechnology facilitates the development in drug delivery strategy to explain the delivery problems of protein based drugs including oligonucleotides and recombinant proteins.

1.4 Innovations in Nanotechnology

Revolution has started in 1989, when IBM researchers established a scientific invention by building 35-atom depiction of the company's logo at nano scale. Nanomaterials are the objects having size range of at least one dimension of

100 nm or less. This size range is approximate size of your average virus. Nanotechnology field encompasses creation, manipulation, and application of materials at the nanoscale. Additionally it may also involve the capability to engineer, control, and exploit the unique chemical, physical, and electrical properties of these nano scale particles. As far as the their properties are concerned nanoparticles neither behave like solids, liquids, nor gases and exist in the world of quantum physics, and small enough escape the laws of Newtonian physics. This feature makes them more conductive, reactive and optically sensitive among others. Nanotechnology is considered as the most powerful technology to bring "the next Industrial Revolution." "That's why nanomaterials are useful and interesting and so hot right now," says Kristen Kulinowski, executive director for education and policy at the Rice University Center for Biological and Environmental Nanotechnology (CBEN). Being in this quantum regime enables new properties to emerge that are not possible or not exhibited by those same chemicals when they're much smaller or much larger. These include different colors, electronic properties, magnetic properties, mechanical properties depending on the particle; any or all of these can be altered at the nanoscale. That's the power of nanotech. The National Nanotechnology Initiative, federal government program for the science, engineering, and technology research and development for nanoscale projects, has predicted the field will be worth $1 trillion to the U.S. economy alone by 2015 or sooner. Noticeably, nanotech is balanced to develop into a major factor in the world's economy and part of our everyday lives in the near future. In future, draft science is going to be very giant, shortly. Owing to its widespread it's very difficult to trace the application of nanotechnology, although we can consider some of the recent events of nanotechnology and the most considerable achievements. Nanotechnology solutions are already beginning to shape industries like electronics, medical technologies, advanced materials, or pharmaceuticals. Nanotechnology can be connected to world's most serious advance issues/problems. Nevertheless there has been no organized precedence of applications of nanotechnology that can be directed towards the most critical issues face by the five billion people from developing world.

In order to strengthen their capacity and sustain economic growth [106], several developing countries have launched nanotechnology initiatives. Department of Science and Technology, India invested $20 million from (2004–2009) for their Nanomaterials Science and Technology Initiative [107]. China ranks third in number of nanotechnology patent applications whereas United States and Japan ranked still saving its first two positions [108]. According to previous report Brazil, projected budget for nanoscience during the 2004–2007 period was found to be is $25 million [109]. Organizations that belong to South African Nanotechnology Initiative are involved in areas such as nanophase catalysts, nanofiltration, nanowires, nanotubes, and quantum dots. Some other developing countries such as the Philippines, Chile, Argentina, Thailand, and Mexico, are also practicing nanotechnology [106]. It's very difficult to address all the events progressing in nanotechnology, though it's possible rank the ten applications of nanotechnology most

likely to benefit developing countries, and demonstrate that these applications can contribute to the attainment of the United Nations Millennium Development Goals (MDGs) as suggested by Salamanca-Buentello et al. [110]. To encourage more advance innovations in nanotechnology Salamanca-Buentello et al. also proposed a way for the international community to accelerate the use of these top nanotechnologies by less industrialized countries to meet critical sustainable development challenges [110]. Salamanca-Buentello and his coworkers explored Correlation between the Top Ten Applications of Nanotechnology for Developing Countries and the UN Millennium Development Goals [110]. Salamanca-Buentello and his coworkers identified and ranked the ten applications of nanotechnology most likely to benefit developing countries [110]. These applications provide systematic approach with which to address sustainable development issues in the developing world. Salamanca-Buentello and his coworkers used modified Delphi Method to identify and prioritize the applications. They have addressed top ten applications of nanotechnology and compared with the UN Millennium Development Goals. These applications covers energy storage, conversion and production, agricultural productivity enhancement, water treatment and remediation, disease diagnosis and screening, drug delivery system, food processing and storage, air pollution and remediation, construction, health monitoring, vector and pest detection and control.

1.5 Nanotechnology Theory to Applications

Nanotechnology is serving to significantly develop, even reform, many technology and industry areas. It has significant applications in information environmental science, medicine, technology, energy, food safety, homeland security, and transportation, among many others. Nanotechnology act as a significant tools in food industry for detection of pathogen, disease treatment delivery systems, food packaging, and delivery of bioactive compounds to target sites. This wide application generates novel methods to improve safety and the nutritional value of food products [111].

In addition to food industry, nanotechnology also involved in design, production, characterization, and application of materials and such devices that are having significant applications in medicine [112]. Nanotechnology applications to medicine and physiology involve materials and devices that are perfectly designed to interact with the body at subcellular (i.e., molecular) scales with a high degree of specificity. Such application cab effectively transform into targeted cellular and tissue-specific clinical applications considered to attain therapeutic affects with least side effects.

Nanotechnology can also be employed in various surgical procedures to treat human cancers. Additionally nanotechnology is the single most prominent forecaster of patient survival is a complete surgical resection [113]. These innova-

tive technologies can assist the surgeon to describe tumor margins, to identify residual tumor cells and micrometastases, and to verify if the tumor has been entirely removed. Recent advances in nanotechnology and optical instrumentation, advances can be integrated for applications in surgical oncology, achieved by nanometer-sized particles such as quantum dots and colloidal gold. Conjugation of such nanoparticles with targeted ligands such as monoclonal antibodies, peptides, or small molecules allows to target malignant tumor cells and tumor microenvironments with high specificity and affinity. Singhal et al. have reported that the size range of 10–100 nm (mesoscopic) nanoparticles having large surface areas for conjugating to multiple diagnostic and therapeutic agents, opening new possibilities in integrated cancer imaging and therapy. There are so many reports [114].

Saini et al. reported possible mechanism of nanotechnological procedures in relation to medicine, in which he has described various materials and devices that were designed to interact with cells and tissues at a molecular (i.e., sub cellular) level, for applications in medicine and physiology, with a high degree of functional specificity, thus allowing a degree of integration between technology and biological systems not previously attainable [114].

The most appreciable thing of nanotechnology is to unite different traditional sciences, such as, chemistry, physics, materials science and biology, to bring together the required collective expertise needed to develop these novel technologies [114]. To achieve this nanotechnology carry is versatile function by offering not only improvements to the current techniques, but also providing entirely new tools and capabilities.

- Alteration in solubility and blood pool retention time
- Controlled release over short or long durations
- Environmentally triggered controlled release or highly specific site-targeted delivery

Potential of nanotechnology can also be employed in construction industry where amazing chemical and physical features of materials (at the nanometer scale) allows new array of application such as structural strength enhancement, energy conservation to antimicrobial properties and self-cleaning surfaces [115]. Therefore manufactured nanomaterials and nanocomposites are being considered for various uses in the construction and related infrastructure industries. It's essential to consider the lifecycle impacts of manufactured nanomaterials on the health of construction workers and dwellers; therefore it's more important to achieve environmentally responsible nanotechnology in construction. Such development of environmentally friendly nanotechnology products avoids environmentally responsible nanotechnology [115]. Lee et al. reported applications of manufactured nanomaterials that improve conventional construction materials, promote the environmentally friendly nanoproducts with reduced adverse biological and toxicological effects and their mitigation [115]. Various applications of nanotechnology in different areas are mentioned in Table 1.2.

Table 1.2 Applications of nanotechnology in different domain

Area	Applications
Everyday material and processes	• Nanoscale additives in polymer composite materials and for surface treatments of fabrics • Nanoscale thin film • Nanoscale materials in cosmetic products • Nano-engineered materials in the food industry, in automotive products and to make superior household products • Nanostructured ceramic coatings • Nanoparticles are used increasingly in catalysis
Electronic and information technology application	• Applications of nanotechnology in computing and electronic products include, ultraresponsive hearing aids flash memory chips for iPod nanos conductive inks for printed electronics for RFID/ smart cards/smart packaging, flexible displays for e-book readers more life-like video games, antimicrobial/antibacterial coatings on mouse/keyboard/cell phone casings • Magnetic random access memory (MRAM) • Nanoscale transistors • Nanostructured polymer films known as organic light-emitting diodes for the displays for many new TVs, laptop computers, cell phones, digital cameras, and other devices incorporate
Sustainable energy applications	• Prototype solar panels incorporating nanotechnology • Nanotechnology is improving the efficiency of fuel production • Nano-bioengineering of enzymes • Nanotechnology for development of advanced batteries • Nanostructured materials to improve hydrogen membrane and storage materials and the catalysts • Nanotechnology in thin-film solar electric panels to power mobile electronic devices
Environmental remedial applications	• For affordable, clean drinking water • To clean industrial water pollutants in ground water • Nanofabric “paper towel,” woven from tiny wires potentially absorb 20 times its weight in oil for cleanup applications • Nanotechnology-based filters that allow “mechanical filtration” • Novel nanotechnology-enabled sensors and solutions to detect, identify, and Filter out, and/or neutralize harmful chemical or biological agents in the air and soil with much higher sensitivity
Nanobiosystem, medical and health applications	• Quantum dots for enhancing biological imaging for medical diagnostics • For diagnosis of atherosclerosis, or the buildup of plaque in arteries • Gold nanoparticles to detect early-stage Alzheimer’s disease • Molecular imaging for the early detection • Multifunctional therapeutics to facilitate its specific targeting to cancer cells • Microfluidic chip-based nanolabs for monitoring and manipulating individual cells and nanoscale probes, • Nanotechnology to encourage the growth of nerve cells

(continued)

Table 1.2 (continued)

Area	Applications
Future transportation technologies	• Nano-engineering of concrete, steel, asphalt, and other cementitious materials, and their recycled forms • Nanoscale sensors and devices for cost-effective continuous structural monitoring of the condition and performance of bridges, tunnels, rails, parking structures, and pavements over time

1.6 Nanomedicine/Nanoscience/Nano-Engineering and Relationship with Drug Delivery

1.6.1 Nanomedicine and Drug Delivery

Emergence of nanotechnology in medicine led to the exploration of more specified drug delivery systems in pharmaceutical sciences. Currently better understanding has been established between nanomedicine and its drug delivery applications in pharmaceutical sciences to minimize toxicity and side effects of drugs. Exploration of such cell/tissue specific drug delivery system avoids the health hazards that some time occur due to the carrier systems themselves that may impose risks to the patient. In last few years several reports have been come up with a conclusion of health risks associated with nanoparticles and associated their preventive measures. Therefore currently various substances are under examination for drug delivery.

More interestingly type of health risks that are caused by using nanoparticles for drug delivery are ahead of that caused by conventional hazards introduced by chemicals in traditional delivery matrices. More understanding is required to investigate the nanoparticle toxicity and its potential hazards as in the case of inhalation toxicity. Risks associated with nanoparticulate materials may vary from toxicology of substances since the composing chemical(s) may or may not be soluble in biological matrices. Therefore their solubility issues greatly influence their potential exposure against various internal organs. Considering this aspect nanoparticulate systems when administered through the most critical route such as lungs get high exposure in the lungs and a low exposure for other organ systems after inhalation. Since these nanoparticles are more specified therefore accumulation or absorption in undesirable organs may cause potential toxicity. Lungs is an organ where exchange off oxygen occurs, accumulation of drug or nano matrix may influence the potential effects of drug and may generate toxic products at different interface. In contrast the positive facts about nanoparticles is their size which may offer opportunities of crossing the various biological barriers within the body such as their potential to cross the blood brain barrier may open new ways for drug delivery into the brain. Moreover small size of these particles permits their entry into the cell and various cellular compartments such as nucleus. Various natural and synthetic substances are under investigation

for the preparation of nanoparticles for drug delivery such as biological substances (albumin, gelatin and phospholipids), and more substances of a chemical nature like various polymers and solid metal containing nanoparticles. These substances are selected according to the desirable target, diagnosis procedure, and clinical conditions, type of tissue or cell, type, organ, type of drug (physic-chemical aspects), ingredients and delivery device and type of biological barrier. The most understandable fact is that the real composition of the nanoparticle formulation always decides the possible interaction between with tissues and cells, and the potential toxicity.

1.6.1.1 Advantages of Nanoparticles for Nanomedicine

- Nanoparticles used for therapeutic purposes fulfill with the newly projected and current accepted general definition of a size ≤100 nm [44]. Nevertheless, this does not essentially have an influence on their functionality in therapeutic applications. The royal society and the royal academy of engineering. Nanoscience and nanotechnologies: opportunities and uncertainties. London, UK: 2004.
- Nanoparticles are considered as attractive therapy for several medicinal purposes since these nano based particles are having important and exclusive features, such as their quantum properties and their ability to absorb, their surface to mass ratio that is much larger than that of other particles and carry other compounds.
- Nanoparticles have a comparatively large (functional) surface which is able to bind, adsorb and carry other compounds such as drugs, proteins and probes.
- These nanoengineered particles not only used for drug delivery as carrier, but also the drug itself may be formulated at a nanoscale, and then function as its own "carrier" [39, 45–47].

1.6.1.2 Challenges

- More improved understanding of the patho-physiological basis of disease is required which will bring more sophisticated diagnostic opportunities, and certainly increase the whole cost the therapy
- Significant dimensions (below 0.1 μm or 100 nm) of nanoparticles doesn't allow sufficient loading of drug onto the particles.
- Some of the serious health risk associated with nanoparticles need to be resolved such as exposure of biological materials with inhaled nanoparticles as part of the unintended release of ultrafine or nanoparticles by combustion derived processes such as diesel exhaust particles [48, 49, 51–54, 116–118]. Exposure to these combustion derived ultrafine particles/nanoparticles is associated with a wide variety of effects [53] including pulmonary inflammation, immune adjuvant effects [54] and systemic effects including blood coagulation and cardiovascular effects [52, 116].

1.6.2 Nanoengineering and Drug Delivery

Nanoengineered drug delivery systems (nDDS) are those systems in which nanoparticulate system or its environment is modified or manipulated to avoid the in limitations induced by general nanoparticles. Currently nDDS is used as potential clinical tools for modulation of pharmacological drug release profile and for but specific targeting of diseased tissues especially cancer tissue or cells [119]. Till date various anticancer drugs such as doxorubicin, paclitaxel, and vincristin has been the main target of nDDS. Liposomes and polymer-drug conjugates are known to be the most accepted group of nDDS used for this purpose. Nano engineering procedure require careful selection and optimization of the different factors that affect drug release profile (i.e. type of biomaterial, size, system architecture, and biodegradability mechanisms) along with the selection of an appropriate manufacture technique that does not compromise the desired release profile, while it also offers possibilities to scale up for future industrialization [119].

Nano engineering or manipulation of drug delivery system can be achieved at various levels during the formulation of drug within a carrier e.g. modulation of drug environment at molecular level, alteration of physic-chemical characteristics of the drug, control of diffuse mobility, and modification of bulk and surface chemistry of nano carrier [119]. These approaches are successful enough in establishing favorable therapeutic end points such as enhanced drug efficacy for prolonged period, reduced drug toxicity; reduce dosage and cost burden and improved patience compliance [119].

nDDS comprised of various controlled and triggered release systems such as polymeric drug delivery system and lipid based drug delivery systems for sustained and triggered release. This can be achieved by the modulation of drug environment and physico-chemical properties, enhancement of drug stability and duration of activity which may further includes formulation processing strategies and polymeric strategies [119]. For specific targeting of therapeutics to disease sites, for maximizing effective dose and from sparing health risks from various adverse effects, several strategies have been adopted such as active and passive tissue targeting and strategies for transport across the tight endothelial junctions [119].

In 1999 Allen et al. describes the properties of block copolymer micelles which influence their efficiency as drug delivery vehicles for hydrophobic drugs. In this review he has explained properties such as loading capacity, release kinetics, circulation time, biodistribution, size, size distribution and stability to promote researchers to tailor-make block copolymer micelles for a particular application [120].

Various researchers considering nanoengineered drug delivery systems as a potential strategy to overcome limitations associated with antibiotic drug therapy. It has been reported that antibiotics encapsulated into nanodelivery systems play significant role in overcoming the serious global burden of antibiotic resistance and provide improved management to patients with various infectious diseases [121]. According to previous reports there are several antibiotic-loaded nanocarriers that

have been formulated to target drugs to infectious sites, achieve controlled drug release profiles, and address formulation challenges, such as low-drug entrapment efficiencies, poor solubility and stability [121]. Additionally the *in vitro*/*in vivo* performances and physicochemical properties of various antibiotic-loaded delivery systems (polymeric nanoparticles, micelles, dendrimers, liposomes, solid lipid nanoparticles, lipid–polymer hybrid nanoparticles, nanohybirds, nanofibers/scaffolds, nanosheets, nanoplexes, and nanotubes/horn/rods and nanoemulsions) should be evaluated [121]. Future investigations is required to optimize formulation and commercialization of these antibiotic-loaded nanosystems to develop the most effective nanoengineered antibiotic drug delivery systems for enhancing the treatment of patients with a range of infections.

Owing to its unique features such as its potential in the formation of supramolecular biomaterials through inclusion complexes as part of drug delivery systems, moderate drug solubility, drug stability and absorption, cyclodextrin is now considered as excipients in the formulation of drugs and played a pivotal role in designing novel drug delivery systems such as liposomes, dendrimers, nanosponges, microspheres and nanoparticles. Recent reports have proven that molecular complexation involving cyclodextrins is expected to reduce or eliminate some problems associated with novel drug delivery in the field of nanotechnology [122]. Various applications of nanomedicines are highlighted in Fig. 1.1.

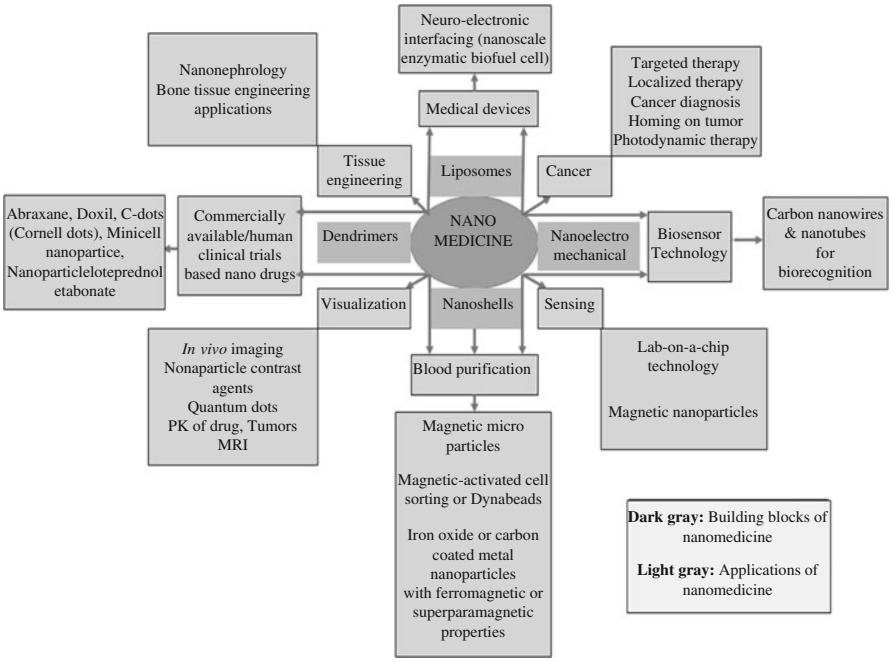

Fig. 1.1 Nano medicine and its applications

1.7 Types of Nanodelivery: Natural or Synthetic

Natural and synthetic are the two types of polymers that can be used for nanodelivery. Natural polymers or biopolymers are directly obtained from natural resources such as green plants, animals, algae, bacteria and fungi are polymers or polymer matrix composites. Synthetic polymers are those polymers that are refined or modified or synthesized in laboratory by using different chemical of more often basic structure of natural polymers can be modified to avoid the limitations incurred during the drug delivery. Collagen while synthetic polymers from the ester family. Currently natural and synthetic polymers are extensively used for nanoparticles design and synthesis. Owing to outstanding advantages of high purity and reproducibility over natural polymers, synthetic polymers such as poly(-caprolactone) (PCL), poly(lactic acid) (PLA), poly(lactide-co-glycolide) (PLGA), poly(glycolic acid) (PGA), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), poly (-hydroxybutyrate) (PHB), or other families such as poly(cyanoacrylates), poly (p-dioxanone) (PPDO), poly(acrylic acid) (PAA), poly(amides) (PA), poly(anhydrides), poly(ethylene glycol) (PEG), poly(ortho esters) (PES), and poly(vinyl alcohol) (PVA), are usually recommended in most of the research. These polymers are suitable for drug delivery due to their special release profiles biodegradability, and biocompatibility [123, 124]. Additionally PLGA has been approved by Food and drug administration (FDA) for human therapy.

1.7.1 Synthetic Polymers

Emulsion techniques and/or solvent exchange, salting out, freeze drying or spray drying of the emulsion and supercritical fluid technology are the principal methods used to formulate and manufacture polymeric particles. Noticeably multiple-emulsion spray-drying technique has potential advantages over freeze-drying technique such as it's more convenient and reproducible, rapid, and can easily be scaled up. Therefore these methodologies can be modified with the objective of encapsulating hydrophilic or hydrophobic drugs into particular polymeric nanoparticles. Utilization of synthetic polymers is encouraged owing to their ability to modify the functional groups and the versatility in the preparation techniques. Additionally polymeric nanoparticles give more response against different stimuli has been of big advantage for targeted delivery systems.

1.7.2 Natural Polymers

Due to some unique and relatively safe features of biocompatibility, biodegradability, abundance in nature, inexpensive than bioresorbable synthetic polymers interest has been generated to produce nanoparticles using natural polymers with aim to

Table 1.3 Advantages and disadvantages of natural and synthetic polymers

Natural polymer	Synthetic polymer
Cellulose, starch, chitosan, carrageenan, aliginates, xantham gum, gellan gum, pectins	Synthetic polymers from the ester family such as poly(lactic acid) (PLA), poly(cyanoacrylates) (PACA), poly(acrylic acid), poly(anhydrides), poly(amides), poly (ortho esters), poly(ethylene glycol), and poly(vinyl alcohol) (PVA) and other like poly(isobutylcynoacrylate) (PIBCA), poly(ethylene oxide) (PEO), poly(åcaprolac-tone) (PCL)
Advantages • Less toxic • Biocompatibility • Biodegradable • Easily available	*Advantages* • Biocompatibility
Disadvantages • High degree of variability in natural materials derived from animal sources • Structurally more Complex • Extraction process very complicated and high cost	*Disadvantages* • Toxic • Non degradable • Synthetic process is very complicated and high cost

encourage their use in drug delivery for exploring more advance pharmaceutical applications against different indications. The most extensively used natural polymers in drug delivery systems include alginate, chitosan, gelatine, albumin and collagen. Different types of procedures are adopted to make natural polymer-based nanodevices. Selection of the technique is based on the nature of the active compound, the polymer and the specifications of the nanoparticles, such as size, zeta potential and encapsulation efficiency. Among all the most extensively used techniques include emulsion-cross linking, coacervation and ionotropic gelation. Different advantages and disadvantages of natural and synthetic polymers are mentioned in Table 1.3.

1.8 Natural and Synthetic Polymeric Nanoparticles

Collective term nanoparticle constitute nanospheres and nanocapsules which are made up of solid carriers that can be either made up of natural or synthetic polymers and whether or not biodegradable. These solid carriers potentially encapsulate Drug/gene by embedding into the matrix or absorbed onto the surface of nanoparticles. Because of their therapeutic potential and greater stability in biological fluids as well as during storage nanoparticles have received more consideration than have liposomes [125]. In addition high encapsulation efficiency, provide fencing drug against degradation from the external environment and use the exclusive

micro-anatomy of the inflamed tissue blood capillaries, which have gaps between the lining of endothelial cells causing vessel leakiness [126]. There are several fabrication methodologies available for nanoparticles however these methods use safe solvents for industrial application. Solvents like DMSO, DMF are known are having the tendency to dissolve many insoluble drugs but there cost and toxicity pose their utilization for any therapeutic purpose especially when the process of nanoparticles preparation conducted at industrial scale. Nanoparticles preparation by polymerization of a monomer or from pre-formed polymers is highly acceptable nowadays. According to our database survey utilization of polymers (synthetic/natural) are highly encouraged since nanoparticles properties can be tailored by using different polymers and co-polymers. According to last 2 years survey it has been discovered that as chitosan (among all natural polymers) is known to be the most leading natural polymer, relatively safe biocompatible, mucoadhesiveness and permeability enhancing properties [127, 128] and its derivates have shown improved characteristics. Additionally it is also used as food additive in food industries. Another example of albumin widely utilized as natural carrier for hydrophobic molecules such as fatty acids, hormones and fat-soluble vitamins has been extensively used as it as non-toxic and non-immunogenic. Natural polymers have their serious concerns in terms of purity and stability and therefore synthetic polymers have been applied. Owing to their biodegradability certain synthetic polymers are highly encouraged in drug delivery such as poly(cyanoacrylates) (PACA), poly(lactic acid) (PLA), poly(anhydrides), poly(ethylene glycol), poly(acrylic acid), poly(amides), poly (ortho esters), and poly(vinyl alcohol) (PVA) and other like poly(isobutyl cynoacrylate) (PIBCA), poly(ε-caprolac-tone) (PCL), poly(ethylene oxide) (PEO). These biocompatible polymers can be conjugated with protein to form different structures with different properties such as controlled release profiles and strong cell biocompatibility. Among all PLGA, is known to be the suitable polymer for biomedical applications such as suture materials [129] and bone fixation nails and screws [130] as well as in diverse drug delivery applications [131, 132], though cost is always a concern for such polymers. Owing to its features of biocompatibility and biodegradability it has extensively studied for various applications. PLGA forms compatible moieties of lactic acid and glycolic acid which are further eliminated by the citric acid cycle. Since this whole procedure is slow so slow it doesn't influence the normal cell function. Owing to the recent innovations in polymeric science, various other polymers such as poly(β-amino ester) (PbAE) has emerged in the spotlight because it demonstrates unique features such as pH sensitive release [133, 134] in which at acid pH it rapidly releases its contents. According to recent reports poly(β-amino ester) has shown to be less toxic than other cationic polymers such as poly(ethyleneimine) and poly(L-lysine) (PLL) [133, 134], and insoluble at physiological pH but solubilized in aqueous media at pH below 6.5. Polymeric agents like poly(β-amino ester) are significant for therapeutics in the surrounding area of tumor mass [133, 134] and for others they must escape endosomal compartmentalization prior to fusion with lysosomes [133, 134]. Various natural/synthetic polymer based commercialized nanomedicines and their applications are highlighted in Table 1.4.

Table 1.4 Commercialization of nano-drug delivery systems using both natural and synthetic polymers

Trade name	Encapsulated drug	Type of nanoparticles	Mode of administration	Application	Manufacturer
Abraxane	Paclitaxel	Albuminbound nanoparticles	IV injection	Metastatic breast cancer	American Biosciences (Blauvelt, NY)
Amphocil	Amphotericin B	Lipocomplex	IV infusion	Serious fungal infections	Sequus Pharmaceuticals
Ambisome	Amphotericin B	Liposome	IV infusion	Serious fungal infections	NeXstar Pharmaceutical (Boulder, CO)
Abelcet	Amphotericin B	Lipid complex	IV infusion	Serious fungal infections	The Liposome Company (Princeton, NJ)
DaunoXome	Daunorubicin citrate	Liposome	Liposome	Kaposi sarcoma in AIDS	NeXstar Pharmaceutical (Boulder, CO)
Doxil	Doxorubicin	Liposome	IV injection	Kaposi sarcoma in AIDS	Sequus Pharmaceuticals
Elestrin	Estradiol	Calciumphosphatebased nanoparticles	Transdermal	Moderated to severe vasomotors Symptoms (hot flashes) in menopausal women	BioSante (Lincolnshire, IL)
Emend	Aprepitant,	MK869 Nanocrystal particles	Oral	To delay nausea and vomiting	Merck/Elan (Whitehouse Station, NJ)
Megace ES	Megaestrol acetate	Nanocrystal particles	Oral	Anorexia, cachexia or unexplained significant weight loss	PAR Pharmaceutical (WoodCliff Lake, NJ)
Rapamune	Sirolimus	Nanocrystal particles	Oral	Immunosuppressant in kidney transplant patients	Wyeth/Elan (Madison, NJ)
Tricor	Fenofibrate	Nanocrystal particles	Oral	Primary hypercholestrolemiamixed lipidemia, hypertriglyceridemia	Abbott (Abbot Park, IL)

References

1. Santamaria A. Historical overview of nanotechnology and nanotoxicology. Methods Mol Biol. 2012;926:1–12.
2. Drexler E. Engines of creation: the coming era of nanotechnology. Anchor Library of Science, 1987.
3. Feynman R. There's plenty of room at the bottom. In: Gilbert HD, editor. Miniaturization. New York: Reinhold; 2004. p. 282–96.
4. Taniguchi N. On the basic concept of 'Nano-technology'. In: Proceedings of the international conference on production engineering, Tokyo, Part II. Japan Society of Precision Engineering, 1974. p. 5–10, 10.
5. Curl RF, Smalley RE, Kroto HW, O'Brien S, Heath JR. How the news that we were not the first to conceive of soccer ball C60 got to us. J Mol Graph Model. 2001;19(2):185–6.
6. Davis T. Biography of Louis E. Brus. Proc Natl Acad Sci U S A. 2005;102(5):1277–9.
7. Drexler KE. Unbounding the future: the nanotechnology revolution. New York: Harper Collins; 1991.
8. Drexler KE. Nanosystems: molecular machinery, manufacturing, and computation. New York: Wiley; 1992.
9. Iijima S. Helical microtubules of graphitic carbon. Nature. 1991;354:56–8.
10. Kresge CT, Leonowicz ME, Roth WJ, Vartuli JC, Beck JS. Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism. Nature. 1992;359:710–2.
11. Murray CB, Norris DJ, Bawendi. Synthesis and characterization of nearly monodisperse CdE (E = sulfur, selenium, tellurium) semiconductor nanocrystallites. J Am Chem Soc. 1993;115:8706–15.
12. Foresight institute first formen prize in nanotechnology. http://www.foresight.org/FI/1997Feynman.html.
13. Foresight institute. http://www.foresight.org/nano/history.html.
14. Seeman NC. Structural DNA nanotechnology: an overview. Methods Mol Biol. 2005;303:143–66.
15. Foresight Update 26, NASA Unit putting major resources into computational nanotechnology. https://www.foresight.org/Updates/Update26/Update26.1.html, by Lew Phelps.
16. Webster TJ. IJN's second year is now a part of nanomedicine history. Int J Nanomed. 2007;2(1):1–2.
17. Nanalyze. The World's Oldest and Biggest Nanotechnology Company, 2014. http://www.nanalyze.com/2014/06/the-worlds-oldest-and-biggest-nanotechnology-company.
18. Seeman NC. From genes to machines: DNA nanomechanical devices. Trends Biochem Sci. 2005;30(3):119–25.
19. Mao C, Sun W, Shen Z, Seeman NC. A nanomechanical device based on the B-Z transition of DNA. Nature. 1999;397:144–6.
20. Kateb B, Heiss JD. The textbook of nanoneuroscience and nanoneurosurgery. Boca Raton, FL: CRC Press; 2013. p. 4.
21. Ginger DS, Zhang H, Mirkin CA. The evolution of dip-pen nanolithography. Angew Chem Int Ed. 2003;43(1):30–45.
22. Freitas RA. Nanomedicine, Vol. I: Basic capabilities. Georgetown, TX: Landes Bioscience; 1999.
23. Paradise J, Wolf SM, Ramachandran G, Kokkoli E, Hall R, Kuzma J. Developing oversight frameworks for nanobiotechnology. Minn J L Sci Tech. 2008;9(1):399–416.
24. Roco MC, Mirkin CA, Hersam MC. Nanotechnology research directions for societal needs in 2020: retrospective. New York: Springer; 2011. p. 12.
25. Lieber C. Nanowires take the prize. Marer Today. 2002;5(2):48.
26. Nikalje AP. Nanotechnology and its applications in medicine. Med Chem. 2015;5:081–9.
27. Gupta SRN. Advances in molecular nanotechnology from premodern to modern era. Int J Mater Sci Eng. 2014;2:2.

28. Wonders S. Endless frontiers: a review of the national nanotechnology initiative. National Academies Press. http://www.ncbi.nlm.nih.gov/books/NBK220670/.
29. Nanoscience and nanotechnologies: opportunities and uncertainties, 2004. https://royalsociety.org/topics-policy/publications/2004/nanoscience-nanotechnologies/.
30. Guston DH. Encyclopedia of Nanoscience and Society. SAGE Publications, 2010, Social Science. p. 598.
31. http://www.foresight.org/nano/history.html.
32. Rothemund PWK, Papadakis N. Winfree E algorithmic self-assembly of DNA Sierpinski triangles. PLoS Biol. 2004;2(12):e424. doi:10.1371/journal.pbio.0020424.
33. Peterson C. Nanotechnology: from Feynman to the grand challenge of molecular manufacturing. IEEE Technology and Society, Winter 2004.
34. Peterson C. Molecular nanotechnology: the next industrial revolution. IEEE Computer, 2000.
35. Regis E, Brown L. Nano: the emerging science of nanotechnology, 1995.
36. Peterson C, Wired H. Nanotechnology: from concept to R&D Goal, 1995.
37. Peterson C. Nanotechnology: evolution of the concept. In: Krummenacker M, Lewis J, editors. Prospects in nanotechnology: toward molecular manufacturing. New York: Wiley; 1995.
38. Geim A, Konstantin. The Nobel Prize in Physics 2010. Novoselov. http://www.nobelprize.org/nobel_prizes/physics/laureates/2010/press.html.
39. Duncan R. The dawning era of polymer therapeutics. Nat Rev Drug Discov. 2003;2:347–60.
40. De Jong WH, Geertsma RE, Roszek B. Possible risks for human health. Report 265001002/2005. Bilthoven, The Netherlands: National Institute for Public Health and the Environment (RIVM); 2005. Nanotechnology in medical applications.
41. European Science Foundation. Policy Briefing (ESF), ESF Scientific Forward Look on Nanomedicine IREG Strasbourg, France, 2005. ISBN: 2-912049-520.
42. European Technology Platform on Nanomedicine. Vision paper and basis for a strategic research agenda for nanomedicine. European Commission Luxembourg, Office for Official Publications of the European Commission, 2005. ISBN: 92-894-9599-5.
43. Ferrari M. Cancer nanotechnology: opportunities and challenges. Nat Rev Cancer. 2005;5:161–71.
44. The Royal Society and The Royal Academy of Engineering. Nanoscience and nanotechnologies: opportunities and uncertainties, London, UK, 2004.
45. Kipp JE. The role of solid nanoparticle technology in the parental delivery of poorly water-soluble drugs. Int J Pharm. 2004;284:109–22.
46. Cascone MG, Lazzeri L, Carmignani C, et al. Gelatin nanoparticles produced by a simple W/O emulsion as delivery system for methotrexate. J Mater Sci Mater Med. 2002;13:523–6.
47. Baran ET, Özer N, Hasirci V. *In vivo* half life of nanoencapsulated L-asparaginase. J Mater Sci Mater Med. 2002;13:1113–21.
48. Oberdörster G. Significance of particle parameters in the evaluation of exposure-dose-response relationships of inhaled particles. Inhal Toxicol. 1996;8:73–89.
49. Donaldson K, Stone V, Clouter A, et al. Ultrafine particles. Occup Environ Med. 2001;58:211–1651.
50. Donaldson K, Stone V, Tran CL, et al. Nanotoxicology. Occup Environ Med. 2004;61:727–2852.
51. Donaldson K, Stone V. Current hypotheses on the mechanism of toxicity of ultrafine particles. Ann Ist Super Sanita. 2003;39:405–10.
52. Borm PJ, Kreyling W. Toxicological hazards of inhaled nanoparticles—potential implications for drug delivery. J Nanosci Nanotechnol. 2004;4:521–31.
53. Donaldson K, Tran L, Jimenez LA, et al. Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. Part Fibre Toxicol. 2005;2:10.
54. Granum B, Lovik M. The effect of particles on allergic immune responses. Toxicol Sci. 2002;65:7–17.
55. LaVan DA, McGuire T, Langer R. Small scale systems for in vivo drug delivery. Nat Biotechnol. 2003;21:1184–91.

56. Borm PJ, Muller-Schulte D. Nanoparticles in drug delivery and environmental exposure: same size, same risks? Nanomedicine. 2006;1:235–49.
57. Gibaud S, Demoy M, Andreux JP, et al. Cells involved in the capture of nanoparticles in hematopoietic organs. J Pharm Sci. 1996;85:944–50.
58. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target specific nanoparticles: theory and practice. Pharmacol Rev. 2001;53:283–318.
59. Demoy M, Gibaud S, Andreux JP, et al. Splenic trapping of nanoparticles: complementary approaches for in situ studies. Pharm Res. 1997;14:463–8.
60. Bazile D, Prud'Homme C, Bassoullet M-T, et al. Stealth PEG-PLA nanoparticles avoid uptake by the mononuclear phagocytes system. J Pharm Sci. 1995;84:493–8.
61. Peracchia MT, Fattal E, Desmaele D, et al. Stealth PEGylated polycyanoacrylate nanoparticles for intravenous administration and splenic targeting. J Control Release. 1999;60:121–8.
62. Niidome T, Yamagata M, Okamoto Y, et al. PEG-modified gold nanorods with a stealth character for in vivo applications. J Control Release. 2006;114:343–7.
63. Gupta AK, Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials. 2005;26:3995–4021.
64. Seki J, Sonoke S, Saheki A, et al. A nanometer lipid emulsion, lipid nano-sphere (LNS), as a parenteral drug carrier for passive drug targeting. Int J Pharm. 2004;273:75–83.
65. Saez A, Guzman M, Molpeceres J, et al. Freeze-drying of polycaprolactone and poly(D,L-lactic-glycolic) nanoparticles induce minor particle size changes affecting the oral pharmacokinetics of loaded drugs. Eur J Pharm Biopharm. 2000;50:379–87.
66. Fishbein I, Chorny M, Banai S, et al. Formulation and delivery mode affect disposition and activity of tyrphostin-loaded nanoparticles in the rat carotid model. Arterioscler Thromb Vasc Biol. 2001;21:1434–9.
67. Shim J, Seok Kang H, Park WS, et al. Transdermal delivery of minoxidil with block copolymer nanoparticles. J Control Release. 2004;97:477–84.
68. Fang C, Shi B, Pei YY, et al. In vivo tumor targeting of tumor necrosis factor-alpha-loaded stealth nanoparticles: effect of MePEG molecular weight and particle size. Eur J Pharm Sci. 2006;27:27–36.
69. Zhang L, Hu Y, Jiang X, et al. Camptothecin derivative-loaded poly(caprolactone-co-lactide)-b-PEG-b-poly(caprolactone-co-lactide) nanoparticles and their biodistribution in mice. J Control Release. 2004;96:135–48.
70. Senior J, Gregoriadis G. Is half-life of circulating small unilamellar liposomes determined by changes in their permeability? FEBS Lett. 1982;145:109–14.
71. Na K, Lee KH, Lee DH, et al. Biodegradable thermo-sensitive nanoparticles from poly(L-lactic acid)/poly(ethylene glycol) alternating multiblock copolymer for potential anti-cancer drug carrier. Eur J Pharm Sci. 2006;27:115–22.
72. Ricci-Junior E, Marchetti JM. Preparation, characterization, photocytotoxicity assay of PLGA nanoparticles containing zinc(II) phthalocyanine for photodynamic therapy use. J Microencapsul. 2006;23:523–38.
73. Gomes AJ, Lunardi LO, Marchetti JM, et al. Indocyanine green nanoparticles useful for photomedicine. Photomed Laser Surg. 2006;24:514–21.
74. Crommelin DJ, Storm G. Liposomes: from the bench to the bed. J Liposome Res. 2003;13:33–6.
75. Metselaar JM, Storm G. Liposomes in the treatment of inflammatory disorders. Expert Opin Drug Deliv. 2005;2:465–76.
76. Minko T, Pakunlu RI, Wang Y, et al. New generation of liposomal drugs for cancer. Anticancer Agents Med Chem. 2006;6:537–52.
77. Win KY, Feng SS. In vitro and in vivo studies on vitamin E TPGS-emulsified poly(D,L-lactic-co-glycolic acid) nanoparticles for paclitaxel formulation. Biomaterials. 2006;27:2285–91.
78. Albrecht C, Knaapen AM, Becker A, et al. The crucial role of particle surface reactivity in respirable quartz induced reactive oxygen/nitrogen species formation and APE/Ref-1 induction in rat lung. Respir Res. 2005;6:129.

79. Tomazic-Jezic VJ, Merritt K, Umbreit TH. Significance of the type and size of biomaterial particles on phagocytosis and tissue distribution. J Biomed Mater Res. 2001;55:523–9.
80. Xia T, Kovochich M, Brant J, et al. Comparison of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. Nano Lett. 2006;6:1794–807.
81. Nemmar A, Hoylaerts MF, Hoet PH, et al. Size effect of intratracheally instilled particles on pulmonary inflammation and vascular thrombosis. Toxicol Appl Pharmacol. 2003;186:38–45.
82. Dyer M, Hinchcliffe M, Watts P, et al. Nasal delivery of insulin using novel chitosan based formulations: a comparative study in two animal models between simple chitosan formulations and chitosan nanoparticles. Pharm Res. 2002;19:998–1008.
83. Edetsberger M, Gaubitzer E, Valic E, et al. Detection of nanometer-sized particles in living cells using modern fluorescence fluctuation methods. Biochem Biophys Res Commun. 2005;332:109–16.
84. Shenoy D, Fu W, Li J, et al. Surface functionalization of gold nanoparticles using heterobifunctional poly(ethylene glycol) spacer for intracellular tracking and delivery. Int J Nanomed. 2006;1:51–7.
85. Konan YN, Chevallier J, Gurny R, et al. Encapsulation of p-THPP into nanoparticles: cellular uptake, subcellular localization and effect of serum on photodynamic activity. Photochem Photobiol. 2003;77:638–44.
86. Panyam J, Zhou WZ, Prabha S, et al. Rapid endo-lysosomal escape of poly(DL-lactide-co-glycolide) nanoparticles: implications for drug and gene delivery. FASEB J. 2002;16:1217–26.
87. Bourges JL, Gautier SE, Delie F, et al. Ocular drug delivery targeting the retina and retinal pigment epithelium using polylactide nanoparticles. Invest Ophthalmol Vis Sci. 2003;44:3562–9.
88. Åkerman ME, Chan WCW, Laakkonen P, et al. Nanocrystal targeting in vivo. Proc Natl Acad Sci U S A. 2002;99:12617–21.
89. Ballou B, Lagerholm BC, Ernst LA, et al. Non-invasive imaging of quantum dots in mice. Bioconjug Chem. 2004;15:79–86.
90. Gupta AK, Curtis ASG. Surface modified supermagnetic nanoparticles for drug delivery: interaction studies with human firbroblasts in culture. J Mater Sci Mater Med. 2004;15:493–6.
91. Weissenbock A, Wirth M, Gabor F. WGA grafted PLGA-nanospheres: preparation and association with Caco-2 cells. J Control Release. 2004;99:383–92.
92. Nobs L, Buchegger F, Gurny R, et al. Poly(lactic acid) nanoparticles labeled with biologically active Neutravidin™ for active targeting. Eur J Pharm Biopharm. 2004;58:483–90.
93. Prinzen L, Miserus R-JJHM, Dirksen A, et al. Optical and magnetic resonance imaging of cell death and platelet activation using annexin A5-functionalized quantum dots. Nano Lett. 2007;7:93–100.
94. Pardridge WM. Blood-brain barrier delivery. Drug Discov Today. 2007;12:54–61.
95. Olivier JC, Fenart L, Chauvet R, et al. Indirect evidence that drug brain targeting using polysorbate 80-coated polybutylcyanoacrylate nanoparticles is related to toxicity. Pharm Res. 1999;16:1836–42.
96. Kreuter J, Ramge P, Petrov V, et al. Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs of the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles. Pharm Res. 2003;20:409–16.
97. Koziara JM, Lockman PR, Allen DD, et al. The blood brain barrier and brain drug delivery. J Nanosci Nanotechnol. 2006;6:2712–35.
98. Kreuter J. Nanoparticulate systems for brain delivery of drugs. Adv Drug Deliv Rev. 2001;47:65–81.
99. Michaelis K, Hoffmann MM, Dreis S, et al. Covalent linkage of apolipoprotein E to albumin nanoparticles strongly enhances drug transport into the brain. J Pharmacol Exp Ther. 2006;317:1246–53.
100. Girardin F. Membrane transporter proteins: a challenge for CNS drug development. Dialogues Clin Neurosci. 2006;8:311–21.

101. Alyautdin RN, Petrov VE, Langer K, et al. Delivery of loperamide across the blood-brain barrier with polysorbate 80-coated polybutylcyanoacrylate nanoparticles. J Pharm Res. 1997;14:325–8.
102. Koziara JM, Lockman PR, Allen DD, et al. Paclitaxel nanoparticles for the potential treatment of brain tumors. J Control Release. 2004;99:259–69.
103. Oberdörster G, Sharp Z, Atudorei V, et al. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol. 2004;16:437–45.
104. Elder A, Gelein R, Silva V, et al. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect. 2006;114:1172–8.
105. Gao X, Tao W, Lu W, et al. Lectin-conjugated PEG-PLA nanoparticles: preparation and brain delivery after intranasal administration. Biomaterials. 2006;27:3482–90.
106. Court E, Daar AS, Martin E, Acharya T, Singer PA. Will Prince Charles et al. diminish the opportunities of developing countries in nanotechnology? 2004.
107. U.S., Indian high technology will benefit through cooperation, 2003. Available: http://newdelhi.usembassy.gov/wwwhpr0812a.htm.
108. China's nanotechnology patent applications rank third in world, 2003. http://www.investorideas.com/Companies/Nanotechnology/Articles/China'sNanotechnology1003,03.asp.
109. Meridian Institute Report of the international dialogue on responsible research and development of nanotechnology, 2004.
110. Salamanca-Buentello F, Persad DL, Court EB, Martin DK, Daar AS, Singer PA. Nanotechnology and the developing world. PLoS Med. 2005;2(5):e97. doi:10.1371/journal.pmed.0020097.
111. Rashidi L, Khosravi-Darani K. The applications of nanotechnology in food industry. Crit Rev Food Sci Nutr. 2011;51(8):723–30. doi:10.1080/10408391003785417.
112. Silva GA. Introduction to nanotechnology and its applications to medicine. Surg Neurol. 2004;61(3):216–20.
113. Singhal S, Nie S, Wang MD. Nanotechnology applications in surgical oncology. Annu Rev Med. 2010;61:359–73.
114. Saini R, Saini S, Sharma S. Nanotechnology: the future medicine. J Cutan Aesthet Surg. 2010;3(1):32–3.
115. Lee J, Mahendra S, Alvarez PJ. Nanomaterials in the construction industry: a review of their applications and environmental health and safety considerations. ACS Nano. 2010;4(7):3580–90.
116. Oberdörster G, Maynard A, Donaldson K, et al. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol. 2005;2:8.
117. Borm PJ. Particle toxicology: from coal mining to nanotechnology. Inhal Toxicol. 2002;14:311–24.
118. Kreyling WG, Semmler M, Möller W. Dosimetry and toxicology of ultrafine particles. J Aerosol Med. 2004;17:140–52.
119. Felicea B, Prabhakaranc MP, Rodrígueza AP, Ramakrishnac S. Drug delivery vehicles on a nano-engineering perspective. Mater Sci Eng C. 2014;41:178–95.
120. Allen C, Maysinger D, Eisenberg A. Nano-engineering block copolymer aggregates for drug delivery. Colloids Surf B Biointerfaces. 1999;16:1–4. 3–27.
121. Kalhapure RS, Suleman N, Mocktar C, Seedat N, Govender T. Nanoengineered drug delivery systems for enhancing antibiotic therapy. J Pharm Sci. 2015;104(3):872–905.
122. Lakkakula J, Krause RWM. Cyclodextrin-based nanoengineered drug delivery system. In: Mishra AK, editor. Nanomedicine for drug delivery and therapeutics. Hoboken, NJ: Wiley; 2013.
123. Barratt GM. Therapeutic applications of colloidal drug carrier. Pharm Sci Technol Today. 2000;3:163–71.

124. Ghosh S. Recent research and development in synthetic polymer-based drug delivery systems. J Chem Res. 2004;4:241–6.
125. Reis CP, Neufeld RJ, Ribeiro ANJ, Veiga F. Nanoencapsulation II. Biomedical applications and current status of peptide and protein nanoparticulate delivery systems. Nanomed Nanotechnol Biol Med. 2006;2(2):53–65.
126. Alvarez-Roman R, Naik A, Kalia YN, Guy RH, Fessi H. Enhancement of topical delivery from biodegradable nanoparticles. Pharm Res. 2004;21(10):1818–25.
127. Nafee N, Schneider M, Schaefer UF, Lehr CM. Relevance of the colloidal stability of chitosan/PLGA nanoparticles on their cytotoxicity profile. Int J Pharm. 2009;381(2):130–9.
128. Reis ACBP. Encapsulação de Fármacos Peptídicos Pelo Método de Emulsificação/Gelificação Interna. Ph.D. Thesis, Faculdade Farmácia Universidade de Coimbra, 2007.
129. Lim TY, Poh CK, Wang W. Poly(lactic-co-glycolic acid) as a controlled release delivery device. J Mater Sci Mater Med. 2009;20(8):1669–75. doi:10.1007/s10856-009-3727-z.
130. Park JK, Yeom J, Oh EJ, Reddy M, Kim JY. Guided bone regeneration by poly(lactic-co-glycolic acid) grafted hyaluronic acid bi-layer films for perio-dontal barrier applications. Acta Biomater. 2009;5(9):3394–403.
131. Vij N, Min T, Marasigan R, Belcher CN, Mazur S. Development of PEGylated PLGA nanoparticle for controlled and sustained drug delivery in cystic fibrosis. J Nanobiotechnol. 2010;8:22.
132. Csaba N, Sanchez A, Alonso MJ. PLGA: Polox-amer and PLGA: poloxamine blend nanostructures as carriers for nasal gene delivery. J Control Release. 2006;113(2):164–72.
133. Little SR, Lynn DM, Ge Q, Anderson DG, Puram SV. Poly-beta amino ester-containing microparticles enhance the activity of nonviral genetic vaccines. Proc Natl Acad Sci U S A. 2004;101(26):9534–9.
134. Shenoy D, Little S, Langer R, Amiji M. Poly(ethylene oxide)-modified poly(beta-amino ester) nanoparticles as a pH-sensitive system for tumor-targeted delivery of hydrophobic drugs. In Vitro Eval Mol Pharm. 2005;2(5):357–66.

Chapter 2
Nanoparticles Types, Classification, Characterization, Fabrication Methods and Drug Delivery Applications

Abstract The most emerging branch in pharmaceutical sciences known as "*Pharmaceutical nanotechnology*" presents new tools, opportunities and scope, which are expected to have significant applications in disease diagnostics and therapeutics. Recently nano-pharamceuticals reveal enormous potential in drug delivery as carrier for spatial and temporal delivery of bioactive and diagnostics. Additionally it also provides smart materials for tissue engineering. This discipline is now well-established for drug delivery, diagnostics, prognostic and treatment of diseases through its nanoengineered tools. Some nanotech based products and delivery systems are already in market. Pharmaceutical nanotechnology comprised of nano-sized products which can be transformed in numerous ways to improve their characteristics. Drugs that are transformed in to nano range offer some unique features which can lead to prolonged circulation, improved drug localization, enhanced drug efficacy etc. Various pharmaceutical nanotechnology based systems which can be termed as nanopharmaceuticals like polymeric nanoparticles, magnetic nanoparticles, liposomes, carbon nanotubes, quantum dots, dendrimers, metallic nanoparticles, polymeric nanoparticles, etc. have brought about revolutionary changes in drug delivery as well as the total medical service system. With the aid of nanopharmaceuticals, Pharmaceutical nanotechnology could have a profound influence on disease prevention to provide better insights into the molecular basis of disease. However some recently found health risk evidences limits their utilization in pharmaceutical industry. Some concerning issues like safety, bioethical issues, toxicity hazards, physiological and pharmaceutical challenges get to be resolved by the scientists. Current researchers are still lacking sufficient data and guidelines regarding safe use of these nanotechnology based devices and materials. Therefore pharmaceutical nanotechnology is still in infancy. The present chapter summarizes the types of nanopharmaceuticals with the most important applications and nanoparticles associated health risk related information available till present.

Keywords Nanotechnology • Nanoparticles • Types • Applications • Fabrication

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2.1 Introduction

Delivering therapeutic compound to the desirable site is a major problem in treatment of many diseases. Conventional utilization of drugs is characterized by poor biodistribution, limited effectiveness, undesirable side effects, and lack of selectivity. Strategies like controlling drug delivery can potentially overcome these limitations by transporting drug to the place of action. Moreover drug delivery system provides protection against rapid degradation or clearance. It also enhances drug concentration in target tissues; therefore, lower doses of drug are required. Such type of therapy is required when there is a discrepancy between a dose or concentration of a drug and its therapeutic results or toxic effects. Targeting cell or specific tissue by the means individually designed carriers that are attached to drugs is a more reliable approach in drug delivery system. Such approach is known as cell or tissue specific targeting. Size reduction of targeted formulation and designing its pathways for suitable drug delivery system is a more fundamental and successful approach that forms the basis of nanotechnology. Recent advancement in nanotechnology has proven that nanoparticles acquire a great potential as drug carriers. Size reduction methods and technologies yields different types of nanostructures that exhibit unique physicochemical and biological properties. These methods make the nanostructures favorable material for biomedical applications and thus acquire the significance importance in pharmaceutical sciences. In addition these methods help in reducing toxicity, enhancing release, improving solubility and bioavailability and provide better formulation opportunities for drugs. Nanotechnology offers drugs in the nanometer size range which enhances the performance in a variety of dosage forms. Various advantages of nano sizing are mentioned below:

- Decreased fed/fasted variability
- Decreased patient-to-patient variability
- Enhanced solubility
- Increased oral bioavailability
- Increased rate of dissolution
- Increased surface area
- Less amount of dose required
- More rapid onset of therapeutic action

Nano word is originated from Latin word, which means dwarf. Ideal size range offered by nanotechnology refers to one thousand millionth of a particular unit thus nanometer is one thousand millionth of a meter (i.e. 1 nm = 10^{-9} m). The branch nanotechnology is the science that particularly deals with the processes that occur at molecular level and of nano length scale size. Nanotechnology is now become an allied science which is most commonly used in other fields of science like electronic, physics and engineering since many decades. Recent exploration of nanotechnology in biomedical and pharmaceutical science results in successful improvement of conventional means of drug delivery system. This multidisciplinary science also covers several applications in other disciplines such as biophysics, molecular biology, and

bioengineering. Nanotechnology has created potential impact in various fields like medicine including immunology, cardiology, endocrinology, ophthalmology, oncology, pulmology etc. In addition it's highly utilized in specialized areas like brain targeting, tumor targeting, and gene delivery. Nanotechnology also provides significant systems, devices and materials for better pharmaceutical applications.

Nanotechnology is the science of material featuring between 10^{-9} and 10^{-7} of a meter [1]. Or in another words it's the science of materials and devices whose structures and constituents demonstrate novel and considerably altered physical, chemical and biological phenomenon due to their nanoscale size. Thus nanotechnology is defined as the manipulation of matter on an atomic, molecular, and supramolecular scale involving the design, production, characterization and application of different nanoscale materials in different potential areas providing novel technological advances mainly in the field of medicine. This forms an independent branch of nanostructures, referred as nanomedicine which is specifically utilized for medicines. Nanomedicine involves utilization of nanotechnology for the benefit of human health and well being. Nanomedicine was defined by European Science Foundation as 'the science and technology of diagnosing, treating and preventing disease and traumatic injury, of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body [1]. This definition was revised by the US NIH as: 'Nanomedicine refers to highly specific medical intervention at the molecular scale for curing diseases or repairing damaged tissues, such as bone, muscle, or nerve'. The European Science Foundation specified five sub-disciplines of nanomedicines [1]:

- Analytical tools
- Nanoimaging tools
- Nanomaterials and nanodevices
- Clinical and toxicological issues
- Novel therapeutics and drug delivery systems

These specified disciplines are overlapping which in many ways. The use of nanotechnology in various sectors of therapeutics has revolutionized the field of medicine where nanoparticles are designed and used for therapeutics, diagnostics, and as biomedical tools for research. With the help of nanotechnology it's now possible to provide therapy at a molecular level which may further help in treating and pathogenesis of disease. Major limitations of conventional drugs (such as non specificity of drug action) urgently requires the developed system of nanomaterials which can be easily used in the diagnosis and treatment of various diseases especially cancer (have major limitations such as poor sensitivity or specificity and drug toxicities). Recently various novel and advance methods of cancer detection based on nanoparticles are being developed. These designed nanostructures are used as fluorescent materials, contrast agents, drugs with targeting antibodies and for molecular research tools. Recent modifications of nanoparticulate systems such as paramagnetic nanoparticles, quantum dots, nanoshells and nanosomes are widely used for diagnostic purposes. Nanotechnology provides the better safety profile against drugs with high toxic potential and these nanoforms can be directed to act specifi-

cally at the target tissue by active as well as passive means. In addition other modalities of therapy such as heat induced ablation of cancer cells by nanoshells and gene therapy are also being developed. Optimization of nanoparticles based drug delivery approaches concerns the early detection of cancer cells and/or specific tumor biomarkers, and the enhancement of the efficacy of the treatments applied. Prominent applications of nanomaterials in biomedical sciences are demonstrated in Figs. 2.1 and 2.2. Potential of nanomedicines in cancer is dependent on passive targeting (due to the enhance of the permeability and retention effect promoted by angiogenic vessels) which can be reinforced by specific targeting (based on multifunctional nanomaterials that bypass the biological barriers and reach cancer cells). Nanoparticles based specific drug targeting and delivery platforms reduce toxicity and other side effects and also improve the therapeutic index of the targeted drug. In the primary objective of nanotechnology especially in cancer therapy is the development of suitable targeting delivery systems which has been taking the lead in what concerns overcoming the MDR problem. Such targeted delivery systems that are based 'Nanosizing' of drugs:

- Decrease drug resistance
- Decrease toxicity [2]
- Enhance oral bioavailability [3]
- Enhance rate of dissolution
- Enhance solubility [4]
- Increase the stability of drug and formulation [5]
- Increase drug targeting ability [6–8]
- Increase patient compliance [5]
- Increase surface area
- Reduce the dose needed [9]

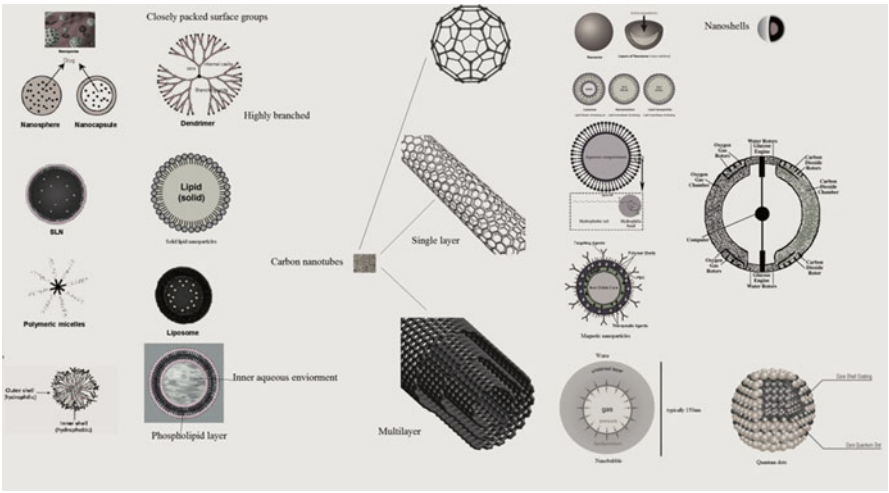

Fig. 2.1 Various nanoforms and their morphological features

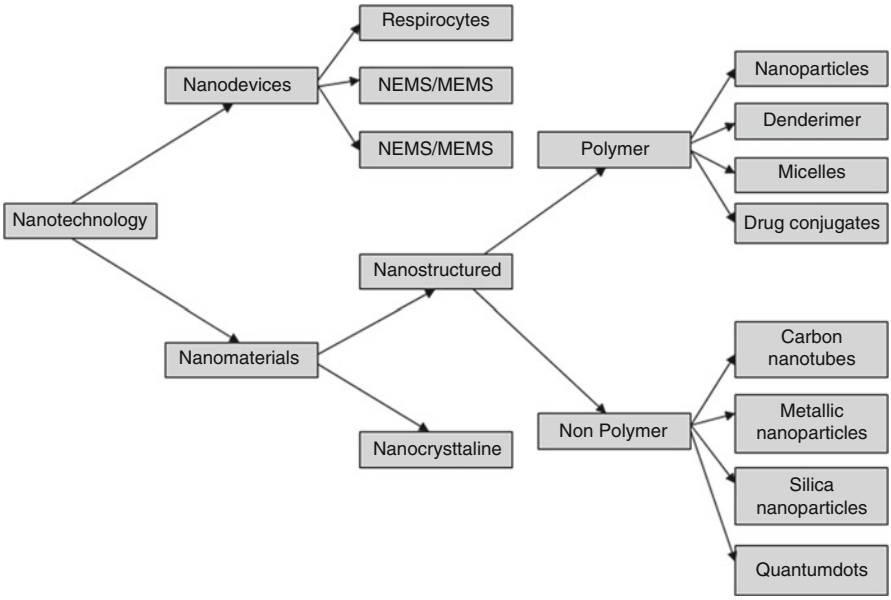

Fig. 2.2 Illustrations demonstrating various types of pharmaceutical nanosystems

Such advantages lead to the development of most efficient targeted therapeutic nanoparticle which is the potential to revolutionize the drug development process and change the landscape of the pharmaceutical industry [10, 11]. Considering the unique physicochemical properties, nanoparticles have shown promise in delivering a range of molecules to desired sites in the body. These targeted nanomedicines may improve the therapeutic index of drugs by enhancing their efficacy and/or increasing their tolerability in the body. Nanotechnology is also efficient in improving the bioavailability of water-insoluble drugs, protect the therapeutic agents from physiological barriers, enable the development of novel classes of bioactive macromolecules as well as carry large payloads. In addition integration of imaging contrast agents within nanoparticles can allows the drug delivery site visible to us and examination of *in vivo* efficacy of the therapeutic agent [12]. So far various nanotechnology products have been approved by the US Food and Drug Administration (FDA) for clinical use, and many are under clinic and preclinic development [13]. Among these clinically approved products the first-generation nanotechnology products are liposomal drugs and polymer–drug conjugates, which are relatively simple and generally lack active targeting or controlled drug release components. While designing therapeutic nanoparticles the ultimate goal of nanotechnology is to develop safer and more effective therapeutic nanoparticles. Therefore the main focus of current researchers is to design novel multifunctional nanoparticle platforms for cell/tissue-specific targeting, sustained or triggered drug delivery, co-delivery of synergistic drug combinations, etc.

This chapter discuss the various platforms of nanotechnology that are being used in different aspects of medicine with special focus on targeted drug delivery systems and novel therapeutics based on nanotechnology. Here, we have also discussed the recent applications on nanoparticles as platforms for anticancer therapy, emphasizing strategies for targeted delivery for gene silencing focusing on the optimal pathways to test these therapeutics in vitro and in vivo. It's very essential to report toxicological aspects of these nano materials. Therefore potential toxicities of the nanoparticles are also described in addition to the safety of nanomedicine is not fully defined yet. However, it is possible that nanomedicine in future would play a crucial role in the treatment of human diseases and also in enhancement of normal human physiology.

One of the emerging branches among biomedical sciences is pharmaceutical technology. Pharmaceutical nanotechnology covers the applications of nanotechnology to pharmacy as nanomaterials, and as devices like drug delivery, diagnostic, imaging and biosensor. Pharmaceutical nanotechnology has provided more fine-tuned diagnosis and focused treatment of disease at a molecular level. Pharmaceutical nanotechnology offers various opportunities to fight against many diseases. It helps in detecting the antigen associated with diseases such as cancer, diabetes mellitus, neurodegenerative diseases, as well as detecting the microorganisms and viruses associated with infections. It is expected that in next 10 years market will be flooded with nanotechnology devised medicine. Applications of nanotechnology to pharmacy that provide intelligent and smart drug delivery systems is expected to emerge as most important and powerful tool as alternate to conventional dosage form. These nano-intelligent drug delivery systems need little investment while expected to be a high profit making deal due to new patent protection for current or soon-to-be off-patent drugs. A recent report claimed that 23 major pharmaceutical patents would expire by 2008 leading to revenue loss of US $ 46 billion and by 2011, US $ 70–80 billion loss is expected as various drugs go off-patent. Pharmaceutical Nanotechnology based systems presents two basic types of nano tools viz. nanomaterials and nanodevices, which play a key role in realm of pharmaceutical nanotechnology and related fields. Nanomaterials are biomaterials used, for example, in orthopedic or dental implants or as scaffolds for tissue-engineered products. Their surface modifications or coatings might greatly enhance the biocompatibility by favoring the interaction of living cells with the biomaterial. These materials can be sub classified into nanocrystalline and nanostructured materials. Nano crystalline materials are readily manufactured and can substitute the less performing bulk materials. Raw nanomaterials can be used in drug encapsulation, bone replacements, prostheses, and implants. Nanostructured materials are processed forms of raw nanomaterials that provide special shapes or functionality, for example quantum dots, dendrimers, fullerenes and carbon nanotubes. Nanodevices are miniature devices in the nanoscale and some of which include nano- and microelectromechanical systems, microfluidics, and microarrays. Examples include biosensors and detectors to detect trace quantities of bacteria, airborne pathogens, biological hazards, and disease signatures and some intelligent machines like respirocyte (Figs. 2.1 and 2.2). Various prominent features and applications of nanosystems are mentioned Table 2.1.

Table 2.1 Various characteristics and brief applications of nanosystems [14]

Types of Nanosystems	Size (nm)	Characteristics	Applications
Carbon nanotubes	0.5–3 diameter and 20–1000 length	Third allotropic crystalline form of carbon sheets either single layer (single walled nanotube, SWNT) or multiple layer (multi-walled nanotube, MWNT). These crystals have remarkable strength and unique electrical properties (conducting, semi conducting, or insulating)	Functionalization enhanced solubility, penetration to cell cytoplasm and to nucleus, as carrier for gene delivery, peptide delivery
Dendrimer	<10	Highly branched, nearly monodisperse polymer system produced by controlled polymerization; three main parts core, branch and surface	Long circulatory, controlled delivery of bioactives, targeted delivery of bioactives to macrophages, liver targeting
Liposome	50–100	Phospholipid vesicles, biocompatible, versatile, good entrapment efficiency, offer easy	Long circulatory, offer passive and active delivery of gene, protein, peptide and various other
Metallic nanoparticles	<100	Gold and silver colloids, very small size resulting in high surface area available for functionalization, stable	Drug and gene delivery, highly sensitive diagnostic assays, thermal ablation and radiotherapy enhancement
Nanocrystals Quantum dots	2–9.5	Semi conducting material synthesized with II-VI and III-V column element; Size between 10 and 100 Å; Bright fluorescence, narrow emission, Broad UV excitation and high photo stability	Long term multiple color imaging of liver cell; DNA hybridization, immunoassay; receptor mediated endocytosis; labeling of breast cancer marker HeR_2 surface of cancer cells
Polymeric micelles	10–100 nm	Block amphiphilic copolymer micelles, high drug entrapment, payload, biostability	Long circulatory, target specific active and passive drug delivery, diagnostic value
Polymeric nanoparticles	10–1000	Biodegradable, biocompatible, offer complete drug protection	Excellent carrier for controlled and sustained delivery of drugs. Stealth and surface modified nanoparticles can be used for active and passive delivery of bioactives

2.2 Classification of Nanoparticles

Nanoparticles are broadly classified in to three classifications [15]

- **One dimension nanoparticles**
  One dimensional system (thin film or manufactured surfaces) has been used for decades. Thin films (sizes 1–100 nm) or monolayer is now common place in the field of solar cells offering, different technological applications, such as chemical and biological sensors, information storage systems, magneto-optic and optical device, fiber-optic systems.
- **Two dimension nanoparticles**
  Carbon nanotubes
- **Three dimension nanoparticles**
  Dendrimers, Quantum Dots, Fullerenes (Carbon 60), (QDs)

2.3 Characterization of Nanoparticles

Characterization of nanoparticles is based on the size, morphology and surface charge, using such advanced microscopic techniques as atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Properties such as the size distribution, average particle diameter, charge affect the physical stability and the in vivo distribution of the nanoparticles. Properties like surface morphology, size and overall shape are determined by electron microscopy techniques. Features like physical stability and redispersibility of the polymer dispersion as well as their *in vivo* performance are affected by the surface charge of the nanoparticles. Different characterization tools and methods for nanoparticles are mentioned in Table 2.2. Therefore it's very important to evaluate the surface charge during characterization of nanoparticles.

2.3.1 Particle Size

Characterizations of nanoparticles are primarily evaluated by the particle size distribution and morphology. With the aid of electron microscopy it's now possible to ascertain the morphology as well as the size of nanoparticles. Application of nanoparticles in drug release and drug targeting can be conveniently determined by various tools. It has already been reported that particle size of nanoparticles has profound effect on the drug release.

Table 2.2 Various characterization tools and methods for nanoparticles [16]

Parameter	Characterization method
Carrier-drug interaction	Diffential scanning calorimetry
Charge determination	Laser Doppler Anemometry
	Zeta potentiometer
Chemical analysis of surface	Static secondary ion mass spectrometry
	Sorptometer
Drug stability	Bioassay of drug extacted from Nanoparticles
	Chemical analysis of drug
Nanoparticle dispersion stability	Critical flocculation temperature (CFT)
Particle size and distribution	Atomic force microscopy
	Laser defractometry
	Photon correlation spectroscopy (PCS)
	Scanning electron microscopy
	Transmission electron microscopy
Release profile	In vitro release characteristics under physiologic and sink conditions
Surface hydrophobicity	Rose Bengal(dye) binding
	Water contact angle measurement
	X-ray photoelectron spectroscopy

Smaller the size of nanoparticles larger surface area, which results in to fast drug release. Loaded drug when exposed to the particle surface area causes significant drug release. In contrast, inside the nanoparticles drugs slow diffusion of larger particles occurs. Consequently smaller particles tend to aggregate during storage and transportation of nanoparticle dispersion. Therefore there is a mutual compromise between maximum stability and small size of nanoparticles [17]. In addition degradation of the polymer can also be affected by the particle size e.g. the extent of poly (lactic-co-glycolic acid) degradation was found to increase with increasing particle size *in vitro* [18]. With the advancement in analytical tools various techniques are now available for determining nanoparticle size as discussed below:

2.3.1.1 Photon-Correlation Spectroscopy (PCS) or Dynamic Light Scattering (DLS)

Current research demands the fastest and most popular method of determining particle size. The fastest and most popular techniques like photon-correlation spectroscopy (PCS) or dynamic light scattering (DLS), widely used to determine the size of Brownian nanoparticles in colloidal suspensions in the nano and submicron ranges. In this technique solution of spherical particles in Brownian motion causes a Doppler shift when they are exposed against shining monochromatic light (laser). Such monochromatic light exposure hits the moving particle which results in

changing the wavelength of the incoming light. Extent of this change in wavelength determines the size of the particle. This parameter assists in evaluation of the size distribution, particle's motion in the medium, which may further assists in measuring the diffusion coefficient of the particle and using the autocorrelation function. Dynamic light scattering (DLS) offer the most frequently used technique for accurate estimation of the particle size and size distribution [19].

2.3.1.2 Scanning Electron Microscopy (SEM)

This electron microscopy based technique determines the size, shape and surface morphology with direct visualization of the nanoparticles. Therefore scanning electron microscopy offer several advantages in morphological and sizing analysis. However they provide limited information about the size distribution and true population average. During the process of SEM characterization, solution of nanoparticles should be initially converted into a dry powder. This dry powder is then further mounted on a sample holder followed by coating with a conductive metal (e.g. gold) using a sputter coater. Whole sample is then analyzed by scanning with a focused fine beam of electrons [20]. Secondary electrons emitted from the sample surface determine the surface characteristics of the sample. This electron beam can often damage the polymer of the nanoparticles which must be able to withstand vacuum. Average mean size evaluated by SEM is comparable with results obtained by dynamic light scattering. In addition these techniques are time consuming, costly and frequently need complementary information about sizing distribution [21].

2.3.1.3 Transmission Electron Microscope

Experimental difficulties in studying nanostructures stem from their small size, which limits the use of traditional techniques for measuring their physical properties. Transmission electron microscopy techniques can provide imaging, diffraction and spectroscopic information, either simultaneously or in a serial manner, of the specimen with an atomic or a sub-nanometer spatial resolution. TEM operates on different principle than SEM, yet it often brings same type of data. The sample preparation for TEM is complex and time consuming because of its requirement to be ultra thin for the electron transmittance. High-resolution TEM imaging, when combined with nanodiffraction, atomic resolution electron energy-loss spectroscopy and nanometer resolution X-ray energy dispersive spectroscopy techniques, is critical to the fundamental studies of importance to nanoscience and nanotechnology. During the TEM characterization nanoparticles dispersion is deposited onto support grids or films. After dispersion they are fixed using either a negative staining material (phosphotungstic acid or derivatives, uranyl acetate, etc., or by plastic embedding). This is done to make nanoparticles withstand against the instrument vacuum and facilitate handling. Alternatively nanonoparticles sample can also be exposing to liquid nitrogen temperatures after embedding in vitreous ice. When a

beam of electrons is transmitted through an ultra thin sample it interacts with the sample as it passes through The surface characteristics of the sample are obtained [21]. TEM imaging mode has certain benefits compared with the broad-beam illumination mode:

- Collection of the information about the specimen using a high angular annular dark field (HAADF) detector (in which the images registered have different levels of contrast related to the chemical composition of the sample)
- It can be utilized for the analysis of biological samples is its contrast for thick stained sections, since high angular annular dark field images (samples with thickness of 100–120 nm) have better contrast than those obtained by other techniques.
- Combining HAADF-TEM imaging leads to imaging the atomistic structure and composition of nanostructures at a sub-angstrom resolution.
- Availability of sub-nanometer or sub-angstrom electron probes in a TEM instrument, due to the use of a field emission gun and aberration correctors, ensures the greatest capabilities for studies of sizes, shapes, defects, crystal and surface structures, and compositions and electronic states of nanometer-size regions of thin films, nanoparticles and nanoparticle systems.

2.3.1.4 Atomic Force Microscopy

This technique is also known as scanning force microscopy (technique that forms images of surfaces using a prob that scans the specimen), very high resolution type of scanning probe microscopy, with reported resolution on the order of fractions of a nanomerter, more than 100 times better than the optical diffraction limit. The atomic force microscopy is based on a physical scanning of samples at sub-micron level using a probe tip of atomic scale and offers ultra-high resolution in particle size measurement [22]. Depending upon properties, samples are usually scanned in contact or noncontact mode. During contact mode, the topographical map is generated by tapping the probe on to the surface across the sample and probe hovers over the conducting surface in non-contact mode. One of the prime advantage of AFM is its ability to image non-conducting samples without any specific treatment. This feature allows the imaging of delicate biological and polymeric nano and microstructures [23]. Moreover AFM (without any mathematical calculation) provides the most accurate description of size, size distribution and real picture which helps in understanding the effect of various biological conditions [24].

2.3.2 Surface Charge

Surface charge and intensity determines the interaction of nanoparticles with the biological environment as well as their electrostatic interaction with bioactive compounds. Stability of colloidal material is usually analyzed through zeta potential of

nanoparticles. Zeta potential is an indirect measure of the surface charge. It can be obtained by evaluating the potential difference between the outer Helmholtz plane and the surface of shear. Thus zeta potential of colloidal based dispersion assists in directly evaluating its storage stability. Zeta potential values (high zeta potential values, either positive or negative) are achieved in order to ensure stability and avoid aggregation of the particles. Zeta potential values can be utilized in evaluating surface hydrophobicity and the nature of material encapsulated within the nanocapsules or coated onto the surface [25].

2.3.3 Surface Hydrophobicity

Techniques such as hydrophobic interaction chromatography, biphasic partitioning, adsorption of probes, contact angle measurements etc. can be utilized for the determination of surface hydrophobicity. Recent advancement in research offers several sophisticated analytical tools for surface property analysis of nanoparticles. modern technique such as X-ray photon correlation spectroscopy not only determine surface hydrophobicity but also permits the identification of specific chemical groups on the surface of nanoparticles [26].

2.3.4 Drug Release

It's very essential to determine extent of the drug release and in order to obtain such information most release methods require that the drug and its delivery vehicle be separated. drug loading capacity of the nanoparticles is defined as the amount of drug bound per mass of polymer or in another term it is the moles of drug per mg polymer or mg drug per mg polymer or it could also be given as percentage relative to the polymer. Various techniques such as UV spectroscopy or high performance liquid chromatography (HPLC) after ultracentrifugation, ultra filtration, gel filtration, or centrifugal ultrafiltration are used to determine this parameter. Methods that are employed for drug release analysis are also similar to drug loading assay which is more often assessed for a period of time to evaluate the drug release mechanism [27, 28].

2.4 Preparation of Nanoparticles

The selection of appropriate method for the preparation of nanoparticles depends on the physicochemical character of the polymer and the drug to be loaded. Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including [29]:

- Antigenicity of the final product.
- Biocompatibility and toxicity

- Degree of biodegradability
- Drug release profile desired
- Inherent properties of the drug (aqueous solubility and stability)
- Size of nanoparticles required
- Surface characteristics (charge and permeability)

Nanoparticles have been usually prepared by three methods:

- Dispersion of preformed polymers
- Ionic gelation or coacervation of hydrophilic polymers
- Polymerization of monomers

However, other methods such as supercritical fluid technology [30] and particle replication in non-wetting templates [31] have also been described in the literature for production of nanoparticles. The latter was claimed to have absolute control of particle size, shape and composition, which could set an example for the future mass production of nanoparticles in industry.

Dispersion of preformed polymers: This technique is based on the preparation of biodregerable nanoparticles via dispersion of biodegradable polymers such as poly (D, L-glycolide), poly (lactic acid) (PLA); poly (cyanoacrylate) (PCA), and PLG; poly (D, L-lactide-co-glycolide) (PLGA) [32–34]. Dispersion of preformed polymers to prepare the nanoparticles can be used in various ways:

2.4.1 Solvent Evaporation Method

Solvent evaporation method is one of the most frequently used methods for the preparation of nanoparticles. This method involves two steps (first is emulsification of the polymer solution into an aqueous phase and second is evaporation of polymer solvent, inducing polymer precipitation as nanospheres). This method is based on the solubility of polymer and hydrophobic drug since both polymer and hydrophobic drug are dissolved in an organic solvent (dichloromethane, chloroform or ethyl acetate) which is also used as the solvent for dissolving the. Mixture obtained from polymer and drug solution is then emulsified in an aqueous solution. This aqueous solution contains surfactant or emulsifying agent to form oil in water (o/w) emulsion. Once the stable emulsion forms, the organic solvent is evaporated either by continuous stirring or by reducing the pressure. Size range of nanoparticles was found to be influenced by the concentrations and type of stabilizer, polymer concentration and homogenizer speed [35]. Ultrasonication or high-speed homogenization may be often employed in order to produce small particle size [36]. The nano particles are collected by ultracentrifugation and washed with distilled water to remove stabilizer residue or any free drug and lyophilized for storage [37]. Modification of this method is known as solvent evaporation method and high pressure emulsification [38]. This method involves preparation of a emulsion which is then subjected to homogenization under high pressure followed by overall stirring to remove organic solvent [39]. The size can be controlled by adjusting the stirring rate, type and amount of dispersing agent, viscosity of organic and aqueous phases and

temperature [40]. However this method can be applied to liposoluble drugs and limitation are imposed by the scale up issue. Polymers used in this method are PLGA [41], PLA [42], cellulose acetate phthalate [43], EC [44], Poly (β -hydroxy-butyrate) (PHB) [45], Poly (β-caprolactone)\ (PCL) [46].

2.4.2 Spontaneous Emulsification or Solvent Diffusion Method

This method is developed from solvent evaporation method [47], in which the water miscible solvent along with a small amount of the organic solvent (water immiscible) is used as an oil phase. During the spontaneous diffusion of solvents between the two phases an interfacial turbulence is generated which may ultimately leads to the formation of small particles. Smaller particle size can be achieved by increasing the concentration of water miscible solvent increases. This method can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase.

2.4.3 Double Emulsion and Evaporation Method

Most of the emulsion and evaporation based methods suffer from the limitation of poor entrapment of hydrophilic drugs. Therefore to encapsulate hydrophilic drug the double emulsion technique is employed, which involves the addition of aqueous drug solutions to organic polymer solution under vigorous stirring to form w/o emulsions. This w/o emulsion is added into second aqueous phase with continuous stirring to form the w/o/w emulsion. The emulsion then subjected to solvent removal by evaporation and nano particles can be isolated by centrifugation at high speed. The formed nanoparticles must be thoroughly washed before lyophilisation [48]. In this method the amount of hydrophilic drug to be incorporated, the concentration of stabilizer used, the polymer concentration, the volume of aqueous phase are the variables that affect the characterization of nanoparticles [48, 49].

2.4.4 Salting Out Method

Method involves the separation of a water-miscible solvent from aqueous solution via a salting-out effect [50]. It's based on the on the separation of a water miscible solvent from aqueous solution via a salting-out effect. During the initial process polymer and drug are dissolved in a solvent which is subsequently emulsified into an aqueous gel containing the salting out agent and a colloidal stabilizer. Various types of salting out agents (electrolytes, such as magnesium chloride and calcium chloride, or non- electrolytes such as sucrose) and colloidal stabilizer (such as

polyvinylpyrrolidone or hydroxyethylcellulose) have been used. This lead to formation of oil/water emulsion which is further diluted with a sufficient volume of water or aqueous solution to enhance the diffusion of solvent into the aqueous phase, ultimately induce the formation of nanospheres. Parameters such as stirring rate, internal/external phase ratio, concentration of polymers in the organic phase, type of electrolyte concentration and type of stabilizer in the aqueous phase can be varied in this process [43]. Salting out method is reported for preparation of ethyl cellulose and PLA, Poly(methacrylic) acids nanospheres. leads to high efficiency and is easily scaled up [51, 52].

2.4.4.1 Advantages

- Does not require an increase of temperature and therefore may be useful when heat sensitive substances have to be processed [53].

2.4.4.2 Disadvantages

- Limited application to lipophilic drug and the extensive nanoparticles washing steps

2.4.5 Emulsions-Diffusion Method

This is another widely used method to prepare nanoparticles. The encapsulating polymer is dissolved in a partially water-miscible solvent (such as propylene carbonate, benzyl alcohol), and saturated with water to ensure the initial thermodynamic equilibrium of both liquids. Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of nanospheres or nanocapsules, according to the oil-to-polymer ratio. Finally, the solvent is eliminated by evaporation or filtration, according to its boiling point. This technique presents several advantages, such as high encapsulation efficiencies (generally 70 %), no need for homogenization, high batch-to-batch reproducibility, ease of scaleup, simplicity, and narrow size distribution.

2.4.5.1 Disadvantages

Disadvantages are the high volumes of water to be eliminated from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase during emulsification, reducing encapsulation efficiency [54]. Several drug-loaded nanoparticles were produced by the technique, including mesotetra

(hydroxyphenyl) porphyrin-loaded PLGA (p-THPP) nanoparticles [55], doxorubicin-loaded PLGA nano particles, and cyclosporine (cy-A-); loaded sodium glycolate nanoparticles [56].

2.4.6 Solvent Displacement/Precipitation Method

In this method preformed polymer is precipitated in an organic solution and organic solvent is diffused in the aqueous medium. Diffusion of organic solvent can be achieved in the presence or absence of surfactant. Semi polar water miscible solvent such as acetone or ethanol can be used to dissolve the polymers, drug, and or lipophilic surfactant. After their complete dissolution, solution is then poured or injected into an aqueous solution containing stabilizer under magnetic stirring. Nano particles are formed immediately by the rapid solvent diffusion. This step is followed by the removal of solvent from the suspensions under reduced pressure. Particles size is dependent on the extent of addition of the organic phase into the aqueous phase. It was also found that decrease in both particles size and drug entrapment occurs as the mixing rate of the two phase increases [57]. This method is more suitable for poorly soluble drugs. Optimization of various parameters (preparation parameters) can effectively control size, drug release and yield of nanosphere.

Nanosphere size, drug release and yield were shown to be effectively controlled by adjusting preparation parameters. Regulation the concentration of polymer in the organic phase was reported to be useful in the production of smaller sized nanospheres. However size range is restricted to minimum range of the polymer to drug ratio.

2.4.7 Coacervation or Ionic Gelation Method

Recent exploration of biodegradable polymers such as gelatin and sodium alginate has been focused now to yield biodegradable nanoparticles having features like biocompatibility and low toxicity. Methods such as ionic gelation can be used for preparing hydrophilic polymer based nanoparticles. Calvo and co-workers developed method for preparing chitosan based nanoparticles by ionic gelation method [58, 59]. In this method two different aqueous phases are prepared for polymer [chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO)] and the other is for polyanion sodium tripolyphosphate. This method is based on the strong electrostatic interaction between positively charged amino group of chitosan and negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Existence of strong electrostatic interaction between two aqueous phases leads to the formation of coacervates. In contrast ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature.

2.4.8 Polymerization Method

This method involves polymerization of monomers to form nanoparticles in an aqueous solution. In polymerization drug is incorporated at two different stages (either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed) [60, 61]. Ultracentrifugation can be used to purify nanoparticle suspension by removing various stabilizers and surfactants employed for polymerization, followed by the re-suspension of particles in an isotonic surfactant-free medium. This technique is reported for making poly (alkylcyanoacrylate) or polybutylcyanoacrylate nanoparticles. Desirable size of nanocapsule can be achieved by optimization of concentration of the surfactants and stabilizers [62].

2.4.9 Production of Nanoparticles Using Supercritical Fluid Technology

Above mentioned conventional methods (such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods) obligatory use organic solvents which are hazardous to the environment as well as to physiological systems. Therefore, there is an urgent requirement of suitable technology which avoid the usage of organic solvents or any other ingredient hazardous to health. Since supercritical fluids are environmentally safe, therefore, the supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro- and nanoparticles [63]. Supercritical fluid technology technique, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive. Supercritical fluids are those fluids which are at a temperature above its critical temperature remains in a single phase regardless of pressure [63]. CO_2 (SC CO_2) is the most widely used supercritical fluid because of its mild critical conditions, non-flammability, low price and nontoxicity. Among the various processing techniques involving supercritical fluids, supercritical anti-solvent (SAS) and rapid expansion of critical solution (RESS) are the most common one. In former process a liquid solvent (methanol) is selected on the basis of it's completely miscibility with the supercritical fluid (SC CO_2). This is done to dissolve the solute to be micronized at the process conditions. Since the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, results in the formation of nanoparticles. This process is reported for formation of hydrophilic drug dexamethasone phosphate drug nanoparticles for microencapsulation purpose. In later process called as RESS, solute is dissolved in a supercritical fluid such as supercritical methanol and then the solution is rapidly expanded through a small nozzle into a region lower pressure [63]. This dramatically affects the solvent power of supercritical fluids which is ultimately decreases and the solute eventually precipitates. RESS and its modified process have been used for the product of polymeric nanoparticles [64].

2.5 Most Favorable Requirements for Designing Therapeutic Nanoparticles

One of the most primary requirements before designing therapeutic nanoparticles is the rapid clearance during systemic delivery. After entering the blood stream nanoparticles surface may experience nonspecific protein adsorption called as opsonization. This process makes them more visible to phagocytic cells. These opsonized nanoparticles could be easily cleared from the bloodstream through phagocytosis by the mononuclear phagocyte system (MPS) in the liver and by spleen filtration. Factors that govern the clearance and biodistribution of nanoparticles should be considered before designing therapeutic nanoparticles. nanoparticle size plays an important role in controlling circulation and biodistribution of nanoparticles during its journey through physiological parameters such as hepatic filtration, tissue extravasation/diffusion, and kidney excretion. Naoparticles size range <10 nm can be rapidly cleared by the kidneys or through extravasation, while larger particles size may have higher tendency to be cleared by cells of the mononuclear phagocyte system (MPS also referred to as reticuloendothelial system, RES). It was also found that PEGylated spherical nanoparticles (<100 nm, 100–200 nm, and >200 nm) showed different protein absorption rate since particle size influence its uptake by murine macrophages, and blood clearance kinetics. It was reported that nanoparticles <100 nm remain in the blood for long periods of time and experience reduced hepatic filtration. Additionally nanoparticle size also affects their accumulation rate in tumor or its surroundings. This accumulation is achieved through the EPR effect. Thus to take advantage of the EPR effect and to efficiently escape from the physiological barriers, many studies advocate the optimal nanoparticle size range of approximately 10–250 nm. Second factor that could affect nanoparticles uptake by the MPS cells is their surface charge. Positively charged nanoparticles generate a higher immune response compared to neutral or negatively charged nanoparticle formulations. Similarly neutrally charged particles have demonstrated much lower opsonization rates than charged particles. For reduced phagocytosis and minimized nonspecific, it has been demonstrated optimal range of nanoparticle is between −10 and +10 mV. Third factor, PEGylation, referred to the surface modification of nanoparticles with PEG, which has favorable intrinsic physicochemical properties which was found to reduce nanoparticle accumulation in off-target organs. A hydrophobic or charged particles PEG shell on the nanoparticle, leads to prolonged circulation half-life compared to non-PEGylated nanoparticles. Several factors such as length, shape, and density of PEG chains on the nanoparticle surface largely affect its surface hydrophilicity and phagocytosis. Brush configuration in PEGylated nanoparticles would create more effective blocking or repulsion of opsonins than the mushroom one. Fourth factor i.e. ligand functionalization is the conjugation of targeting ligands to the surface of PEGylated nanoparticles which has also been proven to affect their biodistribution. However targeting ligands could improve the cell- or tissue-specific delivery of nanoparticles. They may compromise the particle surface properties by masking the PEG layer and adversely affecting the nanoparticles' antibiofouling properties *in vivo*.

2.6 Types of Pharmaceutical Nanosystems

2.6.1 Carbon Based Structures

Carbon nano tubes are carbon based tubular structures that are discovered in 1991 [65]. These structures are arranged in fashion like a graphite sheet rolled up into a cylinder and capped at one or both ends by a buckyball. These are hexagonal networks of carbon atoms having diameter of one nanometer and length from 1 to 100 nm. These carbon networks are arranged layer of graphite rolled up into a cylinder. There are two carbon based configuration that have received much attention recently: single-walled nanotubes (SWNTs) and multiwalled nanotubes (MWNTs). In addition to these types C_{60} fullerenes is also a part of common configurations. These are hollow, carbon-based, cage-like architectures (nanotubes and fullerenes), also known as bucky balls, which are differ in the arrangement of their graphite cylinders. The size, geometry, and surface characteristics of these macromolecules make them appealing for drug carrier usage and have remarkable physical proper. SWNTs and C60 fullerenes have internal diameters range of 1–2 nm. This dimension is equivalent to about half the diameter of the average DNA helix, whereas MWNTs have diameters ranging from several nanometers to tens of nanometers with 0.36 nm distance between layers of MWCNT, depending on the number of walls in the structure. Size may vary in their length ranging from 1 μm to a few micrometers [66]. As far as their architecture is concerned fullerenes and carbon nanotubes are typically fabricated using laser ablation, chemical vapor deposition, electric arc discharge, or combustion processes. Characterization of these concentric forms is based on their strength and stability so that they can be used as stable drug carriers. Cellular entry of nanotubes may be mediated by endocytosis or by insertion through the cell membrane. Fullerenes have also shown drug targeting capability. Tissue-selective targeting and intracellular targeting of mitochondria have been shown with use of fullerene structures. Furthermore, experiments with fullerenes have also shown that they exhibit antioxidant and antimicrobial behavior. Carbon nanotubes and their applications are highlighted in Table 2.3. Some of well known examples of carbon nantubes and their respective applications are highlighted in Table 2.3.

Table 2.3 Carbon nanotubes and their applications [67]

Drug	Type of disease	Type of CNTs
	Breast cancer	MWCNTs
Amphotericin B	Leishmania donovani (parasite)	Not specified
Carboplatin	Bladder cancer	Not specified
Daunorubicin	Leukemia	SWCNTs
Doxorubicin	Lymphoma	SWCNTs
Gemcitabine	Ovarian cancer	SWCNTs
Methotrexate	Breast cancer	MWCNs
Paclitaxel	Breast cancer	SWCNTs

2.6.1.1 Applications

- **Cell specificity**
  Enhancement of cell specificity by conjugating antibodies to carbon nanotubes with fluorescent or radiolabelling [68]
- **Internalization**
  Internalization within mammalian cells can be achieved by surface-functionalized carbon nanotubes
- **Vaccine delivery**
  Conjugation with peptides may be used as vaccine delivery structures
- **Gene delivery**
  With the advancement in molecular dynamics simulations, the flow of water molecules through surface-functionalized carbon nanotubes has been modeled in such a way so that they can be conveniently utilized as small molecule transporters in transporting DNA, indicating potential use as a gene delivery tool. The ability of nanotubes to transport DNA across cell membrane is used in studies involving gene therapy. During this therapy DNA can be attached to the tips of nanotubes or can be incorporated within the tubes. It has been found that gene therapy with β galactosidase marker gene nanotubes showed greater expression compared to transfer of naked DNA. This assures the advantage of non immunogenicity in contrast to viral vectors used for gene transfer.
- **Transport of peptides, nucleic acids and other drug molecules**
  Incorporation of carboxylic or ammonium groups to carbon nanotubes enhances their solubility which makes them more suitable for the transport of peptides, nucleic acids and other drug molecules. Pristine carbon nanotubes is a common example of water insoluble forms having high in vitro toxicity compared to modified water dispersible forms of nanotubes. It has been also proven that the extent of toxicity decreases with functionalization. However functionalization also affects the elimination of the nanotube. Single-walled nanotubes without conjugation to monoclonal antibody have a high renal uptake. Whereas modest liver uptake as compared to single-walled nanotubes with conjugation to monoclonal antibody which is having higher liver uptake and lower renal uptake. In addition it was also reported that carbon nanotubes, except acetylated ones, when conjugated with peptide produce a higher immunological reactions when compared to free peptides. Such a property of inducing immunological responses can be utilized in vaccine production to enhance the efficacy of vaccines.
- **Reduced toxicity and increases the efficacy**
  Carbon nanotubes enhance drug delivery, efficacy and reduces the toxicity as found in the case of Amphotericin B nanotubes. It has been found that Amphotericin B nanotubes has shown enhanced drug delivery to the interior of cells, increased antifungal efficacy and reduced toxicity to mammalian cells when compared to amphotericin B administration without nanotubes [69]. The efficacy of amphotericin B nanotubes was also effective on strains of fungi which are usually resistant to amphotericin B alone [69].

- **Gene silencing**
  Highly selective therapy is required for cancer therapy where tumor cells will be selectively modulated. In this case gene silencing has been done with small interfering RNA. This can be achieved by targeting functionalized single walled carbon nanotubes with siRNA to silence targeted gene expression in the targeted cell [70].
- **In diagnostics**
  It was reported that compounds that are bound to nanotubes enhance the efficiency of diagnostic methods. This property of functionalization and high length to diameter aspect ratio (which provides a high surface to volume ratio), assists in designing the highly efficient biosensors [71].

Thus carbon nanotubes offer diverse advantages over other drug delivery and diagnostic systems due to very interesting physicochemical properties such as ordered structure with high aspect ratio, high electrical conductivity, high mechanical strength, ultra-light weight, high thermal conductivity, metallic or semi-metallic behavior and high surface area [72].

2.6.2 Fullerenes

They are also known as bucky balls, that are the carbon allotrope discovered in 1985 [72] having dimensions near around 7 Å in diameter and composed of 60 carbon atoms that are arranged in a shape known as truncated icosahedrons [73]. Its shape is quite similar to soccer ball with 20 hexagons and 12 pentagons and is highly symmetrical [74]. There are various types of fullerenes such as Alkali doped fullerenes, endohedral fullerenes, endohedral metallofullerenes, exohedral fullerenes and heterofullerenes. Alkali doped fullerenes are the carbon allotropic structures that contains alkali metal atoms in between fullerenes contributing valence electrons to neighboring fullerenes [75]. Similarly endohedral fullerenes have another atom enclosed inside the buckyball. If they are enclosed with metallic atom then they are called as metallofullerenes [76, 77]. Owing to the very small size of C-60 fullerene, it is difficult to synthesize endohedral C_{60} fullerenes. Therefore larger fullerenes (C_{82} or C_{84}) fullerenes are used for synthesizing endohedral fullerenes. Another type of fullerenes called as exohedral fullerenes or fullerene derivatives or functionalized fullerenes which are synthesized by chemical reaction between the fullerene and other chemical groups. Last class of fullerene compounds are heterofullerenes where one or more carbon atoms are replaced by other atoms like nitrogen or boron.

2.6.2.1 Applications

- **Diagnostics**
  Endohedral metallofullerenes can be used for diagnostic purposes as radio contrast media in magnetic resonance imaging and other imaging procedures. Since

the radioactive metal is enclosed within the buckyball, these are less toxic and safer. This method can also be employed for imaging organs as radioactive tracers [77]. Animal studies with C_{60} fullerene conjugated with thyroglobulin have produced a C_{60} specific immunological response which can be detected by ELISA with IgG specific antibodies. This can be used to design methods of estimation of fullerene levels in the body when used for therapeutic or diagnostic purposes [78].

- **Drug transport**
  Fullerenes are being investigated for drug transport of antiviral drugs, antibiotics and anticancer agents [79–82].
- **Free radical scavengers**
  Due to presence of high number of conjugated double bonds in the core structure fullerenes can also be used as free radical scavengers. They also provide protection to the mitochondria against injury induced by free radicals [83], owing to this property they can be used in cancer therapy [84].
- **Photosensitizers**
  Fullerenes especially exohedral fullerenes can be used as photosensitizers in photodynamic therapy against various types of malignancies. These fullerenes potentially generate reactive oxygen species when stimulated by light and kills the target cells. This method is now also being investigated for antimicrobial property as these cause cell membrane disruption especially in Gram positive bacteria and mycobacterium [79–82].
- **Stimulate host immune response and production of antibodies**
  Fullerenes are efficient in stimulating host immune response and production of fullerene specific antibodies.

2.6.2.2 Toxicity

After its parenteral administration through intravenous injection, these fullerenes get distributed to various parts of the body and finally get excreted unchanged through the kidney. In comparison to non soluble derivatives, soluble derivates of fullerenes are safer and biocompatible. They are also having low toxic potential even at higher doses [78]. Cost of fullerenes is dependent on its degree of purification. Purified fullerenes are very expensive, restricting its application in medical field.

2.6.3 Quantum Dots

Quantumdots (QDs) are nanocrystals of semiconducting materials measuring around 2–10 nm, consisting of a semiconductor inorganic core (CdSe), an aqueous organic coated shell (e.g., ZnS) to improve optical properties, and can be made to fluorescence when stimulated by light. Quantumdots bear a cap which enables them in improving their solubility in aqueous buffers. They are neither atomic nor bulk semiconductors. Core of the quantumdots determines the color emitted and outer

Table 2.4 Various examples of drugs that are delivered via Quantum Dots

Therapeutics	Target cells/ diseases	Type of QDs	Efficacy
5-Fluorouracil	Breast cancer	ZnS QDs	Targeting and controlled drug delivery to cancer cells.
Daunorubicin	Leukaemia	CdTe QDs	Enhanced drug uptake
Daunorubicin	Leukemia K562 cells	CdS QDs	Inhibit multidrug resistance
Doxorubicin	Ovarian cancer	Mucin1- aptamer QD	Higher accumulation on target
Saquinavir	HIV-1	Carboxyl-terminated QDs	High site-specificity and can cross BBB

aqueous shell is available for conjugation with biomolecules. Biomolecular conjugation of the quantum dots can be modified according to target various biomarkers [85]. Their properties originate from their physical size, which ranges from 2 to 10 nm in radius. Owing to the to their narrow emission, bright fluorescence, high photostability and broad UV excitation QDs have been adopted for tracking of intracellular process for longer time, for in vitro bioimaging and for real time monitoring (Fig. 2.1). As far as the its applications are concerned QDs covers medical areas as a diagnostic as well as therapeutic tool for *in vitro* and *in vivo* detection and analysis of biomolecules, immunoassays, DNA hybridization, diagnostic tools (magnetic resonance imaging, MRI), time graded fluorescence imaging of tissue, development of non-viral vectors for gene therapy, labeling of cells, as therapeutic tools for cancer treatment and transport vehicles for DNA, protein, drugs or cells [86]. In addition they can be also tagged with biomolecules and used as highly sensitive probes. Quantum dots and their therapeutic applications are highlighted in Table 2.4.

2.6.3.1 Applications

- **Cancer therapy**
  In one report it was proven that quantum dots are accumulated in prostate cancer developed in nude mice by enhanced permeability and retention [87]
- **Bioconjugation with polymer**
  It was reported that its conjugation with polyethylene glycol (PEG) and antibody and targeting to prostate specific membrane antigen enhances its accumulation and retention [87] in the tumor tissue
- **Imaging**
  They can also be utilized for imaging of sentinel node in cancer patients for tumor staging and planning of therapy. This application assists in detecting suitable therapy and stage for various malignancies like melanoma, breast, lung and gastrointestinal tumors [87]. In addition quantum dot probes provide real time imaging of the sentinel node with Near Infra Red (NIR) fluorescence system which is having the potential to produce reduced [88] background noise and deeper penetration of rays into the biological sample.

2.6.3.2 Toxicity

QDs utilization in clinical practice is limited since it has serious elimination problems which cause extreme toxicity. After functionalization of the QDs size increases. This size range is greater than the pore size of endothelium and renal capillaries, thus reducing its elimination and resulting in toxicity. Additionally there are very less reports available on the metabolism and excretion of quantum dots making their utilization more difficult clinically [86].

2.6.4 Nanoshells

Nanoshells are the new modified forms of targeted therapy, having core of silica and a metallic outer layer [89]. These thin coated core particles of different material have gained considerable attention now days. The properties of nanoshells can be altered by simply tuning the core to shell ratio. With the recent advancement in new techniques it is now possible to synthesize these nanostructures in desired shape, size and morphology. Nanoshells are synthesized to create novel structures with different morphologies, since not possible to synthesize all the materials in desired morphologies. For obtaining desirable morphology core particles of different morphologies such as rods, wires, tubes, rings, cubes, etc. can be coated with thin shell in core shell structures. These shells are inexpensive as precious materials can be deposited on inexpensive cores. Therefore while synthesizing nanoshells expensive material is required in lesser amount than usual. Targeting of nanoshells can be achieved by using immunological methods. Nanoshells occupies variety of applications in diverse areas such as providing chemical stability to colloids, enhancing luminescence properties, engineering band structures, biosensors, drug delivery, etc. Synthesis of nanoshells can be useful for creating.

2.6.4.1 Applications

- **Cancer therapy**
  This technology is being evaluated for cancer therapy. Nanoshells are tuned to absorb infra red rays when exposed from a source outside the body and get heated and cause destruction of the tissue. This has been studied in both *in vitro* and *in vivo* experiments on various cell lines [89]
- **Diagnostic purposes**
  They are useful for diagnostic purposes in whole blood immunoassays e.g. coupling of gold nanoshells to antibodies to detect immunoglobulins in plasma and whole blood
- **Hydrogel mediated delivery**
  Nanoshells can be easily embedded in hydrogel polymer containing the drug. Such type of delivery system can be used for targeting tumor cells. Mechanism

of action is based on the targeting of gel to tumor tissue by immunological methods and exposed under infrared laser beam to heat the polymer which facilitates the release of the drug at the desirable site.

- **Micro metastasis and diabetes**
  Nanoshells are currently studied for micro metastasis of tumors and also for treatment of diabetes [90]

2.6.5 Nanobubbles

Nanobubbles (NBs) are nanoscaled bubble like structures that are generated in the interface of hydrophobic surfaces in liquids. These nanobubbles remain stable at room temperature and when heated to physiological temperature within the body coalesce to form microbubbles. The mechanism of NB formation is based on the nucleation of gas at the hydrophobic surface from a supersaturated solution, leading to trap atmospheric gases. However the formation of NBs is thermodynamically forbidden, but the life time of NBs is reached event to the orders of hours. There are four types of nanobubbles: bulk, interfacial, plasmonic and oscillating nanobubbles. Cancer therapeutic drugs can be incorporated into these nanoscaled bubbles like structures. Nanobubbles potentially exhibit advantages in targeting the tumor tissue and delivering the drug selectively under the influence of ultrasound exposure. This may enhance the intracellular uptake of the drug by the tumor cells. Additionally these nanobubbles can be easily visualized in tumor by means of various ultrasound methods [91, 92].

2.6.5.1 Applications

- **Delivery of drugs**
  NBs can be potentially utilized in delivery of drugs like doxorubicin *in vitro* and *in vivo*. These NBs reach the tumor and get accumulated which is followed by formation of microbubbles by coalescing of nanobubbles. Disruption of the microbubbles occurs when the site is focused with high intensity focused ultrasound, which ultimately causes release of the drug. This may results in accumulation of higher levels of drug in the target cells and reduced toxicity and increased efficacy. This method needs further exploration for its utility in treatment of various malignancies.
- **Gene therapy**
  Liposomal nanobubbles and microbubbles are also being studied for effective non viral vectors for gene therapy. Nanobubbles combined with ultrasound exposure have shown improved transfer of gene in both *in vitro* and *in vivo* studies [93, 94].
- **Thrombolysis**
  Nanobubbles are also being investigated for removal of clot in vascular system in combination with ultrasound. This process is called as sonothrombolysis. This method is non invasive and causing less damage to endothelium [95].

- Toxicity
  NBs are not that much toxic since the disruption of the microbubbles occurs only at the targeted site when it's being exposed to ultra sound waves. Therefore drug is released at a particular site.

2.6.6 Paramagnetic Nanoparticles

Magnetic drug targeting is conceptualized with an objective to target magnetic drug carrier particles at a specific site in the body using an externally applied magnetic field. Magnetic nanoparticles are a class of particulate materials of less than 100 nm size that can be manipulated under the magnetic field. These particles are composed magnetic elements such as cobalt, nickel, iron and their respective oxides such as magnetite, maghemite, cobalt ferrite and chromium dioxide. The classification of these particles is based on their magnetic susceptibility which is defined as ratio of induced magnetization to the applied field. Paramagnetic nanoparticles have a greater magnetic susceptibility than conventional contrast agents. They are investigated for both diagnostic and therapeutic purposes. For diagnostic purpose paramagnetic iron oxide nanoparticles are used as contrast agents in magnetic resonance imaging. Targeting with paramagnetic nanoparticles enables identification of specific organs and tissues [96].

2.6.6.1 Applications

- **Cancer therapy**
  Conjugation of paramagnetic nanoparticles with antibodies and their expression in breast cancer cells have been used with MRI to detect breast cancer cells *in vitro* [97]. Moreover conjugation of paramagnetic nanoparticles with luteinizing hormone (releasing hormone as breast cancer cells express LHRH receptors) studied for the detection of breast cancer cells in vivo. Magnetic nanoprobes such as iron nanoparticles coated with monoclonal antibodies are used for cancer therapy. These nanoparticles are targeted towards tumor cells to generate high levels of heat after their accumulation at the target site by means of an alternating magnetic field applied externally. Such procedure kills the cancer cells selectively [98].
- **Eliminate plasma opsonins and increase in circulation time**
  On intravenous administration of decoy of nanoparticle plasma opsonins can be eliminated and reduces uptake of the nanoparticles. Moreover alteration in surface charge of the nanoparticle to neutral by covalent coupling to chemicals leads to an increase in circulation time
- **For internalization**
  Paramagnetic nanoparticles internalization by macrophages can be overcome by treatment with drugs like lovastatin which reduce macrophage receptor expression for the nanoparticle.

- **Identification of proteins**
  Magnetic microparticle probes with nanoparticle probes have been used for identification of proteins like prostate specific antigen. Here magnetic microparticles coated with antibodies together with nanoprobes with similar coating and a unique hybridized DNA barcode are used [99]
- **Imaging**
  Utilization of Iron oxide in MRI imaging faces limitations like specificity and internalization by macrophages. Monocrystalline iron oxide nanoparticles have been studied for magnetic resonance imaging of brain. These are rapidly taken up by the tumor cells [100]. Hence give long lasting contrast enhancement of tumor. The remaining nanoparticles are removed from the circulation by reticuloendothelial system [100]
- **Targeted action**
  Conjugation of antibodies with paramagnetic nanoparticles to direct the nanoparticle to the target site helped to overcome problems with specificity of action.

2.6.7 Nanosomes

Nanosomes are currently being used for medical applications such as targeting, diagnosis and therapy.

- **Brain targeting**
  These nanosomes are being developed for treatment of various tumors (CNS tumors) e.g. silica coated iron oxide nanoparticles coated with polyethylene glycol used to access specific areas of brain involved with tumor [101].
- **Tumor targeting**
  Nanosomal delivery with magnetic resonance imaging and laser assist in targeting the nanoparticle specifically to the tumor cells and destroy the cells loaded with these nanoparticles by the heat generated by iron oxide particles by absorbing the infra red light.
- **ROS production**
  Their stable integration with photocatalyst produces reactive oxygen species when stimulated by light and destroy the target tissue. These nanoform exhibit advantages over conventional drugs in being much safer without the adverse effects of cancerchemotherapy drugs and also the absence of development of drug resistance.

2.6.8 Pharmacyte

Pharmacyte is an ideal nanotechnology-based drug delivery system, which is a self-powered, computer controlled medical nanorobot system capable of digitally precise transport, timing, and targeted delivery of pharmaceutical agents to specific cellular and intracellular destinations within the human body. This nanotechnology

may be constructed using future molecular manufacturing technologies such as diamond mechanosynthesis. Pharmacytes will have many applications in nanomedicine such as initiation of apoptosis in cancer cells and direct control of cell signaling processes [102].

2.6.8.1 Niosome

Niosome is a class of molecular cluster formed by self-association of non-ionic surfactants in an aqueous phase. The unique structure of niosome presents an effective novel drug delivery system (NDDS) with ability of loading both hydrophilic and lipophilic drugs. Niosomes are vesicles composed of non-ionic surfactants, which are biodegradable, relatively nontoxic, more stable and inexpensive, an alternative to liposomes. Niosomes behave in vivo like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability. As with liposomes, the properties of niosomes depend on the composition of the bilayer as well as method of their production. It is reported that the intercalation of cholesterol in the bilayers decreases the entrapment volume during formulation, and thus entrapment efficiency. However, differences in characteristics exist between liposomes and niosomes, especially since niosomes are prepared from uncharged single-chain surfactant and cholesterol, whereas liposomes are prepared from double-chain phospholipids (neutral or charged). The concentration of cholesterol in liposomes is much more than that in niosomes. As a result, drug entrapment efficiency of liposomes becomes lesser than niosomes. Besides, liposomes are expensive, and its ingredients, such as phospholipids, are chemically unstable because of their predisposition to oxidative degradation; moreover, these require special storage and handling and purity of natural phospholipids is variable. Current opinions for the utilization of niosomes in the delivery of biomolecules can be unsubstantiated with a wide scope in encapsulating toxic drugs such as anti-AIDS drugs, anticancer drugs, and anti-viral drugs. Niosomes offers a promising carrier system in comparison with ionic drug carriers, which are relatively toxic and unsuitable. However, the technology utilized in niosomes is still in pipeline. Therefore researches are going on to develop a suitable technology for large production because it is a promising targeted drug delivery system.

2.6.9 Dendrimers

Dendrimers are a unique class of polymers, are hyperbranched, tree-like structures, whose size and shape can be precisely controlled and have compartmentalized chemical polymer. Dendrimers are fabricated from monomers using either convergent or divergent step growth polymerization. Size of these regular branching polymeric nanostructures is dependent on the number of branching which can be controlled. These nanostructures arise several branches from the core in shape of a spherical structure by means of polymerisation, resulting in formation of cavities

within the dendrimer molecule which can be used for drug transport. Free ends of dendrimer can be utilized for conjugation or attachment to other molecule. These end groups that can be tailored according to requirements. Such interconnecting networks transport the attached molecules at desirable site and give dendrimers various functional applications [103]. These well defined nanostructures are equipped with surface functionalization capability, monodispersity of size, and stability properties that make them attractive drug carrier candidates. Incorporation of drug molecule can be easily achieved via either complexation or encapsulation. As far as the construction is concerned it contains three different basic regions: core, branches, and surface (Fig. 2.1). Branches or end groups can be tailored or modified into biocompatible compounds with low cytotoxicity and high biopermeability. Such branches or networks assist in delivery of bioactive ranging from vaccines, drugs, genes and metal to desired sites. Hollow networks present in dendrimers presents space to incorporate drugs and other bioactive physically or by various interactions to act as drug delivery vehicles. Dendrimers covers various distinct applications mainly are solubilization, gene therapy, dendrimer based drug delivery, immunoassay and MRI contrast agent. List of the various drugs that can be delivered through dendrimers are highlighted in Table 2.5. This hollow branched nanostructure is an ideal carrier for drug delivery due to several advantages like:

- Can be modulated for target-specific drug delivery
- Feasibility to develop with defined molecular weight
- Good entrapment efficiency
- Offering surface for functionalization
- Very low polydispersity index
- Very low size (1–5 nm)

Variety of polymers (single or in combinations) are used for designing drug delivery system in form of dendrimers:

- Andpoly(ethylene glycol)
- Chitin
- Melamine, poly(L-glutamic acid)
- Poly(propyleneimine),
- Polyamidoamine
- Polyethyleneimine

2.6.9.1 Applications

- **Drug and gene delivery**
  Dendrimers are being investigated for both drug and gene delivery, as carriers for penicillin, and for use in anticancer therapy.
- **Imaging, targeting & diagnosis of disease**
  Complexes of dendrimers such as tectodendrimers with each dendrimer module of the complex performing different functions such as targeting, diagnosis of disease state, delivery of drug and imaging.

Table 2.5 List of drugs that can be delivered through Dendrimers [67]

Drugs/therapeutics	Type of dendrimers/ conjugates	Target cells/indications/ functions	Advantages/ features
Boron	EGF-carrying PAMAM dendrimers	Neuron capture technology	Intratumoral injection or CED
Doxorubicin	2,2 bis(hydroxymethyl) propanoic acid-based dendrimers	Colon carcinoma cells of rat	In vitro and in vivo, dendrimer product was ten times less toxic
Efavirenz	Tuftsin-conjugated PPE dendrimers	HIV	Targeted delivery to macrophages
EGFR siRNA	Dendriworms	Knockdown EGFR expression	IV or CED
Galactosylceramide analogues	Multivalent phosphorus-containing catanionic dendrimers	HIV-1	Higher stability and anti-viral property, lower toxicity
Lamivudine	Mannose-capped PPE dendrimers	HIV	Increased cellular uptake, reduced cytotoxicity
Plasmid pEGFP-N2	Angiopep-carrying PEGylated PAMAM dendrimer G5.0	Encode green fluorescence protein	IV
siRNA	Amino-terminated carbosilane dendrimers	Lymphocytes	Reduced HIV infection, in-vitro
SN38	G3.5 PAMAM dendrimers	Hepatic colorectal cancer cells	Increase oral bioavailability and decrease gastrointestinal toxicity
Sulphated oligosaccharides	Polylysine dendrimers	HIV	Higher activity due to dendrimer product

PAMAM Poly(amido amine), *PPE* poly (propyleneimine)

- **Chemotherapy**
  Tectodendrimers are the nano device that acquires potential applications in cancer chemotherapy as a mode of targeted drug therapy [104]
- **Gene therapy**
  Dendrimers can be used for gene therapy where these can replace conventional viral vectors. They enter the cells by endocytosis and the DNA gets transported into nucleus for transcription of the applied gene.
- **Stimulation of immune reaction**
  The advantage of dendrimer based therapy is absence of stimulation of immune reaction.

- **Gene transfer**
  It was studied that the potential use of transferring conjugated gene transfer for tumors of various tumors PEG modified polyamidoamine dendrimers and magnetic nanoparticle modified dendrimers for targeted gene delivery to the brain and in transfer of antisense surviving oligonucleotides in tumor cell lines. These methods provide an effective alternative to viral vectors of gene transfer for treatment of various tumors [105]
- **Transfection**
  Various dendrimer based DNA transfection kits (NanojuiceTM Transfection Kit produced by EMD Chemicals Inc. and Superfect® Transfection Reagent of Qiagen) are used for delivering DNA into the cell. These are claimed to have improved transfection efficacy and low toxicity to cells [106]
- **Antiretroviral therapy**
  Dendrimer based drugs are being tried for antiretroviral therapy. Some of the dendrimer based drugs was found to successfully prevent simian HIV infection
- **Treatment of cancer**
  Treatment of cancer by conjugating with anti-cancer drugs like cisplatin, adriamycin or methotrexate [107]. PAMAM dendrimers can also be used in treatment of cancer.
- **Reduces the cytotoxicity**
  While antibacterial investigation it was observed that PEG coating of the dendrimer reduces the cytotoxicity of unmodified PAMAM dendrimers. Hover reduces the efficacy against Gram positive bacteria without change in efficacy against Gram negative bacteria like *Pseudomonas*
- **Contrast agents for imaging**
  Dendrimers are also used as contrast agents for imaging. The 1, 4-diaminobutane core dendrimer and the PAMAM dendrimer are well studied commercially available dendrimers for imaging studies. Renal excretion is the main route of clearance and is dependent on the size of the particle and more than 60 % of injected DAB or PAMAM dendrimer is cleared from circulation within 15 min
- **Rapid clearance**
  Smaller sized dendrimers undergo rapid renal clearance whereas dendrimers with charged surface or hydrophobic surfaces are rapidly cleared by the liver. Those dendrimers with a hydrophilic surface escape renal clearance and have a greater circulation time

2.6.9.2 Toxicity

Toxicity profile of dendrimers renders them not very popular system for use as delivery means. Cationic dendrimers have a greater potential to cause cytotoxicity compared to anionic dendrimer or PAMAM dendrimers. It is proposed to cause cell membrane instability and cell lysis. The toxicity of dendrimer is dependent on the size of the particle and increase with size. It can be reduced by means of surface modification of the dendrimers with incorporation of PEG or fatty acids

2.6.10 Nanopores

Nanopores were designed in 1977, consist of wafers with highly dense pore of size 20 nm (diameter). Main advantage of these nanopores that they doesn't allow the entry of oxygen glucose and other products. They can be potentially utilized to protect transplanted tissues from the host immune system.

2.6.10.1 Application

- **DNA sequencing**
  Currently several researchers are working on modified nanopores that have the ability to differentiate DNA strands based on differences in base pair sequences. Nanopores are also being developed with ability to differentiate purines from pyrimidines.
- **Pharmacogenomics in drug development process**
  DNA sequencing via nanopores could possibly read a thousand bases per second per pore. These can be used for low cost high throughput genome sequencing which would be of great benefit for application of pharmacogenomics in drug development process.
- **Treatment for insulin dependent diabetes mellitus**:
  β cells of pancreas can be enclosed within the nanopore device and implanted in the recipient's body.

2.6.11 Microbivores

Function of these nano based hypothetical forms is to trap circulating microbes just like the function of white blood cells in the blood stream. They are designed in such a way so that they acquire greater efficacy than cellular blood cells in phagocytosis. Their surface is arranged in such a fashion which can extend in length and secure the microbe which gets in contact with it. Entrapped microbe will be gradually transferred to the ingestion port and undergoes the process of morcellization and enzymatic degradation. Degraded products are ultimately released as amino acids, fatty acids, nucleotides and sugars.

2.6.11.1 Application

- **Clear the blood circulation**
  Microbivores could theoretically clear the blood stream in septicaemia at a much greater rate than the natural defense mechanism with antibiotics [108]

2.6.12 Nanocrystals and Nanosuspension

These are aggregated structures that are formed by the combination of various particles of drug in crystalline form coated with surfactant or combination of surfactants. To achieve static and electrostatic surface stabilization a minimum quantity of surfactants needs to be added in nanocrystals. These aggregated forms reduce limitations of several drugs that are suffering from bioavailability and absorption problems. In addition problems of preparing the parenteral dosage form may be resolved by formulation as nanocrystals. Loading capacity especially in carrier-based nanoparticles is quite low however administration of high drug levels with depot release can be achieved if dissolution is sufficiently slow. Nanocrystal technology can be utilized for many dosage forms.

2.6.12.1 Applications

- **Drugs in pipeline**
  Nanocrystals such as Rapamune®, containing sirolimus which is an immunosuppressant drug and Emend®, which contains aprepitant, MK 869, are in pipeline
- **Favorable drug delivery system**
  Serve as a favorable delivery system for drugs like amphotericin B, tacrolimus, etc.
- **Safe and effective passage**
  The size of nanocrystals allows for safe and effective passage through capillaries.
- **Targeting**
  Nanoparticles offer the potential for targeting the mucosa of the gastrointestinal tract after oral administration, and targeting the cells of the mononuclear phagocytic system (MPS) to treat infections of the MPS such as fungal mycobacterial infections and leishmaniasis.

2.6.12.2 Toxicity

Since pure drug is used and no carrier is needed, eliminating potential toxicity issues associated with the carrier molecule.

2.6.13 Solid Lipid Nanoparticles

Solid lipid nanoparticles were developed as an alternative carrier system to liposomes, polymeric nanoparticles and emulsions as a colloidal carrier system for controlled drug delivery. Solid lipid nanoparticles carry distinct advantages that make them unique carriers systems than others like liposomes and polmeric nanoparticles. This type of nanoparticles constitute solid lipid matrix with an average diameter below 1 μm. Drug is normally incorporated in this matrix. These nanoparticles can

also be produced by high pressure homogenization. Different surfactants are used to avoid aggregation and to stabilize the dispersion. These surfactants have an accepted GRAS (Generally Recognized as Safe) status.

2.6.13.1 Applications

- **Can be used for diverse route system**
  SLN have been developed and investigated for parenteral, pulmonal and dermal application routes.
- **Non-viral transfection**
  SLN have been considered as new transfection agents using cationic lipids for the matrix lipid composition. Cationic solid lipid nanoparticles for gene transfer can be formulated using the same cationic lipids as for liposomal transfection agents. Cationic lipid composition seems to be more dominant for *in vitro* transfection performance than the kind of colloidal structure it is arranged in. Hence, cationic SLN extend the range of highly potent non-viral transfection agents by one with favorable and distinct technological properties.

2.6.14 Silicon-Based Structures

These silicon-based structures can be fabricated by techniques such as etching, photolithography, and deposition commonly used in the manufacture of and microelectromechanical systems and semiconductors. Among various silicon-based materials, porous silicon and silica, or silicon dioxide are the most materials that are architecture in form of calcified nanopores, platinum materials containing nanopores, porous nanoparticles, and nanoneedles. Nanopores size (diameter) and density can be accurately controlled to achieve a constant drug delivery rate through the pores. There are various forms (porous hollow silica nanoparticles) that are fabricated in a suspension containing sacrificial nanoscale templates. This followed by the addition of silica precursors, such as sodium silicate, into the suspension, which is then dried and calcinated. Template material is then dissolved further leaving behind the porous silica shell. These nanoparticles mixed with the drug molecule and subsequently drying the mixture to coalesce the drug molecules to the surface of the silica nanoparticles.

2.6.14.1 Applications

Various examples of therapies being studied for use with silicon-based delivery systems include

- **For delivery of antitumor agent**
  Porous silicon embedded with platinum is reported

- **Act as an artificial growth factor**
  Calcified porous silicon designed is reported
- **For antibody delivery**
  Silicon nanopores are reported
- **For antibiotics, enzymes, and DNA delivery**
  Porous silica nanoparticles are reported

2.6.15 Metallic Nanoparticles

Currently these nanoparticles are emerging as good delivery carrier for drug and biosensor. For the synthesis of metallic nanoparticles diverse metals have been explored though silver and gold nanoparticles are of prime importance for biomedical use (Fig. 2.1). Surface functionalization on these nanonarticles can easily been done and various ligands have been decorated onto the surface. Variety of ligands such as sugars, peptide, protein and DNA has been linked to nanoparticles.

2.6.15.1 Applications

Metallic nanoparticles have been used for active delivery of bioactive, drug discovery, bioassays, detection, imaging and many other applications due to surface functionalization ability, as an alternative to quantum dots.

2.6.16 Liposomes

Liposomes are lipid based vesicles that are extensively explored and most developed nanocarriers for novel and targeted drug delivery. Drugs that can deliver through liposomal delivery system are highlighted in Table 2.6. These vesicles are synthesized by hydration of dry phospholipids. Depending upon on their size and number of bilayers they are classified into three basic types:

- **Multilamellar vesicles**
  These vesicles consist of several lipid bilayers separated from one another by aqueous spaces. These entities are heterogeneous in size, often ranging from a few hundreds to thousands of nanometers in diameter.
- **Small unilamellar vesicles**
  Small unilamellar vesicles consist of a single bilayer surrounding the entrapped aqueous space having size range less than 100 nm.
- **Large unilamellar vesicles**
  These vesicles consist of a single bilayer surrounding the entrapped aqueous space having diameters larger than 100 nm.

Table 2.6 Liposomes and their respective model drugs [67]

Therapeutics	Type of liposome	Indications
Amiloride hydrochloride	Small molecular liposome	Cystic fibrosis
Budesonide	Small molecular liposome	Asthma
Doxorubicin + Verapamil	Transferrin- (Tf-) conjugated PEG-Liposome	MDR-leukemia
Insulin	Protein liposome	Diabetes
Interleukin-2	Protein liposome	Lung cancers
Irinotecan + Cisplatin	Mixture of two liposomes	Small-cell lung cancer
Ketotifen	Small molecular liposome	Asthma
siRNA + Doxorubicin	PEG-Liposome	MDR-breast cancer
Tobramycin	Small molecular liposome	Pulmonary infections
Topotecan + Vincristine	PEG-Liposome	Brain cancer
VEGF gene	Gene liposme	Pulmonary hypertension

VEGF vascular endothelial growth factor

Based on the physicochemical characteristics drug molecules can be entrapped in the aqueous space or intercalated into the lipid bilayer of liposomes. Liposomes are prepared with distinct structure, composition, size, flexibility with variety of surface modification. Such availability of liposomes with enormous diverse properties makes them most intelligent carrier system for both active and passive delivery of bioactive.

2.6.16.1 Applications

They have been successfully exploited in cancer therapy, carrier for antigens, pulmonary delivery, leishmaniasis, ophthalmic drug delivery etc. Some of liposome-based formulations are already in market (Table 2.5).

2.6.17 Polymeric Micelles

Polymeric micelles contains amphiphilic block copolymers assemble to form nanoscopic supramolecular core-shell structures called as ‘polymeric micelles’. These micelles are formed in solution as aggregates in which the component molecules are generally arranged in a spheroidal structure with hydrophobic cores shielded from water by a mantle of hydrophilic groups (Fig. 2.1). There are several examples of component molecule such as Amphiphilic AB-type or ABA-type block copolymers, where A and B are hydrophobic and Hydrophilic components, respectively. These polymeric micelles are usually <100 nm and are used for the systemic delivery of water-insoluble drugs. Their hydrophilic surface of these dynamic systems protects their nonspecific uptake by reticuloendothelial system. Polymeric micelle

carries advantage in trapping drugs or contrast agents physically within the hydrophobic cores or can be linked covalently to component molecules of the micelle. Additionally they are proved as an excellent novel drug delivery system due to their high stability in physiological conditions, high and versatile loading capacity, high accumulation of drug at target site, possibility of functionalization of end group for conjugation of targeting ligands and slower rate of dissolution.

2.6.18 Polymer Drug Conjugate

Polymer drug conjugate formed by the conjugation of low molecular weight drugs with polymer. This interaction/conjugation causes drastic change in pharmacokinetic disposition of drug in whole body and at cellular level. They are designed to increase the overall molecular weight, which facilitates their retention in cancer cells through enhanced permeation and retention effect using passive delivery approach.

2.6.19 Polyplexes/Lipopolyplexes

Polyplexes/Lipopolyplexes are the assemblies which are used in transfection protocols. These assembies are formed by spontaneous interaction between nucleic acids and polycations or cationic liposomes (or polycations conjugated to targeting ligands or hydrophilic polymers). Usually composition and charge ratio of nucleic acid to that of cationic lipid/polymer determines the shape, size distribution, and transfection potential of these complexes. Current research offers various types of polycations that have been used in gene transfer/therapy protocols:

- Cationic cyclodextrin
- Linear- and branched-poly (ethyleneimine)
- Poly (amidoamine)
- Poly-amino esters
- Poly-L-lysine

2.6.20 Respirocytes

These are hypothetical nanodevices or called as artificial red blood cells 51 and function as red blood cells but with greater efficacy. Respirocytes are having higher capacity to deliver oxygen to tissues. Their oxygen supplying capacity is 236 times more oxygen per unit volume than natural red blood cells. Respirocytes equipped with sensors on their surface which can detect changes in the environment. There is

also a provision to regulate the intake and output of the oxygen and carbon dioxide molecules. According to past investigation an infusion of 1 L dose of 50 % respirocytes saline suspension in a human can theoretically keep the patient oxygenated up to four hours following cardiac arrest [109]. According to FDA these devices are regulated under the provisions of the Medical Device Amendments of 1976, Safe Medical Devices Act of 1990, and the Medical Device Amendments of 1992 [110].

2.6.21 Polymeric Nanoparticles

The main objective of our book is to explore the recent nano application of wide array of natural polymers obtained from different sources. Natural polymers based nano-conjugates and their advance applications are discussed in this chapter. In addition various drug delivery and targeting based considerations are also discussed. Natural polymer based nanoparticles are usually biocompatible and non toxic, although often suffer from stability problems when delivered across the various biological membranes. Such delivery exposed nanoparticles against various pH. This variation in pH and certain other problems limit their use sometime.

Polymeric nanoparticles consist of a biodegradable polymer which is biocompatible and non toxic. Feature such as biocompatibility is required for potential application in tissue engineering, drug and gene delivery and new vaccination strategies.

Recently research explore some advance modification of natural polymers which consists of synthetic polyesters like poly(D, L-lactide) or polycyanoacrylate and related polymers like poly(lactide-co-glycolide) PLA or poly(lactid acid). Among natural polymers the most widely used polymer which is used now days is chitosan. In addition to chitosan many other such as gelatin, and sodium alginate overcome some toxicological problems with the synthetic polymers. Natural polymer based nanoparticles offers a significant improvement over traditional oral and intravenous methods of drug delivery system in terms of efficiency and effectiveness. The various natural polymers like gelatin, albumin and alginate are used to prepare the nanoparticles. However they have some inherent disadvantages like poor batch-to batch reproducibility, prone to degradation and potential antigenicity. Various polymeric nanoparticles and their respective model drugs are highlighted in Table 2.7. Synthetic polymers used for nanoparticles preparation may be in the form of preformed polymer e.g. polyesters like polycaprolactone (PCL), poly lactic acid (PLA) or monomers that can be polymerized in situ e.g. polyalkyl cyanoacrylate. There are many advantages of using polymeric nanoparticles in drug delivery:

- Biocompatable and biodregerable
- Increase the stability of any volatile pharmaceutical agents
- Less toxic
- They are easily cheaply fabricated in large quantities by a multitude of methods
- Have engineered specificity, allowing them to deliver a higher concentration of pharmaceutical agent to a desired location
- Nonimmunogenicity and nontoxicity

Table 2.7 Different types of Polymer used for the preparation of nanoparticle [16]

Polymer	Model drug	Technique
Hydrophilic Albumin, Gelatin	Hydrophilic and protein affinity	Desolvation and cross linking in water
Hydrophilic Albumin,Gelatin	Hydrophilic	Heat denaturation and cross linking in w/o emulsion
Hydrophilic Alginates and chitosan	Hydrophilic and protein affinity	Cross-linking in water
Hydrophilic Dextran	Hydrophilic	Polymer precipitation in an organic solvent
Hydrophobic Poly(alkylcyanoacrylates)	Hydrophilic	Emulsion polymerization
Hydrophobic Poly(alkylcyanoacrylates)	Hydrophobic	Interfacial O/W polymerization
Polyesters Poly (lactic acid), poly(caprolactone)	Hydrophilic and Hydrophobic Soluble in polar solvent	Solvent extraction evaporation
Polyesters Poly (lactic acid), Poly (lactide-co-glycolide),	Hydrophilic and Hydrophobic Soluble in polar solvent	Solvent displacement
Polyesters Poly (lactic acid), Poly (lactide-co-glycolide)	Soluble in polar solvent	Salting out .

Various polymers that have been used recently for the preparation of nanoparticles are mentioned in Table 2.8.

Polymeric nanoparticles (Fig. 2.1) provide an alternative to abovementioned nanosystems due to some inherent properties like biocompatibility, nonimmunogenicity, nontoxicity and biodegradability. These are colloidal carrier, 10 nm^{-1} μm in size, consisting of synthetic or natural polymers. Polymeric nanoparticles are a broad class comprised of both vesicular systems (nanocapsules) and matrix systems (nanospheres). Nanocapsules are systems in which the drug is confined to a cavity surrounded by unique polymeric membrane whereas nanospheres are systems in which the drug is dispersed throughout the polymer matrix. Polymeric nanoparticles are considered as a matrix system in which the matrix in uniformly dispersed. It should be mentioned, that besides of these spherical vesicular systems nanocapsules are also known, where a polymeric membrane surrounds the drug in a matrix core. The candidate drug is dissolved, entrapped, attached or encapsulated throughout or within the polymeric shell/matrix. The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics. Moreover, polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering, and into drug delivery for species other than humans. Depending on the method of preparation, the release characteristic of the incorporated drug can be controlled. Polymeric nanoparticulate systems are attractive modules for intracellular and site specific delivery.

Table 2.8 Various examples of drugs those can be delivered through polymeric nanoparticles [67]

Therapeutics	Type of polymer/ functionalization	Indication/activity	Effects
Amphotericin B	PLA-b-PEG	Neurodegenerative diseases	Improved transport across the BBB
Cisplatin	Aptamer- PEG-PLGA	Prostate cancer	Higher efficiency
Doxorubicin + Cyclosporine A	PACA	Various cancers	Synergistic effect.
Lamivudine	Methylmethacrylate-sulfopropylmethacrylate	HIV/AIDS	100 % increased BBB permeability
Nerve growth factor (NGF)	Polysorbate 80 coated PBCA	Parkinsonism	Improved transport across the BBB
Paclitaxel	Aptamer- PEG-PLGA	Gliomas	Enhanced delivery
Stavudine	Polybutylcyanoacrylate (PBCA)	HIV/AIDS	8–20 times higher Permeability
Vincristine + Verapamil	PLGA	Hepatocellular carcinoma	Reduced multidrug resistance
Zidovudine	Poly (isohexyl cyanate)	Targeting lymphoid tissue	Drug levels is four times higher
Zidovudine	Polyhexylcyanoacrylate	Targeting lymphoid tissue	Higher Zidovudine levels in the body

PACA Polyalkylcyanoacrylate, *PLA-b-PEG* Polysorbate 80 coated poly(lactic acid)-b-poly(ethylene glycol), *PLGA* poly (lactide-co-glycolide)

Nanoparticles can be made to reach a target site by virtue of their size and surface modification with a specific recognition ligand. Their surface can be easily modified and functionalized. From the polymer chemistry viewpoint, there will be in the future a challenging field to create new polymers matching hydrophilic and lipophilic properties of upcoming drugs for smart formulation.

2.6.22 Applications of Nanoparticulate Delivery Systems

Targeting of the drug to cells or tissue of choice is the potential area in drug delivery. With the assistance of proficient drug targeting systems it's now possible to decide the fate of a drug entering in the body. Modern drug delivery systems and technologies are far away from the model of magic bullet (proposed by Paul Ehrlich) in which the drug is precisely targeted to the exact side of action. Nanotechnology present challenge to achieve this goal (to deliver the drug in the right place at the right time) a bit closer [111]. Branch of nanotechnology is anticipated to bring a fundamental transformation in manufacturing and have an enormous impact on Life Sciences especially in delivery, diagnostics, nutraceuticals and the production of biomaterials. In delivery systems targeting is the ability to direct the drug-loaded system to desirable site. There are two major mechanisms (addressing the desired sites for drug release) involved in this process (Table 2.9):

Table 2.9 Various applications of nanotechnology in the different field

Applied field	Application
Agriculture	Atomic force, microscopic and scanning tunnelling microscope
Chemical and Cosmetics	Nanoscale chemicals and compounds, paints, coatings etc.
Electronics	Semiconductors chips, memory storage, photonica, optoelectronics
Environment and Energy	Water and air purification filters, fuel cells, Photovoltaic
Food Sciences	Processing, nutracetical food, nanocapsules
Materials	Nanoparticles, carbon nanotubes, biopolymers, points, coatings
Military and Energy	Biosensors, weapons, sensory enhancement
Nanomedicines	Nano drugs, Medical devices, Tissue Engineering
Scientific Tools	Atomic force, microscopic and scanning tunnelling microscope

2.6.23 Passive Targeting

Most popular example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumors. This result in to the enhanced vascular permeability of tumor tissues compared with healthy tissue. In passive targeting ligand–receptor interactions can be highly selective; hence precise targeting at the site of interest [112] is possible. In this process targeting with nanoparticles encounters multiple obstacles on the way to their target. These include mucosal barriers, nonspecific uptake of the particle and non-specific delivery of the drug (as a result of uncontrolled release).

2.6.24 Active Targeting

Active targeting allows the surface functionalization of drug carriers with ligands that are selectively recognized by receptors on the surface of the cells of interest. Therefore, two most important aspects of nanoparticle drug delivery must be:

- **Specific targeting of the diseased tissue with nanoparticles**
  Appropriate size and functionalization with antibodies or other means of selective binding provides means of enhanced delivery of drugs and reduced nonspecific toxicity. This issue can be resolved by functionalization of the nanoparticles with recognition elements on their surfaces towards receptors present on the particular diseased tissue. Conjugation with short chain variable fragments (scFvs) or antibodies will provide selective binding to the specific cell's surface, and their endocytosis will be enhanced with suitably adjusted binding affinities.
- **Timed release of the drug**
  To prevent nonspecific toxicity the drug must not diffuse out of the particle while it is still in the circulatory system, and must remain encapsulated until the particle

binds to the target. For addressing this issue, nanoparticles with multilayeres can be engineered, where each layer will contain one drug from the cocktail, and their release will be sequenced in accordance with the appropriate timing of the delivery of each drug for combination therapy.

Nanoparticles can be significantly used in targeted drug delivery at the site of disease

- Improve the drug bioavailability
- Targeting of drugs to a specific site
- To improve the uptake of poorly soluble drugs

Chemotherapeutic agents such as dexamethasone, doxorubicin 5-fluorouracil and paclitaxel have been successfully formulated using nanomaterials. To encapsulate dexamethasone (a glucocorticoid with an intracellular site of action) polylactic/glycolic acid (PLGA) and polylactic acid (PLA) based nanoparticles have been used. Dexamethasone potentially binds to the cytoplasmic receptors and the subsequent drug-receptor complex is transported to the nucleus resulting in the expression of certain genes that control cell proliferation [113]. Site-specific-targeted drug delivery is important for such class of drugs in the therapeutic modulation of effective drug dose and disease control. NPs have been reported for their potential use in targeting to improve the bioavailability, reducing side effects, decreasing toxicity to other organs. This less costly NP-based drug delivery is feasible in hydrophilic and hydrophobic states through variable routes of administration such as oral, vascular, and inhalation. Advantages of using nanoparticles in drug development and discovery

- Nanoparticles can better deliver drugs to tiny areas within the body.
- Engineering on this scale enables researchers to exercise exquisite and previously unthinkable control over the physical attributes of polymers and other biomaterials.
- Nanocarriers holds promise to deliver biotech drugs over various anatomic extremities of body such as blood brain barrier [114]
- Nanoparticles aid in efficient drug delivery to improve aqueous solubility of poorly soluble drugs that enhance Bioavailability for timed release of drug molecules, and precise drug targeting.
- Nanoparticles overcome the resistance offered by the physiological barriers in the body
- Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
- Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
- Targeted nano drug carriers reduce drug toxicity and provide more efficient drug distribution.
- The surface properties of nanoparticles can be modified for targeted drug delivery for e.g. small molecules, proteins, peptides, and nucleic acids loaded nanoparticles are not recognized by immune system and efficiently targeted to particular tissue types.

- The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc. [115]
- They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects because efficient delivery of drug to various parts of the body is directly affected by particle size.

2.6.25 Tumor Targeting Using Nanoparticulate Delivery Systems

The rationale of using nanoparticles for tumor targeting is based on one of the most efficiency of nanoparticles is delivering drug in the area of the tumor targets via the enhanced permeability and retention effect. This can also be achieved by active targeting by ligands on the surface of nanoparticles

Nanoparticles limits the drug distribution to target organ, hence reduces the drug exposure against healthy tissues. It was reported that mice treated with doxorubicin based poly (isohexylcyanoacrylate) nanopsheres showed higher concentrations of doxorubicin in the liver, spleen and lungs than in mice treated with free doxorubicin [116]. It was also demonstrated that polymeric composition of nanoparticles such as biodegradation profile of the polymer along with the associated drug's molecular weight, polymer type, its localization in the nanospheres and mode of incorporation technique, adsorption or incorporation and hydrophobicity have a great influence on the drug distribution pattern *in vivo*. In addition nanaoparticles are advantageous in their rapid nanoparticles rate, within 1/2 h to 3 h, and it likely involves MPS and endocytosis/phagocytosis process [117].

Earlier report suggested the biodistribution and pharmacokinetics (PK) pattern of a cyclic RGD doxorubicin-nanoparticle formulation in tumor bearing mice [118]. During this biodistribution study it was revealed that drug concentrations over time in the heart, lung, kidney and plasma was decreases and drug accumulation has been found in the liver, spleen and tumor. Maximum of the injected dose was observed in the liver (56 %) and only 1.6 % in the tumor at 48 h post injection. This study ensures that nanoparticles have a great tendency to be captured by liver. This and several other studies indicates the greatest challenge of using nanoparticles for tumor targeting is to avoid particle uptake by mononuclear phagocytic system (MPS) in liver and spleen. Such tendency of mononuclear phagocytic system for endocytosis/phagocytosis of nanoparticles provides an opportunity to effectively deliver therapeutic agents to these cells. This biodistribution can be of benefit for the chemotherapeutic treatment of mononuclear phagocytic system rich organs/tissues localized tumors

- Brochopulmonary tumors
- Gynaecological cancers
- Hepatic metastasis arising from digestive tract
- Hepatocarcinoma,

- Mall cell tumors
- Myeloma and leukemia
- Primitive tumors and metastasis

According to earlier report it has been proven that the utilization of doxorubicin loaded conventional nanoparticles was effective against hepatic metastasis model in mice. Moreover it was also discovered that greater reduction in the degree of metastasis than when free drug was used. This is possible due to the allocation of drug reservoir to the malignant tissues and transfer of doxorubicin from healthy tissue, resulting in increased therapeutic efficacy of the formulation [119]. Several other parameters such as histological examination of tissue ensures considerable accumulation of nanoparticles in the lysosomal vesicles of Kupffer cells, whereas nanoparticles could not be clearly identified in tumoral cells [119].

Following a massive uptake of nanoparticles by phagocytosis, Kupffer cells, potentially induce the release of doxorubicin, resulting in to drug concentration gradient, favorable for a prolonged diffusion of the free and still active drug towards the neighboring metastatic cells [119].

When conventional nanoparticles are used during chemotherapy, toxicity up to certain extent against the Kupffer cells can be expected would result in deficiency of Kupffer cells. This may finally resulted in to the reduced liver uptake and decreased therapeutic effect [120]. In addition, conventional nanoparticles can also be a excellent target for bone marrow. Bone marrow site is an important but unfavorable site of action for most anticancer drugs since treatment with chemotherapeutic agents at this site increase myelosuppresive effect. Thus potential of conventional nanoparticles to improve anticancer drugs efficacy is limited to targeting tumors at the level of mononuclear phagocytic system -rich organs. Furthermore, targeting anticancer drug-loaded nanoparticles to other tumoral sites is not feasible if a rapid clearance of nanoparticles occurs shortly after intravenous administration.

2.6.26 Long-Circulating and Target-Specific Nanoparticles

Nanoparticles are known to be the most successful way of delivering drug at desirable site. They can be used to target tumors which are localized outside mononuclear phagocytic system rich organs. Instantaneous identification of colloidal carriers (liposomes and polymeric nanospheres) from the blood by Kupffer cells, has begin a surge of development for “Kupffer cell-evading” or long-circulating particles. These carriers have major applications in vascular drug delivery and release, site-specific targeting (passive as well as active targeting), as well as transfusion medicine. So much effort has been done to develop so-called “stealth” particles (PEGylated nanoparticles), which are invisible to macrophages or phagocytes [121].

Utilization of hydrophilic polymers (poloxamines, poloxamers, polyethylene glycol, and polysaccharides) has begun a major breakthrough in the nanotechnol-

ogy field to efficiently coat conventional nanoparticle surface produced an opposing effect to the uptake by the mononuclear phagocytic system [122]. coating around these nanoparticles provide a active "cloud" of hydrophilic and neutral chains at the particle surface which repel plasma proteins [123], resulting in to the invisibility of coated nanoparticles against mononuclear phagocytic system, therefore, remained in the circulation for a longer period of time. Introduction of hydrophilic polymers can be achieved in two ways, either by adsorption of surfactants or by use of block or branched copolymers for production of nanoparticles.

Several studies also proved that PEG coated nanoparticles have a prolonged half-life in the blood compartment. However they can be selectively extravasate in pathological sites such as tumors or inflamed regions with a leaky vasculature, resulting in increase in potential of long-circulating nanoparticles directly towards targeted tumors located outside MPS-rich regions. Several characteristics such as size of the colloidal carriers and surface characteristics are critical to the biological fate of nanoparticles.

Nanoparticle size (less than 100 nm) and surface characteristics (hydrophilic surface) obligations are required in achieving the reduction of opsonisation reactions and subsequent clearance by macrophages. Surface coating of conventional nanoparticles with PEG or surfactants to obtain a long-circulating carrier has now been used as a standard strategy for drug targeting *in vivo*. Various researches have been contributed to achieve "active targeting" of nanoparticles in order to deliver drugs to the right targets. This is particularly based on molecular recognition processes such as ligand-receptor or antigen-antibody interaction. Based on earlier reports related with overexpression of folate receptors on the surface of some human malignant cells and the cell adhesion molecules (selectins and integrins), it was concluded that nanoparticles bearing specific ligands such as folate may be used to target ovarian carcinoma while specific peptides or carbohydrates may be used to target integrins and selectins [124]. In one more report it was suggested that the benefits of folate ligand coating were to facilitate tumor cell internalization and retention of Gd-nanoparticles in the tumor tissue [125]. Small ligands based targeting emerges more likely to be successful since they are easier to handle and manufacture.

In addition they could be more significant when the active targeting ligands are used in combination with the long-circulating nanoparticles. Such combination can be used to maximize the likelihood of the success in active targeting of nanoparticles. Since cancer cells are able to develop mechanisms of resistance, deterioration of multidrug resistance anticancer drugs in tumor cells or in the tumor interstitium occurs, resulting in to their limited efficacy against numerous solid tumors. Such mechanism facilitates tumors to evade chemotherapy. One of the most serious problems in chemotherapy is multidrug resistance, mainly due to the over expression of the plasma membrane pglycoprotein (Pgp), which is capable of extruding various positively charged xenobiotics, including some anticancer drugs, out of cells. Several strategies including the use of colloidal carriers have been applied in order to restore the tumoral cells' sensitivity to anticancer drugs by circumventing Pgp-mediated MDR. The underlying principle behind the drugs association with colloidal carriers (such as nanoparticles), against drug resistance derives from the fact that

Pgp probably recognizes the drug to be effluxed out of the tumoral cells. This can be achieved only when this drug is present in the plasma membrane, and not when it is located in the cytoplasm or lysosomes after endocytosis [126].

2.6.27 *Nanoparticles for Oral Delivery of Peptides and Proteins*

Exploration of more antigenic substances in biotechnology and biochemistry field persist the production of vaccines. Owing to the recent advancement biotechnology various bio macromolecules and vaccines are explored. Thus there is an urgent requirement of their suitable carriers system which still remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymer based nanoparticles facilitates the encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation e.g. insulin-loaded polymeric nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration. It's universally known that the surface area of human mucosa extends to 200 times that of skin [127]. Protein or peptide based drug delivery encountered a variety of physiological and morphological barriers:

- Bacterial gut flora
- Mucus layer and epithelial cell lining itself
- Proteolytic enzymes at the brush border membrane (endopeptidases)
- Proteolytic enzymes in the gut lumen like pepsin, trypsin and chymotrypsin

Histological feature of intestinal mucosa is designed in such a way to efficiently prevent uptake of particulate matter from the environment. To overcome the gastrointestinal barrier, drug can be delivered in a colloidal carrier system (nanoparticles), which can potentially enhance the interaction mechanisms of the drug delivery system and the epithelia cells in the GI tract. Nanoparticles based targeting to epithelial cells using ligands is an potential strategy to improve the interaction of nanoparticles with adsorptive enterocytes and M-cells of Peyer's patches in the GI tract. Such type of targeting can be classified into those utilizing specific binding to ligands or receptors and those based on nonspecific adsorptive mechanism. M cells display cell-specific carbohydrates and enterocytes surface may serve as binding sites to colloidal drug carriers containing appropriate ligands. Several glycoproteins and lectins efficiently bind to this type of surface structure by specific receptor-mediated mechanism. Lectins such as tomato lectin and bean lectin have been studied to enhance oral peptide adsorption [128]. For an instance vitamin B-12 absorption from the gut under physiological conditions (occurs via receptor-mediated endocytosis) can be enhanced by covalent coupling with peptides such as granulocyte colony stimulating factor, erythropoietin, resulting in enhancement of oral bioavailability [129]. For making process more efficient and selective mucoprotein is required,

which is prepared by the mucus membrane in the stomach and binds specifically to cobalamin. After reaching to the ileum mucoprotein resorption is mediated by selective binding to specific receptors which can further enhance the absorption. Absorption can also be enhanced by using non-specific interactions. Generally, absorption of macromolecules and particulate materials in the gastrointestinal tract is achieved by either paracellular route or endocytotic pathway. Less than 1 % of mucosal surface area is utilized for the paracellular route of absorption of nanoparticles having polymeric material such as starch or chitosan, poly(acrylate) [130–132]. Such polymers enhance the paracellular permeability of macromolecules. Absorption of nanoparticles mediated by endocytotic pathway can be accomplish by receptor-mediated endocytosis (active targeting) or adsorptive endocytosis which does not need any ligands. Existence of electrostatic forces (hydrogen bonding or hydrophobic interactions) between the cell surface and absorbed material encourages this whole process. Adsorptive endocytosis primarily dependent on the size and surface properties of the material e.g. positive surface charged nanoparticles provide an affinity to adsorptive enterocytes though hydrophobic, whereas negative surface charged nanoparticles and hydrophilic shows greater affinity to adsorptive enterocytes and M cells. So it has been concluded that of size, surface charge and hydrophilicity play a major role in absorption of material.

2.6.28 Nanoparticles for Gene Delivery

Several vaccines based nanomedicines functions to deliver genes to host cells and show their expression by production of antigenic protein to initiate immune response. One of the recent example of polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells (where they are expressed) producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such types of vaccines are responsible for the both humoral and cell-mediated immunity since intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system. Polynucleotide vaccines are composed of a key ingredient called as DNA, can be produced economically and has much better storage and handling properties than the ingredients of the majority of protein-based vaccines. Based on the potential immunotherapy these polynucleotide vaccines are set to supersede many conventional vaccines. Nevertheless these polynucleotides based vaccine suffers from several issues which limit their application. These issues include efficient delivery of the polynucleotide to the target cell population and its localization to the nucleus of these cells, and ensuring that the integrity of the polynucleotide is maintained during delivery to the target site are still in consideration. Owing to the rapid escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment, plasmid DNA based nanoparticulate system serve as a potential sustained release gene delivery system. Previous finding suggested the intracellular uptake and endolysosomal escape of these nanoparticles offer sustained release of DNA

resulting in sustained gene expression. Such strategy could be utilized to facilitate bone healing by using PLGA nanoparticles containing therapeutic genes such as bone morphogenic protein [133, 134].

2.6.29 Nanoparticles for Drug Delivery into the Brain

Nervous system is one of the most delicate microenvironments of the body which is protected by the blood–brain barrier (BBB) regulating its homeostasis. The blood–brain barrier (BBB) is the most important factor limiting the development of new drugs for the central nervous system. BBB is a highly complex structure that tightly regulates the movement of ions of a limited number of small molecules and of an even more restricted number of macromolecules from the blood to the brain, protecting it from injuries and diseases. The BBB is characterized by relatively impermeable endothelial cells with tight junctions, enzymatic activity and active efflux transport systems. It effectively prevents the passage of water-soluble molecules from the blood circulation into the CNS, and can also reduce the brain concentration of lipid-soluble molecules by the function of enzymes or efflux pumps. BBB only permits selective transport of molecules that are essential for brain function, consequently, the BBB also significantly precludes the delivery of drugs to the brain, thus, preventing the therapy of a number of neurological disorders. As a consequence, several strategies are currently being sought after to enhance the delivery of drugs across the BBB. For example polysorbate 80/LDL, transferrin receptor binding antibody (such as OX26), lactoferrin, cell penetrating peptides and melano transferrin have been shown capable of delivery of a self non transportable drug into the brain via the chimeric construct that can undergo receptor-mediated transcytosis [135–139]. It has been discovered that poly(butylcyanoacrylate) based nanoparticles was able to deliver dalargin, hexapeptide, doxorubicin and other agents into the brain which is significant because of the great difficulty for drugs to cross the BBB. In addition to several reports which are based on the success of polysorbate coated NPs, this system does have many shortcomings including rapid NP degradation, toxicity caused by presence of high concentration of polysorbate and desorption of polysorbate coating. In addition to some reports OX26 MAbs (anti-transferrin receptor MAbs), the most studied BBB targeting antibody, have been used to enhance the BBB penetration of lipsosomes. Presently, there are no effective therapies for many diseases include neurodegeneration (e.g., amyotrophic lateral sclerosis, Alzheimer's, Parkinson's, Huntington disease, and Prion disease), genetic deficiencies (e.g. lysosomal storage diseases, leukodystrophy), and several types of brain cancer. Even if candidate drugs for therapy of such diseases may be already available in line of principle, they cannot be currently utilized because of their insignificant access to the central nervous system (CNS), due to the presence of the blood–brain barrier (BBB) preventing the passage from blood to the brain. It is possible soon we will see these BBB specific molecules used for targeting nanoparticles to the brain.

2.6.30 Anthrax Vaccine Uses Nanoparticles to Produce Immunity

Anthrax is caused by the spore-forming, Gram-positive bacterium *Bacillus anthracis*. The toxic effects of *B. anthracis* are predominantly due to an AB-type toxin made up of the receptor-binding subunit protective antigen (PA) and two enzymatic subunits called lethal factor and edema factor. Protective immunity to *B. anthracis* infection is conferred by antibodies against PA, which is the primary component of the current anthrax vaccine. A vaccine against anthrax that is more effective and easier to administer than the present vaccine has proved highly effective in tests in mice and guinea pigs, report University of Michigan Medical School scientists in the August issue of Infection and Immunity. Although the vaccine is safe and effective, it requires multiple injections followed by annual boosters. The scientists were able to trigger a strong immune response by treating the inside of the animals' noses with a "nanoemulsion" a suspension of water, soybean oil, alcohol and surfactant emulsified to create droplets of only 200–300 nm in size. The oil particles are small enough to ferry a key anthrax protein inside the nasal membranes, allowing immune-system cells to react to the protein and initiate a protective immune response. That primes the immune system to promptly fight off infection when it encounters the whole microbe. It would take about 265 of the droplets lined up side by side to equal the width of a human hair. The oil particles are small enough to ferry a key anthrax protein inside the nasal membranes, allowing immune-system cells to react to the protein and initiate a protective immune response. That primes the immune system to promptly fight off infection when it encounters the whole microbe. Besides eliminating the need for needles, the nanoemulsion anthrax vaccine has another advantage, the researchers say: It is easy to store and use in places where refrigeration is not available. An effective and easy-to-administer vaccine would be a valuable tool for health authorities dealing with any future attack in which a terrorist might spread anthrax microbes. The researchers say a nasal nanoemulsionbased anthrax vaccine, if it proves effective in humans, could be given easily to people even after they are exposed in an anthrax attack, along with antibiotics. With some diseases, vaccines given after exposure are used to boost the speed of the immune response.

2.6.31 Stem Cell Therapy

Nanotechnology presents efficient tools for improving stem cell therapy. The synergy between size, structure and physical properties of NPs makes them key players in revealing the fate and performance of stem cell therapy. Clearly NPs have much to offer in stem cell research and therapy. Stem cell therapies offer great potentials in the treatment for a wide range of diseases and conditions. With so many stem cell replacement therapies going through clinical trials currently, there is a great need to understand the mechanisms behind a successful therapy, and one of the critical

points of discovering them is to track stem cell migration, proliferation and differentiation in vivo. To be of most use tracking methods should ideally be noninvasive, high resolution and allow tracking in three dimensions. Magnetic resonance imaging is one of the ideal methods, but requires a suitable contrast agent to be loaded to the cells to be tracked, and one of the most wide-spread in stem cell tracking is a group of agents known as magnetic nanoparticles. Researchers have successfully used nanoparticles to improve stem cells potential in stimulating the regeneration of damaged vascular tissue and reduce muscle degeneration in mice (published online on October 5 in Proceedings of the National Academy of Sciences). In addition researchers investigating stem cells role in stimulating new blood vessel formation. This was investigated after their implantation into a living organism. Cells may not continue to renew tissue effectively enough to keep the tissue alive long-term. Hence cells can benefit from help with performance-enhancing genes, which promote growth in the target tissue. Researchers usually rely on viral vectors to deliver these therapeutic genes to stem cells. It has been now investigated that (Chemistry researchers at the University of Warwick) tiny nanoparticles can be utilized for delivering this therapeutic gene since tiny nanoparticles could be twice as likely to stick to the interface of two non mixing liquids than previously believed. This research open gateways for the utilization of nanoparticles in living cells, polymer composites, and high-tech foams, gels, and paints.

2.6.32 Gold Nanoparticles Detect Cancer

Metallic nanostructures are more flexible particles compared to other nanomaterials owed to the possibility of controlling the size, shape, structure, composition, assembly, encapsulation and tunable optical properties. Between the metallic nanostructures possible applied, AuNPs appears of great interest in the medical field, 3showing great efficiency towards cancer therapy [140–143]. The continuous interest in AuNPs is based in their tunable optical properties that can be controlled and modulated for the treatment and diagnosis of diseases. Various researchers have utilized gold nanoparticles as ultrasensitive fluorescent probes to detect cancer biomarkers in human blood. High sensitivity of this approach surpass the current methods by several orders of magnitude and make the process more suitable for direct detection of viral or bacterial DNA. These nanoparticles are promising probes for biomedical applications since they can be easily manufactured and, unlike other fluorescent probes (quantum dots or organic dyes), they don't get heated and burn out after long exposure to light. In one report, china based researchers apply the particles to detect carcinoembryonic antigen (CEA) and alpha foetal protein (AFP) - two important biomarkers in the diagnosis of various cancers, including liver, breast and lung cancer. In this work they have conjugated nanoparticles with antibodies to measure the amount of biomarker levels present in the sample.

2.6.32.1 AuNPs in Cancer Therapy

- **Photothermal Therapy**
  AuNPs presents tunable optical properties that allow the absorption of light at near UV to near infrared, being the last one a characteristic that allows nanoparticles to enter cells, constituting a major breakthrough for its application in photothermal therapy or hyperthermia [144].
- **Radiotherapy**
  AuNPs have been review in radiotherapy experiments in order to overcome the problems associated to the healthy tissue damage imposed by radiotherapy [144].
- **Angiogenesis inhibition**
  The inhibition of angiogenesis, i.e. the formation process of new blood vessels, is also a potent mechanism by which AuNPs can operate for cancer therapy [144].

2.6.32.1.1 AuNPs as Delivery Systems

The well-known application of AuNPs in cancer therapy described above, lead to further investigation of new potential therapeutic strategies and was verified that AuNPs can be used in the design of delivery systems [145]. AuNPs as a potential nanocarrier have the possibility to carry different payloads, such as small drug molecules for drug delivery or biomolecules like DNA, proteins and RNA (siRNAs), being recognized as an attractive gene delivery system

- **Specific targeting**
  The potency of such systems is achieved by the enhancement of cellular accumulation of AuNPs by an active targeting to cancer cells compared to a free drug that passively enters the cells, which simultaneously avoid the biological response and biophysical barriers *in vivo* [146].
- **AuNPs for drug/cargo delivery**
  The construction of DDSs depends on size, charge and surface functionalities of the AuNPs, once they dictate the uptake capacity of such nanovectorization systems as well as its intracellular fate [147].
- **AuNPs for gene therapy**
  Gene therapy is though as a hopeful strategy in cancer therapy being considered as a powerful treatment like chemotherapy and radiotherapy, however the implementation of such systems is based in viral vectors that raise cytotoxic and immune response problems [148]. When conjugated to AuNPs, siRNAs have been shown to exhibit increased stability, cellular uptake and efficacy in physiological conditions, retaining the ability to act through the RNAi pathway

2.6.32.2 Toxicity of AuNPs

One major concern regarding AuNPs application in medical field relies in its toxicity in the biological systems, i.e. the production of a general toxicity response not only in cancer cells but also reaching healthy cells at the vicinity. Taken into account the size, surface modifications and solubility in promoting biocompatibility of the nanovectorization systems, they can be safer to apply in the medical field to the treatment of cancer. In fact, nanoparticles size is an important feature because it turns possible to circumvent the immune response and renal clearance, which maintains the therapeutic capacity of such systems [149].

2.7 Hazards and Toxicity Profile of Nanoparticles

Various reports are available on the toxicity of nanoparticles (Table 2.10) that are originated from the inhalation toxicology including particulate matter with a size below 10 nm.

2.7.1 Health Implication of Nanoparticles

It's very essential to recognize and differentiate 'free' and 'fixed' nano particles. Free nanoparticles exhibit serious health threat since they are more difficult to contain due to airborne and can be inhaled. Nanoparticles can be entered in human body via

- Absorptions by the intestinal tract
- Absorptions by the skin [151]
- Lungs where a rapid translocation through the blood stream to vital organ is possible, including crossing the BBB and

Nanoparticles affect the following organs in several ways:

- **Lungs**
  It has been already observed that titanium dioxide (TiO_2) carbon black and the diesel particles exhibit various adverse effects. Based on previous findings it has been observed that the administration of ultrafine nanoparticles to the lung produce more potent adverse effect in the form of inflammation and subsequent tumors compared with larger sized particles, of identical chemical composition at equivalent mass concentration. Toxicity of these particles is dependent on their surface charachterstics such as surface chemistry [152]

- **Intestinal tract**
  To facilitate the absorption of the food particles, the epithelium of the small and large intestinal is in close contact with ingested material. These food particles are converted in to a mixture of disaccharides, peptides, fatty acids and monoglycerides generated

Table 2.10 Nanoparticles and their related toxicities

Type of nanoparticles	Toxicity
Carbon nanotubes	• MWCNT: Elicit proinflammatory responses in keretenocytes; Platelet aggregation • On a dose per mass basis carbon nanotubes are more toxic then quartz particles which are well known for their lung toxicity • SWCNT: *In vitro* incubation of high dose of SWCNT with keratinocytes and bronchial epithelial cells results in ROS generation, oxidative stress, lipid peroxidation, mitochondrial dysfunction and changes in cell morphology; Platelet aggregation; Intratracheal instillation of high doses of nanotubes causes chronic lung inflammation, foreign body granuloma formation, interstitial fibrosis; *in vivo* studies SWCNT induce lung granuloma
Dendrimers	• Albertazzi et al. [150] demonstrated the *in vivo* distribution and toxicity of PAMAM dendrimers in the central nervous system depend on their surface chemistry • Limited clinical experience using dendrimers make it impossible to designate any particular chemistry intrinsically "safe" or "toxic"
Fullerenes	• Sonicated c-60 fullrenes LC50 was relatively found to be very high, causes lipid peroxidation and related toxicity in brain
Gold nanoparticles	• For gold nanorods the cytotoxicity is attributed to the presence of stablizer CTAB • Gold nanoparticlse can cause cell death in the presence of activated laser light
Quantum dots	• Cadmium-containing QDs can kill cells in culture • QDs undergo design-dependent intracellular localization and they can cause cytotoxicity by releasing free cadmium into solution and by generating free radical species. In animal experiments, QDs preferentially enter the liver and spleens following intravascular injection, undergo minimal excretion if larger than 6 nm, and appear to be safe to the animal • In vitro and *in vivo* studies show an apparent discrepancy with regard to toxicity. Dosing provides one explanation for these findings. Under culture conditions, a cell experiences a constant QD dose, but the *in vivo* QD concentration can vary, and the organ-specific dose may not be high enough to induce detectable toxicity • Surface coating of quantum dotes during in vitro studies might be toxic. In contrast many studies also report that the surface modification decrease the toxicity induced by naked quantum dots
Silica nanoparticles	• For silica nanoparticles both *in vitro* toxic and non toxic responses have been found • Silica exposure results in an increased ROS levels indicating increase in oxidative stress • Cell with long doubling time is more toxic against silica nanoparticles then with short doubling time • Alveolar macrophage cell line is more susceptible against cytotoxicity then lung epithelial cell line

however digestion in small intestine are further transformed and taken in the villi. Particles having charge (e.g. like carboxylated polystyrene nano particles or those composed of positively charged polymer) exhibit poor oral bioavailability through electrostatic repulsions and means entrapment [153]. Smaller the size of particles facilitate the faster penetration (within 2 mints,); 415 nm particles took 30 mints whereas 1000 nm particles were not capable to translocate through this barrier [154].

- **Skin**
  Particles having size range 500–1000 nm, theoretically afar from the area of nanotechnology can infiltrate and reach the lower level of human skin. Size range smaller than 128 are more likely penetrate deeper in to the skin [155].

- **Blood and cardiovascular system**
  Cationic nanoparticles including gold and polystyrene have been shown to cause hemolysis and blood clotting while anionic nanoparticle are non toxic. Combustion and model nanoparticles can gain access to blood following inhalation and can enhance the experimental thrombosis. High exposure to DEP by inhalation cause altered heart rate in hypertensive rats. Inhalation of PM causes atheromatous plaque and destablization in rabbits. Recent data showed that Carbon derived nanomaterials induce platelet aggregation

- **Brain**
  High concentrations of anionic and cationic nanoparticles are toxic to brain. Nanoparticles have been shown to produce reactive oxygen species and oxidative stress. This has been confirmed in the brain after inhalation of MNO_2 nanoparticles. Oxidative stress induced by nanoparticles causes various neurodegenerative disease such as Parkinson and Alzheimer. In addition inhalation of nanoparticles in balb/c mice to particulate matter showed the activation of pro-inflammatory cytokines in the brain

References

1. Martin CR. Welcome to nanomedicine. Nanomedicine. 2006;1(1):5.
2. Lee CC, Gillies ER, Fox ME, Guillaudeu SJ, Fréchet JM, Dy EE, Szoka FC. A single dose of doxorubicin-functionalized bow-tie dendrimer cures mice bearing C-26 colon carcinomas. Proc Natl Acad Sci U S A. 2006;103:16649–54.
3. Goldberg DS, Vijayalakshmi N, Swaan PW, Ghandehari H. G3.5 PAMAM Dendrimers Enhance Transepithelial Transport of SN38 while minimizing Gastrointestinal Toxicity. J Control Release. 2011;150(3):318–25.
4. Lobenberg R, Maas J, Kreuter J. Improved body distribution of 14Clabelled AZT bound to Mataraza nanoparticles in rats determined by radioluminography. J Drug Target. 1998;5(3):171–9.
5. Brewer E, Coleman J, Lowman A. Emerging technologies of polymeric nanoparticles in cancer drug delivery. J Nanomater. 2011;2011:1–10.
6. Liu Z, Fan AC, Rakhra K. Supramolecular stacking of doxorubicin on carbon nanotubes for in vivo cancer therapy. Angew Chem Int Ed Eng. 2009;48:7668–72.

7. Samori C, Li-Boucetta H, Sainz R. Enhanced anticancer activity of multi-walled carbon nanotube-methotrexate conjugates using cleavable linkers. Chem Commun (Camb). 2010, 46: 1494–6.
8. Mahajan SD, Roy I, Xu G, Yong K-T, Ding H, Aalinkeel R. Enhancing the delivery of anti-retroviral drug "Saquinavir" across the blood brain barrier using nanoparticles. Curr HIV Res. 2010;9:396–404.
9. Dutta T, Jain NK. Targeting potential and anti-HIV activity of lamivudine loaded mannosylated poly(propyleneimine) dendrimer. Biochim Biophys Acta. 2007;1770(4):681–6.
10. Wagner V, Dullaart A, Bock AK, Zweck A. The emerging nanomedicine landscape. Nat Biotechnol. 2006;24(10):1211–7.
11. Petros RA, DeSimone JM. Strategies in the design of nanoparticles for therapeutic applications. Nat Rev Drug Discov. 2010;9(8):615–27.
12. Cai W, Chen X. Nanoplatforms for targeted molecular imaging in living subjects. Small. 2007;3:1840–54.
13. Davis ME, Chen Z. Shin DM Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat Rev Drug Discov. 2008;7(9):771–82.
14. Nahar M, Dutta T, Murugesan S, Asthana A, Mishra D, Rajkumar V, Tare M, Saraf S, Jain NK. Functional polymeric nanoparticles: an efficient and promising tool for active delivery of bioactives. Crit Rev Ther Drug Carrier Syst. 2006;23(4):259–318.
15. Hett A. Nanotechnology: small matters, many unknown. 2004.
16. Vyas SP, Khar RK. Targeted and controlled drug delivery.CBS publishers and distributers. New Delhi. 2002;1:331–43.
17. Redhead HM, Davis SS, Illum LJ. Control. Release. 2001;70:353.
18. Betancor L, Luckarift HR. Trends Biotechnol. 2008;26:566. Dunne M, Corrigan.
19. DeAssis DN, Mosqueira VC, Vilela JM, Andrade MS, Cardoso VN. Release profiles and morphological characterization by atomic force microscopy and photon correlation spectroscopy of 99m Technetium—fluconazole nanocapsules. Int J Pharm. 2008;349:152–60.
20. Jores K, Mehnert W, Drecusler M, Bunyes H, Johan C, MAder K. Investigation on the stricter of solid lipid nanopartuicles and oil-loaded solid nanoparticles by photon correlation spectroscopy, fieldflow fractionasition and transmission electron microscopy. J Control Release. 2004;17:217–27.
21. Molpeceres J, Aberturas MR, Guzman M. Biodegradable nanoparticles as a delivery system for cyclosporine: preparation and characterization. J Microencapsul. 2000;17:599–614.
22. Muhlen AZ, Muhlen EZ, Niehus H, Mehnert W. Atomic force microscopy studies of solid lipid nanoparticles. Pharm Res. 1996;13:1411–6.
23. Shi HG, Farber L, Michaels JN, Dickey A, Thompson KC, Shelukar SD, Hurter PN, Reynolds SD, Kaufman MJ. Characterization of crystalline drug nanoparticles using atomic force microscopy and complementary techniques. Pharm Res. 2003;20:479–84.
24. Polakovic M, Gorner T, Gref R, Dellacherie E. Lidocaine loaded biodegradable nanospheres. II. Modelling of drug release. J Control Release. 1999;60:169–77.
25. Pangi Z, Beletsi A, Evangelatos K. PEG-ylated nanoparticles for biological and pharmaceutical application. Adv Drug Del Rev. 2003;24:403–19.
26. Scholes PD, Coombes AG, Illum L, Davis SS, Wats JF, Ustariz C, Vert M, Davies MC. Detection and determination of surface levels of poloxamer and PVA surfactant on biodegradable nanospheres using SSIMS and XPS. J Control Release. 1999;59:261–78.
27. Kreuter J. Physicochemical characterization of polyacrylic nanoparticles. Int J Pharm. 1983;14:43–58.
28. Magenhein B, Levy MY, Benita S. A new in vitro technique for the evaluation of drug release profile from colloidal carriers ultrafiltration technique at low pressure. Int J Pharm. 1993;94:115–23.
29. Kreuter J. Nanoparticles. In: Kreuter J, editor. Colloidal drug delivery systems. New York: Marcel Dekker; 1994. p. 219–342.

30. Reverchon E, Adami R. Nanomaterials and supercritical fluids. J Supercrit Fluids. 2006;37:1–22.
31. Rolland JP, Maynor BW, Euliss LE, Exner AE, Denison GM, DeSimone JM. Direct fabrication and harvesting of monodisperse, shape-specific nanobiomaterials. J Am Chem Soc. 2005;127:10096–100.
32. Kompella UB, Bandi N, Ayalasomayajula SP. Poly (lactic acid) nanoparticles for sustained release of budesonide. Drug Deliv Technol. 2001;1:1–7.
33. Ravi MN, Bakowsky U, Lehr CM. Preparation and characterization of cationic PLGA nanospheres as DNA carriers. Biomaterials. 2004;25:1771–7.
34. Li YP, Pei YY, Zhou ZH, Zhang XY, Gu ZH, Ding J, Zhou JJ. Gao, XJ, PEGylated polycyanoacrylate nanoparticles as tumor necrosis factor-[alpha] carriers. J Control Release. 2001;71:287–96.
35. Kwon HY, Lee JY, Choi SW, Jang Y, Kim JH. Preparation of PLGA nanoparticles containing estrogen by emulsification-diffusion method. Colloids Surf A Physicochem Eng Aspects. 2001;182:123–30.
36. Zambaux M, Bonneaux F, Gref R, Maincent P, Dellacherie E, Alonso M, Labrude P, Vigneron C. Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by double emulsion method. J Control Release. 1998;50:31–40.
37. Song CX, Labhasetwar V, Murphy H, Qu X, Humphrey WR, Shebuski RJ, Levy RJ. Formulation and characterization of biodegradables nanoparticles for intravascular local drug delivery. J Control Release. 1997;43:197–212.
38. Jaiswal J, Gupta SK, Kreuter J. Preparation of biodegradable cyclosporine nanoparticles by high-pressure emulsification solvent evaporation process. J Control Release. 2004;96:169–78.
39. Soppinath KS, Aminabhavi TM, Kulkurni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release. 2001;70:1–20.
40. Tice TR, Gilley RM. Preparation of injectable controlledrelease microcapsules by solvent-evaporation process. J Control Release. 1985;2:343–52.
41. Tabata J, Ikada Y. Protein pre-coating of polylactide microspheres containing a lipophilic immunopotentiator for enhancement of macrophage phagocytosis and activation. Pharm Res. 1989;6:296–301.
42. Ueda H, Kreuter J. Optimization of the preparation of loperamide- loaded poly (l-lactide) nanoparticles by high pressure emulsification solvent evaporation. J Microencapsul. 1997;14:593–605.
43. Allemann E, Gurny R, Doekler E. Drug-loaded nanoparticlespreparation methods and drug targeting issues. Eur J Pharm Biopharm. 1993;39:173–91.
44. Bodmeier R, Chen H. Indomethacin polymeric nanosuspensions prepared by micro- fluidization. J Control Release. 1990;12:223–33.
45. Koosha F, Muller RH, Davis SS, Davies MC. The surface chemical structure of poly (-hydroxybutyrate) microparticles produced by solvent evaporation process. J Control Release. 1989;9:149–57.
46. Lemarchand C, Gref R, Passirani C, Garcion E, Petri B, Muller R. Influence of polysaccharide coating on the interactions of nanoparticles with biological systems. Biomaterials. 2006;27:108–18.
47. Niwa T, Takeuchi H, Hino T, Kunou N, Kawashima Y. Preparation of biodegradable nanoparticles of water-soluble and insoluble drugs with D, Llactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. J Control Release. 1993;25:89–98.
48. Vandervoort J, Ludwig A. Biodegradable stabilizers in the preparation of PLGA nano particles: a factorial design study. Int J Pharm. 2002;238:77–92.
49. Ubrich N, Bouillot P, Pellerin C, Hoffman M, Maincent P. Preparation and characterization of propanolol hydrochloride nano particles: a comparative study. J Control Release. 2004;19:291–300.
50. Couvreur P, Dubernet C, Puisieux F. Controlled drug delivery with Nano particles: current possibilities and future trends. Eur J Pharm Biopharm. 1995;41:2–13.

51. Jung T, Kamm W, Breitenbach A, Kaiserling E, Xiao JK, Kissel T. Biodegradable nano particles for oral delivery of peptides: is there a role for polymer to affect mucosal uptake? Eur J Pharm Biopharm. 2000;50:147–60.
52. Quintanar-Guerrero D, Allemann E, Fessi H, Doelker E. Preparation techniques and mechanism of formation of biodegradable nanoparticles from preformed polymers. Drug Dev Ind Pharm. 1998;24:1113–28.
53. Lambert G, Fattal E, Couvreur P. Nanoparticulate system for the delivery of antisense oligonucleotides. Adv Drug Deliv Rev. 2001;47:99–112.
54. Takeuchi H, Yamamoto Y. Mucoadhesive nanoparticulate system for peptide drug delivery. Adv Drug Del Rev. 2001;47:39–54.
55. Vargas A, Pegaz B, Devefve E, Konan-Kouakou Y, Lange N, Ballini JP. Improved photodynamic activity of porphyrin loaded into nano particles: an in vivo evaluation using chick embryos. Int J Pharm. 2004;286:131–45.
56. El-shabouri MH. Positively charged nano particles for improving the oral bioavailability of cyclosporine-A. Int J Pharm. 2002;249:101–8.
57. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nano capsule formation by interfacial deposition following solvent displacement. Int J Pharm. 1989;55:R1–4.
58. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Novel hydrophilic chitosan-polyethylene oxide nanoprticles as protein carriers. J Appl Polym Sci. 1997;63:125–32.
59. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. Pharm Res. 1997;14:1431–6.
60. Zhang Q, Shen Z, Nagai T. Prolonged hypoglycemic effect of insulin-loaded polybutylcyanoacrylate nanoparticles after pulmonary administration to normal rats. Int J Pharm. 2001;218:75–80.
61. Boudad H, Legrand P, Lebas G, Cheron M, Duchene D, Ponchel G. Combined hydroxypropyl-[beta]- cyclodextrin and poly(alkylcyanoacrylate) nanoparticles intended for oral administration of saquinavir. Int J Pharm. 2001;218:113–24.
62. Puglisi G, Fresta M, Giammona G, Ventura CA. Influence of the preparation conditions on poly(ethylcyanoacrylate) nanocapsule formation. Int J Pharm. 1995;125:283–7.
63. Jung J, Perrut M. Particle design using supercritical fluids: literature and patent survey. J Supercrit Fluids. 2001;20:179–219.
64. Sun Y, Mezian M, Pathak P, Qu L. Polymeric nanoparticles from rapid expansion of supercritical fluid solution. Chemistry. 2005;11:1366–73.
65. Iijima S. Helical microtubules of graphitic carbon. Nature. 1991;354:56–8.
66. Reilly RM. Carbon nanotubes: potential benefits and risks of nanotechnology in nuclear medicine. J Nucl Med. 2007;48:1039–42.
67. Saad MZH, Jahan R, Bagul U. Nanopharmaceuticals: a New perspective of drug delivery system. Asian J Biomed Pharm Sci. 2012;2:14.
68. McDevitt MR, Chattopadhyay D, Kappel BJ, Jaggi JS, Schiffman SR, Antczak C, et al. Tumor targeting with antibody-functionalized, radiolabeled carbon nanotubes. J Nucl Med. 2007;48:1180–9.
69. Prato M, Kostarelos K, Bianco A. Functionalized carbon nanotubes in drug design and discovery. Acc Chem Res. 2008;41:60–8.
70. Zhang Z, Yang X, Zhang Y, Zeng B, Wang S, Zhu T, et al. Delivery of telomerase reverse transcriptase small interfering RNA in complex with positively charged single-walled carbon nanotubes suppresses tumor growth. Clin Cancer Res. 2006;12:4933–9.
71. Sinha N, Yeow JTW. Carbon nanotubes for biomedical applications. IEEE Trans Nanobioscience. 2005;4(2):180–95.
72. Thakral S, Mehta RM. Fullerenes: an introduction and overview of their biological properties. Ind J Pharm Sci. 2006;68:13–9.
73. Kratschmer W, Lamb LD, Fostiropoulos K, Hoffman DR. Solid C 60: a new form of carbon. Nature. 1990;347:354–8.

74. Taylor R, Hare JP, Abdul-Sada AK, Kroto HW. Isolation, separation and characterisation of the fullerenes C60 and C70: the third form of carbon. J Chem Soc Chem Commun. 1990;20:1423–5.
75. Chandrakumar KR, Ghosh SK. Alkali-metal-induced enhancement of hydrogen adsorption in C60 fullerene: an ab initio study. Nano Lett. 2008;8:13–9.
76. Fatouros PP, Corwin FD, Chen ZJ, Broaddus WC, Tatum JL, Kettenmann B, et al. Invitro and invivo imaging studies of a new endohedral metallofullerene nanoparticle. Radiology. 2006;240:756–64.
77. Komatsu K, Murata M, Murata Y. Encapsulation of molecular hydrogen in fullerene C60 by organic synthesis. Science. 2005;307:238–40.
78. Chen BX, Wilson SR, Das M, Coughlin DJ, Erlanger BF. Antigenicity of fullerenes: antibodies specific for fullerenes and their characteristics. Proc Natl Acad Sci U S A. 1998;95:10809–13.
79. Mroz P, Pawlak A, Satti M, Lee H, WhartonT GH, et al. Functionalized fullerenes mediate photodynamic killing of cancer cells: Type I versus Type II photochemical mechanism. Free Radic Biol Med. 2007;43:711–9.
80. Tegos GP, Demidova TN, Arcila-Lopez D, Lee H, Wharton T, Gali H, et al. Cationic fullerenes are effective and selective antimicrobial photosensitizers. Chem Biol. 2005;12:1127–35.
81. Bosi S, Da RT, Castellano S, Banfi E, Prato M. Antimycobacterial activity of ionic fullerene derivatives. Bioorg Med Chem Lett. 2000;10:1043–5.
82. Ji H, Yang Z, Jiang W, Geng C, Gong M, Xiao H, et al. Antiviral activity of nano carbon fullerene lipidosome against influenza virus in vitro. J Huazhong Univ Sci Technolog Med Sci. 2008;28:243–6.
83. Cai X, Jia H, Liu Z, Hou B, Luo C, Feng Z, et al. Polyhydroxylated fullerene derivative C(60)(OH)(24) prevents mitochondrial dysfunction and oxidative damage in an MPP(+)-induced cellular model of Parkinson's disease. J Neurosci Res. 2008;86:3622–34.
84. Markovic Z, Trajkovic V. Biomedical potential of the reactive oxygen species generation and quenching by fullerenes (C60). Biomaterials. 2008;29:3561–73.
85. Iga AM, Robertson JH, Winslet MC, Seifalian AM. Clinical potential of quantum dots. J Biomed Biotechnol. 2007;2007:76087–97.
86. Bailey RE, Smith AM, Nie S. Quantum dots in biology and medicine. Physica E. 2004;25:1–12.
87. Gao X, Cui Y, Levenson RM, Chung LWK, Nie S. In-vivo cancer targeting and imaging with semiconductor quantum dots. Nat Biotechnol. 2004;22:969–76.
88. Amiot CL, Xu S, Liang S, Pan L, Zhao JX. Near-infrared fluorescent materials for sensing of biological targets. Sensors. 2008;8:3082–105.
89. West JL, Halas NJ. Applications of nanotechnology to biotechnology commentary. Curr Opin Biotechnol. 2000;11:215–7.
90. Kherlopian AR, Song T, Duan Q, Neimark MA, Po MJ, Gohagan JK, et al. A review of imaging techniques for systems biology. BMC Syst Biol. 2008;2:74–92.
91. Klibanov AL. Microbubble contrast agents: targeted ultrasound imaging and ultrasound-assisted drug-delivery applications. Invest Radiol. 2006;41:354–62.
92. Gao Z, Kennedy AM, Christensen DA, Rapoport NY. Drugloaded nano/microbubbles for combining ultrasonography and targeted chemotherapy. Ultrasonics. 2008;48:260–70.
93. Negishi Y, Endo Y, Fukuyama T, Suzuki R, Takizawa T, Omata D, et al. Delivery of siRNA into the cytoplasm by liposomal bubbles and ultrasound. J Control Release. 2008;132:124–30.
94. Suzuki R, Takizawa T, Negishi Y, Utoguchi N, Maruyama K. Effective gene delivery with novel liposomal bubbles and ultrasonic destruction technology. Int J Pharm. 2008;354:49–55.
95. Iverson N, Plourde N, Chnari E, Nackman GB, Moghe PV. Convergence of nanotechnology and cardiovascular medicine: progress and emerging prospects. BioDrugs. 2008;22:1–10.
96. Cuenca AG, Jiang H, Hochwald SN, Delano M, Cance WG, Grobmyer SR. Emerging implications of nanotechnology on cancer diagnostics and therapeutics. Cancer. 2006;107:459–66.

97. Artemov D, Mori N, Okollie B, Bhujwalla ZM. MR molecular imaging of the Her-2/neu receptor in breast cancer cells using targeted iron oxide nanoparticles. Magn Reson Med. 2003;49:403–8.
98. Aduro Biotech. Berkeley: Oncologic and Triton BioSystems Merge to Form Aduro BioTech Aduro to Focus on NT™ and TNT™ Systems for Solid Tumor Cancers. 2008. Available from http://www.tritonsys. com/news/Aduro.pdf. Accessed 16 May 2009.
99. Nam JM, Thaxton CS, Mirkin CA. Nanoparticle-based biobar codes for the ultrasensitive detection of proteins. Science. 2003;301:1884–6.
100. Moore A, Weissleder R, Bogdanov Jr A. Uptake of dextrancoated monocrystalline iron oxides in tumor cells and macrophages. J Magn Reson Imaging. 1997;7:1140–5.
101. Xu H, Yan F, Monson EE, Kopelman R. Room-temperature preparation and characterization of poly (ethylene glycol)-coated silica nanoparticles for biomedical applications. J Biomed Mater Res A. 2003;66:870–9.
102. Freitas RA. Pharmacytes: an ideal vehicle for targeted drug delivery. J Nanosci Nanotechnol. 2006;6:2769–75.
103. Moghimi SM, Hunter AC, Murray JC. Nanomedicine: current status and future prospects. FASEB J. 2005;19:311–30.
104. Baker JR, Quintana A, Piehler L, Banazak-Holl TD, Raczka E. The synthesis and testing of anti-cancer therapeutic nanodevices. Biomed Microdevices. 2001;3:61–9.
105. Pan B, Cui D, Sheng Y, Ozkan C, Gao F, He R, et al. Dendrimermodified magnetic nanoparticles enhance efficiency of gene delivery system. Cancer Res. 2007;67:8156–63.
106. Quiagen California: SuperFect Transfection Reagent. Available from http://www1.qiagen.com-Products/Transfection/Transfection Reagents/SuperFectTransfectionReagent.aspx#Tabs=t1. Accessed 3 May 2008.
107. Tomalia DA, Reyna LA, Svenson S. Dendrimers as multipurpose nanodevices for oncology drug delivery and diagnostic imaging. Biochem Soc Trans. 2007;35:61–7.
108. Freitas Jr RA. Microbivores: artificial mechanical phagocytes using digest and discharge protocol. J Evol Technol. 2005;14:1–52.
109. Freitas Jr RA. Exploratory design in medical nanotechnology: a mechanical artifcial red cell. Artif Cells Blood Substit Immobil Biotechnol. 1998;26:411–30.
110. Freitas Jr. RA. A mechanical artificial red cell: exploratory design in medical nanotechnology [serial on the internet]. Available from http ://www.foresight.org/nanomedicine/ Respirocytes4.html#Sec610, Accessed 29 Sept 2008.
111. Kayser O, Lemke A, Hernández-Trejo N. The impact of nanobiotechnology on the development of new drug delivery systems. Curr Pharm Biotechnol. 2005;6(1):3–5.
112. Kaparissides C, Alexandridou S, Kotti K, Chaitidou S. Recent advances in novel drug delivery systems; 2006.
113. Singh Suri S, Fenniri H, Singh B. Nanotechnology-based drug delivery systems. J Occup Med Toxicol. 2007;2:16.
114. Jahanshahi M, Babaei Z. Protein nanoparticle: a unique system as drug delivery vehicles. Afr J Biotechnol. 2008;7(25):4926–34.
115. Rawat M, Deependra S, Saraf S, Saraf S. Nanocarriers: promising vehicle for bioactive drugs. Biol Pharm Bull. 2006;29(9):1790–8.
116. Verdun C, Brasseur F, Vranckx H, Couvreur P, Roland M. Tissue distribution of doxorubicin associated with polyhexylcyanoacrylate nanoparticles. Cancer Chemother Pharmacol. 1990;26:13–8.
117. Couvreur P, Kante B, Lenaerts V, Scailteur V, Roland M, Speiser P. Tissue distribution of antitumor drugs associated with polyalkylcyanoacrylate nanoparticles. J Pharm Sci. 1980;69:199–202.
118. Bibby DC, Talmadge JE, Dalal MK, Kurz SG, Chytil KM, Barry SE, Shand DG, Steiert M. Pharmacokinetics and biodistribution of RGD-targeted doxorubicinloaded nanoparticles in tumor-bearing mice. Int J Pharm. 2005;293:281–90.
119. Chiannilkulchai N, Ammoury N, Caillou B, Devissaguet JP, Couvreur P. Hepatic tissue distribution of doxorubicin-loaded nanoparticles after i.v. administration in reticulosarcoma M 5076 metastasis-bearing mice. Cancer Chemother Pharmacol. 1990;26:122–6.

120. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. Pharmacol Rev. 2001;53:283–318.
121. Storm G, Belliot S, Daemen T, Lasic D. Surface modification of nanoparticles to oppose uptake by the mononuclear phagocyte system. Adv Drug Deliv Rev. 1995;17:31–48.
122. Torchilin V, Trubetskoy V. Which polymer can make nanoparticulate drug carriers long circulating? Adv Drug Deliv Rev. 1995;16:141–55.
123. Jeon SI, Andrade JD. Protein—surface interactions in the presence of polyethylene oxide: II. Effect of protein size. J Colloid Interf Sci. 1991;142:159–66.
124. Stella B, Arpicco S, Peracchia M, Desmaele D, Hoebeke J, Renoir M, d'Angelo J, Cattel L, Couvreur P. Design of folic acid-conjugated nanoparticles for drug targeting. J Pharm Sci. 2000;89:1452–64.
125. Oyewumi MO, Yokel RA, Jay M, Coakley T, Mumper RJ. Comparison of cell uptake, biodistribution and tumor retention of folate-coated and PEG-coated gadolinium nanoparticles in tumor-bearing mice. J Control Release. 2004;95:613–26. Drugs. Eur. J. Cancer Sci 2000. 11:265–83.
126. Larsen AK, Escargueil AE, Skladanowski A. Resistance mechanisms associated with altered intracellular distribution of anticancer agents. Pharmacol Ther. 2000;85:217–29.
127. Brandtzaeg P, Berstad A, Farstad I, Haraldsen G, Helgeland L, Jahnsen F, Johansen F, Natvig I, Nilsen E, Rugtveit J. Mucosal immunity—a major adaptive defense mechanism. Behring Inst Mitt. 1997;98:1–23.
128. Haltner E, Easson J, Lehr C. Lectins and bacterial invasion factors for controlling endo- and transcytosis of bioadhesive drug carrier systems. Eur J Pharm Biopharm. 1997;44:3–13.
129. Hussain N, Jani PU, Florence AT. Enhanced oral uptake of tomato lectin-conjugated nanoparticles in the rat. Pharm Res. 1997;14:613–8.
130. Schipper N, Olsson S, Hoogstrate J, de Boer A, Varum K, Artursson P. Chitosans as absorption enhancers for poorly absorbable drugs. 3: influence of mucus on absorption enhancement. Eur J Pharm Sci. 1999;8:335–43.
131. Lehr C, Bowstra J, Tukker J, Junginer H. Intestinal transit of bioadhesive microspheres in an in situ loop in the rat. J Control Release. 1990;13:51–62.
132. Bjork E, Isakkson U, Edman P, Artursson P. Starch microspheres induce pulsatile delivery of drugs and peptides across the epithelial barrier by reversible separation of the tight junctions. J Drug Target. 1995;6:501–7.
133. Panyam J, Zhou WZ, Prabha S, Sahoo SK, Labhasetwar V. Rapid endo-lysosomal escape of poly(DL-lactide-co-glycolide) nanoparticles: implications for drug and gene delivery. FASEB J. 2002;16:1217–26.
134. Hedley M, Curley J, Urban R. Microspheres containing plasmid-encoded antigens elicit cytotoxic T-cell responses. Nat Med. 1998;4:365–8.
135. Kreuter J. Influence of the surface properties on nanoparticle-mediated transport of drugs to the brain. J Nanosci Nanotechnol. 2004;4:484–8.
136. Pardridge WM. Drug and gene targeting to the brain with molecular Trojan horses. Nat Rev Drug Discov. 2002;1:131–9.
137. Ji B, Maeda J, Higuchi M, Inoue K, Akita H, Harashima H, Suhara T. Pharmacokinetics and brain uptake of lactoferrin in rats. Life Sci. 2006;78:851–5.
138. Scherrmann JM, Temsamani J. The use of Pep: trans vectors for the delivery of drugs into the central nervous system. Int Cong Ser. 2005;1277:199–211.
139. Gabathuler R, Arthur G, Kennard M, Chen Q, Tsai S, Yang J, Schoorl W, Vitalis TZ, Jefferies WA. Development of a potential protein vector (NeuroTrans) to deliver drugs across the bloodbrain barrier. Int Cong Ser. 2005;1277:171–84.
140. Huang X, Jain PK, El-Sayed IH, El-Sayed MA. Gold nanoparticles: interesting optical properties and recent applications in cancer diagnostics and therapy. Nanomedicine. 2007;2(5):681–93.
141. Figueiredo S, Cabral R, Luís D, Fernandes AR, Baptista PV. Integration of gold nanoparticles and liposomes for combined anti-cancer drug delivery. In: Seifalian A. (ed.) Nanomedicine. University College London (UK); 2014. Chapter 3. Available from http://www.onecentralpress.com/nanomedicine/#.

142. Cobley CM, Chen J, Cho EC, Wang LV, Xia Y. Gold nanostructures: a class of multifunctional materials for biomedical applications. Chem Soc Rev. 2011;40(1):44–56.
143. Kumar A, Boruah BM, Liang XJ. Gold nanoparticles: promising nanomaterials for the diagnosis of cancer and HIV/AIDS. J Nanomater. 2011;31:1–17.
144. Boisselier E, Astruc D. Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. Chem Soc Rev. 2009;38(6):1759–82.
145. Wang J, Yao K, Wang C, Tang C, Jiang X. Synthesis and drug delivery of novel amphiphilic block copolymers containing hydrophobic dehydroabietic moiety. J Mater Chem B. 2013;1(17):2324–32.
146. Dreaden EC, Mwakwari SC, Sodji QH, Oyelere AK, El-Sayed MA. Tamoxifen-poly(ethylene glycol)-thiol gold nanoparticle conjugates: enhanced potency and selective delivery for breast cancer treatment. Bioconjug Chem. 2009;20(12):2247–53.
147. Lim ZZJ, Li JEJ, Ng CT, Yung LYL, Bay BH. Gold nanoparticles in cancer therapy. Acta Pharmacol Sin. 2011;32(8):983–90.
148. Hunt KK, Vorburguer SA. Hurdles and hopes for cancer therapy. Science. 2002; 297(5580):415–6.
149. Conde J, Doria G, Baptista P. Noble metal nanoparticles applications in cancer. J Drug Deliv. 2012;2012:1–12.
150. Albertazzi L, Gherardini L, Brondi M, et al. In vivo distribution and toxicity of PAMAM dendrimers in the central nervous system depend on their surface chemistry. Mol Pharm. 2013;10:249–60.
151. Hoet PMH, Brnske HI, Salata OR. Nano particles known and unknown health risk. J Nanobiotecnol. 2004;2:12.
152. Lee KP, Kelly DP, Oneal FO, Kennedy GL. Lung response to ultrafine kertar aramid synthetic fibrils following 2-year inhalation exposure in rats. Findam Appl Toxicol. 1998;11:1–20.
153. Jani P, Halbert GW, Langridge J, Florence AT. Nanoparticles uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. J Pharm Pharmacol. 1990;42(12):812–26.
154. Jani P, Halbert GW, Langridge J, Florence AT. The uptake and translocation of latex nanosphere and microsphere after oral administration to rats. J Pharm Pharmacol. 1989;41(12): 809–12.
155. Lademann J, Weigmann H, Rickmeyer C, Barthelmes H, Schaelmes H, Mueller G, Sterry W. Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. Skin Pharmacol Appl Skin Physiol. 1999;12(5):247–56.

Chapter 3
Natural Polymers vs Synthetic Polymer

Abstract Polymers play an important role as excipients in any dosage form. They influence drug release and should be stable, economic compatible, non-toxic, etc. They are broadly classified as natural polymers and synthetic polymers. Synthetic and natural based biodegradable polymers have received much more attention in the last decades due their potential applications in the fields related to environmental protection and the maintenance of physical health. Biodegradable materials are used in agriculture, medicine packaging, and other areas. In recent years there has been an increase in interest in biodegradable polymers. Two classes of biodegradable polymers can be distinguished: synthetic or natural polymers. Synthetic polymers are widely used in biomedical implants and devices because they can be fabricated into various shapes. Natural polymers are basically polysaccharides so they are biocompatible and without any side effects. In this chapter we have discussed various natural polymers, their advantages over synthetic polymers and role of natural polymers in designing novel drug delivery systems.

Keywords Synthetic polymer • Natural polymer • Polysaccharide • Drug delivery • Toxicity

3.1 Bioengineered Materials: Nano-Engines of Drug Delivery Systems

Engineered materials have been employed for rising smart drug delivery systems. Design and multi-functionalities synthesize efficient smart drug delivery systems are vitally necessary for medicine and healthcare development. In the material science field offers biodegradable, environment-responsive, biocompatible, and highly effective novel polymeric system for targeted delivery. Nanotechnology offers bottom-up and top-down nanofabrication with size controlled and multi-functionality of particulate for targeted delivery. Novel materials invention and advanced technology have been synergistically accomplished in drug delivery so far. The important objectives of medical pharmacology to offer the right medicine, right dosage, and right route at the right time to the right patient, so additional research required to optimize the therapeutic efficacy of the drug. This is the most important principles

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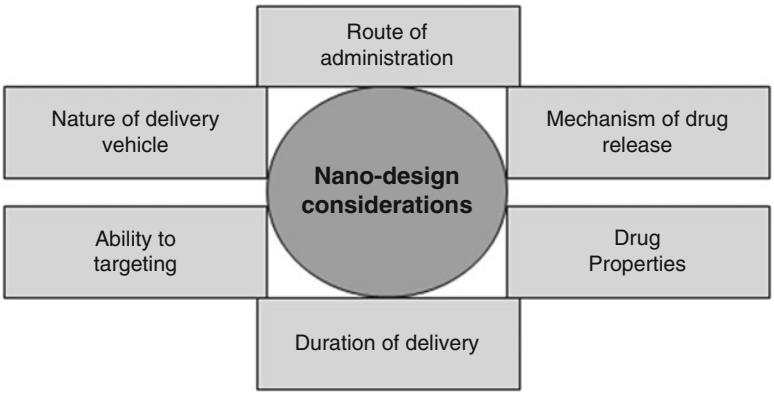

Fig. 3.1 Considerations of various factors for simultaneous reflection to design a polymeric nanoparticle for the smart drug delivery system

behind the smart drug delivery. A smart, controlled delivery system requires synergistic consideration of a number of factors summarized in Fig. 3.1. It is not easy to get all consideration factors in a smart controlled delivery system owing to other influencing factors. Also high efficiency, quality, reliability, and reproducibility are the most considerable issue while designing such a smart system.

3.2 Polymeric Nanoparticles

The polymeric nanoparticles are fabricated from biocompatible and biodegradable polymers in size between 10–1000 nm where the drug is entrapped, dissolved, encapsulated or attached to a nanoparticle matrix. Depending upon the methodology of fabrication nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is restricted to a cavity enclosed by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. The field of polymer nanoparticles (PNPs) is rapidly growing and playing an significant role in a wide spectrum of disciplines ranging from electronics, sensors, medicine, photonics, biotechnology, conducting materials, pollution control and environmental technology. PNPs are promising vehicles for drug delivery by simple manipulation to fabricate carriers with the aim of delivering the drugs to particular target, such an merit advances the drug safety. Polymer based nanoparticles efficiently carry drugs, proteins, and DNA to target cells and organs. Their nanometer size encourages effective permeation via cell membranes and stability in the blood stream. Polymers are very suitable materials for the manufacture of countless and varied molecular designs that can be integrated into exclusive nanoparticle constructs with many potential medical applications. PNPs can be expediently fabricated either from preformed polymers or by direct polymerization of monomers using classical polymerization or polyreactions. Methods like salting-out, dialysis solvent evaporation, and supercritical fluid technology, encompassing the rapid expansion of a supercritical

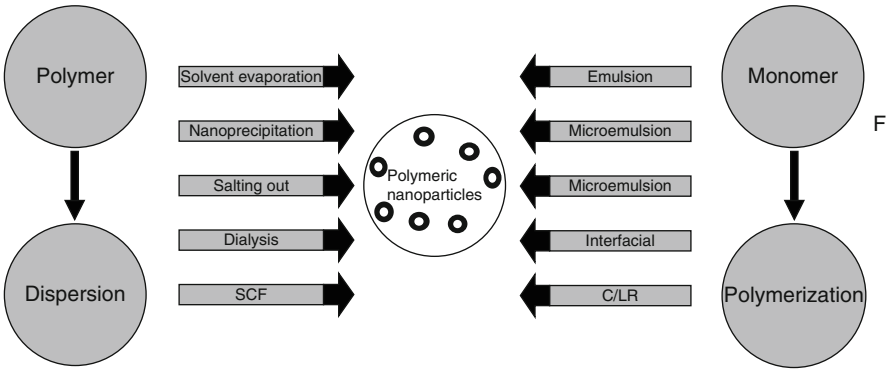

Fig. 3.2 Plan illustration of different techniques for the preparation of polymeric nanoparticles

solution or rapid expansion of a supercritical solution into liquid solvent, can be employed for the fabrication of polymeric nanoparticles from preformed polymers. Alternatively, polymeric nanoparticles can be directly fabricated by the polymerization of monomers using different polymerization techniques e.g. micro-emulsion, surfactant-free emulsion, mini-emulsion, and interfacial polymerization. A representation of various fabrication techniques for polymeric nanoparticles is mentioned in Fig. 3.2. The selection of fabrication method is made on the basis of a number of factors such as the type of polymeric system, area of application, size requirement and others.

3.3 Contemporary Methodologies for Fabrication of Polymeric Nanoparticles

In recent times, the polymeric nanoparticles have appeared as a most potential and viable technology platform for recognizing the targeted, environment-responsive and, multi-functional with navigated controlled drug delivery system. Polysaccharides in smart drug delivery is a fast rising new technological discipline in which different therapeutic applications of nanoproducts are predictable to overcome the patient complaints in healthcare. Smart delivery will offer new keys for therapeutic interventions. There is immense interest from the commencement in smart medicine of advanced and well-characterized bionanotechnological products that will be particularly effective in fighting diseases like cardiovascular diseases, aging, diabetes, cancer, some chronic metabolic syndrome and different degenerative diseases and disorders. For an instance, the innovative smart polymers with nanoparticulate drug-delivery systems can clearly advances in therapeutics by directing the drugs to target cells and reducing the adverse-effect/side-effect on well being. Presently, a number of smart polymer with multi-functioned nanoparticle system strategies in clinical trials, and it demonstrate promising result. Definitely the morbidity and mortality rate of disease affected patients could improve their lifestyle by the initial course of smart therapeutic intervention. This smart

intervention can be achieved by developing high sensitivity and reliable smart drug delivery. The quick development in the above course has been made with the initiation and development of more advanced alternative nanofabrication techniques to offer structures in various nano-scales level of controlled manners. Drug loaded polymeric nanosystems can offer controlled release of both hydrophilic and hydrophobic drugs over a long period of time while reducing undesirable side effects in the body. This encompass the fabrication of various novel biocompatible polymers with well-defined nanometers to a few micro-meters structures using several modern techniques e.g. microfluidic systems, microelectromechanical systems, microneedle based system, advanced high pressure homogenization, electrodropping system, interfacial emulsion polymerization and combined systems. Figure 3.3 explained the small number of modern techniques for polymeric nanoparticles fabrication with various concepts. The physiochemical features of polymeric nanoparticles have to be optimized based on the specific application. A variety of methodologies can be utilized to offer different nano-particulate systems with various polymers. The multifunctional polymeric nanoparticles developments e.g. coreshell nanoparticles, environment-responsive micelles, colloids, nano hydrogel, nano-spheres and coreshell nano-spheres with layer-by-layer assembly for single/dual or multi drug release have been achieved so far. So as to get the preferred features, the mechanism of formulation method plays a vital role. Therefore, it is tremendously beneficial to have synthesis mechanism at hand to approach multi-functional polymeric nanoparticles with exact physiochemical properties for a specific application.

3.4 Activation-Modulated Drug Delivery: Environmental Activation/Stimuli Responsive Smart Delivery System

The smart drug delivery with activation-modulated system has been accomplished by external or environmental stimuli. These environmental responsive smart delivery systems attained a lot more with double and multiple-responsive delivery system. Different activation/stimuli responsive drug delivery vehicles have been prepared and evaluated, in different particle sizes, ranges from nanometers to a few micro-meters sized carriers for various routes of administration. The transdermal electro-activated or electro-modulated drug delivery has been recognized as a competent model. In this assembly of activation-modulated controlled drug delivery system, the release of active agents from the systems is activated by some physical, chemical, electrical, environmental condition or biochemical processes and/or facilitated by an energy supplied externally. The release profile has been regulated by the input energy. Based on the activation/stimulation process applied or energy type used, this activation-modulated controlled drug delivery system can be classified into the different as mentioned in Fig. 3.4. These stimuli-responsive materials display variation in the physicochemical feature while the environmental condition

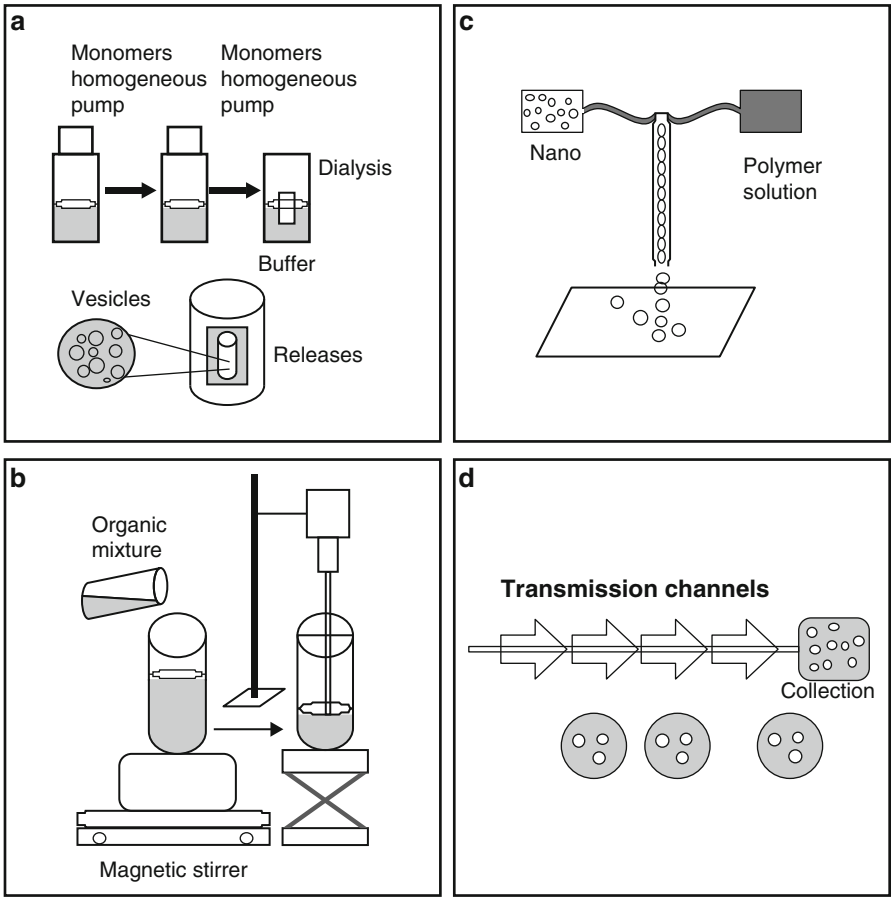

Fig. 3.3 Schematic representation of the advanced techniques of preparation of polymeric nanoparticles. (**a**) Core shell particulate system: in situ semi batch emulsion method. Size of the core controlled via surfactant. Surfactant was removed via dialysis pH-responsive polymer core-shell. (**b**) Sonication based system: mostly probe sonicator is used. Various optimization conditions require ON/OFF cycle to reduce temperature. Ice bath to maintain the temperature. (**c**) Electrodropping system: dual delivery using biocompatible care, homogeneous core shell, layer-by layer assembly possible, difference of release profile possible. (**d**) Well controlled synthesis of particles, tunable nanoparticles possible, reproducible synthesis, distinct nanoparticles for specific target

changes. These variations in features can be entirely utilized in smart delivery system, which definitely alike to the biological response behavior. Various sorts of body organs, different tissues and various types of cellular compartments might have vast dissimilarity in every stimulus with great response. Any definite behavioral changes in the system results in phase transition, these transitions will be key factors for the stimuli-responsive drug delivery system and some selected instances of applications are explained in the Fig. 3.4.

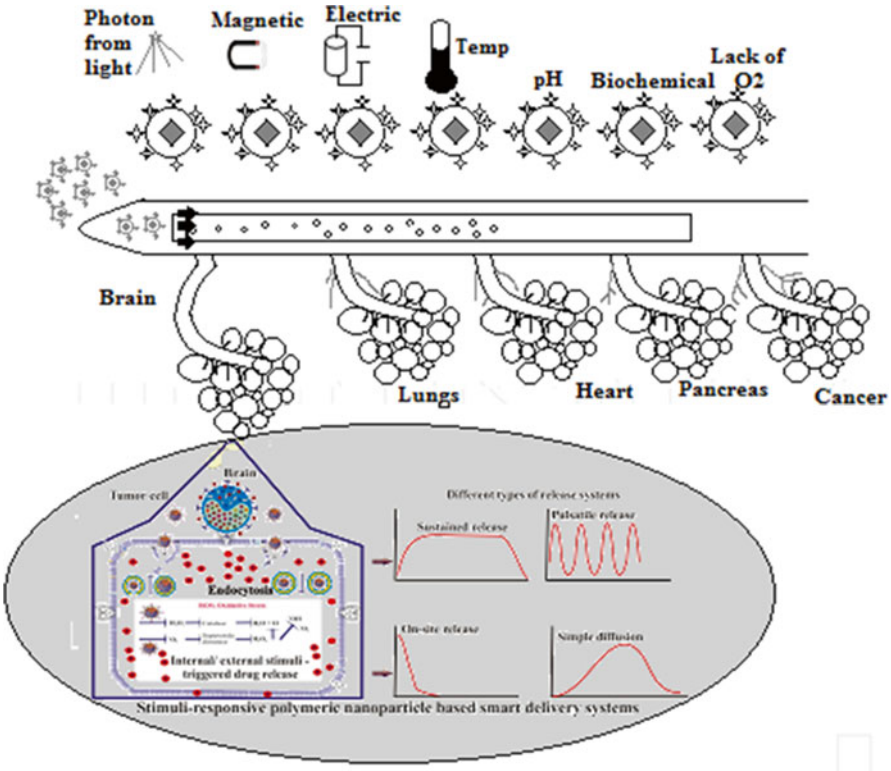

Fig. 3.4 Plan illustration representing the activation-modulated drug delivery systems, which the polymeric nanoparticles activated by different stimuli e.g. physical, chemical, biochemical, environment, and/or a combination of two or more

3.5 Time to Move on Innovative Methods of Administration

Polymers are macromolecules having repeating structural units which are typically connected by covalent chemical bonds. Synthetic and natural polymers are having various applications specifically in the pharmaceutical sector because of their economical, readily available and non-toxic nature. Additionally natural polymers are capable of chemical modifications, potentially biodegradable and with few exceptions, also biocompatible. Various applications of natural and synthetic polymers are mentioned in Fig. 3.5. Most of the pharmaceutical industries primarily manufacture/dispense drugs orally (as solid pills and liquids) or as injectables.

Owing to the recent innovations in pharmaceutical sciences most of the manufacturers are focusing on different strategies for the production of formulations that control the rate and period of drug delivery (i.e., time-release medications) and target specific areas of the body for treatment have become increasingly common and complex. Inclination of current researchers towards development of novel and potential

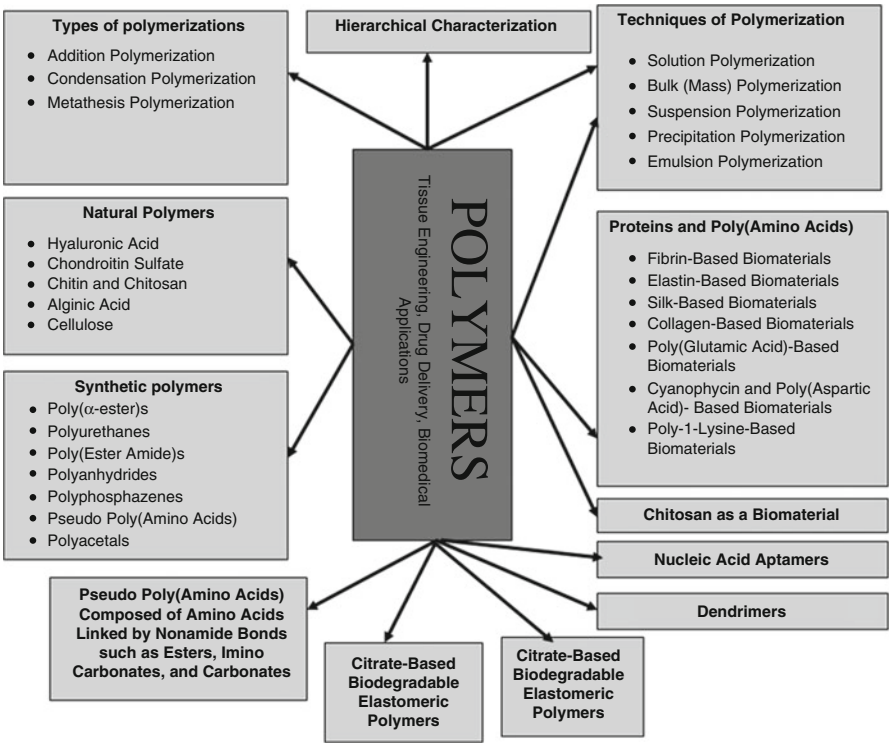

Fig. 3.5 Polymers and their diverse applications in tissue engineering, drug delivery and biomedical science

treatments and discoveries of bioactive molecules lead to exploration of mechanisms and their assisted strategic methods of administration. The most complicated work for current researchers is to design methods of drug delivery that exhibit specific problems such as limited therapeutic effects of certain drugs or their partial degradation that occurs before they reach a desired target in the body. Accountability of factors which directly or indirectly influences the pathway(s) of drug assists in designing the specified or targeted drug delivery system. Such systems not only deliver drug at targeted site but also encourage time dependent release of medications which may provide the relief from symptoms and protection from adverse events solely when necessary. Focus can also be stretched towards development of injectables drugs which could be manufactured less expensively and administered more conveniently if they could simply be dosed orally. Nevertheless such an approach cannot be achieved unless more advanced and scientific methods will be developed to safely direct medications through specific areas of the body, such as the GIT, where pH variation can destroy drug activity, or through an area where healthy bone and tissue might be adversely affected. Objective of all complicated drug delivery systems is to organize medications which can integrate to specifically targeted parts of the body through a medium. Such medium can control the therapy's administration by means

of either a physiological or chemical trigger. This objective can be achieved by designing more advance micro- and nanotechnology in form of polymeric microspheres, polymer micelles, and hydrogel-type materials. Such micro or nano scientific based delivery system can be designed in such a way where it can promote drug specificity, lower systemic drug toxicity, improves treatment absorption rates, and provide protection for pharmaceuticals against biochemical degradation.

3.6 History of Drug Delivery from the Ancient to Date

Treatments obtained from plants and other natural sources were earlier delivered either orally or topically. Various traditional system medicines (e.g. ayurvedic) and their respective formulations were highly recommended in form of oral and topically active preparations. It was later discovered that potential of these preparations is based on the integumentary and gastrointestinal (GI) system integrity. Various natural extracts were supplemented to treat the disease e.g. cinchona tree powdered bark containing quinine administered orally by native Brazilians to treat malaria [1]. Currently inspite of the development of resistant strains, quinine is still one of the core treatments for malaria [2]. Traditional ayurvedic medicines in the form of guttikas, churnas, leha, avleha, bhasmas, arishtas, asavas, and tailas form the foundation of drug delivery system. According to an interview that a pharmacist historian gave the LA Times [1], pills date as far back as 1500 BC. Earliest evidence to pills was found on papyruses in ancient Egypt, and contained bread dough, honey or grease. Pill name was introduced by Roman scholar Pliny (23–79 AD), he called them "pilula," later named as Pill. During medieval period people coated pills with slippery plant substances and gilded them in gold and silver to facilitate their swallowing more easily. The first concrete evidence of active table (sugar-coating and gelatin-coating) was explored in the 1800s and during the same time the compressed tablet also was invented in the 1800s by a Brit named William Brockedon. Later on it was realize that these formulations require more advance, more efficient and effective methods of drug delivery systems. Moreover significant role of drug delivery systems in herbal medicines was explored. For the first in 1656, Sir Christopher Wren reported the use of a syringe [3–5]. The earliest drug delivery systems, first introduced in the 1970s, were based on polymers formed from lactic acid. Later on in 1853, Scottish physician Alexander Wood and French surgeon Charles Pravaz independently publicized two extraordinary functional syringe designs [3–5]. This achievement explored the potential of different types of injectables to directly inject medications with either controlled or spontaneous rate. At last after the development of different designs of injectables, in 1960s, disposable syringes were introduced [6]. This outstanding achievement eliminated the need of boiling or sterilizing glass syringes, which resulted in improved hygiene and convenience. Thus purpose of most of the earlier delivery systems to deliver the therapeutic agent so that it will easily accumulated in target cells in optimal concentrations for a prolonged period. Nevertheless potential drug delivery via topical and systemic routes requires their transport through several

physiological barriers [7, 8]. Till today enteral (entering the body via the GI tract) or parenteral (entering the body by any route other than the GI tract) [7, 8] are the two most primary drug delivery routes used to deliver medications. Most of the manufactures designed different formula or delivery system for same medicines to control their release at the desired site. In spite of the delivery route drug features such as hydrophilic or hydrophobic nature plays an important role in determining the ultimate fate during its transport across lipidic membranes. These membranes are most permeable to lipophilic molecules nevertheless in order for a drug to dissolve in body fluids and be transported, it must also be somewhat hydrophilic [9]. Some additional factors such as low molecular weight and non-polarity improve drug transport across membranes. Strategies were developed to design more advance drug delivery system. In 1997, Glucose-sensitive hydrogel that could be used to deliver insulin to diabetic patients using an internal pH trigger was synthesized by chemical engineers at Purdue University in West Lafayette, IN, under the direction of Nicholas A. Peppas [10]. Development of different types of drug delivery system leads to the germination of new sector where drug release rate can be controlled in a more systematic and effective fashion, exclude side effects and encourages safe delivery of medication. This field is known as controlled drug delivery field began the founders who introduced this exciting and important field, and the prominent researchers who came after them [11]. This section is following the subsequent development phases of the field from its origins in the 1960s to the 1970s and 1980s, when various macroscopic "controlled" drug delivery devices and implants were developed for delivery as mucosal inserts, as implants, as ingestible capsules, as topical patches, and were approved for clinical use. Moreover these historical events section traces various phases of development in the 1960s to the 1980s and 1990s when microscopic degradable polymer depot DD systems (DDS) were commercialized [11]. Lastly the section objectives were set to explore the currently very active and exciting nanoscopic era of targeted nano-carriers, in a sense bringing to life Ehrlich's imagined concept of the "Magic Bullet". In the 1970s nanoscopic period began with systems projected which were first used in the clinic in the 1980s, and which came of age in the 1990s. These are currently emerging as exciting and clinically successful products in the 2000s. Most of these successful products are based on PEGylation and active targeting to specific cells by ligands conjugated to the DDS, or passive targeting to solid tumors via the EPR effect [11]. Key events and pioneers are highlighted in Table 3.1.

While this is of course one of the major reasons for the emergence of this field in the 1960s and 1970s, to me the surge of the field in the last 35 years is a classic example of an early form of "convergence to biomedical science", the idea promoted recently by Sharp and Langer [12]. ALZA Corporation was founded in 1967 by Alejandro Zaffaroni and immediately attracted a distinguished group of scientists from the chemical and pharmaceutical fields. A result of the introduction of mathematical models and molecular design principles in pharmaceutical formulations was the development of a new field setting the foundations, mechanisms and defining the principles of controlled release profiles. The founding of ALZA Corporation of 1967 had a significant effect on the development of the field of "controlled release".

Table 3.1 Historical events occur in drug delivery

Year	Event	Researcher	References
1960s and 1970s	Emergence of drug delivery field "convergence to biomedical science"	Sharp and Langer	[12]
1967	ALZA Corporation: Include the scientists from the chemical and pharmaceutical fields to introduce mathematical models and molecular design principles in pharmaceutical formulations This was done for the development of a new field setting the foundations, mechanisms and defining the principles of controlled release profiles	Alejandro Zaffaroni	[12]
1967	ALZA Corporation had a significant effect on the development of the field of "controlled release"	Alejandro Zaffaroni	[12]
1961	The first pharmaceutical scientist to apply physical chemical principles to the design of controlled release devices	Takeru Higuchi (professor at the University of Wisconsin and then the University of Kansas)	[13]
1960–1985	His classic equation (metamorphosed several times by 1963 to be applied also to porous systems with high or low drug solubility [4]) became the standard of design of drug delivery systems and continues to be widely used in the design of many ethical and generic products, especially of the oral/transmucosal delivery	Takeru Higuchi (professor at the University of Wisconsin and then the University of Kansas)	
1964	Developed systems of medical relevance for the prolonged drug therapy of patients	Folkman and Long	[14]
1981	Offered the first systematic, mechanism-based classification of controlled release systems	Langer and Peppas	[15]
1980	Design of drug delivery systems was a publication: provided simple but accurate solutions and design equations for drug delivery from matrices	Ping Lee	[16]
1990	Developed the first successful biodegradable systems for treatment of brain tumors	Langer and Brem, working with researchers at MIT and Johns Hopkins	[17]
1976	Speiser was the father of pharmaceutical nanotechnology, having written about nanoparticles in drug delivery as early as 1976 [16]	Speiser	[18]

(continued)

Table 3.1 (continued)

Year	Event	Researcher	References
1986	Worked on the enhanced permeability and retention (EPR) effect that explained the mechanisms of macromolecular transport and accumulation to tumors	Hiroshi Maeda	[19]
1960–1970	Resulted in historical development of nanoparticles	Contribution of Paul Ehrlich and then by Ursula Scheffel and colleagues and the extensive work by the group of Professor Peter Speiser at the ETH Zürich in the late 1960s and early 1970s	[20]

3.6.1 Historical Role of Polymers as Plastics

The plastics industry is recognized having its beginning in 1868 with the synthesis of cellulose nitrate. It all started with the shortage of ivory from which billiard balls were made. The manufacturer of these balls, seeking another production method, sponsored a competition. Johny Wesley Hyatt (in the U.S.) mixed pyroxin made from cotton (a natural polymer) and nitric acid with camphor. The result was cellulose nitrate which is called celluloid. It is on record; however that Alexander Parkes, seeking a better insulating material for the electricity industry, had in fact discovered that camphor was an efficient plasticizer for cellulose nitrate in 1862. Hyatt, whose independent discovery of celluloid came later, was the first to take out patents for this discovery. Cellulose nitrate is derived from cellulose a natural polymer. The first truly man made plastic came from 41 years later (in 1909) when Dr. Leo Hendrick Baekeland developed phenol-formaldehyde plastics (phenolics), the source of such diverse materials as electric iron and cookware handles, grinding wheels and electrical plugs. Other polymers-cellulose acetate (toothbrushes, combs, eyeglass frames etc.), urea-formaldehyde (buttons, electrical accessories), poly(vinyl chloride) (flooring, upholstery etc.) and nylon (toothbrush bristles, surgical sutures) followed in the 1920s. It is obvious that the pace of development which was painfully slow up to the 1920s picked up considerable momentum in the 1930s and the 1940s. The first generation of manmade polymers was the result of empirical activities; the main focus was on chemical composition with virtually no attention paid to structure. However during the half of the twentieth century, extensive organic and physical developments led to the first understanding of the structural concept of polymers-long chains or a network of covalently bonded molecules. In this regard the classic work of German chemist Hermann Staudinger on polyoxymethylene and rubber and of the American chemists W.T. Carothers on nylon stand out clearly. Staudinger, first proposed the theory that the polymers are composed of giant molecules and he coined the word macromolecule to describe them. Carothers discovered nylon, and his fundamental research (through which actually nylon was

discovered) contributed considerably to the elucidation of the nature of polymers. His classification of polymers as condensation or addition polymers persists today. The years following World War II (1950s) witnessed great strides in the growth of established plastics and the development of new ones. The Nobel Prize winning development of stereo specific catalysis by professors Karl Ziegler of Germany and Giulio Natta of Italy led to the ability of polymer chemists to order the molecular structure of polymers. As a consequence, a measure of control over polymer properties now exists, polymers can be tailor made for specific purposes. In recent years as a result of better understanding of polymer structure property relationship, introduction of new polymerization techniques and availability of new and low cost monomers, the concept of a true tailor made polymer has become reality. In the years ahead, polymers will continue to grow. The growth from all the indications will be not only from the development of new polymers, but also from the chemical and physical modification of existing ones.

3.7 Shift from Nature to Synthetic (Including the Merits and Demerits of Synthetic Polymers)

Polymers have become a crucial part of life, especially biodegradable polymers are of special interest since they do not accumulate in or nor harm the environment and thus can be considered as green [21].

To date, due to versatility of polymeric materials, specifically biodegradable ones, they are rapidly replacing other biomaterial classes, such as metals, alloys, and ceramics for use in biomedical applications. In 2003 the sales of polymeric biomaterials exceeded $7 billion, accounting almost 88 % of the total biomaterial for that year. The global market for biodegradable polymers increased from 409 million pounds in 2006 to 3 % an estimated 541 million pounds by the end of 2007. It should reach an estimated 1203 million pounds by 2012, a compound annual growth rate of 17 [21].

Biodegradable polymers can be either natural or synthetic. In general synthetic polymers great advantages over natural polymers since they can be tailored in such a way to yield wide array of possibilities with different types of products. Some of the natural polymers have important functional groups that are suitable for applications such as tissue engineering and less prone to produce toxic effects. Nevertheless presence of such functional groups and contaminants present in the material of natural origin may produce undesirable immunological effects [21]. On the other hand synthetic polymers are available with wide range of chemical linkages that can greatly affect the degradation and other derived properties. To obtain the intermediate property two or more polymers can be blended and or chemically linked (copolymerized). This latter approach has basically attracted lot of attention because of the possibility of generating polymers with desired properties without limitation such as phase separation [21].

Polymers are either naturally occurring or purely synthetic. All the conversion process occurring in our bodies are due to the presence of enzymes. Life itself may cease if there is a deficiency of these enzymes [22]. Enzymes, nucleic acid and proteins are polymers of biologic origin. These structures are normally very complex were not understood until very recently. Starch, cellulose and natural rubber are on the other hand example of plant based natural polymers and have relatively simple structure then those of enzymes or proteins. There are large numbers of synthetic polymers consisting of various families: fibers elastomers, plastics, adhesives. Each family itself has subgroups [22].

From last 10 to 15 years much attention has been given to the development of synthetic polymers for drug delivery devices, especially polymers those are fabricated from synthetic polymers that degrade under in vivo conditions. Various databases, literature, and scientific reports in the field polymers science have demonstrated that natural polymers to this field received far less publicity that the synthetic polymers as they play major role in drug delivery science. Three broad classes of natural polymers (proteins, polysaccharides and polyesters) derived from hydroxyacids showed the variable candidates for drug delivery applications [23]. However Kopecek and Ulbrich correctly reported the certain advantages offered by synthetic polymers over natural polymers. He has also suggested that the techniques or methodologies employed to fabricate synthetic polymers simply cannot create the well-designed molecular structures with unique properties featured by many natural polymers [23]. This is the most important reason for considering the latter. Protocol employed in the development of synthetic or natural polymers as drug carrier play a major role in deciding its ultimate applications in drug delivery. Degradation by external factors such as biodegradability by enzymatic hydrolysis or by other means is considered as the dominant feature of natural polymers. This type of degradation eventually causes a molecular weight variation which ultimately affects the molecular weight dependent biological activity of the natural polymer. Process of degradation is more advantageous when the degradation products are the part of normal metabolic process of the body. Potential of natural polymers to show bioactivity offers interesting possibilities that are being explored to some degree, but warrant further study [23]. In such cases, the carrier becomes an active participant in the therapeutic process. Nevertheless antigenicity, a form of bioactivity exhibit by natural polymers like proteins can be very dangerous, though not considered as a major problem to date. Current research cited various references for applications of collagen based drug delivery devices. Consequently it's suitable to review nature for such applications. It is relevant to point out that collagen represent complex family of numerous proteins [23]. Collagens are considered as the most important protein for connective tissue and form a major part of the organic matrix of bones. Although 11 type of collagens with varying features have been discovered and according to researchers many more will be identified in future [23].

Biodegradable synthetic polymer offers a number of advantages for application in tissue engineering and regenerative medicine [24]. The biomaterials can be easily synthesized with reproducible quality and purity and fabricated in to various shapes with desired bulk and surface properties [24]. Specific advantages include the abil-

ity to tailor the mechanical properties and degradation kinetics of these materials to suit various applications. Poly-hydroxy acids such as poly(glycolic acid), poly(L-lactic acid), and their copolymer poly(lactide-co-glycolide) are the most widely used biodegradable synthetic polymers for tissue engineering applications. The polymers have gained popularity due to their processing, consistency, adequate mechanical properties and Biodegradability and they are already FDA approved for human use in variety of applications including as sutures and in drug delivery system [24]. The ester bond in these polymers degrades non enzymatic hydrolysis and their non toxic degradation products are eliminated from the body in form of carbon dioxide and water. The degradation rate of these polymers can be controlled by alteration of their crystalline, initial molecular weight and the copolymer ratio of the lactide and glycolide and the degradation times that can be achieved ranges from several weeks to several months. Since these polymers are thermoplastics, they can be configured in three dimensional structures with a desired microarchitecture, shape and dimension, however synthetic polymer generally lacks intrinsic biological activity and their degradation products may cause adverse effects or alter local microenvironment in vivo. In addition the surface hydrophobicity of synthetic polymer may mediate protein denaturation in the vicinity of the implant and induce fibrous encapsulation [24]. A number of groups have begun to explore the synthesis of biomaterials that unite the advantages of smart synthetic polymers with the biological activities of proteins at the same chemical level. The concept of smart polymers was initially derived from the development of materials that show large conformational changes in response to micro environmental stimuli such as temperature, ionic strength, pH, or light. The responses of the polymer may include precipitation or gelation, reversible adsorption on a surface, collapse of a hydrogel or surface graft, and alteration between hydrophobic and hydrophobic state [24]. In many cases change in the state of the polymer is reversible. Biological application of this technology currently under development span diverse areas including bioseparation, drug delivery, reusable enzymatic catalysts, molecular switches, biosensors, regulated protein folding, microfluides and gene therapy. Smart synthetic polymers may offer promise for revolutionary improvements in tissue engineering scaffolds. Beyond the physical properties of these polymers, a major goal is to impart smart biomaterials with the specific properties of signaling proteins such as growth factors. Natural *polymers* are oxygen-permeable and available in large quantities from renewable sources, while synthetic polymers are produced from non renewable petroleum resources.

Biodegradable synthetic polymer exhibit number of advantages such as easy to process, bioactive molecules can be easily incorporated and mimic natural ECM structure and function. However it also exhibits numerous disadvantages less biocompatible than natural polymer and easily degrades to form bio products. Naturally derived materials are biocompatible, bioactive material can be easily incorporated, mimic natural ECM structure and composition, however its difficult to control biodegradable rate and having poor mechanical stability. Additionally they are temperature sensitive and transfer of pathogen is possible.

In contrast with natural polymers synthetic polymer enjoy the tremendous advantage of versatility. Through creative polymer chemistry, the synthetic polymer can be custom designed to meet specific needs. The toxicology of breakdown products and tissue biocompatibility of the polymers are the major issue in deciding the success of the devices. When intended as a long term delivery systems the cyto-toxicity of degraded products may be less of a problem because of the slow degradation rate and hence the low dose. Any acute inflammatory response to the implant may also have the chance of being resolved as the polymer disappears. However other potential side effects such as carcinogenicity and teratogenicity are difficult to address and evaluate. Nevertheless with the tremendous potential advantages, research in this area is still rewarded with a high benefit to risk ratio.

3.7.1 Natural Polymers and Synthetic Polymers for Scaffolds

Recent report suggested the potential applications of polymers as biomaterials for the fabrication of medical device and tissue-engineering scaffolds [25, 26]. The most preferable features for suggesting the materials as biomaterials are molecular weight, material chemistry, shape and structure, solubility, lubricity, hydrophilicity/hydrophobicity, water absorption degradation, erosion mechanism and surface energy. Owing to their distinctive properties such as high porosity with very small pore size, biodegradation, high surface-to-volume ratio, and mechanical property, polymeric scaffolds are considered for its potential biomedical applications. Their unique characteristics represent distinct advantages of biocompatibility, versatility of chemistry, and the biological properties which are significant in the application of tissue engineering and organ substitution. According to reports various researchers have worked to culture skin and cartilage [27], bone and cartilage [28], liver [29], heart valves and arteries [30], bladder [31], pancreas [32], nerves [33], corneas [34], and various other soft tissues [35]. Depending on the intended use, scaffold materials can be synthetic or biologic, degradable or nondegradable. Based on the properties of polymers such as composition, structure, and arrangement of their constituent macromolecules it can be classified into various types in terms of their structural, chemical, and biological characteristics, for example, ceramics, glasses, polymers, and so forth. In broad terms it can be classified in to naturally occurring polymers, synthetic biodegradable, and synthetic nonbiodegradable polymers used as biomaterials. According to earlier report natural polymers can be considered as the first biodegradable biomaterials used clinically [36]. Since they exhibit bioactive properties and have better interactions with the cells which allow them to enhance the cells' performance in biological system. Natural polymers can be broadly classified as polysaccharides (cellulose, amylose, dextran, chitin, and glycosaminoglycans), proteins (silk, collagen, gelatin, fibrinogen, elastin, keratin, actin, and myosin), or polynucleotides (DNA, RNA) [37]. The most dominating feature of synthetic polymer over natural polymers is that synthetic biomaterial direction provided by biomaterials may assist restoration of structure and function of damaged

or diseased tissues, thus highly considered in biomedical field. In addition their properties (e.g., porosity, degradation time, and mechanical characteristics) can be tailored for specific applications which may further encourage its utilization in biomedical field. In contrast with natural polymer, synthetic polymers are having broad class with defined purity and properties. Additionally they often cheaper than biologic scaffolds; it can be produced in large uniform quantities and have a long shelf time. It has been observed that various commercially available synthetic polymers show physicochemical and mechanical properties comparable to those of biological tissues. As discussed above synthetic polymers correspond to the largest group of biodegradable polymers, and they can be produced under controlled conditions and exhibit, in general, predictable and reproducible mechanical and physical properties such as tensile strength, elastic modulus, and degradation rate [38]. The most commonly used synthetic polymers in tissue engineering [39] are PLA, PGA, and PLGA copolymers. Among various polymers, PHA (belongs to a class of microbial polyesters) is being increasingly considered for applications in tissue engineering [40]. In soft tissue engineering bioactive ceramics (such as HAP, TCP), and certain compositions of silicate and bioactive glasses (such as phosphate glasses) and glass-ceramics (such as apatite-wollastonite) react with physiological fluids and through cellular activity form tenacious bonds to hard [41]. Nevertheless the issues related with their biocompatibility and biodegradability is often inadequate. This may limit their potential use in the clinical side. Researchers are still working on overcoming these issues by developing suitable blend of synthetic and natural polymers or by exploring composite materials that improve the scaffold properties and thereby allowing controlled degradation [42] and improving the biocompatibility in tissue engineering applications [43]. For achieving mechanical and biological performance in hard tissue the suitable blend of degradable polymers and inorganic bioactive particles can be developed. This and many other alike approaches promote their utilization in biomedical field [44].

3.7.2 Natural vs Synthetic Polymer (as Biomaterial)

Natural polymers can be found in living creatures and plants; for example, silk, protein, cotton, linen, wool and DNA. Synthetic polymers, as their name indicates, are synthesized in the lab through a series of chemical reactions. Examples of such polymers are polyvinylchloride, polypropylene, chewing gum, rubber and nylon. There are various biomedical applications of synthetic and natural polymers (Table 3.2). Synthetic and natural polymers have diverse applications in drug delivery. Natural polymers faced many problems like instability, irreproducibility, changes in aesthetics on storage, uncontrollable formulation characteristic etc. therefore novel designs were required to develop in form of synthetic polymer by some chemical processes like polymerization. Development of new structural imprints could require solutions for some of the problems that are usually associated with natural polymers. Most of natural polymers are covered by gums such as

Table 3.2 Biomedical applications of synthetic and natural polymers

Polymer	Biomedical applications
Poly (2-hydroxyethyl methacrylate)	Contact lens
Poly(dimethyl siloxane)	Breast implants, contact lenses, knuckle replacements
Poly(ethylene)	Orthopedic joint implants
Poly(ethylene glycol)	Pharmaceutical fillers, wound dressings
Poly(ethylene terepthalate)	Vascular grafts, sutures
Poly(e-caprolactone)	Drug delivery devices, sutures
Poly(lactic-co-glycolic acid)	Resorbable meshes, sutures
Poly(methyl-methacrylate)	Bone cements, diagnostic contact lenses
Poly(tetrafluoroethylene)	Vascular grafts, sutures
Poly(isoprene)	Gloves
Poly(propylene)	Sutures
Alginate	Wound dressing
Chitosan	Wound dressing
Collagen	Orthopedic repair material, Nerve repair matrices, Tissue engineering matrices
Elastin	Skin repair matrices
Fibrin	Hemostatic products, tissue sealants
Glycosaminoglycan	Orthopedic repair matrices
Hyaluronic acid	Orthopedic repair matrices

acacia, tragacanth, guargum, xanthan, etc. these polysaccharides are having molecular weight which sometime hinders its biological property. Natural polymers those are derived from the animal origin carry antigenicity and therefore interfere with function of model drug by inducing some immune reactions. Microbial based polysaccharides often carries the antigenic property which again interferes with in vivo biological reactions induced by model drug, though they are still use for various pharmaceutical applications. On the other hand synthetic polymers are either synthesized from natural polymers or completely synthesized from synthetic monomers. Synthetic derivatives like cellulose, acryl, vinyl polymers relatively address these issues. In modern day formulations well engineered polymers are gradually replacing the natural polymers. Natural polymers are less toxic, biodegradable and don't contain any synthetic chemical as synthetic polymers and derivatives contains. Though the Synthetic polymers are more stable then the natural polymers therefore synthetic polymers can be readily sterilized. Most of the natural polymers tend to change color due to the process called as auto oxidation. This will lead to the leaching of colorful substance in product which can interfere with the physic-chemical properties of parent product. Therefore plasticizer supplementation is highly recommended while fabricating natural polymer based formulation. Natural polymers have the problem of short shelf life when compared with synthetic polymer. Some of toxicities under *in vivo* and *in vitro* conditions were also highlighted in some of the reports. There are various structural variable used to control

Table 3.3 Structural variable used to control biodegradable polymer properties [45]

Variables	Effects	Examples
Inco-operation of both natural or non natural monomers	May reduce/eliminate immunologic response often found in natural derived polymers	Nonimmunologic PGA and PLA (vs collagen)
Inco-operation of labile groups in polymer chain	Control kinetics of biodegradation	Hydrolysable ester bond in PGA
Inco-operation of functional groups in side chains	Control chemical and physical properties of polymers	Hydrophilic, hydrophobic and amphiphilic polyphosphazenes
Inco-operation of chiral centers in polymeric chains	Control chemical and mechanical properties of polymers	Semi-crystalline I PLA, amorphous di PLA
Possibility of utilizing multiple monomers	Control properties of polymers	Glycolic and lactic acids in PLGA
Use of natural compounds as monomers	Biocompatible breakdown of products	Lactic acid in PLA
Use of different polymer Architectures	Control chemical and mechanical properties of polymers	Branched polymers lower viscosity

bio-degradable polymer properties (Table 3.3). Toxicity assessment of both synthetic and natural polymers can be achieved by considering the biocompatibility and biodegradability parameters which can be further assessed by *in vivo* and *in vitro* cyto-toxicity (e.g. lactate *dehydrogenase* assay).

3.7.3 Natural vs Synthetic Polymer in Tissue Engineering

Natural based polymer can be derived from the sources within the body or outside the body. One of the most common natural biomaterial found in the human body is the protein collagen. Many different type of collagen exist in different tissues and several of these particularly type I and II have been explored as biomaterials. Another protein base biomaterial fibrin, results from the combination of blood clotting factors fibrinogen and thrombin. Both fibrin and collagen have been frequently used in tissue engineering attempts to repair cartilage damage and other orthopedic applications.

In addition to proteins, naturally based polymers may be derived from sugars (carbohydrates). Hyaluronic acid is an example of carbohydrate molecule occurring in human tissue that is often employed as a biomaterial. However the source of other carbohydrate derived materials may be non human. Chitosan, a sugar based substance found in arthropod exoskeletons, agarose which is formed by algae and alginate derived from seaweed, are all currently being investigated as biomaterials for a variety of applications. For example, combination of chitosan and alginate has been examined for wound dressings.

There are advantages and disadvantages of both natural and synthetic polymer and particularly materials may lend themselves to certain applications over others. In many cases natural polymers have composition similar to tissues they are replacing. Therefore they may be more fully integrated in to the surrounding tissue over the time or more easily remolded in response to changes in tissue needs. However concerns about the feasibility of findings large amount of some these materials for clinical applications, their relatively low mechanical properties and the assurance of pathogen removal. In addition regions of these molecules may be recognized as foreign by the body immune system leading to a type of material rejection. Further potential problem arise when the biomaterial is based on not a single naturally occurring polymer, but decellularized tissue. Here unwanted calcification leading to device failure is a particular concern.

In contrast synthetic polymer can be easily mass produced and sterilized so supply issues are not a problem at all. Additionally their physical, chemical, mechanical and degradative properties can be tailored for specific applications. However unless specifically treated, most synthetic materials do not interact with tissue in an active manner and therefore cannot direct or aid in healing around the implant site. Also few synthetic polymers have been approved by regulatory agencies for use in humans in specific applications.

3.7.4 Natural vs Synthetic Polymer Hydrogels

Hydrogel can be prepared from natural or synthetic polymer using various methods. Hydrogels made from natural sources can be derived from polymers such as collagen, hyaluronic acid, fibrin, alginate, agarose, and chitosan. Many natural polymer such as collagen HA and fibrin have been used in tissue engineering applications because they are either components or have macromolecular properties similar to the natural extracellular matrix. Collagens are composed of three polypeptide strands twisted together to form a triple helix and are the main protein of mammalian ECM. Likewise hyaluronic acid an anionic glycosaminoglycan polysaccharide, is also found in nearly all adult animal tissues. Alternatively alginate, agarose and chitosan are hydrophilic, linear polysaccharides derived from marine algae sources (alginate and agarose) or crustaceans (chitosan). Another natural derived gel Matrigel™, is derived from soluble basement membrane extract of mouse tumors. Various natural polymers have specific utilities and properties based on their origin and composition. Advantages of natural polymer based gels include inherent biodegradability and biologically recognizable moieties that support cellular activities. Disadvantages of some of these hydrogels include mechanical weakness and the possibility of evoking immune/inflammatory responses. Synthetic hydrogels are appealing for tissue engineering due to amount of control scientists have over structure, such as cross-linking density and tailored properties such as bio-degradation, mechanical strength, and chemical and biological response to stimuli. Synthetic polymers such as poly(ethylene glycol) (PEG) and other PEG based polymers or

poly(vinyl alcohol) (PVA) can be reproducibly produced with specific molecular weights, block structures, degradable linkages and cross linking density and mechanical and degradation properties of the material. Hydrogels made from synthetic polymers like PVA, PEG or their derivatives do not possess the inherent bioactive properties that gels made from natural polymers do. However, they do have well defined structures and are versatile templates for subsequent modifications that yield taliorable degradability and functionality.

3.8 Natural Polymers (Reasons for Reverting to Nature)

Polymers derived from natural resources have been widely researched as biomaterials for a variety of biomedical applications including drug delivery and regenerative medicine. These molecules have biochemical similarity with human ECM components and hence are readily accepted by the body. Additionally these polymers inherit several advantages including natural abundance, relative ease of isolation and room for chemical modification to meet the technological needs. In addition these polymers undergo enzymatic and hydrolytic degradation in the biological environment with body friendly degradation bye products. Natural polymers include the list of polysaccharides and animal derived proteins. Polysaccharides are an important class of biomaterials with significant research interest for a variety of drug delivery and tissue engineering applications due to their assured biocompatibility and bioactivity. Polysaccharides are often isolated and purified from renewable sources including plants, animals, and microorganisms. Essentially these polymers have structural similarities, chemical versatilities and biological performance similar to ECM components, which often mitigate issues associated with biomaterials toxicity and host immune responses. The building block of carbohydrate monosaccharide's are joined together by o-glycosidic linkages to form polysaccharide chains. Polysaccharides offer a diverse set of physicochemical properties based on monosaccharide's that constitutes the chain, its composition and source. The popular list of polysaccharides used for a variety of biomedical applications includes cellulose, chitin/chitosan, starch, alginates, HAs, pullulan, guar gum, xanthan gum, and GAGs. In spite of many merits as biomaterials, these polysaccharides suffer from various drawbacks including variation in the material properties based on the source, microbial contamination, uncontrolled water uptake, poor mechanical strength and unpredictable degradation pattern. These inconsistencies have limited their usage and biomedical applications related technology development. Numerous synthetic polymers with well defined mechanical and degradation properties have been developed to meet the technological needs in the biomedical applications. However these polymers from the biological standpoint lack much desired bioactivity and biocompatibility and may cause toxicity and immune response. Polysaccharide structure offers freely available hydroxyl and amine functionalities that make it possible to alter its physicochemical properties by chemically modifying polysaccharide structure. For instance grafting synthetic monomers on the

polysaccharide chains offer an easy way to control polymer solubility undesired solvents, water uptake and degradation. These semi synthetic polymers offer best feature of the both natural and synthetic polymers. Various cross linking techniques to restrict the polysaccharide chain movement to control their water uptake, degradation and mechanical properties have also been developed. Polysaccharides based porous scaffolds, fiber matrices, hydrogels, and micro and nanoparticles have been developed for variety of tissue regeneration and drug delivery applications. In the recent years glycochemistry has gained research momentum for understanding carbohydrate biological functions and development of carbohydrate based drugs and vaccines. Engineered carbohydrate based polymeric structures may serve as an alternative material platform for a variety of regenerative medicine and drug delivery applications. A new nonpetroleum based biomaterial platform to meet the versatile needs in biological science and biomedical engineering could be achieved by collaborative efforts between academia, government and industry partnership. The collaborative efforts should include bringing scientist working in different disciplines of chemistry, biology, polymers, materials sciences and engineering to work toward these activities. The collaborative efforts could lead to the development of a methodology for synthesis natural polymer based semi synthetic polymers and provide a greater depth of understanding of carbohydrate biological functions, polymer structure, material properties degradation and mechanical properties. Further the development of modeling tools to predict the structure, property and biological activity of carbohydrates for biomedical applications is a step in this direction. The goal of new initiatives should focus on the development of natural polymer based orthopedic fixation devices, biomedical implants, drug delivery vehicles, carbohydrate based drugs, hydrogels, surfactants, coagulants, and absorbents for a variety of biomedical applications. The research activities in this area could generate commercially available technologies and product from the renewable resources and contribute immensely toward economic development.

3.8.1 Need of Natural Polymers

- **Biodegradable**
  Naturally occurring polymers produced by all living organisms. They show no adverse effects on the environment or human being.
- **Biocompatible and Non-Toxic**
  Chemically, nearly all of these plant materials are carbohydrates in nature and composed of repeating monosaccharide units. Hence they are non-toxic.
- **Economic**
  They are cheaper and their production cost is less than synthetic material.
- **Safe and Devoid of Side Effects**
  They are from a natural source and hence, safe and without side effects.
- **Easy Availability**
  In many countries, they are produced due to their application in many industries.

3.8.2 Disadvantages of Herbal Polymers

- **Microbial Contamination**
  During production, they are exposed to external environment and hence, there are chances of microbial contamination.
- **Batch to Batch Variation**
  Synthetic manufacturing is controlled procedure with fixed quantities of ingredients while production of natural polymers is dependent on environment and various physical factors.
- **The Uncontrolled Rate of Hydration**
  Due to differences in the collection of natural materials at different times, as well as differences in region, species, and climate conditions the percentage of chemical constituents present in a given material may vary [46].
- **Slow Process**
  As the production rate is depends upon the environment and many other factors, it can't be changed, thus natural polymers have a slow rate of production.
- **Heavy Metal Contamination**
  There are chances of Heavy metal contamination often associated with herbal excipients [47].

References

1. Marino Jr R, Gonzales-Portillo M. Preconquest Peruvian neurosurgeons: a study of Inca and pre-Columbian trephination and the art of medicine in ancient Peru. Neurosurgery. 2000;47(4):940–50.
2. Warrell DA. The 1996 Runme Shaw Memorial Lecture: malaria—past, present and future. Ann Acad Med Singapore. 1997;26(3):380–7.
3. Paghdal KV, Schwartz RA. Topical tar: back to the future. J Am Acad Dermatol. 2009;61(2):294–302.
4. Thami GP, Sarkar R. Coal tar: past, present and future. Clin Exp Dermatol. 2002;27(2):99–103.
5. Feldmann H. History of injections. Pictures from the history of otorhinolaryngology highlighted by exhibits of the German History of Medicine Museum in Ingolstadt. Laryngorhinootologie. 2000;79(4):239–46.
6. Kravetz RE. Hypodermic syringe. Am J Gastroenterol. 2005;100(12):2614.
7. Jain RK. Understanding barriers to drug delivery: high resolution in vivo imaging is key. Clin Cancer Res. 1999;5(7):1605–6.
8. Wilding I. Site-specific drug delivery in the gastrointestinal tract. Crit Rev Ther Drug Carrier Syst. 2000;17(6):557–620.
9. Zarowitz BJ. Pharmacologic consideration of commonly used gastrointestinal drugs in the elderly. Gastroenterol Clin N Am. 2009;38(3):547–62.
10. Dorski CM, Doyle FJ, Peppas NA. Preparation and characterization of glucose sensitive p(MAA-g-EG) hydrogels. Polym Mater Sci Eng Proc. 1997;76:281–2.
11. Hoffman AS. The origins and evolution of "controlled" drug delivery systems. J Control Release. 2008;132(3):153–63.
12. Sharp PA, Langer R. Promoting convergence in biomedical science. Science. 2011;333:521–2.
13. Higuchi T. Rate of release of medicaments from ointment bases containing drugs in suspensions. J Pharm Sci. 1961;50:874–5.

14. Folkman J, Long DM. The use of silicone rubber as a carrier for prolonged drug therapy. J Surg Res. 1964;4:139–42.
15. Langer RS, Peppas NA. Present and future applications of biomaterials in controlled drug delivery systems. Biomaterials. 1981;2:201–14.
16. Lee PI. Diffusional release of a solute from a polymeric matrix. J Membr Sci. 1980;7:255.
17. Brem H. Polymers to treat brain tumors. Biomaterials. 1990;11:699–710.
18. Marty JJ, Oppenheim RC, Speiser P. Nanoparticles: a new colloidal drug delivery system. Pharm Acta Helv. 1976;53:17.
19. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent SMANCS. Cancer Res. 1986;46:6387–92.
20. Kreuter J. Nanoparticles—a historical perspective. Int J Pharm. 2007;331(1):1–10.
21. Jain PJ, Ayen WY, Domb AJ, Kumar N. Biodegradable polymers in clinical use and clinical development. NJ: Wiley; 2011.
22. Robert O. Ebewele polymer science and technology. Technology & engineering. Boca Raton, FL: CRC Press; 2000. p. 6.
23. Tarcha PJ. Polymers for controlled drug delivery. Boca Raton, FL: CRC Press; 1990. p. 150.
24. Armiñán A, Sepúlveda P, Vicent MJ. Polymer therapeutics as nano-sized medicines for tissue regeneration and repair. In: Pradas MM, Vicent MJ, editors. Polymers in regenerative medicine: biomedical applications from nano- to macro-structures. Hoboken: Wiley; 2014. doi:10.1002/9781118356692.ch8.
25. Piskin E. Biodegradable polymers as biomaterials. J Biomater Sci Polym. 1994;6:775–95.
26. Ghosh YJK, Shu XZ, et al. Electrospun three-dimensional hyaluronic acid nanofibrous scaffolds. Biomaterials. 2006;27(20):3782–92.
27. Eaglstein WH, Falanga V. Tissue engineering and the development of Apligraf a human skin equivalent. Adv Wound Care. 1998;11(4):1–8.
28. Boyan BD, Lohmann CH, Romero J, Schwartz Z. Bone and cartilage tissue engineering. Clin Plast Surg. 1999;26(4):629–45.
29. Mayer J, Karamuk E, Akaike T, Wintermantel E. Matrices for tissue engineering-scaffold structure for a bioartificial liver support system. J Control Release. 2000;64(1–3):81–90.
30. Mayer JE, Shin'oka T, Shum-Tim D. Tissue engineering of cardiovascular structures. Curr Opin Cardiol. 1997;12(6):528–32.
31. Oberpenning F, Meng J, Yoo JJ, Atala A. *De novo* reconstitution of a functional mammalian urinary bladder by tissue engineering. Nat Biotechnol. 1999;17(2):149–55.
32. Tziampazis E, Sambanis A. Tissue engineering of a bioartificial pancreas: modeling the cell environment and device function. Biotechnol Prog. 1995;11(2):115–26.
33. Mohammad J, Shenaq J, Rabinovsky E, Shenaq S. Modulation of peripheral nerve regeneration: a tissue-engineering approach. The role of amnion tube nerve conduit across a 1-centimeter nerve gap. Plast Reconstr Surg. 2000;105(2):660–6.
34. Germain L, Auger FA, Grandbois E, et al. Reconstructed human cornea produced in vitro by tissue engineering. Pathobiology. 1999;67(3):140–7.
35. Diedwardo CA, Petrosko P, Acarturk TO, Dimilia PA, Laframboise WA, Johnson PC. Muscle tissue engineering. Clin Plast Surg. 1999;26(4):647–56.
36. Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. Prog Polym Sci. 2007;32(8–9):762–98.
37. Yannas IV. Classes of materials used in medicine: natural materials. In: Ratner BD, Hoffman AS, Schoen FJ, Lemons J, editors. Biomaterials science: an introduction to materials in medicine. San Diego, CA: Elsevier Academic Press; 2004. p. 127–36.
38. Gunatillake P, Mayadunne R, Adhikari R. Recent developments in biodegradable synthetic polymers. Biotechnol Annu Rev. 2006;12:301–47.
39. Ma PX. Scaffolds for tissue fabrication. Mater Today. 2004;7(5):30–40.
40. Chen LJ, Wang M. Production and evaluation of biodegradable composites based on PHB-PHV copolymer. Biomaterials. 2002;23(13):2631–9.
41. Hench LL. Bioceramics. J Am Ceram Soc. 1998;81(7):1705–27.

42. Cascone MG, Barbani N, Cristallini C, Giusti P, Ciardelli G, Lazzeri L. Bioartificial polymeric materials based on polysaccharides. J Biomater Sci. 2001;12(3):267–81.
43. Ciardelli G, Chiono V, Vozzi G, et al. Blends of poly-(ε-caprolactone) and polysaccharides in tissue engineering applications. Biomacromolecules. 2005;6(4):1961–76.
44. Roether JA, Boccaccini AR, Hench LL, Maquet V, Gautier S, Jérôme R. Development and in vitro characterization of novel bioresorbable and bioactive composite materials based on polylactide foams and Bioglass® for tissue engineering applications. Biomaterials. 2002;23(18):3871–8.
45. Atala A. Synthetic biodegradable polymer scaffolds. New York: Springer; 1997.
46. Kumbar S, Laurencin C, Deng M. Natural and synthetic biomedical polymers. Burlington: Elsevier; 2014.
47. Jani GK, Shah DP, Prajapati VD, Jain VC. Gums and mucilages: versatile excipients for pharmaceutical formulations. Asian J Pharm Sci. 2009;4(5):309–32.

Chapter 4
Plant Derived Polymers, Properties, Modification & Applications

Abstract Current polymeric research has explored various applications in drug delivery and its related biomedical applications. Natural polymers especially those are derived from plant sources has evidenced for growing interest and attention in biomedical and pharmaceuticals sectors. Owing to their relative abundance, low cost, and biodegradable and eco-friendly profiles, plant derived polysaccharides are more preferred over the synthetic polymers. Present work demonstrates the drug delivery applications of plant based polysaccharides especially in nanotechnology sector. Outstanding features of these polysaccharides attributed to its unique physico-chemical properties. These plants polymer based nanomaterials used or investigated as release retardant in sustained or controlled release drug delivery systems. Nanomaterials of these plant based polysaccharide exhibit high water content, functionality, biocompatibility, tunable size from submicrons to tens of nanometers, large surface area for multivalent bioconjugation, and interior network for the incorporation of therapeutics. These unique properties present great potential for the utilization of polysaccharide-based microgels/nanogels in tissue biomedical implants, engineering, bionanotechnology, and particularly, drug delivery.

Keywords Plant • Nanomaterials • Polysaccharides • Drug delivery • Biomedical • Natural polymer • Nanotechnology • Nanoparticles

4.1 Introduction

Owing to their relative toxicity and stability issues under physiological environment drugs are rarely administered as such since most of them are always formulated into a desirable dosage form with the support of active excipients. Active excipients are those excipients that may mask the toxic or undesirable effect of drug without affecting its significant biological activity. Additionally most of the recent formulations tend to enhance biological activity by increasing its stability profile especially under in vivo environment. According to International Pharmaceutical Excipients Council excipients are those substances, other than the active drug substances of finished dosage form, though some excipients also exert similar or different biological activity then active drug and sometime impart synergistic or cumulative effect

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to the active drug. Objectives of each and every excipients is to either aid the processing of the drug delivery system through its manufacture, protect, bioavailability, support or enhance stability, assist in product identification, or patient acceptability, or enhance any other features of the general safety and efficiency of the drug delivery system throughout storage or use [1]. Therefore excipients plays a major role in deciding the final fate of drug under both in vitro and in vivo environmental conditions. Currently a variety of excipients have been explored as binding, flavoring, suspending, lubricating, gelling, sweetening and bulking agent among others [2]. They also play an important role in preserving the efficiency, safety, and stability of active drug and guarantying that they deliver their assured benefits to the patients. One of the major advantages of excipients is that their utilization at optimal concentration offers enhanced functionality, pharmaceutical manufacturers with cost-savings in drug development and help in drug formulations innovation. Since excipients are the largest components of any pharmaceutical formulation, therefore its essential to determine their stability and toxicological parameters from pre formulation studies. They can be obtained from natural or synthetic origin and in contrast with natural excipients, synthetic excipients are more utilized in pharmaceutical dosage forms [3]. Owing to the exclusive properties and advantages over naturally derived compounds, including a low sensitivity to various ingredients or moisture, resulting in more efficient and effective pharmaceutical products, synthetic and semi-synthetic products are preferred more [3]. Synthetic and semi-synthetics excipients differentiated on the basis of their origin such as pure synthetic organic chemical called as synthetic compound and substance that is naturally derived but has been chemically modified is called as semi-synthetic.

Polymeric materials obtained from lipids, carbohydrates and protein covers a broad class of excipients. Most of them are derived from natural polysaccharides and their derivatives. Polysaccharides of plant origin endow a group of polymers that are widely used in pharmaceutical formulations and play a significant role in evaluating the underlying mechanism and rate of drug release from the dosage form. Currently variety of plant based polysaccharides have been explored as excipients in the formulation of solid, liquid and semisolid dosage forms in which they play distinct functions as film formers, matrix formers or release modifiers, disintegrates, binders, stabilizers, emulsifiers, suspending agents, thickeners or viscosity enhancers and muco adhesives [4, 5]. Additionally plant based natural polymers can also be used in the implants, micro particles, films, nanoparticles, beads, formulation and manufacture of solid monolithic matrix systems, inhalable and injectable systems as well as viscous liquid formulations [5–7]. These plant based polymers are not only considered over synthetic polymers because of its significant features such as biodegradability, biocompatibility, non toxic and low cost and relative abundance compared to their and synthetic counter parts [8, 9], however also as natural resources are renewable and provide constant supply of raw material if cultivated or harvested in a sustainable manner [10]. The most popular plant based natural polymers that are used in pharmacy and other fields are chitosan, ispaghula, acacia, agar, guar gum, carrageenan, gelatin, shellac and gum karaya. These natural polymers are extensively used in pharmaceutical industry as adjuvant, emulsifying agent and adhesive in packaging; and also suitable for cosmetic and pharmaceutical

product development. Moreover various wide ranges of applications in drug delivery have been explored since as polymers, they endow exclusive properties which so far have not been exhibited by any other materials [11]. Natural polymers can be conjugated with small molecular weight proteins, polypeptides, lipids, surfactants, drugs, peptides, metals, nucleic acid, antibody, etc. They can be modified in such a way so that large chains and functional groups can be conjugated with other low and high molecular-weight materials to attain new materials with a variety of physicochemical properties. To overcome their demerits, natural polymers are tailored by chemical modification. Various physico-chemical modification reports are also available on natural gums, mucilages and other polysaccharides suggesting their potential role in pharmaceutical industry especially in drug delivery [12, 13]. Owing to the rising concern towards natural polymeric materials as pharmaceutical excipients, it's very difficult to document all the polymers at one platform, though we tried to cover most of the plant based polymers with its physic-chemical modifications and current applications in pharmaceutical industry.

4.2 Sources of Plant Polymers

Diversity of natural polymers in nature confers variation in their structural and gelling properties. As a matter of fact native polymers show variability and versatility, associated with their complex structures, not found in other classes of polymers. Plant and algal derived polysaccharides are the precursors for the diverse polymers which are widely used in drug delivery industry as advanced therapeutics. From a view of commercial utilization plant derived polymers are at most priority, however more researches are currently enduring on algal polysaccharides because of its complex structure related gelling properties. Moreover mammalian and microbial polysaccharide is another foundation for the polymeric industries because of their unique properties or because they provide a cheaper and superior alternative to other materials derived from plant, animal, or synthetic sources. Throughout the whole literature we found that usage of polysaccharides falls into three distinct areas: food applications, nonfood applications and biological purpose, whereas their growth and evaluation requires considerable investment in time, money, and technology. Many of these native polysaccharides for which potential industrial applications have been claimed have not proved to be of commercial value. Thus latest tools are required for their better study. Further discoveries in polymeric sciences furnish the continuous supply of novel polysaccharide from novel origin which makes a trouble in covering all the polymers under one platform. Combining together, here our attempt addresses the nature's broad class of polysaccharide from diverse origin with their current advancements and contribution in the field of pharmaceutics. Exploration of extensive class of plant based polysaccharides suitable for nanodelivery, chiefly from natural sources with techniques to increase its development in pharmaceutics. Here in this chapter we have covered the following set of objectives to explore the diverse polysaccharides in pharmaceutical sciences.

- To cover the utmost diversity of natural polysaccharides with aim to distinguish their commercial and medicinal utilization according to their structure related gelling properties.
- Broad view of nano applications of these polymers
- Latest equipments and knowledge used in the structural interpretation and gelling properties evaluation of diverse polysaccharides from different foundation.

Natural polysaccharides based polymers guarantee a new class of compounds for the development of a variety of drug delivery systems. They are now distinguished as valuable polymers for their significant pharmaceutical properties. These renewable compounds are extremely advantageous as compared to synthetic polymers in properties like non-toxic, biocompatible and show a number of peculiar physico-chemical properties [14]. Polysaccharides have some common uniqueness which are significant from a view of its applications; they have the ability to form multiple hydrogen bonds implying the local stiffness of the molecules, generally giving them the property of being water-soluble, but they also can be water-insoluble when they form intermolecular hydrogen bonds with each other to give crystals or large, high molecular weight, insoluble crystalline aggregates, granules, or fibers; from this rigidity, they get a high thickener character [15]. Knowledge of solution properties is needed to understand the polysaccharides' behavior in different applications. The main factors affecting the solution properties of polysaccharides are the molecular structures of the polysaccharides themselves, for example, the content of side galactose units and degree of substitution, molar mass, and temperature, pH, and ionic strength circumstances [16].

Polysaccharides are present in all kind of organisms, mammals, plants and microorganisms (Table 4.1). Because of its abundance it's very difficult to furnish absolute classification of polysaccharides. Polysaccharide derived from plants polymers are nowadays of greatest interest. This interest is generated by the features of these natural sources, such as being able to produce biodegradable and biocompatible new products and as value-added materials [16]. Whereas algal galactans like agar, alginate and carrageenan are the major hydrocolloids used as texturing agents for food and non-food applications. Their extraordinary gelling and thickening properties make them more complex then plant polysaccharides [17, 18]. In this context mammalian and microbial polysaccharides also play a great contribution in pharmaceutical field [18–24]. More focusing event in studding the polysaccharides is to establish a relationship between its structural and gelation properties. Various chemical, physical and biochemical tools are now available for their precise chemical and structural characterization.

Recently advance techniques and equipments have provided a more precise view of the interaction between the structural and the gelling characteristics of these complex polysaccharides. The quantitative estimation of all the constituent sugars, moe specifically the acid labile 3,6-anhydrogalactose can be done by methanolysis and reductive acid hydrolysis procedures coupled to different chromatographic separations techniques. This advancement also presents the means of determining sugar linkages, substitutions and sequences using chemical, enzymatic and spectroscopic

Table 4.1 Classification of polysaccharides

Plant polysaccharides	Cellulose and its derivatives, starch (cyclodextrins and amylose) and its derivatives, rosin, inulin, pectin, psyllium and arabinogalactans (larch) Other polysaccharides from different sources like aloe, cereal, psyllium, quince seed and oat brans also play some important role. **Gums and mucilages** Xanthan gum, gellan gum, konjac glucomannan, Xyloglucan, Guar gum (guar beans), Karaya gum (Sterculia gum), Gum tragacanth (Astragalus shrubs), Chicle gum (From Chicle tree), Konjac glucomannan (From Konjac plant), Gum Arabic (Acacia tree), Gum ghatti (sap of Anogeissus tree), Locust bean gum (carub tree), Cashew gum Mastic gum (mastic tree), Tamarind kernel gum, Hakeagibbosa gum, Irvingiagabonensis, Moringaoleifer gum, Kyaha gum, Okra, Grewia, mucilage gum, Mimosa scabrella, Mimosa pudica, Albizia gum, Hupu gum, Lepidium sativum, Gum Copal, Gum Damar, Bhara Gum, Moi gum, Cactus mucilage, Cordia gum, Hakea, Karaya gum, Mucuna gum, Satavari mucilage, Ocimum seed, Mucilage, Leucaena seed gum, Cassia tora, Cashew gum, Asario mucilage, Bavchi mucilage, Abelmoschus mucilage, galactomannans (locust bean, guar, fenugreek and tara gum, hexofuranosides) Gum kondagogu, gum olibanum, Sida acuta gum (SAG), Cashew-nut tree exudate gum, gum from Meryta sinclairii, peach tree gum, angico gum, Laguncularia racemosa, Durian seed gums, Lepidium perfoliatum, Flaxseed gum, Albizia lebbeck gum, seeds of Gleditsia sinensis Lam gum, Mesquite gum (Prosopis spp.), Albizia procera gum, Yanang (Tiliacora triandra) leaves, Mesona Blumes gum, tamarind seed gum, Salvia macrosiphon) seed gum, hsian-tsao leaf gum, flamboyant (Delonix regia) seed gum, Boswellia and Commiphora gum, Angum gum, Gum karaya (Sterculia urens L.), Bael gum
Algal polysaccharides	**Brown algae**: mannitol, Alginates and fucose/fucans/fucoidans, sargassan, Laminaran, Polyuronan, alginic acid **Green algae**: Ulvan Oligo-Ulvans **Red algae**: Agar/Agarose (agarans), carrageenans, hypneans, porphyran, furcellaran, funoran, dulsan, and iridophycan, mannans, crystalline mannas and xylomannans, rhamnanns **Mirco alga**: Spirulan, sacran However certain green algae polysaccharides also play some important role. Cyanobacteria (cyanobacterial polysaccharide) of the genera Aphanocapsa, Cyanothece, Gloeothece, Synechocystis, Phormidium, Anabaena and Nostoc are able to produce sulfated polysaccharides containing uronic acids
Microbial polysaccharides	**Bacterial polysaccharide**: Bacterial cellulose, dextran, bacterial hyaluronic acid, xanthan, emulsan, β-d glucans, curdlan, alignate, gellan and pullulan, Scleroglucan and Schizophyllan. Bacterial Hyaluronic Acid, kefiran, exopolysacharide (EPS). xanthan gum, dextran, welan gum, gellan gum, diutan gum and pullulan. **Fungal polysaccharides** (Chitin, Scleroglucan, Lentinan, Schizophyllan Krestin, galactofurinase) **Yeast polysaccharide**: Zymosan, glucans, glycogen, mannan
Mammalian polysaccharides	Glycosaminoglycans (Hyaluronic acid or hyaluronan, Chondroitin sulphate), gelatin and heparin sulfate. Chitin and chitosan
Others	β 1,3-Glucans derived from a variety of natural sources (such as yeasts, grain, mushroom or seaweed), poly-gamma-glutamate (Aminoacid polymer)

methods. Developments in multi- and low-angle laser-light diffusion detectors coupled to high performance size exclusion chromatography now render the determination of molecular weight and molecular weight distribution of these galactans more accessible. Moreover techniques like NMR, rheology, dissolution techniques, bioadhesion testing methods, DSC, desulfation methods, various carbohydrate determination methods, SEC, freeze-drying, scanning electron microscopy, colorimetry, turbidimetry, X-ray diffraction method, plane polarized microscopy¸ fingerprinting approaches, Chromatographic separations of the fragments by HPLC, HPAEC and/or capillary electrophoresis and mass-spectrometric identification methods using the recently developed ESI-MS-MS and/or MALDITOF-MS technologies. (high performance size exclusion chromatography) coupled to multiple or low angle laser light scattering detectors, various other hydrolysis and chemical modification methods, establish a more clear link between structure and gelation of polysaccharides. From the view of their applications and structural complexicity now a day's polymers from algal sources are getting more magnitude of concern then from plants, mammalian and microbial resources. However here we have targeted certain gelling polysaccharides of natural origin with a objective to study its broad pharmaceutical applications, to testify their effectiveness in curing various human disorders and how current research is employing different tools in studying and improving their native properties [18–32].

Carbohydrate-containing structures are amongst the most complex, heterogeneous, and abundant biomolecules on earth. Diversity of polysaccharides has given germination to modern knowledge to understand its structure related gelling properties. It is essential to classify these compounds to distinguish their role and quality in the drug delivery systems. Throughout our literature survey we comes to conclusion that plant derived polysaccharides has wide applications in pharmaceutics however algal or sulphated polysaccharides gives more advancement to the area of polymeric sciences [5, 33]. Whereas mammalian polysaccharides are considered as non toxic biomolecules having excellent mucoadhesive capacity and many important applications in formulation of bioadhesive drug delivery systems. Besides its mucoadhesive properties, it was found that this biopolymer may enhance the absorption of drugs and proteins via mucosal tissues. Furthermore microbial polysaccharides are more economic and exclusive polymers provide an alternate source to the current polymers exploiting industries.

4.3 Methods of Extractions

4.3.1 Cold Extraction

5 g of dried algal material was dissolved in 250 mL of distilled water and kept in orbital shaking incubator for 12 h at 20–25 °C degree. To obtain polysaccharide fraction the insoluble fraction was removed by centrifugation (15,000 rpm at 4 °C).

The supernatant was separated and treated with ethanol (1:3 v/v). Ethanol precipitated fraction was again dissolved in distilled water and dialyzed. The obtained dialyzed sample was lyophilized weighed (0.38 g) and coded as EC [34].

4.3.2 Hot Extraction [Mild Acidic (EHA), Alkaline (EHB) and Radical Hydrolysis (EHR)]

5 g of dried algal material was extracted with HCl (0.1 M) and maintained at 80 °C with constant mechanical stirring for different periods of time. The acid solublized fraction was separated by centrifugation (15,000 rpm at 4 °C) for 15 min. Similar procedure was again repeated for alkaline hydrolysis using $NaBH_4$ (0.1 M). Both fractions were lyophilized and their yield was denoted as EHA and EHB. For all the procedures the reaction time between sample and hydrolyzing agent was limited to 2 h.

$$\%\,\text{yield of POR} = \frac{\text{Dry weight of Porphyran}\,(\text{g})}{\text{Dry weight of Seaweed}\,(\text{g})} \times 100$$

4.3.3 Radical Hydrolysis (EHR)

Radical hydrolysis was conducted by using ascorbate (0.1 M) and H_2O_2 (0.1 M) at 25 °C for 5 g of dried algal sample and the lyophilized product was denoted as HER [35–37]. The percentage yield was calculated on the basis of following equation:

$$\%\,\text{yield of POR} = \frac{\text{Dry weight of Porphyran}\,(\text{g})}{\text{Dry weight of Seaweed}\,(\text{g})} \times 100$$

4.3.4 Microwave Assisted Extraction (EM)

Domestic Microwave oven (CATA 2R, 140–700 W, Catalyst System, Pune, India) equipped with closed vessel (100 mL), power sensor, temperature sensor and temperature controller was used at conditions specified in the Table 4.1. 5 g of distilled water dissolved algal sample was introduced in to the closed vessel followed by opening of the vessel and cooling in an ice bath shortly to relieve the pressure. Subsequent procedures were similar to those for hot extraction of polysaccharides [29].

4.3.5 Ultrasonic Extraction (EU)

5 g of algal sample was dissolved in distilled water and placed in a 250 mL beaker. The beaker and its contents were placed in to 42 kHz bath (Branson Ultrasonic cleaning bath unit, model 1510 DTH) and extracted under specified conditions (Table 4.1). After this, the beaker was taken out of the sonication bath and subsequent steps were followed as mentioned in Hot extraction for polysaccharides [38].

4.3.6 Enzymatic Hydrolysis (EE)

5 g of algal sample was treated with different percent of weighed amount of *cellulase* in conditions as specified in Table 4.1. After the addition of distilled water pH was adjusted (4.5). Rest procedure was followed in a similar manner as followed in hot extraction method [39].

4.4 Chemical Composition Analysis

Chemical composition of polysaccharides can be determined by these methods. Total sugar content, galactose and 3, 6 anhydrogalactose (AGR) contents were estimated by phenol sulfate [40, 41] resorcinol methods. Furthermore sulfur and protein content were determined by toluidine [42, 43] methods [40–43]. The organic functional groups of the polysaccharides preparations can be identified by using an FTIR spectrophotometer via the KBr 141 pressed-disc method.

4.5 Physical Properties

4.5.1 Determination of Gelling Strength (GS)

5 % polysaccharide solution can be prepared in an autoclave at 100 °C. Gel formation took place in dark place at 25 °C after which the gel was kept at 10 °C overnight in a refrigerator [44]. Strength of the gel was measured at 20 °C using a Model TA-XT2 Texture analyser (Stable Micro System, Surrey, UK).

4.5.2 Determination of Gelling Temperature (GT) and Melting Temperature (MT)

The gelling and melting temperatures were measured according the method described by Craigie and Leigh [45]. For measurement of gelling temperature, 10 mL solution of agar was allowed to cool gradually and a thermometer was

emerged in the sol. The temperature at which the thermometer was fixed to the gel was noted. For melting temperature the gel was heated on a water bath and one iron ball (ca. 1 g of weight) was placed on the surface of the gel. The temperature at which the ball touched the bottom of the tube was noted.

4.5.3 Viscosity Measurement (VS)

Apparent Viscosity of polysaccharide can be measured by Brookfield Viscometer (Synchrolectric Viscometer, Stoughton, MASS 02072). Spindle No. 1 at 60 rpm was used for measuring apparent viscosities of agar samples (5 % in deionized water) at 60 °C.

4.5.4 Molecular Mass Determination (MM)

Owing to structural similarity with many polysaccharides, Agarose of known MW was taken as standard. 5 % polysaccharide solution of all fractions (test samples) and agarose (standard) was prepared in double distilled water. Flow time of the all solutions (test and standard) and solvent (double distilled water) was determined by using Cannon-Ubbeholde viscometer which gives the intrinsic viscosities [η] values of all preparation. Viscosity average MWs were calculated from the intrinsic viscosity using the Mark–Houwink equation for agarose, $[\eta] = 0.07\ M\ 0.72$ where [η] is mL/g [46, 47].

4.6 Physical-Chemical Modification of Plant Based Natural Polymers (PBNPS)

In contrast with synthetic polymers plant based natural polymers have their dominant features that can be physically or chemically modified for improvement in their respective utilization and applications. Owing to the diverse class of natural polymers it's very difficult to cover each and every polymer therefore here in this section we are more emphasizing on starch and cellulose based modification as an ideal tool to endow the strategic platform for other PBNPs. PBNPs can also be modified by grafting or conjugation on either linear or branched backbone or on its active functional groups. This type of tailoring can be achieved by surface modification, polymer-peptide conjugation, polymer-DNA conjugation, polymer-siRNA conjugation, conjugation of polymer-surfactant, polymer-antibody, polymer-gene and polymer-drug. These conjugates can be formulated in to nano or micro forms for their delivery at suitable site.

4.6.1 Chemical Modifications of Plant Based Natural Polymers (PBNPS)

Chemical modification of plant based natural polymer involves the polymer molecules in its native form. Modification is generally achieved through derivatization such as esterification, etherification and oxidation, crosslinking, cationization and grafting. Nevertheless, there has been shortage of new methodologies in chemical modifications since this type of modification endows issues concerning consumers and the environment. Currently polymeric science is adopting combinational treatment using various types of chemical treatments to create new kinds of modifications. In a similar way, chemical methods have been combined with physical modifications such as microwave, radiation and extrusion to produce modified polymer with specific functional properties. Overall merits of these modifications were to reduce the time of modification and encourage production. Owing to the presence of large amount of hydroxyl groups at the surface of PBNPs different chemical modifications have been attempted, including etherification, esterification, oxidation, silylation, polymer grafting, etc. noncovalent Surface modification by adsorbing surfactants and polymer coating has also been reported. Chemical modifications of plant based natural polymers (PBNPs) have been mainly conducted to alter their surface energy characteristics which can further improve compatibility, particularly when employed in conjunction with hydrophobic or nonpolar matrices in nanocomposites. Additionally chemical modifications establish stable negative or positive electrostatic charges on the surface of PBNPs. This introduction of charge provides better dispersion. Conducting this chemical modification or functionalization in a safe mode i.e. only alter the surface characteristics of PBNPs by maintaining the unique morphology. This step may avoid any polymorphic conversion and to preserve the integrity of the crystal. Various polymers especially polysaccharides have been chemically modified such as Kaur et al. [48] reported various surface modifications of starch e.g. microwave radiation with lipase as catalyst [49, 50], hydrophobic reaction of starch and alkenyl ketene dimer [51], esterification of starch nanoparticles with lipase as a catalyst [52], dual modified crosslink-phosphorylated [53], crosslinking coupled with osmotic pressure [54], starch-based hydrogels prepared by UV photopolymerization [55], starch esterified with ferulic acid [56], microwave-assisted synthesis of starch maleate and starch succinates [57, 58], microwave and ultrasound irradiation [59], hydroxypropylation and enzymatic hydrolysis [60], Ozone-oxidised starch [61–63].

4.6.1.1 Noncovalent Surface Chemical Modifications of Plant Based Natural Polymers (PBNPS)

Noncovalent surface chemical modifications of plant based natural polymers (PBNPs) are usually achieved by surface adsorption of surfactants. This was initially studied by Heux et al. [64, 65] who employed surfactant consisting of the

mono- and di-esters of phosphoric acid bearing alkylphenol tails and the obtained surfactant-coated CNs dispersed very well in nonpolar solvents [64]. it was further observed that surfactant molecules formed a thin layer of about 15 Å at the surface of the CNs [66]. Later on various surface modifier (ionic and non-ionic) were used to accelerate the characteristics of whole CNs based formulation [67]. Recently saccharide-based amphiphilic block copolymers were used to induce the surface modification on CNs, which resulted in to the excellent dispersion abilities in nonpolar solvents [68].

4.6.1.2 Tempo-Mediated Oxidation

At present, the more frequently used pre-treatment is TEMPOmediated oxidation. Certainly, the TEMPO-oxidized cellulose nanofibers, confers a complete class of nanocellulose valuable of consideration, in addition to PBNPs and MPBPs. This oxidation based method is the most reliable method for altering the surface modification of natural/raw cellulose, in which functional groups such as carboxylate and aldehyde can be incorporated into solid native cellulose under suitable conditions [69–74]. Additionally in contrast with energy consumption of repeated cycles of a high pressure homogenizer (700–1400 MJ/kg), this oxidation based pre-treatment considerably decline the consumption to values less than 7 MJ/kg [58, 60].

For chemical modification of plant based natural polymers (PBNPs) (2,2,6,6-Tetramethylpiperidine-1-oxyl)-mediated (or TEMPO-mediated) oxidation employed to convert the hydroxylmethyl groups present on their surface to their carboxylic form. Advantage of this reaction is that the oxidation reaction is highly selective for primary hydroxyl groups, thus the whole reaction is "green" and easy to execute. This reaction encompass the application of stable nitroxyl radical, the 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) in the presence of NaBr and NaOCl. This TEMPO-Mediated Oxidation was initially explored by De Nooy et al. In his study he observed that only the hydroxymethyl groups of polysaccharides were oxidized, whereas the secondary hydroxyls remained unaffected. The whole method is based on of pre-treatment consisting cellulose fibers oxidation via the addition of NaClO to aqueous cellulose suspensions in the presence of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and NaBr (at pH 10–11 at room temperature). During this chemical reaction primary hydroxyl groups (C6) selectively transformed to carboxylate groups by means of the C6 aldehyde groups. Additionally only the NaOH and NaClO are consumed [73]. Owing to the presence of the repulsive forces among the ionized carboxylates (which overpower the hydrogen bonds by holding them together) nanofibrils within the fibers separate from each other [75]. Amount of NaClO supplemented in reaction determines number of carboxylate groups formed on the surface of the MPBPs i.e. more carboxylate groups when more amount of NaClO is added. Similarly this supplementation of NaClO allows the oxidation for extended time [76]. It was reported that enhancing the addition concentration of NaClO (3.8–5.0 mmol/g) increases the carboxylate content from 0.2 to 0.3 mmol/g. Additionally this reaction extends the oxidation time period from

Table 4.2 Effectiveness of TEMPO based oxidation reaction in terms of oxidation efficiency of their pre-treatment

Reaction	Raw material	Oxidation efficiency	Reference
TEMPO based oxidation reaction using NaClO at high concentration	Sulfite pulp	70–95 %	[77]
	Cotton linters	62 %	
	Ramie	85 %	
	Spruce holocellulose	96 %	

40–45 min to 115–130 min. TEMPO based oxidation reaction is applied over various sources such as cotton linters, tunicate, wood pulp, ramie, bacterial cellulose and even spruce holocellulose [74, 77]. These researchers have described the effectiveness of this reaction on the basis of their oxidation efficiency of their pre-treatment [77] (Table 4.2).

One more TEMPO-mediated oxidation based methodology reported by Isogai et al., Isogai et al., and Saito et al. [78–80] based on the same principle apart from pH 7, NaClO instead and NaBr, and replacement of the primary oxidant NaClO with $NaClO_2$. In one review Isogai et al. [78, 79] already discussed the dissimilarity among these two processes. During first process oxidation of C_6-primary hydroxyls of wood cellulose achieved by TEMPO/NaBr/NaClO system at pH 10 and at room temperature, however very little amount of aldehyde groups (<0.08 mmol/g) are present in the oxidized cellulose [78, 79]. On contrary no aldehyde groups is obtained by the TEMPO/NaClO/$NaClO_2$ system at pH 5–7, from oxidized wood cellulose with a higher molecular weight. Moreover concentration of carboxylate was found to be 0.8 mmol/g, and the optimum reaction time and temperature required are higher [78, 79]. Recently TEMPO electro-mediated reaction discovered by Isogai et al. [78, 79] was explored as an alternative method to oxidize the C6-primary hydroxyls of cellulose. Electro-mediated oxidation with TEMPO at pH 10, and 4-acetamido-TEMPO at pH 6.8 in a buffer solution were recently applied to softwood bleached kraft pulp explored as new sustainable method to yield MPBPs. Such MPBPs must be having carboxylate and aldehyde groups on its surface so that and it could well replace the first two systems, however longer oxidation times are required, resulting in to high yield more than 80 % and preserves the main characteristics of TEMPO-oxidized MFC produced from bleached softwood kraft pulp [78, 79]. This procedure of pre-treatment with TEMPO oxidation is followed by mechanical treatment, which can be achieved using a cooking blender or an ultra turax system. To remove the partial fibrillated MPBPs, generally centrifugation is employed separation on a laboratory scale. However now day's sonication is employed inspite of blending in order to separate the TEMPO oxidized pulp and it was observed that the sonication time influences the yield of nanofibrils [75, 81]. TEM characterization confirmed the transformation of 97.5 % of the fiber suspension into MPBPs with a width of 3–5 nm. On contrary Li and Renneckar [82], measured an average thickness value of 1.38 nm and a length of

580 nm with the help of AFM after 30 min of sonication. Various researchers established apparent relationship between sonication time and its significant impact on the nanofibril dimensions. It has been reported that with longer sonication time thickness decreased to 0.74 nm and the length to 260 nm.

It was observed that when this TEMPO-mediated oxidation was employed for oxidation of CNs only half of the accessible hydroxymethyl groups are available to react, whereas the other half is buried within the crystalline particle. Araki et al. [83] demonstrated CNs maintained their initial morphological integrity and formed a homogeneous suspension when dispersed in water after the TEMPOmediated oxidation. This is due to the presence of the newly installed carboxyl groups that imparted negative charges at the CN surface and thus induced electrostatic stabilization. Later on similar observations were reported by Montanari et al. [71]. In his study he has observed that excessive TEMPO-mediated oxidation decreases crystal size which results in to the partial delamination of cellulose chains. Similarly various authors have investigated the degrees of oxidation that can be examined by using specific amounts of the primary oxidizing agent (NaOCl). Such an investigation was based on supramolecular structure, morphology, and crystallographic parameters of the CNs. It was observed that many TEMPO-oxidized or carboxylated natural polymers such as CN suspensions when dispersed in water give display birefringence patterns and do not show flocculation or sedimentation. This occurs due to the polyanionic character carried by the negative charges on the CNs surfaces.

4.6.1.3 Cationization of Plant Based Natural Polymers

During this process positive charges are introduced on the surface of plant based natural polymers (PBNPs) e.g. weak or strong ammonium containing groups, such as epoxypropyltrimethylammonium chloride can be grafted onto the plant based natural polymers (PBNPs) surfaces [84]. This can be achieved by the nucleophilic addition of the alkali-activated cellulose hydroxyl groups to the epoxy moiety of epoxypropyltrimethylammonium chloride. Ultimately aforementioned step resulted in stable aqueous suspensions of PBNPs such as CNs with unexpected thixotropic gelling properties. Shear birefringence was reported in some reports while no liquid crystalline chiral nematic phase separation was observed which may lead to high viscosity of the suspension.

4.6.1.4 Esterification, Silylation and Other Surface Chemical Modifications of Plant Based Natural Polymers

Sassi and Chanzy have reported homogeneous and heterogeneous acetylation of plant based natural polymers such as CNs. In this study they have induced homogeneous and heterogeneous acetylation by using acetic anhydride in acetic acid [85]. After TEM and X-ray diffraction analysis of acetylated samples, only a limited reduction in CN length was observed. This was happened because of limited

reduction in CN length was observed, which was further explained by nonswelling mechanism which only affects the cellulose chains localized at the crystal surface. The partially acetylated molecules instantaneously partition into the acetylating medium as they adequately solublize during homogeneous acetylation whereas the cellulose acetate stay insoluble due to the presence of unreacted cellulose chains in surrounded the crystalline core. In some cases of natural plant based polymers concurrent occurrence of hydrolysis and acetylation has been also reported e.g. as found in the case of cellulose. Application of some prominent simultaneous esterification/hydrolysis based reactions were also explored in the case Fischer esterification of amorphous cellulose chains as a viable one-pot reaction methodology that allows isolation of acetylated CNs in a single-step process [86, 87]. Yuan et al. [88] has recently explored the environmentally friendly CN surface acetylation which involves low reagent consumption and simple-to-apply procedure. Another reaction which is recently employed on cellulose matrix was based on alkenyl succinic anhydride (ASA), development of ASA-CA emulsion, to yield acylated CNs with high hydrophobic features. Highly substituted CN esters were recently developed by Berlioz et al. via highly efficient method (fatty acid chains based on dried CNs via a gas-phase process) for an almost complete surface esterification of CNs proceeded from the surface of the substrate to the crystal core. This method yielded fully reacted (esterified) CN without change in its native morphological features. Reaction of natural plant based polymers with organic fatty acid chlorides [having different lengths of the aliphatic chain (C12 to C18)], was also reported which resulted in high density of C18 fatty acid, advantageous enough for further grafting on such lengthy aliphatic chain (C12 to C18) [89]. Silylated based PBNPs modification was observed in case of cellulose whiskers. This has been resulted from acid hydrolysis of tunicate which have been partially silylated by a series of alkyldimethylchlorosilanes. This reaction yielded a product with the carbon backbone of the alkyl moieties ranging from a short carbon length to longer lengths [90]. Degree of silylation (DOS) plays an important role in deciding solubility of cellulose and its dispersion in solvent e.g. DOS between 0.6 and 1, encourages dispersion in solvents of low polarity leading to stable suspensions with preserved morphological integrity whereas at time when DOS >1 leads to deeper silylation (chains in the core of the crystals became silylated) which can further resulted in to disintegration of the crystals and ultimately the loss of original morphology characteristics. However some highly silylated CN was investigated by Roman and Winter as nanocomposites [91]. Lastly, it has been observed that *N*-octadecyl isocyanate based modification assist in improving the stiffness and ductility of the resultant nanocomposites [92].

4.6.1.5 Carboxymethylation and Acetylation

Carboxymethylation is another chemical pre-treatment which increases the anionic charges in the formation of carboxyl groups on the surface of the MPBPs. In previous work carboxymethylated MPBPs was compared its dimensions with

non-pretreated MPBPs [93] and it was observed that carboxymethylation treatment makes the fibrils highly charged and easier to liberate. Moreover it was observed that net specific energy consumption required after carboxymethylation was 2.2 MWh/t per pass through a microfluidizer, whereas 5.5 MWh/t per pass was required to obtain MPBPs without pre-treatment [94]. Zimmermann's research group developed acetylation process in which grafting of acetyl moieties intend to reduce the hydrophilicity of MPBPs and increase the chemical affinity between MPBPs and a nonpolar solvent. For improving compatibility with the PLA matrix Tingaut et al. [95] developed PLA/MFC biocomposites with acetylated MPBPs. Further they observed that concentration of an acetyl content above 4.5 % encourages significant alteration in the crystalline structure of MPBPs. In this study acetylation was done in the inner crystalline regions of the MPBPs and prevents hornification upon drying. Modification with acetyl groups reduces the chances of hydrogen bonding between MPBPs and therefore facilitates improved dispersibility in an apolar polymeric matrix. It was later discovered that MPBPs especially MFC can be stored in a dry form which allow its possible industrial-scale production.

4.6.1.6 Polymer Grafting of Plant Based Natural Polymers

Two main approaches, specifically, the "grafting-onto" and "grafting-from" has been carried on the surface of plant based natural polymers during polymer grafting. First strategy involve the grafting on to the open ends hydroxyl groups at the PBNPs surface of presynthesized polymer chains by using a coupling agent. In second strategy "grafting-from" *in situ* surface-initiated polymerization (from immobilized initiators on the substrate) has been carried out to form the polymeric chains. First approach was initially utilized by Ljungberg et al. [96]. In his work he has grafted maleated polypropylene onto the surface of tunicate-extracted CNs, which was resulted in to nanocrystals with good compatibility and high adhesion when dispersed in atactic polypropylene. Grafting of amine terminated polymers on the surface of TEMPO-mediated oxidized was investigated by Araki et al. [83] and Vignon et al. [97] Similarly grafting of DNA oligomers on the surface of CNs was studied by Mangalam et al. [98] All of these researchers reported high grafting density that was enough for grafted chains to crystallize at the surface of CNs. Cocrystallization phenomenon was first reported by Cao et al. [99] to yield grafted CNs polymer. This research has further promoted cocrystallizations of the free chains of the respective polymer matrices during CN-based nanocomposite processing. Additionally this phenomenon of cocrystallization significantly enhances the interfacial adhesion by inducing the formation of a co-continuous phase between the matrix and filler, resulted in to the highly improved mechanical strength of the resulting nanocomposites. Second approach known as "grafting from" was first reported by Habibi et al. [100], who has utilized stannous octoate (Sn(Oct)2) as a grafting and polymerization agent to graft polycaprolactone onto the surface of CNs via ring-opening polymerization. Pranger et al. [101] studied *in situ* polymerization of furfuryl alcohol from the surface of cellulose whiskers.

Later on various researchers produced thermoresponsive substrates by the polymerization of vinyl monomers from the surface of CNs [102].

4.6.2 *Procedure for the Development of Microfibrillated Plant Based Polymers (MPBPS) by Physical Modification*

MPBPs is currently fabricated from a number of different natural sources e.g. wood, bleached kraft pulp (starting material for MPBPs production) [73, 76, 94, 103–105], and bleached sulfite pulp [106, 107] (Figs. 4.1 and 4.2). Still various sources are needed to explore to fulfill the demand for such raw materials offering environmental benefits owing to their renewable nature and their low energy consumption in production [108]. Considering cellulose, Eucalyptus sulfite wood pulp, Bleached Luffa cylindrica fibers, Bleached sulfite pulp, Bleached sisal pulp, Sisal fibers (Agave sisalana), Elemental chlorine free bleached hardwood kraft pulp from Birch, Mixture of pine and spruce pulps (*Betula pendula*), Bleached and unbleached kraft hardwood pulps, Softwood sulfite pulp of spruce (*Picea abies*) and white fir (*Abies alba*), Wheat straw (*Triticum sp.*) Refined fibrous wheat straw (Vitacel, Rettenmaier & Sohne GmbH & Co.KG), Refined beech wood (Fagus sylvatica) (Mikro-Technik GmbH & Co. KG), Refined fibrous beech wood pulp (Arbocel, Rettenmaier & Sohne GmbH & Co. KG), Bleached sulfite softwood (Domsjo ECO Bright), Elemental chlorine free bleached hardwood kraft pulp from Birch (*Betula pendula*), Domsjo dissolving plus (Sweden), Softwood dissolving pulp (Domsjo), Wood pulp, Softwood dissolving pulp (Domsjo), Softwood dissolving pulp (Domsjo), Bleached kraft bamboo (*P. pubescens*), Domsjo dissolving plus (Sweden), Bleached sulfite pulp, Sisal fibers (*Agave sisalana*), are the main sources as reported by Lavoine et al. [109].

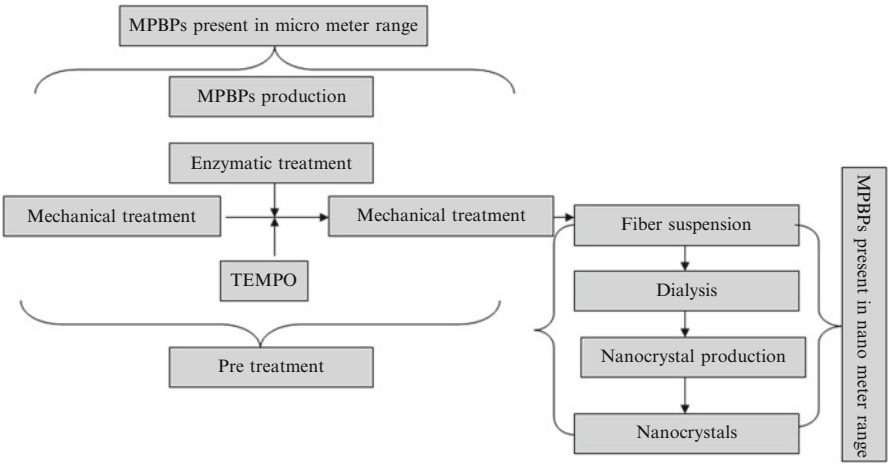

Fig. 4.1 Schematic representation of production of microfibrillated natural polymers

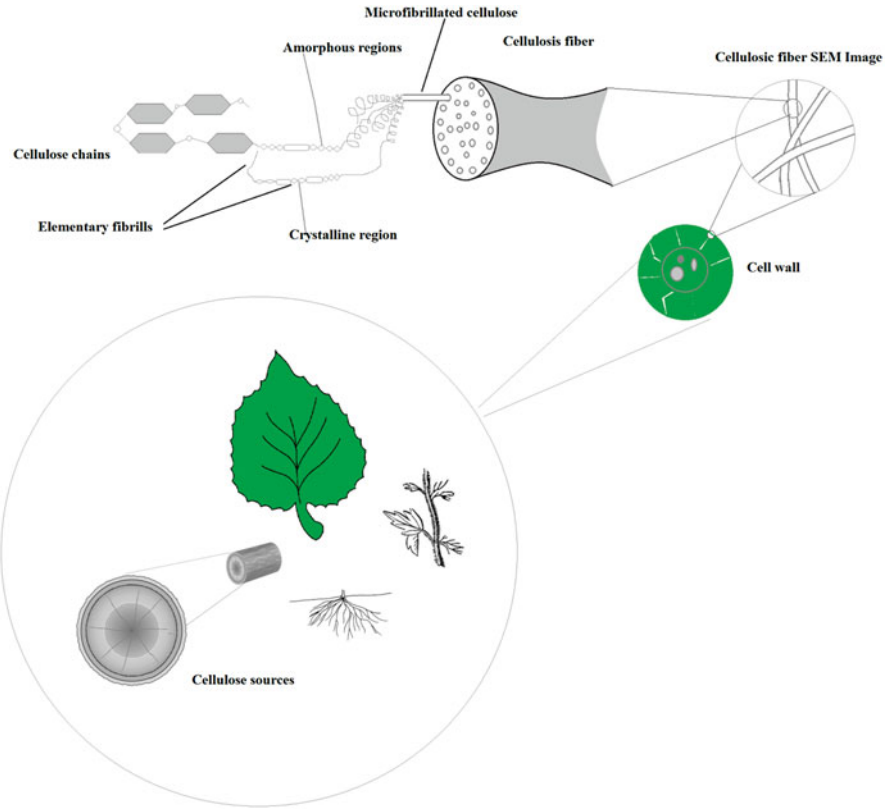

Fig. 4.2 Illustration demonstrating microfibrillated plant based polymers and their arrangement in plants

Irrespective of the source, natural polymers are manufactured from a pulp suspension mainly using a mechanical treatment. There are several types of equipments available to defibrillate the pulp and obtain nanopolymer. Various physical methods have been applied to transform the raw cellulose in micrometer size in to suspension or nano crystal form in nanometer size. Various physical methods that can be applied for this purpose are mentioned below.

4.6.2.1 Mechanical Treatments

Homogenizer and Microfluidizer

With the help of Gaulin homogenizer, Turbak et al. [110], for the first time applied mechanical treatment produce to manufacture all kinds of microfibrillated cellulose [93, 103, 111–115]. During this type of modification slurry of selected natural polymer is forced at elevated pressure and fed via a spring-loaded valve assembly. Fibers

are subjected to a huge pressure dive under elevated shearing forces. This was carried out by allowing the valve to open and close in quick progression. Combined force aggravates high degree of fibrillation of the cellulose fibers [115]. Equipment, Gaulin homogenizer most frequently used the microfluidizer since it makes it possible to obtain more uniformly sized fibers [111]. This equipment function when the masses of wood pulp passes through thin z-shaped chambers under high pressure [116] which results in the formation of very thin cellulose nanofibers. During this mechanical process chambers of different sizes are employed to increase the degree of fibrillation. This equipment used various time supplementation of raw material passage from chamber which consequently increases the number of passes. This factor limits the likely scaling-up of production and consequently creates negative environmental force with high energy consumption.

Grinding Process

Transformation of raw micro natural polymer in to fibrillated polymer can also be achieved by grinding process. There are various equipments used for grinding raw plant based polymer. Using equipment known as grinder, Masukoc was the first researcher to build and sell apparatus. Grinder generates shearing forces that help in breakdown of the cell wall structure. During this process raw material in form of pulp is passed through a static grind stone and a rotating grind stone revolving at about 1500 rpm. Grinder promotes separation of microfibrill by breakdown the cell wall which contains nanofibers in a multilayer structure. This step encourages individualized nanofibers from the pulp when material passes from the grinder after multiple attempts. It has been observed that multiple passes of raw fibers from *Pinus radiate* influence MPBPs morphology. After sequential one to three passes, maximum raw material turned into sub-micron-size and nano-sized fibers, whereas after five passes, most of the fibers became nanosized fibers. No significant changes were observed in the fiber morphology after passing raw material from grinder at higher number thus it was concluded after that five passes through the grinder the fibrillation of pulp fibers was roughly complete. In contrast with homogenization process, this grinding require only few passes to obtain plant based fibrillated natural polymer in micro or nano range. No. of passing usually determine the size or dimension of cellulose fiber. Nevertheless pulp degradation caused by grinding process in terms of reduction in length might affect the strengthening and physical properties of PNMPs [117]. As discussed above its very difficult to determine the length and suitable analysis of related characteristics therefore they cannot be monitored in comprehensive manner. Several authors have attempted to evaluate the chain length which was based on the results obtained from the intrinsic viscosity in cupriethylene diamine. Pohler et al. [118] proven that microfluidizer reduces the chain length more than the grinder. Couple of years before Uetani and Yano [115] used blender (with an ABS-BU motor, Vita Mix, and a CAC90B X-TREME 2 L bottle, WARING) with a well equipped tamper to produce microfibrillated cellulose. This equipment has efficiency to defibrillated different wood fiber suspensions

(0.1–1.5 wt%) at different stirring speeds, from 5000 to 37,000 rpm. However it's difficult to deduce best mechanical treatment since product obtain from this blender is not very homogeneous, even it doesn't acquire the capacity to transform whole plant based raw fibers in to micro fibrils. It was reported that it induces less damage then grinder treatment but still it's difficult to conclude which treatment can known to be the best treatment.

Cryocrushing

One rarely used and the most expensive method which cannot be scale up called as cryocrushing as suggested by Dufresne et al. [119]. He has crushed frozen pulp with liquid nitrogen [120, 121]. Owing supercritical nature of nitrogen, liquid nitrogen enter inside the cell, this penetration cause the crystallization of cell (Ice crystals within the cells are then formed), and eventually mechanical crushing is employed to breakdown the cellular wall and release wall fragments. So far this method is applied over many pulps and other plant material (sugar beet pulp, wheat straw and soy hulls, flax, hemp, and rutabaga fibers and soybean stock) to yield microfibrill polymer with varying nano dimensions [120–122].

Electrospinning

In contrast to all these methods electrospinning methods can be employed to obtain fibrillated PNMPs process of cellulose regeneration. However this process require more fundamental studies are currently dedicated to this method [123–126].

Energy Consumption and New Processes

Above mentioned methods require mechanical treatments with high energy utilization. Energy consumption (70,000 kWh/t) in case of homogenizer estimated was initially estimated by Eriksen et al., whereas energy consumption in case of microfluidizer estimated by Zimmermann et al., reaches up to 8.5 kWh [127, 128]. As discussed above in case of microfluidizer fibrillation depends on the number of times raw material passes through the assembly therefore for 10 L of PBNPs pulp it takes about 15 min to pass through a microfluidizer once and let there are overall four passes during this process then consumed energy can be calculated accordingly (8.5 kW, and its value increased to 14,875 kW with only three passes more). Since the most difficult thing that need further research is variation in mass during each pass (i.e. mass doesn't remain ideal) so for estimating single pass energy each time mass should be calculated. Process become more difficult when pulp solution obtained is less homogenous. Later on various authors' studied the factors (e.g. number of passes, the pressure, and the speed) that influence the rate of formation of microfibrills and films, mainly the nature of material (bleached and unbleached

kraft hardwood pulps) on consumption of energy [129, 130]. These authors' have done the comparative study of the energy consumption and physical properties of MFC produced by different processing methods, namely a homogenizer, a microfluidizer, and a grinder. From this study it was deduced that, in spite of its high energy consumption, homogenizer resulted in MFC with the highest specific surface area and films with the lowest water vapor transmission rate. Furthermore films produced during both process (microfluidizer and a grinder) offered better optical, physical, and water interaction properties. This unique features obtained from these processes suggested that these materials could be created with additional cost-effective approach for packaging purposes. Alternatively exploration of other less energy-consuming disintegration methods also turn into precedence in protecting the industrialization of MPBPs production. Presently combinations of various pre-treatments and mechanical methods have been employed to yield suitable microfibrill. Additionally in order to obtain MFC with low energy consumption or via a faster process, every year new equipment is being studied or developed. Heiskanen, Harlin, Backfolk, and Laitinen [131] has modified the process and tested with extrusion. Later on SUNPAP (2009), Papiertechnische Stiftung (PTS) in Germany, developed commercial process, Cavitron®. Recently novel fractionation devices have been developed to classify different MPBPs qualities [132].

4.6.3 Pre-treatment

Various approaches have been employed in order to obtain fibers that are less stiff and cohesive. These approached help in reducing the energy required for fibrillation. Among these approaches three approaches (avoiding the hydrogen bonds, adding a repulsive charge, reducing the DP or the amorphous link between individual PNMPs) are more preferred during pre-treatment. Protocols that are more favorable according to these considerations are:

4.6.3.1 Enzymatic Pre-treatment

Enzymatic modification has mainly used hydrolyzing enzymes in its modification and one of its products is syrup be it glucose syrup or high fructose corn syrup. With research, there are more enzymes being identified for use in modification of polymer. Combinational treatment is employed by combining enzymatic hydrolysis with mechanical shearing and high pressure homogenization (105 and 170 MPa) to obtain PNMPs in desirable nano range. Paakko et al. [133] conducted the whole process by introducing the enzymatic treatment with endoglucanase between two refining steps. Step is achieved by before passing the pulp slurry through the microfluidizer. Main positive point of enzymatic hydrolysis than acid hydrolysis it is less aggressive and allows selective hydrolysis of the non-crystalline cellulose, which facilitates the mechanical disintegration [133, 134]. Enzyme supplementation

promotes cell wall degradation and prevents the blockage in Z-shaped chamber of microfluidizer [135]. It has been observed that in some cases more specific enzymatic treatment is required e.g. pre-treatment with C-type endoglucanase enzyme studied by Henriksson et al. [135], to treat some disorder in the structure in order to attack the cellulose and attain its conformation that encourage its desirable properties.

After the mechanical treatment with Gaulin homogenizer, these researchers examined non-pretreated PNMPs in contrast with enzyme-pretreated MFC, as well as enzyme-pretreated PNMPs. This pre-treatment with endoglucanase allows the disintegration of cellulosic wood fiber pulp by enhancing its swelling in water, additionally this eco-friendly pretreatment present more favorable structure on the MFC, since it decreases the fiber length and enhances the amount of fine material, in contrast to end product obtained from acid hydrolysis pre-treatment. It was also studied that pretreatment with endoglucanase appeared to be a very promising method for industrial applications and important for first pilot production of PNMPs [134].

4.6.4 Post-treatments

In addition to the pretreatment, post-treatments in combination with pretreatment are progressively carried out in order to increase the features of microfibrillated natural polymer. In contrast with various pre-treatment approaches utilization of post-treatment still remains small. Goal of these two protocols are very distinctive since the primary objective of pre-treatment is to decrease the energy consumption of PNMPs production, while the post-treatment mainly aims to improve the PNMPs or to incorporate new features, from the viewpoint of new probable applications. In order to develop PNMPs films with good barrier properties, Rodionova et al. [136] carried out the acetylation of MFC from kraft pulp. Based on results [136], it was observed that hydroxyl groups replacement by acetyl groups occurs at surface of the PNMPs as well as amorphous regions for long reaction times. Later on it was studied that acetylation is an essential tool for bacterial cellulose to improve the optical properties of nanocomposite films [137]. Nogi et al. [138] studied that acetylation was also used to improve the thermal properties. In order to obtain hydrophobic MFC, Andresen et al. [139] modified the surface of MFC by means of silylation with chlorodimethyl isopropylsilane. It has been seen among some natural polymers that when the silylation conditions were too strong, PNMPs lost its microfibrillar structure and consequently it was concluded that with a degree of surface silylation (between 0.6 and 1), some natural polymer e.g. MFC could be dispersed into an organic solvent without losing its characteristics or properties. Some post-treatments among PNMPs e.g. MFC, encourage nanocomposite applications after their grafting with suitable coupling agents. Most of the post-treatments (Table 4.3) provide MFC with a hydrophobic character in order to improve its compatibility with non-polar polymers and thus play a major role in the elaboration of nanocomposites. However some post-treatments emerge to endow

Table 4.3 Post-treatments among MFC

Coupling agents	Purpose & applications	References
MFC+ titanate	To enhance the adhesion between MFC and epoxy resin matrix	[140]
Titanate + MFC	MFC with enhanced hydrophobic surface property	[106]
MFC oxidation with cerium IV	MFC with a hydrophobic surface layer, nanoscale electronic and optoelectronic devices	[106]
Grafting of hexamethylene diisocyanate.	MFC with more hydrophobic surface layer, nanoscale electronic and optoelectronic devices	[106]
Succinic and maleic acids coupled to the MFC	MFC with a hydrophobic surface layer, nanoscale electronic and optoelectronic devices	[106]
N-octadecyl isocyanate onto MFC	To improve the MFC's compatibility with polycaprolactone, using an in situ solvent exchange	[92]
N-octadecyl isocyanate onto MFC	To improve the MFC's compatibility with polycaprolactone	[141, 142]
Grafting cellulose with octadecyldimethyl (3-trimethoxysilylpropyl) ammonium chloride (ODDMAC)	MFC films with antibacterial property	[143]

microfibrillated cellulose with some new functionality. Owing to the unique features MFC that are attractive in different fields. Primary aim of this work is to reduce the energy consumption of MFC production so as to fulfill with a political agenda and gain market interest and second goal is to improve MFC properties in order to endorse a novel biomaterial with unique characteristics that can compete against the current non-biopolymers.

4.6.5 Dual Modifications

Recommendation for physical modification is sometime preferred over chemical since it can be safely used as a modification process in food products as it does not involve any chemical presence which some causes harm to biological tissue. Various physical methods have been employed for different plant based natural polymer using a combination of chemical and physical or chemical and enzymatical methods (Table 4.4).

Owing to the diverse class of PBNPs, here in this section we have only discussed the starch based physical or chemical modifications. Deetae et al. [53] combined methodology (physical as well as chemical) using crosslinking with sodium trimeta-

Table 4.4 Physical methods reported for starch modifications

Treatments	References
Corona electrical discharges	[144]
Deep freezing	[145, 146]
Instantaneous controlled pressure drop (DIC) process	[147, 148]
Iterated syneresis	[149]
Mechanical activation-with stirring ball mill	[150]
Micronization in vacuum ball mill	[151]
Multiple deep freezing and thawing	[146]
Osmotic-pressure treatment	[152]
Pulsed electric fields treatment	[153]
Superheated starch	[154]
Thermally inhibited treatment (dry heating)	[151, 155, 156]

phosphate and phosphorylation on rice starch, provided modified rice starch with good freeze-thaw stability. Whole procedure was conducted in the presence of osmotic-pressure enhancing salts [53]. These salts caused an increase final viscosity with a sharp decline in breakdown. It has been observed that trigger in osmotic pressure enhances the activity of the crosslinking agent [157]. UV induced polymerization is used to prepare starch-based hydrogels. During this procedure polymerization performed by treatment acryloylated starch with zwitterionic monomer 3-dimethyl (methacryloyloxyethyl) ammonium propane sulfonate (DMAPS). It was observed that this type of polymerization induces a unique salt-tolerant swelling behavior in modified starch [55]. Ou et al. [56] developed modified starch via esterification with ferulic acid which yielded in to starch ferulate. In contrast to native, starch ferulated starch exhibited higher water holding capacity, lower viscosity and much less retrogradation during low storage temperature [56]. Similarly Xing et al. [57] developed efficient method in esterifying starch, microwave-assisted esterification, to produce starch maleate using the dry method had a reaction efficiency of up to 98 %. Jyothi et al. [58] developed efficient method of producing succinylated cassava starch with microwave assistance to decrease the use of chemicals and enhance production. Later on it was observed that microwave and ultrasound irradiation can be employed for the esterification of carboxymethyl cold-water-soluble potato starch with octenylsuccinic anhydride which consequently shorten the esterification time from a few hours to a few minutes. Derivates produced during this process present outstanding emulsifying and surfactant performance properties [59]. For achieving more successful dual modification process, Karim et al. [60] utilized native starch in form of corn and mung bean starch. Native starch was modified by partial enzymatic treatment followed by hydroxypropylation with propylene oxide. Modified starch proved to have significant functional properties in contrast with hydroxypropyl starch prepared with untreated native starch [60].

4.6.6 Ozonation

In addition to above mentioned methods various other methods such as ozonation have been developed with more development in polymeric sciences and its allied fields. This process carries extra oxygen atom therefore act as powerful oxidant and can be applied for process of ozonation. According to previous reports this process enhances the carboxyl and carbonyl contents and concentration keep on increasing with time of exposure to ozone. In contrast hypochlorite oxidation process where large amount of salts are produced this powerful oxidant (ozone) is a clean and leaves no residues behind unlike [61].

Chan et al. [62] observed that there was a difference in the rate of starch oxidation among starches from various sources. An and King [63] found that starch those are produced in the presence of amino acids were more suitable alternatives in contrast to highly chemically oxidized starch and found useful as thickening agents.

As mentioned above physical modifications are safer for processing of food products since it does not involve any chemical presence. Owing to the diversity of plant based polymers, here in this section we have only discuss starch based modification. Some new strategies developed in physical modification for PBNPs are highlighted in Table 4.4. Pukkahuta et al. [152] utilized "Osmotic-pressure treatment" (OPT) in the presence of high salt solutions to obtain a uniform starch suspension and heat distribution. After treatment with the gelatinization temperatures potato-starch treated changed from a B to a A type. A uniform heat distribution is an advantage of this method in contrast to heat-moisture treatment which helps in production of modified starch at large scale. Similarly Szymonska et al. [145] reported the deep freezing and thawing of moistened starch to increase the crystallinity of the granules, however Szymonska et al. [146] also reported that multiple deep freezing and thawing caused an irreversible disruption of the crystalline order. Process of deep freezing and thawing was repeated until the moisture content in the solid phase was less than 20 %. It was observed that most of the starch showed conversion towards B-type X-ray diffraction pattern suggesting a disruption of the crystalline property [149]. Since there is no involvement of any type of chemicals therefore there is no concern for the effect on the environment and safety issues to be addressed. Process called as iterated syneresis was similar to multiple deep freezing and thawing. Other physical modification including instantaneous controlled pressure drop and DIC lead to increase in gelatinization transition temperatures and enzymatic hydrolysis while gelatinization enthalpy decreased after treatment. During this procedure saturated steam at a fixed pressure and predetermined time was injected before it drops towards vacuum to pursue the short pressurization. Other mechanical actions such as collision, friction, impingement, shear, etc can also be employed to modify the crystalline structures and properties of the starch granule. This process is called as mechanical activation or micronization. During this process large particle breakdown to form smaller particles however the tiny particles agglomerate and form large particles, resulting in to the decrease in gelatinization temperature and viscosity of the treated sample [150,

151]. Han et al. [153] reported the pulsed electric field (PEF) technology (non-thermal food preservation method) to study the effect of the treatment on starch. They have observed that starch molecules rearranged and destructed resulting in to the constant decrease in gelatinization properties, viscosity and crystallinity. Nemtanu and Minea [144] also reported that with increase of exposure time to corona electrical discharges, solubility, gel consistency and clarity of starches decreased. Dehydration of starch is done to achieve thermal inhibition which is carried out (a) by dehydrating starch until it becomes anhydrous, (b) treating it to a temperature of hundred degree Celsius for a period of time. The effect of heating can also be increased by an alkaline condition. Chiu et al. [155] suggested that pastes obtained from theses starches had increased resistance to viscosity breakdown and a non-cohesive texture. Ionic gums such as sodium alginate, CMC and xanthan act as crosslinking agents to form graft copolymers through ester formation. Such type of gums induced thermal inhibition [156]. Production of spreadable particle gels with spherulite morphology and creamlike texture upon cooling can be obtained by heating a starch solution to a temperature between 180 and 220° to form superheated starches. It was observed that in contrast with native starch dry superheated starches when mix with cold water gives immediate gel-like texture [154]. Two processes fluidized bed heating and extrusion heating were applied over on amaranth starch-rich fraction. Treatment with fluidized bed heating lead to some loss in crystallinity but granule integrity was preserved whereas extrusion heating caused a high degree of granule disruption and almost complete loss of crystallinity [158].

4.7 Genetic/Biotechnology Modification

Development in genetic engineering allows genetic modification of various natural plant based polymers probably by targeting the enzymes of the biosynthetic pathways of the respective NPBPs. Owing to the diverse classification of PBNPs, here in this section we have only discussed starch which can present the better ideology/platform for the modification of others. Current development in genetic engineering science allows transgene technology to produce genetically modified starches which can prevent the environmentally harmful post-harvest chemical or enzymatic modification [159]. It was observed that activity of these enzymes plays an important role in affecting the functionality, reactivity and applicability in non food and food applications of these modified starches. Traditional plant-breeding techniques or modern biotechnology can be applied to accomplish genetic modification in a more successful way [160]. Some of the key modifications of starches that have been done genetically are mentioned below. For alteration of specific structural motifs in potato starch, repression of starch phosphorylating enzyme R1 was used in the of potato starch [161]. Similarly modification for potato cell lines was achieved by treatment with an *Escherichia coli* glg B encoding a glycogen branching enzyme [162]. Starch obtained after this treatment contains higher amount of short amylopectin chains

with lower content of phosphate which can be used to give hard and adhesive gels. Alike to potato cell line total cassava root biomass can be treated with ADP-glucose pyrophosphorylase for enhancing the total cassava root biomass by 2.6 fold [163]. Modification of starch was also investigated when a full length cDNAs encoding a second starch branching enzyme isoform was isolated and an antisense starch branching enzyme A RNA was produced on transgenic potato plants. During this study complete reduction in starch branching enzyme A was observed. The average chain length of amylopectin was greater in modified starch and it was observed that the composition and structure of the potato starch was completely altered. Additionally higher levels of phosphorous were also reported [164]. Similarly Safford et al. [165] reported the same study by the modification of starch obtained from potato (showed altered amylopectin branch patterns). Verhoeven et al. [166] explored the tree mutagenised grains of the diploid oat (Avena strigosa): mutants lam-1, lam-2 and sga-1. It was investigated that two mutagens (lam-1 and lam-2) lacked in GBSS activity and amylose component thus endow mutations of the waxy type [166].

4.8 Applications of Plant Based Polysaccharides

Plant based polysaccharides are having various established applications in biomedical, tissue engineering, pharmaceutical sciences and other areas. Our current concern is to cover plant based natural polymers and their nanotechnological applications in different field. Table 4.5 covered various modifications of natural polymers and its nanotechnological application in different drug delivery systems.

4.8.1 Cellulose

In 1838 French chemist Anselme Payen discovered cellulose by isolating it from plant matter and determined its chemical formula. Cellulose is a linear unbranched organic polysaccharide with the molecular formula $(C_6H_{10}O_5)n$, consisting of $\beta(1 \rightarrow 4)$ linked D-glucose units from several hundred to over ten thousand (Fig. 4.3). Among all plant cell wall polysaccharides e.g. hemicelluloses, pectin, cellulose forms a vital structural component in higher plants and corresponds to the most abundant organic polymer [359, 360]. Linearly arranged various parallel cellulose molecules form crystalline microfibrils which are mechanically strong and highly resistant to enzymatic attack and are aligned with each other to provide structure to the cell wall. This organic polysaccharide is insoluble in water and indigestible by the human body [361, 362], however digested by herbivores and termites. Fibrous materials (such as wood and cotton) derived cellulose such as wood and cotton, can be mechanically treated (disintegrated) to produce powdered cellulose. This powdered form has been used in the pharmaceutical industry as filler in tablets. Treatment of high quality powdered cellulose with hydrochloric acid produces

Table 4.5 Various modification of plant based natural polysaccharides and its nano applications

Polymer	Modification	Nano applications	Drug delivery applications	References
Cellulose	(a) Sulfonation (b) Oxidation by TEMPO (c) Ester linkages via acid chlorides (d) Cationization via epoxides (e) Ester linkages via acid anhydrides (f) Urethane linkages via isocyanates (g) Silylation (h) SURFUNCELL (Surface functionalisation of cellulose with noble metals NPs through a selective nucleation)	(a) Nanocrystalline cellulose (NCC) (b) Nano-fibrils (c) Nano fibrillar cellulose matrices (d) Au, Ag, Pt, Fe_2O_3, Cds, PbO NPs (e) CA/TiO_2 hybrid membranes (f) Micrometer-long hybrid nanofibers (CdS NPs/bacterial cellulose hybrid nanofibers) (g) Hybrid Fe_3O_4@Amino cellulose NPs (h) Ag–Pd alloy nanoparticles (i) Cellulose whiskers (j) Magnetic NPs (k) pH-sensitive core–shell NPs (l) Cellulose-chitosan NPs (m) Fluorescent CMC NPs (n) Cellulose based semiconductors NCs	(a) Multi-particulate drug delivery of Aphidicolin nanosuspension (b) Transversal drug delivery of primaquine cellulose based niosomes (c) Various cellulose based formulations: PQ/- Eudragit® RL 100-, ethyl cellulose polymers with various penetration enhancers, PQ/ethyl cellulose polymer mixed with Mygliol® 840 plus the antioxidant alpha-tocopherol)	[167–187]
Xyloglucan	(a) Degalatosylation (b) β-galactosidase degradation (c) Synthesizing thiolated xyloglucan (TXG)	(a) pH dependent xylogucan nano aggregates (b) Self-assembled polystyrene/xyloglucan nanospheres	Xyloglucan mucoadhesive polymer is suitable for anti-protozoal and antimicrobial drug delivery	[188–195]
Galactomannan	Enzymic oxidation on the C-6 of the galactose side units (a) Oxidation (TEMPO or TEMPO-NaBr-NaClO system) (a) Etherification, (b) Esterification, (c) hydroxypropylation, (d) carboxymethylation	(a) Galactomannan and chitosan Nps (b) Gold Nps (c) Guar gum Nps (Cationic, carboxymethyl, hydroxylpropyl, and carboxymethylhydroxypropyl galactomannans)	Lichen galactomannan and its vanadyl (IV) complex on peritoneal macrophages and leishmanicidal activity.	[196–204]

(continued)

Table 4.5 (continued)

Polymer	Modification	Nano applications	Drug delivery applications	References
konjac glucomannan KGM	(e) Phosphorylated (f) glucomannan (g) By alkali and sodium (h) carboxymethylcellulose (enzymatic, alkali and acid hydrolysis) (i) Hydrogen bonds formation (j) Number of junction zones Length of connecting chains (k) Deacetylation process	(a) Mannose receptors targeted GM Nps (b) Galactomannan and chitosan (protein particular carrier) (KGM, KGM-KOH, KGM-CMC and KGM-CMC-KOH) (c) Conjugate with kappa carrageenan, acetan (xylinan), gellan gum, alginate and chitosan		[205–208]
Rosin	Polymerized rosin (PR)	Rosin NPs(hydrocortisone)	Rosin transparent wax preparation	[209–212]
Cyclodextrin	(a) Aqueous organometallic catalysis in aqueous media (hydrogenation, hydroformylation, oxidation, reduction and carbon-carbon coupling reactions) (b) α-cyclodextrin-dodeca (2, 3) benzoate (c) hexakis (6-amino-6-deoxy)-α-cyclodextrin hexahydrochloride (d) hexakis (6-amino-6-deoxy)-dodeca (2, 3)-*O*-methyl-α-cyclodextrin hexahydrochloride (e) hexa (6)-*O*-methyl-α-cyclodextrin (f) heptakis (6-azido-6-deoxy)-β-CD-tetradeca (2, 3)acetate	(a) Meglumine antimoniate-beta-cyclodextrin conjugates (b) Paclitaxel loaded Nonsurfactant Cyclodextrin Nanoparticles (c) Enhanced antiviral activity of Acyclovir loaded into β- cyclodextrin -poly(4-acryloylmorpholine) conjugate nanoparticles (d) aminated β-cyclodextrin silver nanoparticles (e) cyclodextrin–poly(anhydride) nanoparticles (f) β-cyclodextrin-modified TiO2 nanoparticles (f) Tamoxifen citrate loaded amphiphilic β-cyclodextrin nanoparticles (g) Camptothecin loaded amphiphilic β-cyclodextrin nanoparticles (h) Cyclodextrin-covered gold nanoparticles	(a) Oral delivery of meglumine antimoniate-beta-cyclodextrin complex (b) Beta-cyclodextrin as an absorption enhancer of the water-soluble drug meglumine antimoniate (c) Meglumine antimoniate-beta-cyclodextrin conjugates	[213–233]

Starch	Microwave radiation with lipase as catalyst a) Hydrophobic reaction of starch and alkenyl ketene dimer b) Esterification of starch nanoparticles with lipase as a catalyst c) Dual modified crosslink-phosphorylated d) Crosslinking coupled with osmotic pressure e) Starch-based hydrogels prepared by UV photopolymerization f) Starch esterified with ferulic acid g) Microwave-assisted synthesis of starch maleate and starch succinates h) Microwave and ultrasound irradiation i) Hydroxypropylation and enzymatic hydrolysis j) Ozone-oxidised starch	(a) Starch-Stabilized Silver Nanoparticles (b) Hydroxyl propyl starch NPs (c) starch-coated iron oxide nanoparticles (d) Nasal starch insulin NPs starch capped water soluble copper (e) idarubicin propylated starch NPs (f) supramagnetic carboxymethyl starch NPs (g) amylomaize coenzyme Q10 NPs	Primaquine-Conjugated Gum Arabic Microspheres oxidized starch imine derivatives	[52, 234–240]
Xanthan gum	α-Galactosidase enzyme treatment Modified with formaldehyde to improve the dissolution rate.	Starch–xanthan gum Chitosan/xanthan Gum/montmorillonite nanocomposites Konjac glucomannan/xanthan gum	Amphotericin B-Gum Arabic Conjugates To improve the bioavailability of Berbrine, antiprotozoal drug Colon targeted metanidazole by xanthan gum	[241–249]
Guar gum	Silanization of guar gum (trimethylsilyl chloride) derivatization, grafting and network formation Carboxymethylation	Polyacrylamide/guar gum graft copolymer silver nanoparticles Tamoxifen loaded Guar gum Acrylic acid grafted guargum–nanosilica	Guar gun for Acyclovir delivery Colon targeted metanidazole by guar gum	[201, 241, 250–261]

(continued)

Table 4.5 (continued)

Polymer	Modification	Nano applications	Drug delivery applications	References
Arabinogalactan	With 5-amino salicyclic acid with isonicotinic acid hydrazide	gold nanocomposites (Au, Ag, Pd, and Pt protected by natural polymer arabinogalactan) Core-shell colloidal structure of nanobiocomposites of gold nanoparticles	Efficacious treatment of experimental leishmaniasis with amphotericin B-arabinogalactan water-soluble derivatives. Deaxtran and arabinogalactan conjugates	[241, 262–294]
Inulin	Tosylated and azidated inulins O-(aminoethyl)inulin β-hydroxyalkyl ethers of inulin inulin with amidoxime groups and coordination with copper(II) ions poly(acrylic acid) grafted inulin carboxy methylation of inulin Modified inulin 2 6 using tetramethylsilane (TMS)	Hydroxyapatite Nps (carboxymethyl inulin derivative) Inulin multi-methacrylate (IMMA) Nps Inulin PEG-ylated Nps		[295–309]
Locust beam	β-mannanase, β-mannosidase and α-galactosidase cleavage carboxyl, hydroxyl, and phosphate derivatives of these polymers	Locust bean based polymer-lipid NPs		[310–314]
Gum tragacanth	Using epichlorhydrine. Effect of gamma irradiation Lactose addition Mechanical degradation (microfluidization)	Fabricated silver nanoparticles	Binding agent in antiprotozaol drug delivery	[272–277, 279, 280]

Gum Arabic	Methylation β-D-mannanase degradation acetylation Addition of monovalent metal	Nanoparticles with affinity ligands specific for antibodies Magnetic nanoparticles with oleylamine Maltose and gum arabic hybrid gold nanoparticles Silver nanoparticles in gum arabic based semi-IPN hydrogen	Amphotericin B-gum arabic conjugates Self-gelling primaquine-gum arabic conjugate for primaquine	[241, 281, 282, 284–289, 291, 294, 315, 316]
Gum khatti	Methylation Acylation deionized water treatment Microwave assisted synthesis of polyacrylamide grafted gum ghatt	Size-controlled silver nanoparticles Insulin nanoparticles using chitosan and Arabic gum Noble metal nanoparticles Beta-lactoglobulin-polysaccharide nanoparticles	Trichostatin as an antiprotozoal agent	[317–322]
Pectin	Pectins except glycine Methyl ester (glycylglycinemodified pectin) Deesterified by base, plant or fungal pectin esterase or esterified inacid methanol Arabinase, pectin esterase and pectin acetyl esterase degradation UV irradiation Heat inactivated pectin methylesterase and NaCl Endo-arabinanase and α-L-arabinofuranosidase Modification in their degrees of methylation and acetylationCalcium binding efficacy modification Citrus pecin (potassium pectate), were modified with a low amount of UV-absorbing substituents Protease, arabinanase/galactanase mixture, polygalacturonase structural modifications	Thiolated pectin nanoparticles pectin-based nanoparticles for poorly soluble drugs β-lactoglobulin–pectin nps pectin–iron oxide magnetic nanocomposite Formation of nano-hydroxyapatite crystal in situ in chitosan–pectin polyelectrolyte complex network	Metronidazole loaded pectin microspheres Nystatin, clotrimazole, amphotericin B, miconazole, ketoconazole or griseofulvin oral pectin based delivery Zn/pectin beads with a eudragit coating	[323–341]

(continued)

Table 4.5 (continued)

Polymer	Modification	Nano applications	Drug delivery applications	References
Gellan (ion activated gelling polymer)	Methacrylated Gellan Gum esterified Gellan Gum Gellan gum grafted cinnamate photo crosslinkable polymer Fermentation mediated esterification Ionotropic gelation of gellan with trivalent Al+3 ions and covalent cross-linking with glutaraldehyde (GA) for Al+3/gellanbeads Peptide- modification of gellan gum Gellan gum films with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide cross-linker Gellan gum and egg albumin preparation Microwave assisted synthesis of acrylamide grafted gellan Monovalent cation salts addition for Commercial gellan preparation TEMPO oxidation Deacylation of gellan Gellan forms coupled networks with konjac glucomannan and tamarind xyloglucan, Phase-separated networks with kappa carrageenan and calcium alginate Interpenetrating networks with agarose and gelling maltodextrin Complex coacervates with gelatin under acidic conditions	Gellan gel beads containing magnetic nanoparticle Gellan gum blended PEI nanocomposites	Swellable gellan gum for acyclovir deliver Gellan gum for antiprotozaol drug delivery Chitosan and gelln gum based delivery of clindamycin In situ gellan gum gel for secnidazole delivery	[342–358]

(2,2,6,6-Tetramethylpiperidine-1-oxyl)-mediated (orTEMPO-mediated)

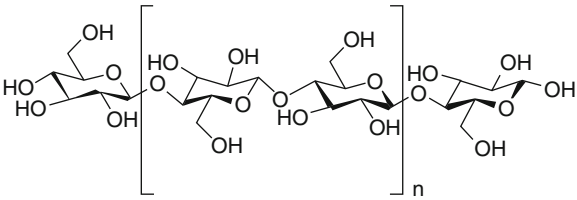

Fig. 4.3 Chemical structure of a powdered cellulose (n ≈ 500) or microcrystalline cellulose (n ≈ 220)

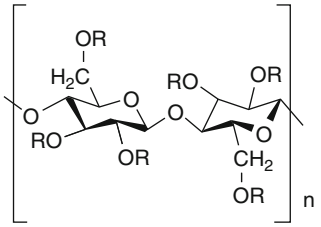

Fig. 4.4 Chemical structure of hydroxypropylmethylcellulose

Where R is H, CH_3 or $[CH_3CH(OH)CH_2]$.

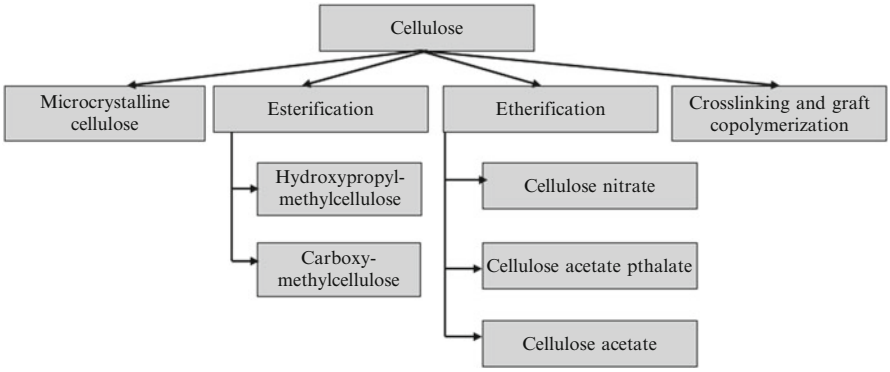

Fig. 4.5 Derivatization and modification of cellulose

microcrystalline cellulose. Microcrystalline cellulose is preferred over powdered cellulose because it is more free-flowing containing non-fibrous particles and therefore used in the pharmaceutical industry as a diluent/binder in tablets for both the granulation and direct compression processes [363]. Derivatization of cellulose can be achieved by replacing/modifying open hydroxyl moieties on the D-glucopyranose units of the cellulose polymer to give a variety of derivatives (Fig. 4.5). This process involves various physical/chemical modification which are fixed according to post and pre treatment procedures during which chief chemical reactions such as etherification, esterification, cross-linking or graft copolymerization are conducted. Esterification results in derivatives which include cellulose nitrate, cellulose acetate and cellulose acetate phthalate whereas etherification yields derivatives such as

hydroxyl-propyl-methylcellulose and carboxyl-methyl-cellulose. These all chemical products have outstanding application in membrane controlled release systems or monolithic matrix systems such as film or enteric coating and the use of semi-permeable membranes in osmotic pump delivery systems. Partly O-methylated and O-(2-hydroxypropylated) cellulose ether derivative known as hydroxypropylmethylcellulose has been extensively studied as an excipient in controlled release drug delivery systems due to its gel forming ability. These derivatives are having wide use and applications in monolithic matrix systems. Various reports and investigations have proven their potential to sustain the release of medicaments and most of these derivatives have been employed for this purpose [364].

4.8.2 Hemicellulose

A hemicellulose is a heteropolymer (matrix polysaccharides), such as arabinoxylans, consisting xyloglycans, xylans, mannans and glucomannans, and β-(1 → 3, 1 → 4)-glucans (Fig. 4.4) [365].

4.8.2.1 Arabinoxylans

These polysaccharides are bound to the surface of cellulose microfibrils which themselves do not form micro fibrils can be extracted from the plant cell wall with the aid of strong alkali. In contrast with cellulose (crystalline, unbranched strong, and resistant to hydrolysis), hemicellulose has a random, amorphous structure with little strength. Unlike cellulose, hemicelluloses have β-(1 → 4)-linked backbones with an equatorial configuration, consists of shorter chains—500–3000 sugar units. In addition, hemicellulose is a branched polymer, while cellulose is unbranched. Though the xyloglycans have alike backbone as cellulose, they contain xylose branches on 3 out of every 4 glucose monomers, while the β-1,4-linked D-xylan backbone of arabinoxylan contains arabinose branches [366].

4.8.2.2 Glucomannans

Glucomannan is mainly a straight-chain hydrocolloidal polysaccharide of the mannan family, consisting of β-1,4 linked D-mannose and D-glucose monomers (with acetyl side branches on some of the backbone units), with about 8 % branching through β-(1 → 6)-glucosyl linkages. The component sugars are β-(1 → 4)-linked D-mannose and D-glucose in a ratio of 1.6:1, but the mannose:glucose ratio may differ depending on the source [367]. The acetyl groups contribute to its solubility and swelling capacity of the glucomannans and enhance the solubility of the glucomannans by supporting glucomannans in making a soluble natural polysaccharide with the highest viscosity and water-holding capacity. It is diversely

present very in nature and specifically derived from tubers, softwoods, roots and plant bulbs. Glucomannan is called as konjac Glucomannan as it is the most commonly used type of Glucomannan which is extracted from the tubers of *Amorphophallus konjac* (Ulmaceae) and act as a potential excipient in controlled release drug delivery devices in combination with other polymers or by modifying its chemical structure. As mentioned, it is a very promising polysaccharide for incorporation into drug delivery systems. According to previous investigation konjacglucomannan and xanthan in combination efficiently slow down drug release by stabilization of the gel phase of the tablets. Stabilization is achieved by a network of intermolecular hydrogen bonds between the two polymers [368].

4.8.3 Starches

Starch or amylum (Fig. 4.6) is a storage and structural polysaccharide consisting of a large number of glucose units joined together by glycosidic bonds, chiefly present in plants as energy source. Various sources of starches have been used for pharmaceutical purpose such as maize (*Zea mays*), rice (*Oryza sativa)*, wheat (*Triticum aestivum*), and potato (S*olanum tuberosum*) [369]. Generally starch present in two forms modified and native starch. Starch whether in the native or modified form has been used as one of the key pharmaceutical excipients in pharmaceutical tablet and capsule formulations. Modified Starch is evaluated as pregelatinized starch product in directly compressible controlled-release matrix systems which can be prepared by enzymatic degradation of potato starch followed by precipitation (retrogradation), filtration and washing with ethanol whereas native starch may not be suitable in controlled release drug delivery systems due to substantial swelling and rapid enzymatic degradation resulting in too fast release of

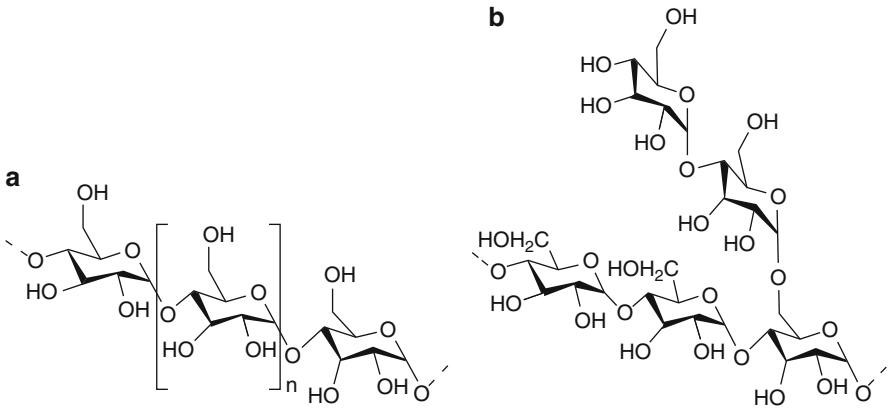

Fig. 4.6 Chemical structure of starch, with (**a**) amylose and (**b**) amylopectin

many drugs. Owing to this derivatives of native starch that are more resistant to enzymatic degradation as well as crosslinking and formation of co-polymers. Moreover most of them serve various functions such as binder, aiding drug delivery, bulking or disintegrant. Currently starch based micro-capsulated delivery systems can be used to deliver proteins or peptidic drugs orally [369]. Recently starch was modified using interfacial cross-linking agent, terephthaloyl chloride, to form starch/bovine serum albumin mixed walled microcapsules. During this procedure native or amino-protected aprotinin was loaded in microcapsules by supplementing protease inhibitors in the aqueous phase. This procedure was performed with the help of cross linking agent. Modified starch in form of microcapsules showed protective effect for bovine serum albumin. It was observed that acetylation of starch significantly reduces its swelling and enzymatic degradation [370]. According to previous findings, acetylation of potato starch considerably delay drug release compared to that of natural potato starch film. During the investigation on Amylose-rich maize starch (Hylon VII™) for tablet film coating it was observed that the temperature of the coating pan did not influence the roughness of the coated tablet at low spray rates, whereas at high spray rates increases the temperatures, resulted in to the smooth films. This was resulted in to the rapid dissolution rate Hylon VII™ coated tablets in an acid medium (releasing 75 % of the drug). Various other reports on the amylose and native starches as film forming agent were explored [371–375]. In another work ethyl-cellulose was employed with amylase in combination to generate aqueous and non-aqueous based coatings for colon drug delivery has been reported [376].

4.8.4 Pectin

Pectin (Fig. 4.7) is the purified non-starch, linear polysaccharides extracted from the plant cell walls especially by acid hydrolysis from the inner portion of the rind of citrus peels i.e. *Citrus Simon* or *Citrus Aurantium*, (Rutaceae). This water soluble linear galacturonic acid polysaccharide mainly composed of α-1,4-linked Dgalacturonic acid residues interrupted by 1,2-linked L-rhamnose residues with a few hundred to about one thousand building blocks per molecule, equivalent to an average molecular weight of about 50,000 to about 1,80,000 [377]. since galacturonic acid polysaccharides contains different neutral sugars such as arabinose, rhamnose, xylose, glucose, and galactose, therefore composition of pectin varies according to the botanical source, e.g. pectin from citrus contains lower amount of neutral sugars and has a lesser molecular size in comparison to the pectin obtained from apples [378, 379]. Owing to its water solubility, this polysaccharide is not able to protect its drug load efficiently for the period of its passage from the stomach and small intestine [377]. Against this pH variable *in vivo* environment, drug core should be protected with significant thickness or layers of polymer, thus the whole center of attention was inclined to the progress of low water soluble pectin derivatives which get degraded by the colonic micro flora. Derivatization or modification of pectin reduces their solubility e.g. calcium derivatives of pectin were found to reduce the

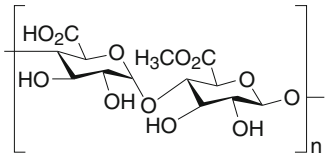

Fig. 4.7 Chemical structure of pectin

solubility by forming an eggbox configuration. Ethyl-cellulose was also examined as a coating material for colon-specific drug delivery. This combination was developed to overcome the drawback of high solubility of pectin and provide colon specific degradation properties of pectin with the protective properties of the water insoluble ethyl cellulose [380]. Pectin based hydrogels widely investigated used as controlled-release matrix tablets. Pectin in form of high-methoxy-pectin showed their potential in controlled-release matrix formulations and the drug release from compressed pectin based matrix tablets can be modified by altering the concentration and nature of pectin in the matrix tablets. It was observed pectinic acid (degree of methoxylation 4 %) with retarded solubility was suitable as an excipient for pelletisation by extrusion/spheronisation. According to previous work presence of 20 % pectinic acid in formulations is suitable for the formation of approximately all spherical beads which were mechanically stable and showed drug release at physiological pH 6.8 [381]. Pectin based micro particulate polymeric delivery systems have been investigated as a possible approach to improve the low bioavailability characteristics [382]. Spray dried pectin microspheres of piroxicam showed a considerable improvement of piroxicam bioavailability when compared with marketed piroxicameye drops. Modification of pectin such as amidated pectin was investigated to mask the bitter taste of chloroquine when orally administered [383]. Study showed potential applications of pectin-chloroquine patch matrix for the transdermal delivery of chloroquine. Similarly calcium pectinate nanoparticles were prepared to deliver insulin in colon [384]. Additionally pectin gel formulations has wide application in cosmetics for the prolonged release of cosmetic compounds such as citronellal responsible for the fragrance and proved as promising materials for controlled fragrance release [385].

4.8.5 Inulin

Inulin (Fig. 4.8) is a naturally occurring storage polysaccharide obtained from Dehlia, *Inula Helenium* (Compositae), *Saussurea lappa* (Compositae) or chicory roots, Dendelion, *Taraxacum officinale* (Compositae). Burdock root, *Cichonium intybus* (Compositae) [386]. This polysaccharide contains mixture of oligomers and belongs to the group of gluco-fructans, widely present in plants such as artichoke, garlic, onion, and chicory. As far as structural features are concerned inulin molecules contain from two to more than 60 fructose molecules linked by β-2,1-bonds. According to previous investigation inulin is not digested properly in the upper gastrointestinal tract, however this polysaccharide hydrolyzed by colonic

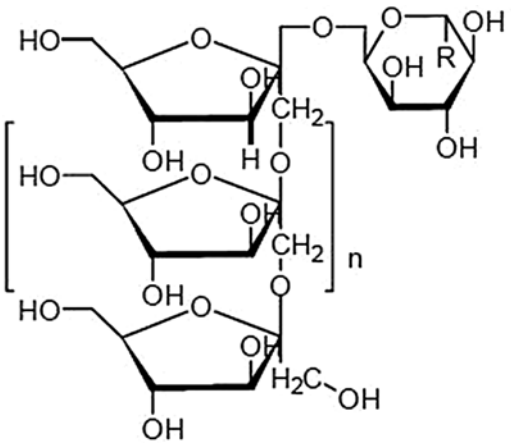

Fig. 4.8 Chemical structure of inulin

microflora [387, 388]. For delivering drug or any other therapeutic substance in gastric and intestinal fluids, inulin with a high degree of polymerization was used in combination with Eudragit® RS (that could withstand break down by the gastric and intestinal fluids) to prepare biodegradable colon-specific films [387]. In an alike study it was reported that using the combination of two different grades of Eudragit® RS and Eudragit® RL when mixed with inulin showed better swelling and permeation properties in colonic medium rather than other gastrointestinal media [389]. Earlier investigation on inulin based hydrogels for colon-specific drug delivery systems proven that methylated inulin hydrogels exhibit comparatively high rate of water uptake and anomalous dynamic swelling behavior pH sensitive hydrogel by UV irradiation were investigated by derivatization of inulin with methacrylic anhydride and succinic anhydride [388]. This pH sensitive hydrogel system exhibit exhibited a reduced swelling and low chemical degradation in acidic medium. However it shows good swelling and degradation properties in simulated intestinal fluid especially in the presence of its specific enzyme called inulinase [295].

4.8.6 Rosin

Rosin (Fig. 4.9) also called colophony or Greek pitch (*Pix græca*), is a low molecular weight (400 Da) natural polymer obtained from the oleoresin of various *Pinus sp.* by heating fresh liquid resin to vaporize the volatile liquid terpene components. It contains abietic and pimaric acids and exhibit excellent film-forming properties. According to recent report, Rosin and its derivatives investigated as potential biopolymer for various pharmaceutical applications such as film-forming and coating properties, matrix materials in the tablets for sustained and controlled release and microencapsulation [390]. Recent report explored its improved drug release properties matrix tablets and pellets [391] when its synthesized by a reaction with polyethylene glycol 200 and maleic anhydride. This work lead to the

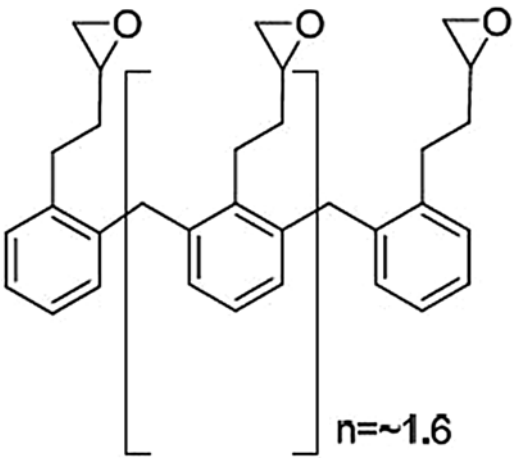

Fig. 4.9 Structure of Rosin

development of potential derivatives which can be suitable for sustain release of the tablets. Similarly high polymerization of rosin films showed excellent potential as coating materials for the development of sustained release dosage forms [392]. Various investigations on rosin derivatives (glycerol ester of maleic rosin) and their film forming and coating properties established their potential as potential coating materials for pharmaceutical products as well as in sustained release drug delivery systems. It was observed that rosin concentration has profound effect on the release of the hydrocortisone from rosin based nanoparticles which has demonstrated its potential as effective nanoparticulate drug delivery systems [209].

4.8.7 Plant Based Gums

Plant has its own established mechanism to secrete viscous, sticky fluid exudate gums in order to seal-off infected sections of the plant and prevent loss of moisture due to physical injury or fungal attack [393]. These exudates have their own unique property to convert in to brittle, translucent, glassy, hard mass. This property recently explored as a potential tool in retaining various drugs and other therapeutic candidates for their sustained, controlled or specified release from the desired dosage forms. There are various types of gums (mentioned below) secreted by plants currently investigated for their pharmaceutical applications.

4.8.7.1 Gum Arabic

Acacia gum or gum Arabic is the dried gummy exudation obtained from the *Acacia arabica* (Leguminosae) and other related African species of acacia [241, 394]. The gum has been recognized as a branched molecule of 1, 3-linked β-D-galactopyranosyl

units. It is an acidic polysaccharide containing D-galactose, L-arabinose, L-rhamnose, and D-glucuronic acid and is mainly used in oral and topical pharmaceutical formulations as a suspending and emulsifying agent. Previous study has explored gum Arabic as a matrix microencapsulating agent for the enzyme, endoglucanase [394], shown the slow release endoglucanase from the formulation. Gum Arabic was used as an osmotic suspending and expanding agent to prepare a monolithic osmotic tablet system. The optimum system delivered the water-insoluble drug, naproxen, at a rate of approximately zero order for up to 12 h at a pH of 6.8.58 Sustained release of ferrous sulphate was achieved for 7 h by preparing gum Arabic pellets. An increase in the amount of gum Arabic in the pellets decreased the rate of release.

4.8.7.2 Tragacanth Gum

This gum is obtained from the branches of *Astragalus gummifer* (Leguminosae). 22 and anionic carbohydrate which consists of two major fractions: tragacanthin (water-soluble) and bassorin (water-swellable) [395]. Tragacanthic acid is composed of D-galacturonic acid, D-xylose, L-fructose, D-galactose, and other sugars. It's very difficult to understand the clear physical or chemical relationship between tragacanthin and bassorin since both fractions can be easily separated. As far as the chemical nature and composition are concerned bassorin and tragacanthin differ mainly in terms of their uronic acid and methoxyl content [396]. It has been demonstrated that bassorin is a complex structure of polymethoxylated acids and on demethoxylation, perhaps yields tragacanthin [397]. Tragacanth when employed as the carrier in the formulation of 1- and 3-layer matrices produced suitable release prolongation either alone or in combination with other polymers. It has been suggested that supplementation of tragacanth in aqueous media results in the improvement of rheological behavior at very low concentration, thus considered as potential as a suspending agent, stabilizer, and emulsifier [398]. Only few reports have been explored on the functional properties of gum tragacanth and its application in various fields. Recent investigation has demonstrated the flow behavior of Iranian gum tragacanth at different concentration dispersions, showed that all of the gum dispersions had shear-thinning natures and exhibit significant physicochemical properties [399].

4.8.7.3 Mucilage Gums

Recently various mucilage gums have been explored from seed by different extraction procedures such as guar gum from the ground endosperms or seeds of the plant *Cyamopsistetragonolobus* (Fam. Leguminosae) and locust bean gum from the endosperms of the hard seeds of the locust bean tree (Carob tree), *Ceratoniasiliqua* (Fam. Caesalpiniaceae) [400]. These polysaccharides have been investigated for their potential role in drug delivery and other pharmaceutical applications.

4.8.7.4 Locust Bean Gum

Locust bean gum (Fig. 4.10) or carob gum, irregularly shaped molecule with branched β-1, 4-D-galactomannan units, derived from the seeds of the carob, *Ceratoniasiliqua* Linn (Fam. Caesalpiniaceae). For achieving full solubility with complete hydration and highest viscosity this neutral charged polymer requires heat. This feature encourages its coating strength and imparts protection to several drug candidates against several degradation factors *in vivo*. Colon-specific drug delivery systems based on polysaccharides using locust bean gum and chitosan was developed. It was observed that when these coating materials was applied over core tablet it endow better shielding affect by protecting the drug from being released in the physiological environment of stomach and small intestine. Additionally it also provides protection against colonic bacterial enzymatic actions with resultant drug release in the colon.

4.8.7.5 Guar Gum

Guar gum (Fig. 4.11), high molecular weight hydrocolloidal polysaccharide consist of linear chain of β-D-mannopyranosyl units linked (1 → 4) with single member α-D-galacto-pyranosyl units occurring as side branches. This Cyamposistetragonolobus endosperms derived polysaccharide contains glycosidic linked galactan and mannan

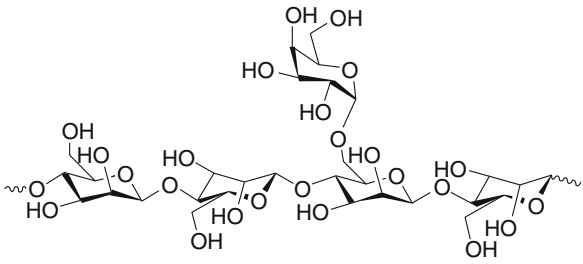

Fig. 4.10 Chemical structure of locust bean gum

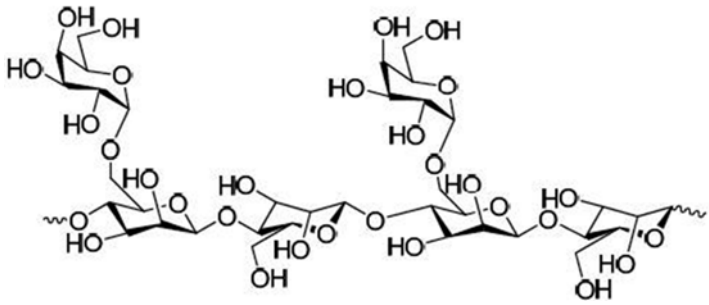

Fig. 4.11 Chemical structure of guar gum

units and shows degradation in the large intestine due the presence of microbial enzymes. Guar gum has high molecular weight with high apparent viscosity in solution and shows solubility in cold water with its complete swift hydration to yield viscous pseudo plastic solutions. However it was observed that its shear-thinning usually have better low-shear viscosity than other hydrocolloids. This high viscosity and molecular weight dependent gelling property delays the drug from its dosage form, and become more vulnerable against susceptible to degradation in the colonic environment. This galactomannan based non-ionic polysaccharide that abundantly present in nature and endow many significant properties desirable for drug delivery applications. Nevertheless owing to its elevated swelling features in water, the utilization of this galactomannan based polysaccharide is limited. Recently various physico-chemical methods have been employed to improve its property such as derivatiztion, grafting and network formation. Such modification allows its more utilization for biomedical applications. Additionally this plant based natural polymer can be exploited in various forms such as matrix tablets, hydrogels, nano/micro particles and coatings can be exploited as potential carriers for targeted drug delivery [401]. One report suggested the significant role of guar gum in oral controlled drug delivery systems for highly water-soluble metroprolol as a carrier in the form of a three-layer matrix tablet. During this study it was observed that three-layer guar gum matrix tablets provide the desirable release rate for metoprolol tartrate formulations with no change either in physical appearance, drug content or in dissolution pattern and did not show any possibility of metoprolol tartrate/guar gum interaction with the formulation excipients used in the study [402]. Same results were obtained in guar gum based three layer tablet of tri-metazidinedihydrochloride controlled release formulation [403]. In another report where guar gum potential was explored as a carrier for colon-specific drug delivery in form of a novel tablet formulation for oral administration using indo-methacin as a model drug. It was observed that this galactomannan based polysaccharide protects the drug from being released entirely in the physiological environment of stomach and small intestine. Based on these investigations it was concluded that 4% w/v of rat caecal contents in PBS offers the best conditions for in vitro assessment of guar gum [404]. In one more investigation guar gum was modified by using acrylamide grafting in which amide groups of these grafted copolymers were transformed into carboxylic functional groups and finally tablets were prepared by incorporating diltiazem hydrochloride. Based on in-vitro drug release data it was observed that that the drug release dissolution was controlled in case of unhydrolyzed copolymer in contrast with hydrolyzed copolymers, drug release was swelling-controlled at first, however at later stage, it became dissolution-controlled. This report suggested potential role of pH sensitive hydrolyzed pAAm-*g*-GG matrices for intestinal drug delivery [405].

4.8.7.6 Grewia Gum

Grewia genus which was formerly categorized under linden Family (Tiliaceae) or the Spermamanniaceae and has been now merged into the Malvaceae [406]. Its increasing citations in International Pharmaceutical Abstracts database, EBSCO

and in PubMed augment its interest on *Grewia mollis*as as a potential pharmaceutical excipient [407]. In concern with its polysaccharide based pharmaceutical evaluation, the first report was documented in the early 2000s [408–416], in which researchers have explored some physicochemical and rheological characteristics as well as water vapor permeability of the aqueous-based films. During this investigation effect of gum derived from *Grewia mollis*as, on the binding properties in sodium salicylate tablets [413] and its influence on the granulating fluid on the release profile of drug [414] were evaluated. Later on in a study researchers have observed that method of incorporating the gum into tablet formulation had significant effect on tablet properties. Additionally it was discovered that introduction by activation with water produced improved tablet properties than when incorporate by wet granulation or direct compression [415]. It was observed that acidic hydrolysis of the gum and some chemical modifications showed reduced viscosity and improved drug release from tablets [416–418]. In recent work some workers have explored the significant role of gum in binding in contrast with both untreated gum and gelatin in paracetamol tablet formulations [419]. It was observed that tablet formulations containing treated grewia gum exhibited low onset of plastic deformation, enhanced friability was found with increase in acid concentration and treatment time and decrease in crushing strength, disintegration and dissolution times with increase in acid concentration and treatment time was observed. Other researchers concluded that acid treated grewia gum, depending on the desired onset of action of medicament, can be used in formulation of conventional tablets especially if the formulation does not require sustained release [420]. With the advancement in analytical tools it's now possible to characterize the natural polymers in much better way then the conventional technologies. Technologies such as gel permeation chromatography, gas chromatography, differential scanning calorimetry, scanning electron microscopy, thermo gravimetric analysis, ^{1}H and ^{13}C-NMR, solid-state nuclear magnetic resonance, Fourier transformed infrared, x-ray photoelectron spectroscopy, and NIR techniques have been utilized to characterize the gum [421, 422]. Based on the analysis it was observed that polysaccharide gum is a normally amorphous polysaccharide gum containing rhamnose, glucose, arabinose, galactose, and xylose as neutral sugars with an average molecular weight of 5925 kDa expressed as the pullulan equivalent. As far as its physical properties are concerned gum gradually hydrated in water. This dispersion swells to form a highly viscous dispersion having pseudo plastic flow behavior. Technique like centrifugation gives better molecular weights range between 230 and 235 kDa of gum when centrifuged successively at 4500 rpm for 30 min. Such processes improve the aqueous solubility and useful in delivering more solids to the substrate when used as a film coating agent [422].

4.8.7.7 Okra Gum

Okra gum is obtained from plant which is widely cultivated and grown in most tropical part of Nigeria known as *Abelmoschusesculentus* (Fam. Malvaceae). This plant is widely consumed as food in Asia and Africa and therefore considered as

subject of research in agriculture [423, 424]. Okra is popular for its viscous mucilaginous solution which is formed when heated and extracted in water [425]. This high molecular weight polysaccharide gum is reported as pharmaceutical excipient in various reports as a suspending [425–427], control release [428], film coating [429], binder [430, 431], and bio-adhesive [432] agent. Okra gum is documented as controlled-release agent in modified release matrices, in contrast with sodium carboxymethyl- cellulose and hydroxyl-propyl-methyl-cellulose, using Paracetamol as a model drug. Results showed that an okra gum matrix was useful in the formulation of sustained-release tablets for up to 6 h [428].

4.8.7.8 Kyaha Gum

Recent report explored the relative binding effects of khaya gum obtained from *Khayasenegalensis* and *Khayagrandifoliola* (Fam. Meliaceae) in paracetamol tablet formulation were evaluated [433]. In one report mechanical properties of the tablets using khaya gum were assessed using the tensile strength, brittle fracture index and friability of the tablets while the drug release properties were evaluated by means of disintegration and dissolution times. It was observed that the brittle fracture index and friability decreased whereas tensile strength, disintegration and the dissolution times of tablets increased with the increase in binder concentration. It was also concluded that gum obtained from *K. senegalensis* produced strong tablets with extended disintegration and dissolution times in contrast with those obtained from *K. grandifoliola* gum. Based on reports it was finally suggested that gums obtained from *K. senegalensis* will be more suitable as a binding agent than *K. grandifoliola* when high mechanical strength and slow release profiles of tablets are required.

4.8.7.9 Moringaoleifer Gum

Moringaoleifera derived gum was investigated for its physical features such as loss on drying, swelling index, solubility, and pH in form of gel based formulations using Diclofenac sodium as model. It was studied that 8.0 % mucilage gels prepared were found to be ideal and equivalent with a marketed preparation [434].

4.8.7.10 Irvingiagabonensis

Seeds of *Irvingia gabonensis,* commonly known as ‘African mango’ or ‘bush mango’ contains large amount of lipids and polymeric constituents [423, 435–440] which are very important to pharmaceutical scientists as excipients. Mucilage isolated from the kernel is of great pharmaceutical significance and has been used as binding agent in tablet formulation [441], as emulsifying and suspending agent [442]. Moreover the lipid has been employed in sustained release ingredient

[443–445], as suppository base [446–448], tableting as lubricant, microencapsulation [449], and as a part of film coating process [446]. Polysaccharide extraction can be achieved by using aqueous dispersion in petroleum ether or diethyl ether [450], however simultaneous extraction processes for both lipis and polysaccharide content has not been reported yet [451]. Irvingiagabonensis mucilage was investigated for use as suspending and emulsifying agent and its rheological behavior was evaluated against tragacanth. It was observed that formulated suspensions of Irvingia mucilage at all concentrations gave higher final sedimentation height and sedimentation volume values. Furthermore it was also documented that 2.0 % w/v Irvingia mucilage (as an emulsifying agent) when compared against tragacanth and acacia gum showed stability throughout the 6 weeks of study. This observation led to conclusion that Irvingia mucilage presents superior properties than acacia and tragacanth. This performance was found to be much better, even at lower concentrations in the formulation of emulsions and suspensions. Iirvingia lipid based suppository base has also been investigated and it was observed that irvingia fat can be actively employed as suppository base. Furthermore the potential binding effects of mucilage on sustained release tablets, metronidazole tablets and as lubricating potential in tablet formulations have been investigated.

4.8.7.11 Hakeagibbosa Gum

This water-soluble gum is obtained from Hakeagibbosa (hakea), having considerable muco adhesive and sustained-release properties especially for the formulation of buccal tablets. Hakeagibbosa gum is recently investigated in flat-faced tablets using chlorpheniramine maleate as model drug [452]. It was observed that hakea coated tablet significantly extend the in vitro release up to several hours and characterization results suggested the absence of chemical interactions. Additionally force of detachment for directly compressed and wet granulated tablets was increased as the amount of hakea per tablet was increased. Researchers also reported that hakea, might not only be employed to prolong the chlorpheniramine maleate release from a buccal tablet, however it also displayed excellent mucoadhesive properties. The underlying mechanism behind this is the slow relaxation of the hydrated hakea which can cause chlorpheniramine maleate release in a sustained manner.

4.8.7.12 Psyllium Mucilage

Mucilage derived from Psyllium, has been investigated for its tablet binding properties [453]. This mucilage is derived from the seed coat of *Plantagoovata* (Fam. Plantaginaceae) [454]. Isolation is achieved by milling of the outer layer of the seeds yield smooth texture mucilage. Psyllium hydrogels were investigated by insulin as model drug and N, N'-methylene-bis-acryl-amide as cross-linker. Formulation showed controlled release of the active ingredient [455].

4.8.7.13 Miscellaneous Gums and Mucilage

Various gums have been explored for pharmaceutical significance such as *Colocassiaesculenta* [456], seeds of *Linumusitatissimum* [457], malva nut gum [458] and *Sterculiafoetida* gum for swelling and erosion modulator in controlled release matrix tablets of diltiazem hydrochloride.

References

1. IPECFED. The world unites for safer medicines, 2011.
2. Parrott EL. Pharmaceutical technology: fundamental pharmaceutics. Minneapolis: Burgess Publishing Company; 1971.
3. Russell R. Synthetic excipient challenge all-natural organics offer advantages/challenges to developer and formulators. Pharm Tech. 2004;27:38–50.
4. Guo J, Skinner GW, Harcum WW, Barnum PE. Pharmaceutical applications of naturally occurring water-soluble polymers. Pharm Sci Technol Today. 1998;1:254–61.
5. Beneke CE, Viljoen AM, Hamman JH. Polymeric plant-derived excipients in drug delivery. Molecules. 2009;14:2602–20.
6. Pandey R, Khuller GK. Polymer based drug delivery systems for mycobacterial infections. Curr Drug Deliv. 2004;1:195–201.
7. Chamarthy SP, Pinal R. Plasticizer concentration and the performance of a diffusion-controlled polymeric drug delivery system. Colloids Surf A Physiochem Eng Asp. 2008;331:25–30.
8. Malafaya PB, Silva GA, Reis RL. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. Adv Drug Deliv Rev. 2007;59:207–33.
9. Malviya R, Srivastava P, Kulkarni GT. Applications of mucilages in drug delivery – a review. Adv Biol Res. 2011;5:1–7.
10. Perepelkin KE. Polymeric materials of the future based on renewable plant resources and biotechnologies. Fibre Chem. 2005;37:417–30.
11. Raizada A, Bandari A, Kumar B. Polymers in drug delivery: a review. Int J Pharm Res Dev. 2010;2:9–20.
12. Verbeken D, Dierckx S, Dewettinck K. Exudate gums: occurrence, production and applications. Appl Microbiol Biotechnol. 2003;63:10–21.
13. Maneesh K, Gaurav S, Ravinder K, Kapil K, Paramjot K, et al. Applications of novel excipients in the allopathic and herbal formulations. J Chem Pharm Res. 2010;2:851–60.
14. Paulsen BS. Biologically active polysaccharides as possible lead compounds. Phytochem Rev. 2002;1:379–87.
15. Coviello T, Matricardi P, Marianecci C, Alhaique F. Polysaccharide hydrogels for modified release formulations. J Control Release. 2007;119:5–24.
16. Robyt JF. General properties, occurrence, and preparation of carbohydrates. In: Reid BF, Tatsuta K, Thiem J, editors. Glycoscience. Berlin: Springer; 2008. 10.1007/978-3-540-30429-6_2.
17. Lahaye M. Developments on gelling algal galactans, their structure and physico-chemistry. J Appl Phycol. 2001;13:173–84.
18. Cardozo KHM, Guaratini T, Barros MP, Falcao VR, Tonon AP, Lopes NP, Campos S, Torres MA, Souza AO, Colepicolo P, Pinto E. Metabolites from algae with economical impact. Comp Biochem Physiol C Toxicol Pharmacol. 2007;146:60–78.
19. Holst O, Loennies SM. Microbial polysaccharide structures. Borstel: Research Center Borstel-Leibniz-Center for Medicine and Biosciences; 2007.

20. Sutherland IW. Bacterial exopolysaccharides. Edinburgh: Edinburgh University; 2007.
21. McDonald JA, Camenisch TD. Hyaluronan: genetic insights into the complex biology of a simple polysaccharide. Glycoconj J. 2003;19:331–9.
22. Kogan G, Soltes L, Stern R, Gemeiner P. Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. Biotechnol Lett. 2007;29:17–25.
23. Girish KS, Kemparaju K. The magic glue hyaluronan and its eraser hyaluronidase: a biological overview. Life Sci. 2007;80:1921–43.
24. Harrington JC, Morris ER. Conformational ordering and gelation of gelatin in mixtures with soluble polysaccharides. Food Hydrocoll. 2009;23:327–36.
25. Velde FV, Knutsen SH, Usov AI, Rollemay HS, Cerezo AS. ^{1}H and ^{13}C high resolution NMR spectroscopy of carrageenans: application in research and Industry. Trends Food Sci Technol. 2002;13:73–92.
26. Kazłowski B, Pan CL, Ko YT. Separation and quantification of neoagaro- and agaro-oligosaccharide products generated from agarose digestion by b-agarase and HCl in liquid chromatography systems. Carbohydr Res. 2008;343:2443–50.
27. Jones C, Mulloy B. The application of nuclear magnetic resonance to structural studies of polysaccharides. In: Jones C, Mulloy B, Thomas AH, editors. Spectroscopic methods and analyses NMR – Mass spectrometry, and metalloprotein techniques, Methods in molecular biology, vol. 17. Totowa, NJ: Humana Press; 1993. p. 6.
28. Tuvikene R, Truus K, Kollist A, Volobujeva O, Mellikov E, Pehk T. Gel-forming structures and stages of red algal galactans of different sulfation levels. J Appl Phycol. 2007;20:527–35. doi:10.1007/s10811-007-9229-9.
29. Navarro DA, Flores ML, Stortz CA. Microwave-assisted desulfation of sulfated polysaccharides. Carbohydr Poly. 2007;69:742–7.
30. Chou BAM, Koenig JL. A review of polymer dissolution. Prog Polym Sci. 2003;28:1223–70.
31. Talukdar MM, Vinckier I, Moldenaers P, Kinget R. Rheological characterization of Xanthan Gum and hydroxypropylmethyl cellulose with respect to controlled-release drug delivery. J Pharm Sci. 1996;85:5.
32. Phillips HM, Craig DQM, Royall PG, Hill VL. Characterisation of the glass transition of HPMC using modulated temperature differential scanning calorimetry. Int J Pharm. 1999;180:83–90.
33. Rinaudo M. Seaweed polysaccharides. Grenoble: Centre de Recherches sur les Macromole´ cules Ve´ ge´ tales, CNRS; 2007.
34. Maciel JS, Chaves LS, Souza BWS, Teixeira DIA, Freitas ALP, Feitosa JPA, et al. Structural characterization of cold extracted fraction of soluble sulfated polysaccharide from red seaweed *Gracilaria birdiae*. Carbohydr Polym. 2008;71(4):559–65.
35. Zhao T, Zhang Q, Qi H, Zhang H, Niu X, Xu Z, et al. Degradation of porphyran from *Porphyra haitanensis* and the antioxidant activities of the degraded porphyrans with different molecular weight. Int J Biol Macromol. 2006;38:45–50.
36. Noseda MD, Viana AG, Duarte MER, Cerezo AS. Alkali modification of carrageenans. Part IV. Porphyrans as model compounds. Carbohydr Polym. 2000;42:301–5.
37. Karlsson A, Singh SK. Acid hydrolysis of sulphated polysaccharides. Desulphation and the effect on molecular mass. Carbohydr Polym. 1999;38:7–15.
38. Zhou C, Yu X, Zhang Y, He R, Ma H. Ultrasonic degradation, purification and analysis of structure and antioxidant activity of polysaccharide from *Porphyra yezoensis* Udea. Carbohydr Polym. 2012;87:2046–51.
39. Denis C, Jeune HL, Gaudin P, Fleurence J. An evaluation of methods for quantifying the enzymatic degradation of red seaweed *Grateloupia turuturu*. J Appl Phycol. 2009;21(1):153–9.
40. Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F. A colorimetric method for the determination of sugars. Nature. 1951;168:167.
41. Yaphe W, Arsenault GP. Improved resorcinol reagent for the determination of fructose, and of 3, 6-anhydrogalactose in polysaccharides. Anal Biochem. 1965;13:143–8.

42. Nader HB, Dietrich CP. Determination of sulfate after chromatography and toluidine blue complex formation. Anal Biochem. 1977;78:112–8.
43. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265–75.
44. Hutardo-Ponce AQ, Umezaki I. Physical properties of agar gel from Gracilaria (Rhodophyta) of the Philippines. Botanica marina. 1988;31:171–4.
45. Craigie JS, Leigh C. Carrageenans and agars. In: Hellebust JA, Craigie JS, editors. Hand book of phycological methods. Cambridge: Cambridge University Press; 1978. p. 109–31.
46. Rochas C, Lahaye NI. Average molecular weight and molecular weight distribution of agarose and agarose-type polysaccharide. Carbohydr Polym. 1989;10:289–98.
47. Kilp T, Guillet JE. A rapid procedure for the determination of viscosity – MW relations. Macromolecules. 1977;10:90–4.
48. Kaur B, Ariffin F, Bhat R, Karim AA. Progress in starch modification in the last decade. Food Hydrocoll. 2012;26:398–404.
49. Rajan A, Prasad VS, Abraham TE. Enzymatic esterification of starch using recovered coconut oil. Int J Biol Macromol. 2006;39:265–72.
50. Rajan A, Sudha JD, Abraham TE. Enzymatic modification of cassava starch by fungal lipase. Ind Crops Prod. 2008;27:50–9.
51. Qiao L, Gu Q-M, Cheng HN. Enzyme-catalyzed synthesis of hydrophobically modified starch. Carbohydr Polym. 2006;66:135–40.
52. Chakraborty S, Sahoo B, Teraoka I, Miller LM, Gross RA. Enzyme catalyzed regioselective modification of starch nanoparticles. Macromolecules. 2005;38:61–8.
53. Deetae P, Shobsngob S, Varanyanond W, Chinachoti P, Naivikul O, Varavinit S. Preparation, pasting properties and freeze-thaw stability of dual modified crosslink-phosphorylated rice starch. Carbohydr Polym. 2008;73:351–8.
54. Varavinit S, Paisanjit W, Tukomane T, Pukkahuta C. Effects of osmotic pressure on the cross-linking reaction of tapioca starch. Starch/Stärke. 2007;59:290–6.
55. Li JM, Zhang LM. Characteristics of novel starch-based hydrogels prepared by UV photopolymerization of acryloylated starch and a zwitterionic monomer. Starch/Stärke. 2007;59:418–22.
56. Ou S, Li A, Yang A. A study on synthesis of starch ferulate and its biological properties. Food Chem. 2001;74:91–5.
57. Xing GX, Zhang SF, Ju BZ, Yang JZ. Microwave-assisted synthesis of starch maleate by dry method. Starch/Stärke. 2006;58:464–7.
58. Jyothi AN, Rajasekharan KN, Moorthy SN, Sreekumar J. Microwave assisted synthesis and characterization of succinate derivatives of cassava (Manihot esculenta Crantz) starch. Starch/Stärke. 2005;57:556–63.
59. Cízová A, Sroková I, Sasinková V, Malovíková A, Ebringerová A. Carboxymethyl starch octenylsuccinate: microwave- and ultrasound-assisted synthesis and properties. Starch/Stärke. 2008;60:389–97.
60. Karim AA, Sufha EH, Zaidul ISM. Dual modification of starch via partial enzymatic hydrolysis in the granular state and subsequent hydroxypropylation. J Agric Food Chem. 2008;56:10901–7.
61. Kesselmans RPW, Bleeker IP. Method for oxidizing dry starch using ozone. World Intellectual Property Organization, WO/1997/032902, 1997.
62. Chan HT, Bhat R, Karim AA. Physicochemical and functional properties of ozone-oxidized starch. J Agric Food Chem. 2009;57:5965–70.
63. An HJ, King JM. Using ozonation and amino acids to change pasting properties of rice starch. J Food Sci. 2009;74:278–83.
64. Heux L, Chauve G, Bonini C. Nonflocculatingand chiral-nematic self-ordering of cellulose microcrystals suspensions in nonpolar solvents. Langmuir. 2000;16:8210.
65. Heux L, Bonini C. International Patent WO 2000/077088, 2000.
66. Bonini C, Heux L, Cavaille JY, Lindner P, Dewhurst C, Terech P. Rodlike cellulose whiskers coated with surfactant: a small-angle neutron scattering characterization. Langmuir. 2002; 18:3311.

67. Rojas OJ, Montero GA, Habibi Y. J Appl Polym Sci. 2009;113:927.
68. Zhou Q, Brumer H, Teeri TT. Macromolecules. 2009;42:5430.
69. Gert EV, Torgashov VI, Zubets OV, Kaputskii FN. Preparation and properties of enterosorbents based on carboxylated microcrystalline cellulose. Cellulose. 2005;12(5):517–26.
70. Kitaoka T, Isogai A, Onabe F. Chemical modification of pulp fibers by TEMPO-mediated oxidation. Nordic Pulp Paper Res J. 1999;14(4):279–84.
71. Montanari S, Roumani M, Heux L, Vignon MR. Topochemistry of carboxylated cellulose nanocrystals resulting from TEMPO-mediated oxidation. Macromolecules. 2005;38(5): 1665–71.
72. Saito T, Isogai A. TEMPO-mediated oxidation of native cellulose. The effect of oxidation conditions on chemical and crystal structures of the water-insoluble fractions. Biomacromolecules. 2004;5(5):1983–9.
73. Saito T, Isogai A. Introduction of aldehyde groups on surfaces of native cellulose fibers by TEMPO-mediated oxidation. Colloids Surf A Physicochem Eng Asp. 2006;289(1–3): 219–25.
74. Saito T, Nishiyama Y, Putaux JL, Vignon M, Isogai A. Homogeneous suspensions of individualized microfibrils from TEMPO-catalyzed oxidation of native cellulose. Biomacromolecules. 2006;7(6):1687–91.
75. Eichhorn SJ, Dufresne A, Aranguren M, Marcovich NE, Capadona JR, Rowan SJ, et al. Review: Current international research into cellulose nanofibres and nanocomposites. J Mater Sci. 2010;45(1):1–33.
76. Saito T, Kimura S, Nishiyama Y, Isogai A. Cellulose nanofibers prepared by TEMPO-mediated oxidation of native cellulose. Biomacromolecules. 2007;8(8):2485–91.
77. Saito T, Okita Y, Nge TT, Sugiyama J, Isogai A. TEMPO-mediated oxidation of native cellulose: microscopic analysis of fibrous fractions in the oxidized products. Carbohydr Polym. 2006;65(4):435–40.
78. Isogai A, Saito T, Fukuzumi H. TEMPO-oxidized cellulose nanofibers. Nanoscale. 2011;3(1):71.
79. Isogai T, Saito T, Isogai A. Wood cellulose nanofibrils prepared by TEMPO electro-mediated oxidation. Cellulose. 2011;18(2):421–31.
80. Saito T, Hirota M, Tamura N, Kimura S, Fukuzumi H, Heux L, et al. Individualization of nano-sized plant cellulose fibrils by direct surface carboxylation using TEMPO catalyst under neutral conditions. Biomacromolecules. 2009;10(7):1992–6.
81. Johnson RK, Zink-Sharp A, Renneckar SH, Glasser WG. A new bio-based nanocomposite: fibrillated TEMPO-oxidized celluloses in hydroxypropylcellulose matrix. Cellulose. 2008;16(2):227–38.
82. Li Q, Renneckar S. Molecularly thin nanoparticles from cellulose: isolation of submicrofibrillar structures. Cellulose. 2009;16(6):1025–32.
83. Araki J, Wada M, Kuga S. Langmuir. 2001;17:21.
84. Hasani M, Cranston ED, Westmana G, Gray DG. Soft Matter. 2008;4:2238.
85. Sassi JF, Chanzy H. Cellulose. 1995;2:111.
86. Braun B, Dorgan JR. Biomacromolecules. 2009;10:334.
87. Sobkowicz MJ, Braun B, Dorgan JR JR. Green Chem. 2009;11:680.
88. Yuan H, Nishiyama Y, Wada M, Kuga S. Biomacromolecules. 2006;7:696.
89. Junior de Menezes A, Siqueira G, Curvelo AAS, Dufresne A. Extrusion and characterisation of functionalised cellulose whiskers reinforced polyethylene nanocomposites. Polymer. 2009;50:4552.
90. Gousse C, Chanzy H, Excoffier G, Soubeyrand L, Fleury E. Polymer. 2002;43:2645.
91. Grunnert M, Winter WT. Polym Mater Sci Eng. 2000;82:232.
92. Siqueira G, Bras J, Dufresne A. Biomacromolecules. 2009;10:425.
93. Aulin C, Ahola S, Josefsson P, Nishino T, Hirose Y, Osterberg M, et al. Nanoscale cellulose films with different crystallinities and mesostructures—their surface properties and interaction with water. Langmuir. 2009;25(13):7675–85.

94. Taipale T, Osterberg M, Nykanen A, Ruokolainen J, Laine J. Effect of microfibrillated cellulose and fines on the drainage of kraft pulp suspension and paper strength. Cellulose. 2010;17(5):1005–20.
95. Tingaut P, Zimmermann T, Lopez-Suevos F. Synthesis and characterization of bionanocomposites with tunable properties from poly(lactic acid) and acetylated microfibrillated cellulose. Biomacromolecules. 2009;11(2):454–64.
96. Ljungberg N, Bonini C, Bortolussi F, Boisson C, Heux L, Cavaille JY. Biomacromolecules. 2005;6:2732.
97. Vignon M, Montanari S, Habibi Y. (Centre National de la Recherche Scientifique CNRS, Fr.) FR 2003/5195, 2004.
98. Mangalam AP, Simonsen J, Benight AS. Biomacromolecules. 2009;10:497.
99. Cao X, Habibi Y, Lucia LAJ. Mater Chem. 2009;19:7137.
100. Habibi Y, Goffin AL, Schiltz N, Duquesne E, Dubois P, Dufresne AJ. Mater Chem. 2008;18:5002.
101. Pranger L, Tannenbaum R. Macromolecules. 2008;41:8682.
102. Zoppe J, Habibi Y, Rojas OJ. Abstr. Pap., ACS Natl. Meet. 2008, 235, CELL-057.
103. Iwamoto S, Nakagaito AN, Yano H, Nogi M. Optically transparent composites reinforced with plant fiber-based nanofibers. Appl Phys A. 2005;81(6):1109–12.
104. Spence KL, Venditti RA, Habibi Y, Rojas OJ, Pawlak JJ. The effect of chemical composition on microfibrillar cellulose films from wood pulps: mechanical processing and physical properties. Bioresour Technol. 2010;101(15):5961–8.
105. Spence KL, Venditti RA, Rojas OJ, Habibi Y, Pawlak JJ. The effect of chemical composition on microfibrillar cellulose films from wood pulps: water interactions and physical properties for packaging applications. Cellulose. 2010;17(4):835–48.
106. Stenstad P, Andresen M, Tanem BS, Stenius P. Chemical surface modifications of microfibrillated cellulose. Cellulose. 2007;15(1):35–45.
107. Ahola S, Osterberg M, Laine J. Cellulose nanofibrils-adsorption with poly(amideamine) epichlorohydrin studied by QCM-D and application as a paper strength additive. Cellulose. 2007;15(2):303–14.
108. Alemdar A, Sain M. Biocomposites form wheat straw nanofibers: morphology, thermal and mechanical properties. Compos Sci Technol. 2008;68(2):557–65.
109. Lavoine N, Desloges I, Dufresne A, Bras J. Microfibrillated cellulose – its barrier properties and applications in cellulosic materials: a review. Carbohydr Polym. 2012;90:735–64.
110. Turbak AF, Snyder FW, Sandberg KR. Micro-fibrillated cellulose and process for producing it. Patent CH 648071 (A5), 1985.
111. Aulin C. Novel oil resistant cellulosic materials (Pulp and paper technology). Stockholm: KTH Chemical Science and Engineering; 2009.
112. Dinand E, Maureaux A, Chanzy H, Vincent I, Vignon MR. Microfibrillated cellulose and process for making the same from vegetable pulps having primary walls, especially from sugar beet pulp. Patent EP 0726356 B1, 2002.
113. Leitner J, Hinterstoisser B, Wastyn M, Keckes J, Gindl W. Sugar beet cellulose nanofibril-reinforced composites. Cellulose. 2007;14(5):419–25.
114. Nakagaito AN, Yano H. The effect of morphological changes from pulp fiber towards nanoscale fibrillated cellulose on the mechanical properties of high-strength plant fiber based composites. Appl Phys A. 2004;78(4):547–52.
115. Uetani K, Yano H. Nanofibrillation of wood pulp using a high-speed blender. Biomacromolecules. 2011;12(2):348–53.
116. Siqueira G, Bras J, Dufresne A. Cellulosic bionanocomposites: a review of preparation, properties and applications. Polymer. 2010;2(4):728–65.
117. Iwamoto S, Nakagaito AN, Yano H. Nano-fibrillation of pulp fibers for the processing of transparent nanocomposites. Appl Phys A. 2007;89(2):461–6.
118. Pohler T, Lappalainen T, Tammelin T, Eronen P, Hiekkataipale P, Vehniainen A, et al. Influence of fibrillation method on the character of nanofibrillated cellulose (NFC). Presented

at the TAPPI international conference on nanotechnology for the forest products industry, Espoo, 2010.
119. Dufresne A, Cavaille JY, Vignon MR. Mechanical behavior of sheets prepared from sugar beet cellulose microfibrils. J Appl Polym Sci. 1997;64(6):1185–94.
120. Chakraborty A, Sain M, Kortschot M. Cellulose microfibrils: a novel method of preparation using high shear refining and cryocrushing. Holzforschung. 2005;59(1):102–7.
121. Alemdar A, Sain M. Isolation and characterization of nanofibers from agricultural residues – wheat straw and soy hulls. Bioresour Technol. 2008;99(6):1664–71.
122. Bhatnagar A, Sain M. Processing of cellulose nanofiber-reinforced composites. J Reinforced Plastics Compos. 2005;24(12):1259–68.
123. Huang ZM, Zhang YZ, Kotaki M, Ramakrishna S. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. Compos Sci Technol. 2003;63(15):2223–53.
124. Li D, Xia Y. Electrospinning of nanofibers: reinventing the wheel? Adv Mater. 2004;16(14):1151–70.
125. Diez I, Eronen P, Osterberg M, Linder MB, Ikkala O, Ras RHA. Functionalization of nanofibrillated cellulose with silver nanoclusters: fluorescence and antibacterial activity. Macromol Biosci. 2011;11(9):1185–91.
126. Walther A, Timonen JVI, Diez I, Laukkanen A, Ikkala O. Multifunctional high-performance biofibers based on wet-extrusion of renewable native cellulose nanofibrils. Adv Mater. 2011;23:2924–8.
127. Zimmermann T, Bordeanu N, Strub E. Properties of nanofibrillated cellulose from different raw materials and its reinforcement potential. Carbohydr Polym. 2010;79(4):1086–93.
128. Eriksen O, Syverud K, Gregersen OW. The use of microfibrillated cellulose produced from kraft pulp as strength enhancer in TMP paper. Nordic Pulp Paper Res J. 2008;23(3):299–304.
129. Spence KL, Venditti RA, Rojas OJ, Habibi Y, Pawlak JJ. A comparative study of energy consumption and physical properties of microfibrillated cellulose produced by different processing methods. Cellulose. 2011;18(4):1097–111.
130. Spence KL, Venditti RA, Rojas OJ, Pawlak JJ, Hubbe MA. Water vapor barrier properties of coated and filled microfibrillated cellulose composite films. Bioresources. 2011;6(4):4370–88.
131. Heiskanen I, Harlin A, Backfolk K, Laitinen R. Process for production of microfibrillated cellulose in an extruder and microfibrillated cellulose produced according to the process. Patent No WO 2011051882, 2011.
132. Tanaka A, Sneck A, Seppanen V, Houni J, Pirkonen P. Possibilities to characterize NFC: NFC fractionation. Presented at the SUNPAP Workshop 2011.
133. Paakko M, Ankerfors M, Kosonen H, Nykanen A, Ahola S, Osterberg M, et al. Enzymatic hydrolysis combined with mechanical shearing and high-pressure homogenization for nanoscale cellulose fibrils and strong gels. Biomacromolecules. 2007;8:1934–41.
134. Engstrom A, Ek M, Henriksson G. Improved accessibility and reactivity of dissolving pulp for the viscose process: pretreatment with monocomponent endoglucanase. Biomacromolecules. 2006;7(6):2027–31.
135. Henriksson M, Henriksson G, Berglund LA, Lindstrom T. An environmentally friendly method for enzyme-assisted preparation of microfibrillated cellulose (MFC) nanofibers. Eur Polym J. 2007;43(8):3434–41.
136. Rodionova G, Lenes M, Eriksen O, Gregersen O. Surface chemical modification of microfibrillated cellulose: improvement of barrier properties for packaging applications. Cellulose. 2010;18(1):127–34.
137. Ifuku S, Nogi M, Abe K, Handa K, Nakatsubo F, Yano H. Surface modification of bacterial cellulose nanofibers for property enhancement of optically transparent composites: dependence on acetyl-group DS. Biomacromolecules. 2007;8(6):1973–8.

138. Nogi M, Ifuku S, Abe K, Handa K, Nakagaito AN, Yano H. Fiber-content dependency of the optical transparency and thermal expansion of bacterial nanofiber reinforced composites. Appl Phys Lett. 2006;88(13):133124.
139. Andresen M, Johansson LS, Tanem BS, Stenius P. Properties and characterization of hydrophobized microfibrillated cellulose. Cellulose. 2006;13(6):665–77.
140. Lu J, Askeland P, Drzal LT. Surface modification of microfibrillated cellulose for epoxy composite applications. Polymer. 2008;49(5):1285–96.
141. Missoum K, Belgacem MN, Bras J. Ionic liquids: green process for modifying nanofibrillated cellulose surface chemistry. Presented at the SUNPAP Workshop, Espoo, 2011.
142. Missoum K, Belgacem N, Krouit M, Martin C, Tapin-Lingua S, Bras J. Influence of fibrillation degree & surface grafting of micro-fibrillated cellulose on their rheological behavior in aqueous suspension. Presented at the 2010 TAPPI nanotechnology conference for the forest product industry, Espoo, 2010.
143. Andresen M, Stenstad P, Moretro T, Langsrud S, Syverud K, Johansson LS, et al. Nonleaching antimicrobial films prepared from surface-modified microfibrillated cellulose. Biomacromolecules. 2007;8(7):2149–55.
144. Nemtanu MR, Minea R. Functional properties of corn starch treated with corona electrical discharges. Macromol Symp. 2006;245–246:525–8.
145. Szymonska J, Krok F, Tomasik P. Deep-freezing of potato starch. Int J Biol Macromol. 2000;27:307–14.
146. Szymonska J, Krok F, Komorowska Czepirska E, Rebilas K. Modification of granular potato starch by multiple deep-freezing and thawing. Carbohydr Polym. 2003;52:1–10.
147. Zarguili I, Maache-Rezzoug Z, Loisel C, Doublier JL. Influence of DIL hydrothermal process conditions on the gelatinization properties of standard maize starch. J Food Eng. 2006;77:454–61.
148. Maache-Rezzoug Z, Maugard T, Zarguili I, Bezzine E, El Marzouki MN, et al. Effect of instantaneous controlled pressure drop (DIC) on physicochemical properties of wheat, waxy and standard maize starches. J Cereal Sci. 2009;49:346–53.
149. Lewandowicz G, Soral Smietana M. Starch modification by iterated syneresis. Carbohydr Polym. 2004;56:403–13.
150. Huang ZQ, Lu JP, Li XH, Tong ZF. Effect of mechanical activation on physico-chemical properties and structure of cassava starch. Carbohydr Polym. 2007;68:128–35.
151. Che LM, Li D, Wang LJ, Chen XD, Mao ZH. Microniation and hydrophobic modification of cassava starch. Int J Food Properties. 2007;10:527–36.
152. Pukkahuta C, Shobsngob S, Varavinit S. Effect of osmotic pressure on starch: new method of physical modification of starch. Starch/Stärke. 2007;58:78–90.
153. Han Z, Zeng X, Zhang B, Yu S. Effect of pulsed electric fields (PEF) treatment on the properties of corn starch. J Food Eng. 2009;93:318–23.
154. Steeneken PAM, Woortman AJJ. Superheated starch: a novel approach towards spreadable particle gels. Food Hydrocoll. 2009;23:394–405.
155. Chiu CW, Schiermeyer E, Thomas DJ, Shah MB. Thermally inhibited starches and flours and process for their production. U.S. Patent 5,725,676, 1998.
156. Lim ST, Han JA, Lim HS, BeMiller JN. Modification of starch by dry heating with ionic gums. Cereal Chem. 2002;79:601–6.
157. Vartiainen J, Pohler T, Sirola K, Pylkkanen L, Alenius H, Hokkinen J, et al. Health and environmental safety aspects of friction grinding and spray drying of microfibrillated cellulose. Cellulose. 2011;18(3):775–86.
158. González R, Carrara C, Tosi E, Añón MC, Pilosof A. Amaranth starch rich fraction properties modified by extrusion and fluidized bed heating. LWT Food Sci Technol. 2007;40:136–43.
159. Davis JP, Supatcharee N, Khandelwal RL, Chibbar RN. Synthesis of novel starches in planta: opportunities and challenges. Starch/Stärke. 2003;55:107–20.
160. Johnson LA, Baumel CP, Hardy CL, White PJ. Identifying valuable corn quality traits for starch production. A project of the Iowa Grain Quality Initiative Traits Task Team. Ames, IA: Center for Crops Utilization Research, Iowa Agriculture & Home Economics Experiment Station, Iowa State University; 1999.

161. Viksø-Nielsen A, Blennow A, Jørgensen K, Jensen A, Møller BL. Structural, physicochemical, and pasting properties of starches from potato plants with repressed r1-gene. Biomacromolecules. 2001;23:836–43.
162. Wischmann B, Blennow A, Madsen F, Jørgensen K, Poulsen P, Bandsholm O. Functional characterisation of potato starch modified by specific in planta alteration of the amylopectin branching and phosphate substitution. Food Hydrocoll. 2005;19:1016–24.
163. Ihemere U, Arias-Garzon D, Lawrence S, Sayre R. Genetic modification of cassava for enhanced starch modification. Plant Biotechnol J. 2006;4:453–65.
164. Jobling SA, Schwall GP, Westcott RJ, Sidebottom CM, Debet M, Gidley MJ, et al. A minor form of starch branching enzyme in potato (*Solanum tuberosum* L.) tubers has a major effect on starch structure: cloning and characterization of multiple forms of SBE A. Plant J. 1999;18:163–71.
165. Safford R, Jobling SA, Sidebottom CM, Westcott RJ, Cooke D, Tober KJ, et al. Consequences of antisense RNA inhibition of starch branching enzyme activity on properties of potato starch. Carbohydr Polym. 1998;35:155–68.
166. Verhoeven T, Fahy B, Leggett M, Moates G, Denyer K. Isolation and characterisation of novel starch mutants of oats. J Cereal Sci. 2004;40:69–79.
167. Lam E, Male KB, Chong JH, Leung ACW, Luong JHT. Applications of functionalized and nanoparticle-modified nanocrystalline cellulose. Trends Biotechnol. 2012;30(5):283–90.
168. Kayser O. Nanosuspensions for the formulation of aphidicolin to improve drug targeting effects against Leishmania infected macrophages. Int J Pharm. 2000;196(2):253–6.
169. Valo H, Kovalainen M, Laaksonen P, Häkkinen M, Auriola S, Peltonen L, Linder M, Järvinen K, Hirvonen J, Laaksonen T. Immobilization of protein-coated drug nanoparticles in nanofibrillar cellulose matrices—enhanced stability and release. J Control Release. 2011;156(3):390–7.
170. Boufi S, Ferraria AM, Rego AMB, Battaglini N, Herbst F, Vilar MR. Surface functionalisation of cellulose with noble metals nanoparticles through a selective nucleation. Carbohydr Polym. 2011;86(4):1586–94.
171. Bilbao-Sainz C, Bras J, Williams T, Sénechal T, Orts W. HPMC reinforced with different cellulose nano-particles. Carbohydr Polym. 2011;86(4):1549–57.
172. Liu S, Tao D, Zhang L. Cellulose scaffold: a green template for the controlling synthesis of magnetic inorganic nanoparticles. Powder Technol. 2012;217:502–9.
173. Abedini R, Mousavi SM, Aminzadeh R. A novel cellulose acetate (CA) membrane using TiO2 nanoparticles: preparation, characterization and permeation study. Desalination. 2011;277(1–3):40–5.
174. Gutierrez J, Tercjak A, Algar I, Retegi A, Mondragon I. Conductive properties of TiO2/bacterial cellulose hybrid fibres. J Colloid Interface Sci. 2012;377(1):88–93.
175. Yang J, Yu J, Fan J, Sun D, Tang W, Yang X. Biotemplated preparation of CdS nanoparticles/bacterial cellulose hybrid nanofibers for photocatalysis application. J Hazard Mater. 2011;189(1–2):377–83.
176. Zhang Y, Jin Q, Zhao J, Wu C, Fan Q, Wu Q. Facile fabrication of pH-sensitive core–shell nanoparticles based on HEC and PMAA via template polymerization. Eur Polym J. 2010;46(7):1425–35.
177. Kaihara S, Suzuki Y, Fujimoto K. *In situ* synthesis of polysaccharide nanoparticles via polyion complex of carboxymethyl cellulose and chitosan. Colloids Surf B Biointerfaces. 2011;85(2):343–8.
178. Li S, Yang W, Chen M, Gao J, Kang J, Qi Y. Preparation of PbO nanoparticles by microwave irradiation and their application to Pb(II)-selective electrode based on cellulose acetate. Mater Chem Phys. 2005;90(2–3):262–9.
179. Generalova AN, Sizova SV, Oleinikov VA, Zubov VP, Artemyev MV, Spernath L, et al. Highly fluorescent ethyl cellulose nanoparticles containing embedded semiconductor nanocrystals. Colloids Surf A Physicochem Eng Asp. 2009;342(1–3):59–64.
180. Aswathy RG, Sivakumar B, Brahatheeswaran D, Raveendran S, Ukai T, Fukuda T, Yoshida Y, et al. Multifunctional biocompatible fluorescent carboxymethyl cellulose nanoparticles. J Biomater Nanobiotechnol. 2012;3:254–61.

181. Fidale LC, Nikolajski M, Rudolph T, Dutz S, Schacher FH, Heinze T. Hybrid Fe3O4@amino cellulose nanoparticles in organic media – heterogeneous ligands for atom transfer radical. J Colloid Interface Sci. 2013;390:25–33.
182. Guilminot E, Gavillon R, Chatenet M, Berthon-Fabry S, Rigacci A, Budtova T. New nanostructured carbons based on porous cellulose: elaboration, pyrolysis and use as platinum nanoparticles substrate for oxygen reduction electrocatalysis. J Power Sources. 2008;185(2):717–26.
183. Liu H, Wang D, Shang S, Song Z. Synthesis and characterization of Ag–Pd alloy nanoparticles/carboxylated cellulose nanocrystals nanocomposites. Carbohydr Polym. 2011;83(1):38–43.
184. Dorlo TPC, Balasegaram M, Beijnen JH, Vries PJ. Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. J Antimicrob Chemother. 2012;67:2576–97.
185. Mayorga P, Puisieux F, Puisieux F, Couarraze G. Formulation study of a transdermal delivery system of primaquine. Int J Pharm. 1996;132(1–2):71–9.
186. Uchegbu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int J Pharma. 1998;172(1–2):33–70.
187. Murambiwa P, Masola B, Govender T, Mukaratirwa S, Musabayane CT. Anti-malarial drug formulations and novel delivery systems: a review. Acta Trop. 2011;118(2):71–9.
188. Jó TA, Petri DFS, Beltramini LM, Lucyszyn N, Sierakowski MR. Xyloglucan nano-aggregates: physico-chemical characterisation in buffer solution and potential application as a carrier for camptothecin, an anti-cancer drug. Carbohydr Polym. 2010;82(2):355–62.
189. Lubambo AF, Lucyszyn N, Petzhold CL, Camargo PC, Sierakowski MR, Schreiner WH, Saul CK. Self-assembled polystyrene/xyloglucan nanospheres from spin coatingevaporating mixtures. Carbohydr Polym. 2011;84:126–32.
190. Itoh K, Yahaba M, Takahashi A, Tsuruya R, Miyazaki S, Dairaku M, et al. In situ gelling xyloglucan/pectin formulations for oral sustained drug delivery. Int J Pharm. 2008;356(1–2):95–101.
191. Miyazaki S, Suisha F, Kawasaki N, Shirakawa M, Yamatoya K, Attwood D. Thermally reversible xyloglucan gels as vehicles for rectal drug delivery. J Control Release. 1998;56(1–3):75–83.
192. Mahajan HS, Tyagi VK, Patil RR, Dusunge SB. Thiolated xyloglucan: synthesis, characterization and evaluation as mucoadhesive in situ gelling agent. Carbohydr Polym. 2013;2:618–25.
193. Freitas RA, Busato AP, Mitchell DA, Silveira JLM. Degalatosylation of xyloglucan: effect on aggregation and conformation, as determined by time dependent static light scattering. HPSEC–MALLS and viscosimetry. Carbohydr Polym. 2011;83(4):1636–42.
194. Lubambo AF, Lucyszyn N, Petzhold CL, Camargo PC, Sierakowski MR, Schreiner WH, Saul CK. Mucoadhesive xyloglucan-containing formulations useful in medical devices and in pharmaceutical formulations. U.S. Patent Application 20120088726, 2012.
195. Lubamboa AF, Lucyszynb N, Petzhold CL, Camargo PC, Sierakowski MR, Schreinera WH, Saula CK. Self-assembled polystyrene/xyloglucan nanospheres from spin coating evaporating mixtures. Carbohydr Polym. 2011;84(1):126–32.
196. Cao Y, Gu Y, Ma H, Bai J, Liu L, Zhao P, He H. Self-assembled nanoparticle drug delivery systems from galactosylated polysaccharide-doxorubicin conjugate loaded doxorubicin. Int J Biol Macromol. 2010;46(2):245–9.
197. Noleto GR, Mercê AL, Iacomini M, Gorin PA, Soccol VT, Oliveira MB. Effects of a lichen galactomannan and its vanadyl (IV) complex on peritoneal macrophages and leishmanicidal activity. Mol Cell Biochem. 2002;233(1-2):73–83.
198. Il'ina AV, Mestechkina NM, Kurek DV, Levov AN, Semenyuk PI, Orlov VN, Shcherbukhin VD, Varlamov VP. Preparing, studying, and prospects of using nanoparticles based on chitosan and galactomannan. Nanotechnol Russia. 2011;6(1–2):154–60.
199. Lesnichaya MV, Aleksandrova GP, Feoktistova LP, Sapozhnikov AN, Sukhov BG, Trofimov BA. Formation kinetics of gold nanoparticles in the galactomannan polysaccharide matrix. Doklady Chem. 2011;440(2):282–5.

200. Cerqueira MA, Bourbon AI, Pinheiro AC, Martins JT, Souza BWS, Teixeira JA, Vicente AA. Galactomannans use in the development of edible films/coatings for food applications. Trends Food Sci Technol. 2011;22:662–71.
201. Soumya RS, Ghosh S, Abraham ET. Preparation and characterization of guar gum nanoparticles. Int J Biol Macromol. 2010;46(2):267–9.
202. Sierakowski MR, Milas M, Desbrieres J, Rinaudo M. Specific modifications of galactomannans. Carbohydr Polym. 2000;42:51–7.
203. Srivastava M, Kapoor VP. Seed galactomannans: an overview. Chem Biodivers. 2005;2(3):295–317.
204. Silveira JLM, Bresolin TMB. Pharmaceutical use of galactomannans. Quim Nova. 2011;34(2):292–9.
205. Matji JA et al. Products for receptor mediated activation and maturation of monocyte-derived dendritic cells by a phosphorylated glucomannane polysaccharide. U.S. Patent Application 20080171002, 2008.
206. Cheng LH, Abd Karim A, Norziah MH, Seow CC. Modification of the microstructural and physical properties of konjac glucomannan-based films by alkali and sodium carboxymethylcellulose. Food Res Int. 2002;35(9):829–36.
207. Alonso-Sande M, Teijeiro-Osorio D, Remuñán-López C, Alonso MJ. Glucomannan, a promising polysaccharide for biopharmaceutical purposes. Eur J Pharm Biopharm. 2009;72:453–62.
208. Xu J, Hou Z, Li T. Novel sample preparation method of polymer emulsion for SEM observation. Microsc Res Tech. 2007;70(10):847–50.
209. Lee CM, Lim S, Kim GY, Kim DW, Joon HR, Lee KY. Rosin nanoparticles as a drug delivery carrier for the controlled release of hydrocortisone. Biotechnol Lett. 2005;27:1487–90.
210. Baek W, Nirmala R, Barakat NAM, El-Newehy MH, Al-Deyab SS, Kim HY. Electrospun cross linked rosin fibers. Appl Surf Sci. 2011;258(4):1385–9.
211. Do HS, Park JH, Kim HJ. UV-curing behavior and adhesion performance of polymeric photoinitiators blended with hydrogenated rosin epoxy methacrylate for UV-crosslinkable acrylic pressure sensitive adhesives. Eur Polym J. 2008;44(11):3871–82.
212. Gupta SK. Transparent cold-wax and hot-wax depilatory compositions with three-dimensional suspended particles. U.S. Patent 7,438,897, 21 Oct 2008.
213. Demicheli C, Ochoa R, da Silva JB, Falcão CA, Rossi-Bergmann B, de Melo AL, Sinisterra RD, Frézard F. Oral delivery of meglumine antimoniate-beta-cyclodextrin complex for treatment of leishmaniasis. Antimicrob Agents Chemother. 2004;48(1):100–3.
214. Berman JD. Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clin Infect Dis. 1997;24(4):684–703.
215. Martins PS, Ochoa R, Pimenta AM, Ferreira LA, Melo AL, da Silva JB, Sinisterra RD, Demicheli C, Frézard F. Mode of action of beta-cyclodextrin as an absorption enhancer of the water-soluble drug meglumine antimoniate. Int J Pharm. 2006;325(1-2):39–47.
216. Salem II, Düzgünes N. Efficacies of cyclodextrin-complexed and liposome-encapsulated clarithromycin against Mycobacterium avium complex infection in human macrophages. Int J Pharm. 2003;250(2):403–14.
217. Chattopadhyay A, Jafurulla M. A novel mechanism for an old drug: amphotericin B in the treatment of visceral leishmaniasis. Biochem Biophys Res Commun. 2011;416:7–12.
218. Ribeiro RR, Ferreira AW, Martins SP, Neto LR, Rocha GO, Moyec L, Demicheli L, Frezard CF. Prolonged absorption of antimony(V) by the oral route from non-inclusion meglumine antimoniate-beta-cyclodextrin conjugates. Biopharm Drug Dispos. 2010;31:109–19.
219. Date AA, Joshi MD, Patravale VB. Parasitic diseases: liposomes and polymeric nanoparticles versus lipid nanoparticles. Adv Drug Deliv Rev. 2007;59(6):505–21.
220. Bricout H, Hapiot F, Tilloy APS, Monflier E. Chemically modified cyclodextrins: an attractive class of supramolecular hosts for the development of aqueous biphasic catalytic processes. Sustainability. 2009;1:924–45.

221. Badi P, Guégan P, Legrand FX, Leclercq L, Tilloy S, Monflier E. B-Cyclodextrins modified by alkyl and poly(ethylene oxide) chains: a novel class of mass transfer additives for aqueous organometallic catalysis. J Mol Catal A Chem 2010;318:8–14.
222. Cavalli R, Donalisio M, Civra A, Ferruti P, Ranucci E, Trotta F, Lembo D. Enhanced antiviral activity of Acyclovir loaded into β-cyclodextrin-poly(4-acryloylmorpholine) conjugate nanoparticles. J Control Release. 2009;137(2):116–22.
223. Gerebern Petrus Roger Vandecruys. Pharmaceutical compositions comprising a basic drug, a cyclodextrin, a polymer and an acid. EP0998304 B1, 2003.
224. Abou-Okeil A, Amr A, Abdel-Mohdy FA. Investigation of silver nanoparticles synthesis using aminated β-cyclodextrin. Carbohydr Polym. 2012;89(1):1–6.
225. Agüeros M, Areses P, Campanero MA, Salman H, Quincoces G, Peñuelas I, Irache JM. Bioadhesive properties and biodistribution of cyclodextrin–poly(anhydride) nanoparticles. Eur J Pharm Sci. 2009;37(3–4):231–40.
226. Shown I, Masaki U, Imae T. Sensitizing of pyrene fluorescence by β-cyclodextrin-modified TiO2 nanoparticles. J Colloid Interface Sci. 2010;352(2):232–7.
227. Memisoglu-Bilensoy E, Vural I, Bochot A, Renoir JM, Duchene D, Hincal AA. Tamoxifen citrate loaded amphiphilic β-cyclodextrin nanoparticles: in vitro characterization and cytotoxicity. J Control Release. 2005;104(3):489–96.
228. Çirpanli Y, Bilensoy E, Lale DA, Çaliş S. Comparative evaluation of polymeric and amphiphilic cyclodextrin nanoparticles for effective camptothecin delivery. Eur J Pharm Biopharm. 2009;73(1):82–9.
229. Kwon TK, Kim JC. In vitro skin permeation of monoolein nanoparticles containing hydroxypropyl β-cyclodextrin/minoxidil complex. Int J Pharm. 2010;392(1–2):268–73.
230. Badruddoza AZM, Hidajat K, Uddin MS. Synthesis and characterization of β-cyclodextrin-conjugated magnetic nanoparticles and their uses as solid-phase artificial chaperones in refolding of carbonic anhydrase bovine. J Colloid Interface Sci. 2010;346(2):337–46.
231. Kim JW, Nichols WT. Hierarchically assembled titania-cyclodextrin nano-networks. Mater Lett. 2012;67(1):11–3.
232. Nielsen AL, Steffensen K, Larsen KL. Self-assembling microparticles with controllable disruption properties based on cyclodextrin interactions. Colloids Surf B Biointerfaces. 2009;73(2):267–75.
233. Kanwar JR, Long BM, Kanwar RK. The use of cyclodextrins nanoparticles for oral delivery. Cyclodextrin-covered gold nanoparticles for targeted delivery of an anti-cancer drug. J Mater Chem. 2009;19(16):2310–5.
234. Park C, Youn H, Kim H, Noh T, Kook YH, Oh ET, Park HJ, Kim C. J Mater Chem. 2009;19:2310–5.
235. Fanta GF, Kenar JA, Felker FC, Byars JA. Preparation of starch-stabilized silver nanoparticles from amylose-sodium palmitate inclusion complexes. Carbohydr Polym. 2012;92:260–8.
236. Santander-Ortega MJ, Stauner T, Loretz B, Ortega-Vinuesa JL, Bastos-González D, Wenz G, Schaefer UF, Lehr CM. Nanoparticles made from novel starch derivatives for transdermal drug delivery. J Control Release. 2010;141(1):85–92.
237. Ernest V, Shiny PJ, Mukherjee A, Mukherjee A, Chandrasekaran N. Silver nanoparticles: a potential nanocatalyst for the rapid degradation of starch hydrolysis by α-amylase. Carbohydr Res. 2012;352:64.
238. Cole AJ, David AE, Wang J, Galbán CJ, Hill HL, Yang VC. Polyethylene glycol modified, cross-linked starch-coated iron oxide nanoparticles for enhanced magnetic tumor targeting. Biomaterials. 2011;32(8):2183–93.
239. Valodkar M, Rathore PS, Jadeja RN, Thounaojam M, Devkar RV, Thakore S. Cytotoxicity evaluation and antimicrobial studies of starch capped water soluble copper nanoparticles. J Hazard Mater. 2012;201–202:244–9.
240. Jain R, Dandekar P, Loretz B, Melero A, Stauner T, Wenz G, Koch M, Lehr CM. Enhanced cellular delivery of idarubicin by surface modification of propyl starch nanoparticles employing pteroic acid conjugated polyvinyl alcohol. Int J Pharm. 2011;420(1):147–55.

241. Nishi KK, Antony M, Mohanan PV, Anilkumar TV, Loiseau PM, et al. Amphotericin B-Gum arabic conjugates: synthesis, toxicity, bioavailability, and activities against Leishmania and fungi. Pharm Res. 2007;24:971–80.
242. Nishi KK, Jayakrishnan A. Preparation and in vitro evaluation of primaquine-conjugated gum Arabic microspheres. Biomacromolecules. 2004;5(4):1489–95.
243. Ferreira EI, Cruz ML, Korolkovas A. Latentiation of chemotherapeutic agents. Part 1: synthesis of oxidized starch imine derivatives and antimalarials. Starch/starke. 1992;44:21–4.
244. Saboktakin MR, Maharramov A, Ramazanov MA. Synthesis and characterization of superparamagnetic nanoparticles coated with carboxymethyl starch (CMS) for magnetic resonance imaging technique. Carbohydr Polym. 2009;78(2):292–5.
245. Kim EA, Kim JY, Chung HJ, Lim ST. Preparation of aqueous dispersions of coenzyme Q10 nanoparticles with amylomaize starch and its dextrin. Food Sci Technol. 2012;47(2):493–9.
246. Comba S, Sethi R. Stabilization of highly concentrated suspensions of iron nanoparticles using shear-thinning gels of xanthan gum. Water Res. 2009;43(15):3717–26.
247. Liu H, Nakagawa K, Chaudhary D, Asakuma Y, Moses O. Tadé Freeze-dried macroporous foam prepared from chitosan/xanthan gum/montmorillonite nanocomposites. Chem Eng Res Des. 2011;89(11):2356–64.
248. Mundargi RC, Patil SA, Agnihotri SA, Aminabhavi TM. Development of polysaccharide-based colon targeted drug delivery systems for the treatment of amoebiasis. Drug Dev Ind Pharm. 2007;33(3):255–64.
249. Vuddanda PR, Chakraborty S, Singh S. Berberine: a potential phytochemical with multispectrum therapeutic activities. Expert Opin Investig Drugs. 2010;19:10.
250. Sarmah JK, Bhattacharjee SK, Mahanta R, Mahanta R. Preparation of cross-linked guar gum nanospheres containing tamoxifen citrate by single step emulsion in situ polymer cross-linking method. J Incl Phenom Macrocycl Chem. 2009;65(3–4):329–34.
251. Mahantab JKSR, Mahantac SKBR, Deyd A, Guhad P, Biswa A. In-vitro cytotoxicity analysis of tamoxifen citrate loaded cross-linked guar gum nanoparticles on jurkat (human t-cell leukemia) cell line. J Drug Deliv Ther. 2012;2(2).
252. Giri A, Bhunia T, Mishra SR, Goswami L, Panda AB, Pal S, Bandyopadhyay A. Acrylic acid grafted guargum–nanosilica membranes for transdermal diclofenac delivery. Carbohydr Polym. 2013;91(2):492–501.
253. Sarmah JK, Mahanta R, Bhattacharjee SK, Mahanta R, Biswas A. Controlled release of tamoxifen citrate encapsulated in cross-linked guar gum nanoparticles. Int J Biol Macromol. 2011;49(3):390–6.
254. Mao CF, Zeng YC, Chen CH. Enzyme-modified guar gum/xanthan gelation: an analysis based on cascade model. Food Hydrocoll. 2012;27(1):50–9.
255. Su L, Ji WK, Lan WZ. Chemical modification of xanthan gum to increase dissolution rate. Carbohydr Polym. 2003;53(4):497–9.
256. Alvarez-Manceñido F, Landin M, Lacik I, Martínez-Pacheco R. Konjac glucomannan and konjac glucomannan/xanthan gum mixtures as excipients for controlled drug delivery systems. Int J Pharm. 2008;349(1–2):11–8.
257. Dodi G, Hritcu D, Popa MI. Carboxymethylation of guar gum: synthesis and characterization. Cellulose Chem Technol. 2011;45(3-4):171–6.
258. Zhang J, Chen G. Silanization of guar gum to improve the temperature resistance. Heterocycl Lett. 2011;1(4):329–33.
259. Krishnan PN, Saraswathi R, Dilip C, Rama Rao N, Sambasiva Rao KRS, Raghvendra. Formulation and evaluation of acyclovir microcapsules using biodegradable and non-biodegradable polymers. Der Pharm Lett. 2010;2(5):83–94.
260. Tiraferri A, Chen KL, Sethi R, Elimelech M. Reduced aggregation and sedimentation of zero-valent iron nanoparticles in the presence of guar gum. J Colloid Interface Sci. 2008;324(1–2):71–9.
261. Abdel-Halim ES, El-Rafie MH, Al-Deyab SS. Polyacrylamide/guar gum graft copolymer for preparation of silver nanoparticles. Carbohydr Polym. 2011;85(3):692–7.

262. Badykova LA, Mudarisova RK, Khamidullina GS, Aminev KK, Monakov YB. Arabinogalactan with isonicotinic acid hydrazide. Chem Nat Compounds. 2008;44(3):279.
263. Mudarisova RK, Badykova LA, Tolstikova TG, Aleksandrova GP, Borisov IM, Monakova YB. Modification of arabinogalactan and its oxidized fractions with 5-aminosalicylic acid. Russian J Appl Chem. 2005;78(10):1691–4.
264. Gasilova E, Galina A. Core-shell colloidal structure of nanobiocomposites of gold nanoparticles capped with natural polysaccharide arabinogalactan. J Nanomed Nanotechnol. 2012;3.
265. Smith JP. Nanoparticle delivery of anti-tuberculosis chemotherapy as a potential mediator against drug-resistant tuberculosis. Yale J Biol Med. 2011;84(4):361–9.
266. Golenser J, Frankenburg S, Ehrenfreund T, Domb AJ. Efficacious treatment of experimental leishmaniasis with amphotericin B-arabinogalactan water-soluble derivatives. Antimicrob Agents Chemother. 1999;43(9):2209–14.
267. Mucalo MR, Bullen CR, Manley-Harris M, McIntire TM. Arabinogalactan from the Western larch tree: a new, purified and highly water-soluble polysaccharide-based protecting agent for maintaining precious metal nanoparticles in colloidal suspension. J Mater Sci. 2002;37(3):493–504.
268. Nikolaeva MN, Aleksandrova GP, Martynenkov AA. Effect of electrization on molecular mobility in gold nanocomposites based on arabinogalactan. Russian J Phys Chem A. 2012;86(5):812–5.
269. Gasilova E, Aleksandrova G, Sukhov B, Trofimov B. Colloids of gold nanoparticles protected from aggregation with arabinogalactan. Macromol Symp. 2012;317–318(1):1–6.
270. Light Gasilova ER, Toropova AA, Bushin SV, Khripunov AK, Grischenko LA, Aleksandrova GP. Scattering from aqueous solutions of colloid metal nanoparticles stabilized by natural polysaccharide arabinogalactan. J Phys Chem B. 2010;114(12):4204–12.
271. Kora AJ, Beedu SR, Jayaraman A. Size-controlled green synthesis of silver nanoparticles mediated by gum ghatti (Anogeissus latifolia) and its biological activity. Org Med Chem Lett. 2012;2(1):17.
272. Silvestri S, Gabrielson G. Degradation of tragacanth by high shear and turbulent forces during microfluidization. Int J Pharm. 1991;73(2):163–9.
273. Starz KA, Goia D, Koehler J, Bänisch V. Noble metal nanoparticles, a process for preparing these and their use. Patent Number 8071259, 2011.
274. Hatami M, Nejatian M, Mohammadifar MA. Effect of co-solute and gelation temperature on milk protein and gum tragacanth interaction in acidified gels. Int J Biol Macromol. 2012;50(4):1109–15.
275. Hatami M, Nejatian M, Mohammadifar MA, Alijani S, Balaghi S, Mohammadifar MA. Effect of gamma irradiation on rheological properties of polysaccharides exuded by *A. fluccosus* and *A. gossypinus*. Int J Biol Macromol. 2011;49(4):471–9.
276. Borisy A, Keith C, Foley MA, Stockwell BR. Combinations of chlorpromazine and pentamidine for the treatment of neoplastic disorders. U.S. Patent Number 6,569,853, 2003.
277. Skeiky Y, Brannon M, Guderian J. Heterologous fusion protein constructs comprising a Leishmania antigen. U.S. Patent Application 20030175294, 2003.
278. Chenlo F, Moreira R, Pereira G, Silva C. Rheological modelling of binary and ternary systems of tragacanth, guar gum and methylcellulose in dilute range of concentration at different temperatures. Food Sci Technol. 2009;42(2):519–24.
279. Beverley SM, Zhang K. Compositions and methods for inhibiting protozoan growth. U.S. Patent Application 20080119483, 2008.
280. Chenlo F, Moreira R, Silva C. Rheological behaviour of aqueous systems of tragacanth and guar gums with storage time. J Food Eng. 2010;96(1):107–13.
281. Batalha IL, Hussain A, Roque AC. Gum Arabic coated magnetic nanoparticles with affinity ligands specific for antibodies. J Mol Recognit. 2010;23(5):462–71.
282. Wilson Jr OC, Blair E, Kennedy S, Rivera G, Mehl P. Surface modification of magnetic nanoparticles with oleylamine and gum Arabic. Mater Sci Eng C. 2008;28(3):438–42.

283. Livney YD. Beta-lactoglobulin-polysaccharide nanoparticles for hydrophobic bioactive compounds. Patent Application Number 20110038942, 2011.
284. Avadi MR, Sadeghi AMM, Mohammadpour N, Abedin S, Atyabi F, Dinarvand R, Rafiee-Tehrani M. Preparation and characterization of insulin nanoparticles using chitosan and Arabic gum with ionic gelation method. Nanomedicine. 2010;6(1):58–63.
285. Gils PS, Ray D, Sahoo PK. Designing of silver nanoparticles in gum arabic based semi-IPN hydrogel. Int J Biol Macromol. 2010;46(2):237–44.
286. Fent GM, Casteel SW, Kim DY, Kannan R, Katti K, Chanda N, Katti K. Biodistribution of maltose and gum arabic hybrid gold nanoparticles after intravenous injection in juvenile swine. Nanomedicine. 2009;5(2):128–35.
287. Roque ACA, Wilson Jr OC. Adsorption of gum Arabic on bioceramic nanoparticles. Mater Sci Eng C. 2008;28(3):443–7.
288. Williams DN, Gold KA, Holoman TRP, Ehrman SH, Wilson Jr OC. Surface modification of magnetic nanoparticles using gum Arabic. J Nanopart Res. 2006;8(5):749–53.
289. Wu CC, Chen DH. Facile green synthesis of gold nanoparticles with gum arabic as a stabilizing agent and reducing agent. Gold Bull. 2010;43(4):234–40.
290. Mahmoud AE. Viscosity modification of gum Arabic as a means of enhancing marketability. M.Sc. Thesis, Faculty of Forest Products, Virginia Polytechnic Institute and State University, Blacksburg; 1983.
291. Dhar S, Reddy EM, Shiras A, Pokharkar V, Prasad BL. Natural gum reduced/stabilized gold nanoparticles for drug delivery formulations. Chemistry. 2008;14(33):10244–50.
292. Hinrichs WLJ, Manceñido FA, Sanders NN, Braeckmans K, Smedt SCD, Demeester J, Frijlink HW. The choice of a suitable oligosaccharide to prevent aggregation of PEGylated nanoparticles during freeze thawing and freeze drying. Int J Pharm. 2006;311(1–2):237–44.
293. Bibby DC, Talmadge JE, Dalal MK, Kurz SG, Chytil KM, Barry SE, Shand DG, Steiert M. Pharmacokinetics and biodistribution of RGD-targeted doxorubicin-loaded nanoparticles in tumor-bearing mice. Int J Pharm. 2005;293(1–2):281–90.
294. Chockalingam AM, Babu HKRR, Chittor R, Tiwari JP. Gum arabic modified Fe3O4 nanoparticles cross linked with collagen for isolation of bacteria. J Nanobiotechnol. 2010;8:30.
295. Castelli F, Sarpietro MG, Micieli D, Ottim S, Pitarresi G, Tripodo G, Carlisi B, Giammona G. Differential scanning calorimetry study on drug release from an inulin-based hydrogel and its interaction with a biomembrane model: pH and loading effect. Eur J Pharm Sci. 2008;35:76–85.
296. Öner M, Uysal U. Synthesis of hydroxyapatite crystals using carboxymethyl inulin for use as a delivery of ibuprofen. Mater Sci Eng C Mater Biol Appl. 2013;33:482–9.
297. Morros J, Levecke B, Infante MR. Chemical hydrophobic modification of inulin in aqueous media: synthesis of β-hydroxyalkyl ethers of inulin. Carbohydr Polym. 2010;81(3):681–6.
298. Izawa K, Hasegawa T. Tosylated and azidated inulins as key substrates for further chemical modifications to access inulin-based advanced materials: an inulin-based glycocluster. Bioorg Med Chem Lett. 2012;22(2):1189–93.
299. Ren J, Wang P, Dong F, Feng Y, Peng D, Guo Z. Synthesis and antifungal properties of 6-amino-6-deoxyinulin, a kind of precursors for facile chemical modifications of inulin. Carbohydr Polym. 2012;87(2):1744–8.
300. Morros J, Levecke B, Infante MR. Hydrophobically modified inulin from alkenyl succinic anhydride in aqueous media. Carbohydr Polym. 2011;84(3):1110–6.
301. Ren J, Liu J, Dong F, Guo Z. Highly efficient synthesis and antioxidant activity of O-(aminoethyl)inulin. Carbohydr Polym. 2011;83(3):1240–4.
302. Pitarresi G, Giacomazza D, Triolo D, Giammona G, San Biagio PL. Rheological characterization and release properties of inulin-based hydrogels. Carbohydr Polym. 2012;88(3):1033–40.
303. Ren J, Liu J, Dong F, Guo Z. Synthesis and hydroxyl radicals scavenging activity of N-(aminoethyl)inulin. Carbohydr Polym. 2011;85(1):268–71.

304. Ronkart SN, Paquot M, Deroanne C, Fougnies C, Besbes S, Blecker CS. Development of gelling properties of inulin by microfluidization. Food Hydrocoll. 2010;24(4):318–24.
305. Verraest DL, Peters JA, Kuzee HC, Raaijmakers HWC, Bekkum H. Modification of inulin with amidoxime groups and coordination with copper(II) ions. Carbohydr Polym. 1998;37(3):209–14.
306. Kim Y, Faqih MN, Wang SS. Factors affecting gel formation of inulin. Carbohydr Polym. 2001;46(2):135–45.
307. Fares MM, Salem MS, Khanfar M. Inulin and poly(acrylic acid) grafted inulin for dissolution enhancement and preliminary controlled release of poorly water-soluble Irbesartan drug. Int J Pharm. 2011;410(1–2):206–11.
308. Stevens CV, Meriggi A, Booten K. Chemical modification of inulin, a valuable renewable resource, and its industrial applications. Biomacromolecules. 2001;2(1):1–16.
309. Gupta H, Sharma A. Ion activated bioadhesive in situ gel of clindamycin for vaginal application. Int J Drug Deliv. 2009;1:32–40.
310. Braz L, Grenha A, Ferreira D, Rosa da Costa A, Sarmento B. Locust bean gum derivatives for nanometric drug delivery. Rev Port Farm. 2011;52:127–8.
311. Dionísio M, Grenha A. Locust bean gum: exploring its potential for biopharmaceutical applications. J Pharm Bioallied Sci. 2012;4:175–85.
312. Braz L, Grenha A, Sarmento B, Costa AR. XVIII Novel locust bean gum nanoparticles for protein delivery. In: International Conference on Bioencapsulation, Porto, Portugal, 2, 2010.
313. Ngwuluka N, Pillay V, Choonara YE, Claire Du TL. Polymeric matrix of polymer-lipid nanoparticles as a pharmaceutical dosage form. WIPO Patent WO/2012/070031, 2012.
314. Braz L, Grenha A, Ferreira D, Rosa da Costa AM, Luis Braz BS et al. Locust bean gum based nanoparticles for oral antigen delivery. Pharm Anal Acta 3:1. 2nd International Conference on Pharmaceutics & Novel Drug Delivery Systems 3, 1 – 115 Pharm Anal Acta, 2012 San Francisco Airport Marriott Waterfront, USA.
315. Livney YD. Beta-lactoglobulin-polysaccharide nanoparticles for hydrophobic bioactive compounds. Patent Application Number, 20110038942, 2011.
316. Mahmoud AE. Viscosity modification of gum Arabic as a means of enhancing gum quality. In Proceedings of the Meetings of the National Crop Husbandry Committee 40th, 2005, 226–236.
317. Kang J, Cui SW, Guo Q, Chen J, Wang Q, Phillips GO, Nikiforuk J. Structural investigation of a glycoprotein from gum ghatti. Carbohydr Polym. 2012;89(3):749–58.
318. Deshmukh AS, Setty CM, Badiger AM, Muralikrishna KS. Gum ghatti: a promising polysaccharide for pharmaceutical applications. Carbohydr Polym. 2012;87(2):980–6.
319. Kang J, Cui SW, Chen J, Phillips GO, Wu Y, Wang Q. New studies on gum ghatti (Anogeissus latifolia). Part I: Fractionation, chemical and physical characterization of the gum. Food Hydrocoll. 2011;25(8):1984–90.
320. Omura S, Oiwa R. Trichostatin as an antiprotozoal agent. U.S. Patent 4,218,478, 1980.
321. Kang J, Cui SW, Phillips GO, Chen J, Guo Q, Wang Q. New studies on gum ghatti (Anogeissus latifolia). Part II: Structure characterization of an arabinogalactan from the gum by 1D, 2D NMR spectroscopy and methylation analysis. Food Hydrocoll. 2011;25(8):1991–8.
322. Rani P, Sen G, Mishra S, Jha U. Microwave assisted synthesis of polyacrylamide grafted gum ghatt. Carbohydr Polym. 2012;89:275–81.
323. Vaidya A, Jain A, Khare P, Agrawal RK, Jain SK. Metronidazole loaded pectin microspheres for colon targeting. J Pharm Sci. 2009;98(11):4229–36.
324. Majumdar S, Roy S, Ghosh B. Design and gamma scintigraphic evaluation of colon specific pectin-EC pellets of secnidazole prepared by powder layering technology. Pharmazie. 2011;66(11):843–8.
325. Ding D, Zhou Y, Zhang YK, Zhang YK, Plattner JJ, Plattner JJ, Zhou H. Boron-containing small molecules as antiprotozoal agents. Patent Application Number, 20110207701, 2011.
326. Andremont A, Huguet HC. Colonic delivery using zn/pectin beads with a eudragit coating. EP2081557 A1, 2009.

327. Newsham EA, Forrester JW, Rowley DJ. Moistureless oral drug delivery formulation and method for preparing same. Patent Number 4867970, 1989.
328. Kurita O, Miyake Y, Yamazaki E. Chemical modification of citrus pectin to improve its dissolution into water. Carbohydr Polym. 2012;87(2):1720–7.
329. Buchholt HC, Christensen TMIE, Fallesen B, Ralet MC, Thibault JF. Preparation and properties of enzymatically and chemically modified sugar beet pectins. Carbohydr Polym. 2004;58(2):149–61.
330. Schmelter T, Wientjes R, Vreeker R, Klaffke W. Enzymatic modifications of pectins and the impact on their rheological properties. Carbohydr Polym. 2002;47(2):99–108.
331. Matthew JA, Howson SJ, Keenan MHJ, Belton PS. Improvement of the gelation properties of sugarbeet pectin following treatment with an enzyme preparation derived from *Aspergillus niger*. Comparison with a chemical modification. Carbohydr Polym. 1990;12(3):295–306.
332. Kowalonek J, Kaczmarek H, Dąbrowska A. Air plasma or UV-irradiation applied to surface modification of pectin/poly(vinyl alcohol) blends. Appl Surf Sci. 2010;257(1):325–31.
333. Schmelter T, Vreeker R, Klaffke W. Characterisation of a novel gel system containing pectin, heat inactivated pectin methylesterase and NaCl. Carbohydr Polym. 2001;45(3):277–84.
334. Ngouémazong DE, Kabuye G, Fraeye I, Cardinaels R, Loey AV, Moldenaers P, Hendrickx M. Effect of debranching on the rheological properties of Ca2+–pectin gels. Food Hydrocoll. 2012;26(1):44–53.
335. Ralet MC, Crépeau MJ, Buchholt HC, Thibault JF. Polyelectrolyte behaviour and calcium binding properties of sugar beet pectins differing in their degrees of methylation and acetylation. Biochem Eng J. 2003;16(2):191–201.
336. Morris GA, Hromádková Z, Ebringerová A, Malovı´ková A, Alföldi J, Harding SE. Modification of pectin with UV-absorbing substituents and its effect on the structural and hydrodynamic properties of the water-soluble derivatives. Carbohydr Polym. 2002; 48(4):351–9.
337. Funami T, Nakauma M, Ishihara S, Tanaka R, Inoue T, Phillips GO. Structural modifications of sugar beet pectin and the relationship of structure to functionality. Food Hydrocoll. 2011;25:221–9.
338. Sharma R, Ahuja M, Kaur H. Thiolated pectin nanoparticles: preparation, characterization and ex vivo corneal permeation study. Carbohydr Polym. 2012;87(2):1606–10.
339. Jones OG, Lesmes U, Dubin P, McClements DJ. Effect of polysaccharide charge on formation and properties of biopolymer nanoparticles created by heat treatment of β-lactoglobulin–pectincomplexes. Food Hydrocoll. 2010;24(4):374–83.
340. Gong JL, Wang XY, Zeng GM, Chen L, Deng JH, Zhang XR, Niu QY. Copper (II) removal by pectin–iron oxide magnetic nanocomposite adsorbent. Chem Eng J. 2012;185–186(15):100–7.
341. Li J, Zhu D, Yin J, Liu Y, Yao F, Yao K. Formation of nano-hydroxyapatite crystal in situ in chitosan–pectin polyelectrolyte complex network. Mater Sci Eng C. 2010;30(6):795–803.
342. Narayana RC, Harish NM, Gulzar AM, Prabhakara P, Singh AK, Subrahmanyam EV. Formulation and in vitro evaluation of in situ gels containing secnidazole for vaginitis. Yakugaku Zasshi. 2009;129(5):569–74.
343. Survase SA, Annapure US, Singhal RS. Gellan gum as immobilization matrix for production of cyclosporin A. J Microbiol Biotechnol. 2010;20(7):1086–91.
344. Morrisa ER, Nishinarib K, Rinaudoc M. Gelation of gellan – a review. Food Hydrocoll. 2012;28(2):373–411.
345. Britten N. Pharmaceutical dosage form for mucosal delivery. EP1471890 B1. 27 Sept 2006.
346. Coutinho DF, Sant SV, Shin H, Oliveira JT, Gomes ME, Neves NM, Khademhosseini A, Reis RL. Modified gellan gum hydrogels with tunable physical and mechanical properties. Biomaterials. 2010;31(29):7494–502.
347. Silva NA, Cooke MJ, Tam RY, Sousa N, Salgado AJ, Reis RL, Shoichet MS. The effects of peptide modified gellan gum and olfactory ensheathing glia cells on neural stem/progenitor cell fate. Biomaterials. 2012;33(27):6345–54.

348. Hamcerencu M, Desbrieres J, Khoukh A, Popa M, Riess G. Synthesis and characterization of new unsaturated esters of Gellan Gum. Carbohydr Polym. 2008;71(1):92–100.
349. Dreveton E, Monot F, Lecourtier J, Ballerini D, Choplin L. Influence of fermentation hydrodynamics on gellan gum physico-chemical characteristics. J Fermentation Bioeng. 1996;82(3):272–6.
350. Maiti S, Ranjit S, Mondol R, Ray S, Biswanath SA. Al^{+3} ion cross-linked and acetalated gellan hydrogel network beads for prolonged release of glipizide. Carbohydr Polym. 2011;85(1):164–72.
351. Lee MW, Tsai HF, Wen SM, Huang CH. Photocrosslinkable gellan gum film as an anti-adhesion barrier. Carbohydr Polym. 2012;90(2):1132–8.
352. Lee MW, Chen HJ, Tsao SW. Preparation, characterization and biological properties of Gellan gum films with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide cross-linker. Carbohydr Polym. 2010;82(3):920–6.
353. Kulkarni RV, Mangond BS, Mutalik S, Sa B. Interpenetrating polymer network microcapsules of gellan gum and egg albumin entrapped with diltiazem–resin complex for controlled release application. Carbohydr Polym. 2011;83(2):1001–7.
354. Bar-Shalom D, Slot L, Fischer G, Hemmingsen PH. Swellable dosage form comprising gellan gum. Patent Application Number 20120039969, 16 Feb 2012.
355. Vijan V, Kaity S, Biswas S, Isaac J, Ghosh A. Microwave assisted synthesis and characterization of acrylamide grafted gellan, application in drug delivery. Carbohydr Polym. 2012;90(1):496–506.
356. Doner LW, Douds DD. Purification of commercial gellan to monovalent cation salts results in acute modification of solution and gel-forming properties. Carbohydr Res. 1995;273(2): 225–33.
357. Rana V, Rai P, Tiwary AK, Singh RS, Kennedy JF, Knill CJ. Modified gums: approaches and applications in drug delivery. Carbohydr Polym. 2011;83(3):1031–47.
358. Redouan E, Emmanuel P, Christine B, Bernard C, Josiane C, Cédric D. Development of new ulvan-like polymer by regioselective oxidation of gellan exopolysaccharide using TEMPO reagent. Carbohydr Polym. 2010;80:485–90.
359. Nishiyama Y, Langan P, Chanzy H. Crystal structure and hydrogen-bonding system in cellulose Iβ from synchrotron X-ray and neutron fiber diffraction. J Am Chem Soc. 2002;124(31):9074–82.
360. Scheller HV, Jensen JK, Sørensen SO, Harholt J, Geshi N. Biosynthesis of pectin. Physiol Plant. 2007;129:283–95.
361. Aquilera JM, Stanley DW. Microstructural principles of food processing and engineering. Aspen: Springer; 1999. p. 99–103.
362. Cosgrove DJ. Growth of the plant cell wall. Nat Rev Mol Cell Biol. 2005;6:850–61.
363. Dumitriu S. Cellulose and its derivatives: structures, reactions and medical uses. In: Hon DNS, editor. Polysaccharides in medicinal applications. New York, NY: Marcel Dekker, Inc; 1996. p. 87–106.
364. Hon DNS. Cellulose and its derivatives: structures, reactions and medical uses. New York, NY: Marcel Dekker; 1996.
365. Scheller HV, Ulvskov P. Hemicelluloses. Annu Rev Plant Biol. 2010;61:263–89.
366. Lerouxel O, Cavalier DM, Liepman AH, Keegstra K. Biosynthesis of plant cell wall polysaccharides – a complex process. Curr Opin Plant Biol. 2006;9:621–30.
367. Katsuraya K, Okuyamab K, Hatanakab K, Oshimab R, Satoc T, et al. Constitution of konjac glucomannan: chemical analysis and 13C NMR spectroscopy. Carbohydr Polym. 2003;52:183–9.
368. Fan J, Wang K, Liu M, He Z. In vitro evaluations of konjac glucomannan and xanthan gum mixture as the sustained release material of matrix tablet. Carbohydr Polym. 2008;73:241–7.
369. Larionova NV, Ponchel G, Duchene D, Larionova NI. Biodegradable cross-linked starch/protein microcapsules containing proteinase inhibitor for oral protein administration. Int J Pharm. 1999;189:171–8.

370. Tuovinen L, Peltonen S, Jarvinen K. Drug release from starch-acetate films. J Control Release. 2003;91:345–54.
371. Krogars K, Antikainen O, Heinamaki J, Laitinen N, Yliruusi J. Tablet film coating with amylose-rich maize starch. Eur J Pharm Sci. 2002;17:23–30.
372. Milojevic S, Newton JM, Cummings JH, Gibson GR, Botham RL, et al. Amylose, the new perspective in oral drug delivery to the human large intestine. STP Pharma Sci. 1995;5:47–53.
373. Milojevic S, Newton JM, Cummings JH, Gibson GR, Botham RL, et al. Amylose as a coating for drug delivery to the colon: preparation and an in vitro evaluation using 5-aminosalicylic acid pellets. J Control Release. 1996;38:75–84.
374. Milojevic S, Newton JM, Cummings JH, Gibson GR, Botham RL, et al. Amylose as a coating for drug delivery to the colon: preparation and in vitro evaluation using glucose pellets. J Control Release. 1996;38:85–94.
375. Palviainen P, Heinamaki J, Myllarinen P, Lahtinen R, Yliruusi J, et al. Corn starches as film formers in aqueous-based film coating. Pharm Dev Technol. 2001;6:351–61.
376. Siew LF, Basit AW, Newton JM. The potential of organic based myloseethylcellulose film coatings as oral colon specific drug delivery systems. AAPS PharmSciTech. 2000;1, E22.
377. Sinha VR, Kumria R. Polysaccharides in colon-specific drug delivery. Int J Pharm. 2001;224:19–38.
378. Fry SC. Primary cell wall metabolism, tracking the careers of wall polymers in living plant cells. New Phytol. 2004;161:641–75.
379. Cárdenas A, Goycoolea FM, Rinaudo M. On the gelling behaviour of 'nopal' (Opuntia ficus indica) low metholoxyl pectin. Carbohydr Polym. 2008;73:212–22.
380. Sungthongjeen S, Pitaksuteepong T, Somsiri A, Sriamornsak P. Studies on pectins as potential hydrogel matrices for controlled release drug delivery. Drug Dev Ind Pharm. 1999;25:1271–6.
381. Tho I, Sande SA, Kleinebudde P. Pectinic acid: a novel excipient for production of pellets by extrusion/spheronisation: preliminary studies. Eur J Pharm Biopharm. 2002;54:95–9.
382. Giunchedi P, Conte U, Chetoni P, Saettone MF. Pectin microspheres as ophthalmic carriers for piroxicam: evaluation in vitro and in vivo in albino rabbits. Eur J Pharm Sci. 1999;9:1–7.
383. Musabayane CT, Munjeri O, Matavire TP. Transdermal delivery of chloroquine by amidated pectin hydrogel matrix patch in the rat. Ren Fail. 2003;25:525–34.
384. Cheng K, Lim LY. Insulin-loaded calcium pectinate nanoparticles: effects of pectin molecular weight and formulation pH. Drug Dev Ind Pharm. 2004;30:359–67.
385. Liu L, Chen G, Fishman ML, Hicks KB. Pectin gel vehicles for controlled fragrance delivery. Drug Deliv. 2005;12:149–57.
386. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 22nd ed. India: Nirali Prakashan; 2003. p. 133–66.
387. Vervoort L, Kinget R. *In vitro* degradation by colonic bacteria of inulin HP incorporated in Eudragit RS films. Int J Pharm. 1996;129:185–90.
388. Vervoort L, Van den Mooter G, Augustijns P, Kinget R. Inulin hydrogels, I. Dynamic and equilibrium swelling properties. Int J Pharm. 1998;72:127–35.
389. Akhgari A, Farahmand F, Garekani H, Sadeghi F, Vandamme TF. Permeability and swelling studies on free films containing inulin in combination with different polymethacrylates aimed for colonic drug delivery. Eur J Pharm Sci. 2006;28:307–14.
390. Satturwar PM, Fulzele SV, Dorle AK. Biodegradation and *in vivo* biocompatibility of rosin: a natural film-forming polymer. AAPS Pharm Sci Tech. 2003;4:1–6.
391. Nande VS, Barabde UV, Morkhade DM, Patil AT, Joshi SB. Synthesis and characterization of PEGylated derivatives of rosin for sustained drug delivery. React Funct Polym. 2006;66:1373–83.
392. Fulzele SV, Satturwar PM, Dorle AK. Polymerized rosin: novel film forming polymer for drug delivery. Int J Pharm. 2002;249:175–84.

393. Izydorczyk M. Understanding the chemistry of food carbohydrates. Boca Raton, FL: Taylor and Francis; 2006.
394. Ramakrishnan A, Pandit N, Badgujar M, Bhaskar C, Rao M. Encapsulation of endoglucanase using a biopolymer gum arabic for its controlle lease. Bioresour Technol. 2007;98:368–72.
395. Aspinall GO, Baillie J. Gum tragacanth. Part I: Fractionation of the gum and the structure of tragacanthic acid. J Chem Soc (Resumed). 1963:1702–14.
396. Anderson DMW, Bridgeman MME. The composition of the proteinaceous polysaccharides exuded by astragalus microcephalus, A. Gummifer and A. Kurdicusâ—the sources of Turkish gum tragacanth. Phyto Chem. 1985;24:2301–04.
397. Anderson DMW, Grant DAD. The chemical characterization of some Astragalus gum exudates. Food Hydrocoll. 1988;2:417–23.
398. Philips GO, Williams PA. Handbook of hydrocolloids. New York: CRC; 2000.
399. Sima B, Mohammad AM, Azizollaah Z. Physicochemical and rheological characterization of gum tragacanth exudates from six species of Iranian Astragalus. Food Biophys. 2010;5:57–71.
400. Parija S, Misra M, Mohanty AK. Studies of natural gum adhesive extracts - an overview. Polym Rev. 2001;4:175–97.
401. Prabaharan M. Prospective of guar gum and its derivatives as controlled drug delivery systems. Int J Biol Macromol. 2011;49:117–24.
402. Krishnaiah YS, Karthikeyan RS, Satyanarayana V. A three-layer guar gum matrix tablet for oral controlled delivery of highly soluble metoprolol tartrate. Int J Pharm. 2002;241: 353–66.
403. Krishnaiah YS, Karthikeyan RS, Gouri Sankar V, Satyanarayana V. Three-layer guar gum matrix tablet formulations for oral controlled delivery of highly soluble trimetazidine dihydrochloride. J Control Release. 2002;81:45–56.
404. Prasad YV, Krishnaiah YSR, Satyanarayana S. In vitro evaluation of guar gum as a carrier for colon-specific drug delivery. J Control Release. 1998;51:281–7.
405. Toti US, Aminabhavi TM. Modified guar gum matrix tablet for controlled release of diltiazem hydrochloride. J Control Release. 2004;95:567–77.
406. Heywood VH, Brummitt RK, Culham A, Seberg O. Flowering plant families of the world. Richmond Hill, ON: Firefly Books; 2007.
407. Wikipedia, Grewia, Wikipedia encyclopedia 2010.
408. Wikipedia, Carl Linnaeus, Wikipedia encyclopedia, 2010.
409. Sprague TA. The section microcos of Grewia in Africa. Bull Miscellaneous Inf (Royal Botanic Gardens, Kew). 1909;1909:66–8.
410. Onwuliri FC, Mawak JD, Wonang DL, Onwuliri EA. Phytochemical toxicological and histopathological studies of some medicinal plants in Nigeria. Int J Nat Appl Sci. 2006;2:225–9.
411. Okafor IS. Characterization and application of grewia gum in tabletting, Pharmaceutics. Nsukka: University of Nigeria; 2001.
412. Okafor IS. The rheological properties of grewia gum. Nigeria J Polym Sci Technol. 2001;2:169–75.
413. Okafor IS, Chukwu A. Water vapor permeability of aqueous-based grewia gum film. Nigeria J Polym Sci Technol. 2003;3:178–85.
414. Okafor IS, Chukwu A. The binding property of grewia gum in sodium salicylate tablets. West Afr J Biol Sci. 2003;14:9–21.
415. Okafor IS, Chukwu A, Udeala OK. Some physicochemical properties of grewia gum. Nigeria J Polym Sci Technol. 2001;2:161–8.
416. Okafor IS, Danat IM. The influence of granulating solvents on drug release from tablets containing grewia gum. J Pharm Bioresour. 2004;1:76–83.
417. Audu-Peter JD, Gokum BG. Effect of methods of incorporating grewia gum as binder on tablet properties. Nigerian J Pharm Res. 2005;4:68–73.
418. Audu-Peter JD, Isah S. Evaluation of grewia gum as binder in paracetamol tablet. J Pharm Bioresour. 2007;4:68–73.

419. Emeje M, Isimi C, Kunle O. Effect of Grewia gum on the mechanical properties of Paracetamol tablet formulations. Afr J Pharm Pharmacol. 2008;2:1–6.
420. Muazu J, Musa H, Musa KY. Compression, mechanical and release properties of paracetamol tablets containing acid treated Grewia gum. J Pharm Sci Technol. 2009;1:74.
421. Nep EI, Conway BR. Characterization of grewia gum, a potential pharmaceutical excipient. J Excip Food Chem. 2010;1:30–40.
422. Ogaji I. Characterization and application of grewia gum as a film coating agent in theophylline hydrochloride tablets. Pharm Pharm Technol. 2011;308.
423. Ndjouenkeu R, Akingbala J, Oguntimein G. Emulsifying properties of three African food hydrocolloids: okra (Hibiscus esculentus), dika nut (Irvingia gabonensis) and khan (Belschmiedia sp.). Plant Foods Hum Nutr. 1997;51:245–55.
424. Udayasekhara Rao P. Chemical composition and biological evaluation of Okra (Hibiscus esculentus) seeds and their kernels. Plant Foods Hum Nutr. 1985;35:89–396.
425. Nasipuri RN, Igwilo CI, Brown AS, Kunle OO. Mucilage from Abelmoschus esculentus (okra) fruits: a potential pharmaceutical raw material; part I; physicochemical properties. J Pharm Res Dev. 1996;1:22–8.
426. Ogaji I. Some physicochemical properties of acetaminophen pediatric suspensions formulated with okra gums obtained from different extraction processes as suspending agent. Asian J Pharm. 2011;5:15–20.
427. Femi-Oyewo MN, Adedokun MO, Olusoga TO. Evaluation of the suspending properties of Albizia zygia gum on sulphadimidine suspension. Trop J Pharm Res. 2004;3:279–84.
428. Kalu VD, Odeniyi MA, Jaiyeoba KT. Matrix properties of a new plant gum in controlled drug delivery. Arch Pharm Res. 2007;30:884–9.
429. Ogaji I, Nnoli O. Film coating potential of okra gum using paracetamol tablets as a model drug. Asian J Pharm. 2010;4:130–4.
430. Tavakoli N, Ghasemi N, Taimouri R, Hamishehkar H. Evaluation of okra gum as a binder in tablet dosage forms. Iranian J Pharm Res. 2004;2:47.
431. Momoh MA, Akikwu MU, Ogbona JI, Nwachi UE. In vitro study of release of metronidazole tablets prepared from okra gum, gelatin gum and their admixture. Bio-Research. 2009;6:339–42.
432. Attama AA, Adikwu MU, Amorha CJ. Release of indomethacin from bioadhesive tablets containing Carbopol 941 modified with Abelmuschus esculentus (Okra) gum. Boll Chim Farm. 2003;142:298–302.
433. Adenuga YA, Odeku OA, Adegboye TA, Itiola OA. Comparative evaluation of the binding properties of two species of Khaya gum polymer in a paracetamol tablet formulation. Pharm Dev Technol. 2008;13:473–80.
434. Singh AK, Panner Selvam R, Sivakumar T. Isolation, characterisation and formulation properties of a new plant gum obtained from mangifera indica. Int J Pharm Biomed Res. 2010;1:35–41.
435. Eka O. Proximate composition of bush mango tree and some properties of dika fat. Nigerian J Nutr Sci. 1980;1:33–6.
436. Giami SY, Okonkwo VI, Akusu MO. Chemical composition and functional properties of raw, heat-treated and partially proteolysed wild mango (Irvingia gabonensis) seed flour. Food Chem. 1994;49:237–43.
437. Amubode FO, Fetuga BL. Amino acid composition of seeds of some lesser known tree crops. Food Chem. 1984;13:299–307.
438. Lewkowitsch J. Dika fat. Analyst. 1905;30:394–5.
439. Meara ML, Patel CB. The component acids and glycerides of dika fat. J Sci Food Agric. 1950;1:48–51.
440. Abdurahman EM, Rau PP, Shok M, Olurinola PF, Laakso I. Analysis of the fatty acid composition of the seed fat of two varieties of Irvingia gabonensis by high resolution gas chromatography. J Pharm Res Dev. 1996;1:48–9.

441. Odeku OA, Patani B. Evaluation of dika nut mucilage (Irvingia gabonensis) as a binding agent in metronidazole tablet formulation. Pharm Dev Technol. 2005;10:439–46.
442. Isimi CY, Kunle OO, Bangudu AB. Some emulsifying and suspending properties of the mucilage extracted from kernels of Irvingia gabonensis. Boll Chim Farm. 2000;139:199–204.
443. Ofoefule SI, Chukwu A. Effects of polyethyleneglycol 4000 and sodium lauryl sulphate on the release of hydrochlorothiazide embedded in the dika fat matrix. Acta Pharm. 2001;51:233–9.
444. Ofoefule SI, Chukwu A, Okore VC, Ugwah MO. Use of dika fat in the formulation of sustained release frusemide encapsulated granules. Boll Chim Farm. 1997;136:646–50.
445. Umekoli GC, Onyechi JO, Udeala OK. Use of dika fat in the formulation of sustained release theophylline tablets and capsules. Bio-Research. 2009;7:456–60.
446. Okore VC. Evaluation of dika fat as a suppository base: factors which affect the drug release from dika fat-based suppositories. Acta Pharm. 1998;48:39–46.
447. Okore VC. Evaluation of dika fat as a suppository base II: thermal and release characteristics of blended dika fat suppositories. Drug Dev Ind Pharm. 1994;20:93–100.
448. Megwa SA. Evaluation of dika fat as a suppository base. Drug Dev Ind Pharm. 1987;13: 2731–48.
449. Udeala OK, Aly SAS. The effects of microencapsulation with dika wax on the degradation and dissolution of aspirin tablets. Drug Dev Ind Pharm. 1986;12:397–421.
450. Dudu PO, Okiwelu SN, Lale NES. Attractancy of diethyl ether extracts of Arachis hypogaea (Linnaeus) (Papilionaceae), Citrullus lanatus (Thunberg) (Cucurbitaceae) and Irvingia gabonensis var. excelsa (Baillon) (Irvingiaceae) to Oryzaephilus mercator (Fauvel) (Coleoptera: Silvanidae). J Stored Prod Res. 1998;34:237–41.
451. Ogaji I, Anjan N, Hoag SW. A novel extraction method and some physico- chemical properties of extractives of irvingia gabonensis seeds. J Young Pharm. 1996;23:45–9.
452. Alur HH, Pather SI, Mitra AK, Johnston TP. Evaluation of the gum from Hakea gibbosa as a sustained-release and mucoadhesive component in buccal tablets. Pharm Dev Technol. 1999;4:347–58.
453. Tyler VE, Brady LR, Robers JE. Plant gums and mucilage. 8th ed. Philadelphia: Lea and Febiger; 1981.
454. Kulkarni GT, Gowthamrajan K, Rao BG, Suresh B. Evaluation of binding properties of plantago ovate and Trigonella foenum graecum mucilages. Indian Drugs. 2002;38:422–5.
455. Singh B, Chauhan N. Modification of psyllium polysaccharides for use in oral insulin delivery. Food Hydrocoll. 2009;23:928–35.
456. Chukwu KI, Udeala OK. Binding effectiveness of Colocassia esculenta gum in poorly compressible drugs-paracetamol and metronidazole tablet formulations. Boll Chim Farm. 2000;139:89–97.
457. Baveja SK, Ranga Rao KV, Arora J. Examination of natural gums and mucilages as sustaining materials in tablet dosage forms. Indian J Pharm Sci. 1988;50:89–92.
458. Somboonpanyakul P, Wang Q, Cui W, Barbut S, Jantawat P. Malva nut gum. (Part I): Extraction and physicochemical characterization. Carbohydr Polym. 2006;64:247–53.

Chapter 5
Marine Polysaccharides Based Nano-Materials and Its Applications

Abstract Marine polysaccharides and its associated nano-materials are currently considered as an excellent source for nano-technological applications. These applications are broadly categorized in the field of cancer therapy, wound dressing, drug delivery, gene delivery, tissue engineering, water treatment and biosensor. Promising biological functions are due to their structure and physicochemical characteristics, which certainly depend on the source of the organism. Production of marine polysaccharides based nano-materials is simple, economical, biodegradable, and well suggested to be used in the large-scale production of bio-nanomaterials. These polysaccharides are highly biocompatible, biodegradable, nontoxic, low cost, stable, safe, and abundant. Nevertheless the majority of the commercial applications are still at the laboratory level. Moreover in vivo investigations and clinical applications are required to develop industrial nanoproducts. In addition to these applications marine polysaccharide-based nano-materials have various applications biomedical sciences, fabric and food industries, and pharmaceutical industries.

Keywords Marine • Nanomaterial • Nanoparticle • Polysaccharide • Polymer • Alga

5.1 Introduction

Marine polysaccharides especially derived from algal sources offers various potential applications in modern medicine and nanobiotechnology field. These polysaccharides have several applications in drug delivery, gene delivery, wound dressing and tissue engineering [1]. Algae especially seaweeds present an array of therapeutic compounds with diverse structures and remarkable biological activities. These bioactive compounds contain rich source of sulfated polysaccharides such as porphyran, agarose, alginate, fucoidan, carrageenan, and ulvan. Sulfated polysaccharides are derived from various sources as mentioned in Fig. 5.1. In addition to algal sources exoskeleton of marine crustaceans offer some bioactive polysaccharides

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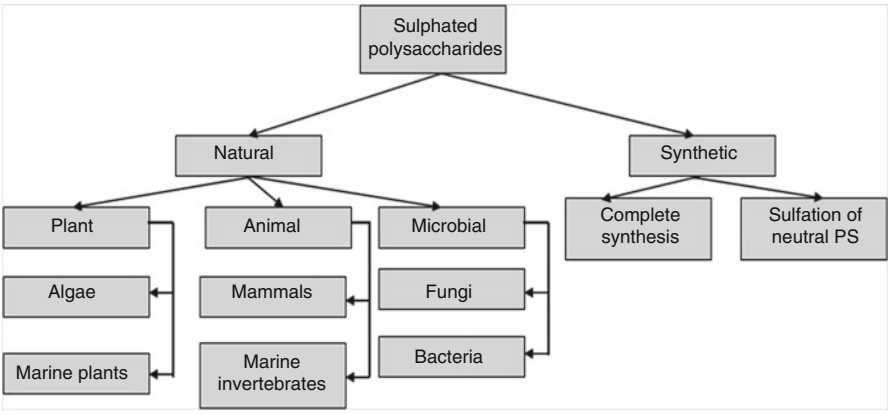


Fig. 5.1 Various sources of sulphated polysaccharides

such as chitosan (CS), chitin, and oligosaccharides [2]. Various marine organisms produce exopolysaccharides (EPS) as an approach for growth adhering to solid surfaces and to survive in adverse conditions. These marine microorganisms producing EPS are a complex mixture of biopolymers chiefly comprised of polysaccharides as well as lipids, proteins, nucleic acids and humic substances [3]. Owing to the various advantages such as nanotoxicity, biocompatibility, low cost, biodegradability, and abundance offered by marine polysaccharide-based nanomaterials they endow significant applications in biomedical and chemical research [4]. Currently pharmaceuticals based on marine bionanoparticles of polymers such as nanomaterials, liposomes, dendrimers, metals or metaloxides or micelles are mainly investigated for fighting against different diseases, including cancer and bacterial infections and for drug delivery, gene delivery, wound healing and tissue engineering [5]. Sulfated polysaccharides and EPS are easily developed in to various nano and micro (nanoparticles, nanofibers, microparticles) membranes, gels, scaffolds, beads, and sponge forms. There pharmaceuticals have been used for variety of biomedical applications in drug delivery, tissue engineering, cancer therapy, wound dressing, biosensors, and water treatment in the area of nanobiotechnology (Table 5.1) [7]. This chapter highlights marine polysaccharide-based nanomaterials for nanotechnological and biomedical applications.

5.2 Polysaccharides Derived from Marine Sources

Among all living organisms marine polysaccharides derived from various resources such as marine algae, crustaceans, and microorganisms [4] forms one of the major components and considered as the most abundant source of

Table 5.1 Description of various sulphated polysaccharides highlighting their own biological activity and applications [6]

Polysacchari-des	Marine organisms (source)	Class	Biological properties	Applications
Heterofucans	*Canistrocarpus cervicornis* a.k.a. *Dictyota cervicornis* *Dictyopteris delicatula*	Chromophyta Dictyotales	Anticoagulant, antioxidant; anti-proliferative, anti-thrombotic, antiviral	Vaccines for immunotherapy Production of nanofibers Gluing and soft tissue closure after surgery Lubricants for bone joints Nanotechnology
S-fucans	*Canistrocarpus cervicornis* a.k.a. *Dictyota cervicornis* *Dictyopteris delicatula* *P. tetrastromatica* *Cladosiphon okamuranus* a.k.a. *Okinawa mozuku* *Spatoglossum schröederi* *Ascophyllum nodosum* Fucus spp. F. vesiculosus Hizikia fusiforme a.k.a. Sargassum fusiforme Sargassum spp.	Chromophyta Dictyotales Ectocarpales Fucales	Anti-proliferative, antiviral, anti-inflammatory, antiadhesive, Antitumor, immunomodulator, angiogenic, gastroprotective, Cardioprotective, restenosis preventive	
S-galactofucans	*D. menstrualis* *Lobophora variegate* *Spatoglossum schröederi* *Adenocystis utricularis* *Costaria costata* *L. japonica* *a.k.a. Saccharina japonica* *Undaria pinnatifida*	Chromophyta Dictyotales Ectocarpales Laminariales	Anti-thrombotic; Peripheral anti-nociceptive; Anti-proliferative, anti-adhesive, antioxidant	
LMW-S-fucans	*Nemacystus decipiens* *P. canaliculata*	Fucales Ectocarpales	Anticoagulant	
S-fucoidan	*C. novae-caledoniae* *Saccharina cichorioides* *a.k.a. Laminaria cichorioides* *Laminaria spp.* *S. horneri* *S. henslowianum*	Fucales Ectocarpales	Antitumor	
Fucoidans	*Eisenia bicyclis* *Lessonia vadosa*	Fucales	Anticoagulant	
S-galactofucans Fucoidan	*Undaria pinnatifida*	Fucales	Antiviral, anticoagulant, antitumor, anti-proliferative, immunomodulatory, anti-inflammatory induced osteoblastic differentiation	
LMW-fucoidan	*S. patens*	Fucales	Antiviral	
S-laminaran	*Laminaria sp. (or Saccharina)*	Fucales	Antitumor, anticoagulant, decreases liver triglyceride, cholesterol and phospholipid levels; serum hypocholesterolaemic, hypotensive, antibacterial, immunomodulator	

(continued)

Table 5.1 (continued)

Polysacchari-des	Marine organisms (source)	Class	Biological properties	Applications
Laminarans	*Eisenia bicyclis*	Fucales	Anti-proliferative, antitumor, anticoagulant; Antitumor, serum hypocholesterolaemic, hypotensive, antibacterial, immunomodulator	Wound healing Burn-wound dressings Tissue regeneration
S-galactan porphyran	*Porphyra spp.*		Antitumor, hypotensive, regulates blood cholesterol	Cell encapsulation Scaffolds for tissue engineering Wound healing and dressings Revascularization
Porphyran	*P. yezoensis* *P. haitanensis*	Rhodophyta Bangiales	Antitumor, immunomodulatory, hypolipidaemic	
S-agarans	*Bostrychia montagnei* *Cryptopleura ramose* *Digenea simplex* *Gloiopeltis complanata* *Aghardiella tenera G. corticata*	Rhodophyta Bangiales Ceramiales Cryptonemiales	Antiviral	
LMW-PS	*Corallina sp.* *C. ocellatus* *Furcellaria lumbricalis* *Hypnea charoides* *Soliera chordalis*	Corallinales Gigartinales	Antiviral; Antitumor	
Agaroid-carrageenan	*G. furcata*	Cryptonemiales		
di-S-galactan	*Gelidium crinale*	Gelidiales		
Hybrid DL-galactans	*Pterocladia capillacea* *Gymnogongrus torulosus*	Gigartinales		
S-λ-carrageenan	*Chondrus crispus* *E. spinosa* *G. skottsbergii* *Phyllophora brodiei* *Stenogramme interrupta*	Gigartinales	Anticoagulant, antithrombotic	
LMW-PS	*Hypnea charoides*		Immunostimulant	
LMW-S-carrageenans	*Kappaphycus striatus*		Antitumor, immunomodulator	
sPS	*Gracilaria caudate* *G. verrucosa* *U. conglobata* *U. fasciata* *U. lactuca* *U. rigida* *C. cupressoides*	Gracilariales Chlorophyta Bryopsidales Ulvales Diatoms Chlorophytes Prasinophyte Prymnesiophyte/ haptophyte	Antiviral, anti-inflammatory, immunomodulator, anti-proliferative, prevention of tumour cell growth	
S-galactans	*G.corticata* *Grateloupia indica* *Schizymenia dubyi* *S. binderi* *U. lactuca* *Caulerpa spp.* *Aghardiella tenera* *Euchema cottonii* *Pterocladia capillacea*	Ulvales Rhodymeniales Nematomatales Halymeniales Gracilariales Gigartinales	Anticoagulant, antithrombotic	

(continued)

Table 5.1 (continued)

Polysacchari-des	Marine organisms (source)	Class	Biological properties	Applications
S-mannans	*Nemalion helminthoides* *Capsosiphon fulvescens*	Ulotrichales Nemaliales	Antiviral Immunomodulator	Drugs carriers Encapsulation, Scaffolds for ligaments and tissue engineering Regeneration of tissues Moulding in dentistry Wound healing and dressings
Xylogalactans S-xylomannans	*Nothogenia fastigiata*	Nemaliales	Antiviral, anticoagulant	
di-S-galactan LMW-sPS	*Botryocladia occidentalis* *Champia feldmannii*	Rhodymeniales	Anticoagulant, anti-venom	
S-xylomannans	*Sebdenia polydactyla*	Nemaliales	Antiviral	
S-arabinogalactans	*Codium spp.*	Chlorophyta Bryopsidales	Anticoagulant, antithrombotic, antiviral	
S-pyrulylated-galactans	*C. isthmocladum*	Chlorophyta Bryopsidales	Antioxidant, anticoagulant, anti-proliferative	
S-rhamnans and LMW-S-rhamnans Rhamnans	*Monostroma latissimum* *M. nitidum* *Enteromorpha intestinalis*	Ulotrichales	Anticoagulant, antithrombotic, hepatoprotective, antitumor, immnunomodulator	
S-ulvans and Derivatives, LMW-S-ulvan or otherwise modified	*E. prolifera* *U. pertusa*	Ulvales	Antioxidant, anti-proliferative, Hypocholesterolaemic	
EPS	*Haslea ostrearia* *Nitzschia closterium* *Skeletonema costatum* *Chaetoceros spp.* *Amphora sp.* *Dunaliella salina* *Ankistrodesmus angustus* *Botryococcus braunii*	Diatoms Chlorophytes Cyanobacteria	Antiviral, antibacterial, prevention of tumour Cell growth	
s-Spirulan	*Arthrospira platensis*	Cyanobacteria	Anti-proliferative, anti-adhesive, Anti-metastatic	

polysaccharides (Table 5.2) (Figs. 5.2 and 5.3). These polysaccharides exhibit various biological and biomedical applications, namely [8], antiangiogenic [9], antimetastatic [10], and anticoagulant [11], antioxidant [11], anti-inflammatory, immunomodulating [8], antiproliferative [11], antitumor [12], antiparasitic antiviral [8] properties. Among all class sulphated polysaccharides have been of great interest because of the presence of sulphur group and their potential to generate new bioactive compounds [10].

Table 5.2 Chief sources of polysaccharides and its description

Sources	Description	Name	Bio-prop.	Biopolymer	Application
Green algae sulfated polysaccharides	(1-3(6))-Linked galactose,(1-3 (4))-linked arabinose,(1-4)- linked glucose and terminal,(1-4)-linked xylose residues. Sulfation soccuron O6 of galactose and O3 of arabinose. Sulfate ester content: 9 %	Green algae, Caulerpa racemosa	Antiviral activity (herpes simplex virus type 1 and 2)	Ulvan	Tissue engineering
	(1-2)-Linked L-rhamnose residues with sulfate groups substitute dat positions of C3 and/or C4. Sulfate ester content: 23 %/25 %	Green algae, Monostroma latissimum	Anticoagulant activity		
Brown algae sulfated polysaccharides	Fucan: (1-3)-linked α-L-fucopyranosyl backbone, mostly sulfated atC4, and branched at C2 with non-sulfated fucofuranosyl and fucopyranosyl units, and 2-sulfated fucopyranosyl units. Galactan: D-galactopyranose units linked on C3 and C6, and sulfation mostly on C4. Sulfate ester content: 30–34 %/21–24 %	Brown algae, Adenocystis utricularis	Antiviral activity (herpes simplex virus type 1 and 2), Antiretroviral activity (HIV-1)	Alginate	Drug delivery, Anti-tubercular and antifungal, Antitumor
	Mainly composed of fucose (82 %), galactose (14 %), and small amounts of xylose and mannose Sulfate ester content: 92 %	Brown algae, Ecklonia cava	Antiproliferative activity, anticancer activity		
	Fucose, galactose. Sulfations occur on position-2 and-3. Sulfate ester content: 41–92 %	Brown algae, Ecklonia cava	Anti-inflammatory activity	Algal fucoidan	Cytotoxic activity, anticancer

Red algae sulfated polysaccharides (porphyran)	Backbone of alternating β-(1-3)- linked D-galactosyl units and α- (1-4)-linked L-galactosyl,(1-6)- sulfate or3,6-anhydro-α-L-galactosyl units. Sulfate ester content: 17 %	Red algae, Porphyra haitanensis	Antioxidant activities, anticoagulant activities	Agarose & porphyran	Cytotoxic activity Antibacterial activity Drug delivery
Shrimp and crab	Biopolymer family of lin-ear polysaccharide that is composed of glucosamine and N-acetyl glucosamine units via ˇ-(1 → 4) linkages randomly or block-spread throughout the polymer chain, depending on the extraction pro-cedures to derive CS from chitin	Shrimp and crab	Gene delivery, Antimicrobial activity, Tissue engineering, Anticancer activity, Wound dressing	Chitosan	Drug delivery
	Water-soluble anionic derivative of chitin containing carboxyl groups		Wound dressing, Antibacterial activity, Drug delivery	Chitin (Carboxymethyl chitin)	Drug delivery
	COS, a low molecular weight depolymerization product of CS,		Anticancer	Chitooligosaccharide	Drug delivery

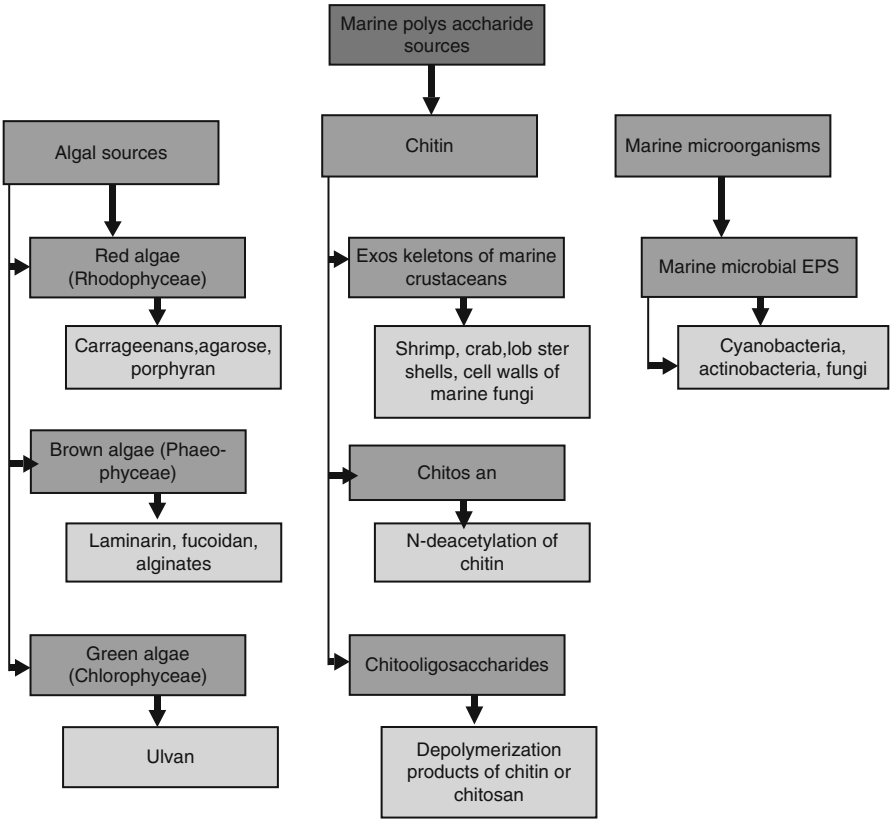


Fig. 5.2 Marine based polysaccharides and its sources

5.2.1 Marine Algae Based Polysaccharides

Marine algae polysaccharides especially sulfated polysaccharides received a greater importance as natural resources of marine natural products. Owing to the potential properties of seaweed and other marine sources derived polysaccharides they have received considerable attention in the cosmeceutical, nutraceutical, and pharmaceutical fields [13]. Marine algae chiefly classified in to three: green algae (Chlorophyceae), brown algae (Phaeo-phyceae) and red algae (Rhodophyceae) (Fig. 5.2).

From the research and commercial point of view there are some prominent sources (such as carrageenans, agarose, and porphyran) of sulfated polysaccharides extracted from red seaweeds [14], ulvan is a natural polysaccharide isolated from green algae [15] and laminarin, fucoidan, and alginates are chiefly derived from marine brown algae.

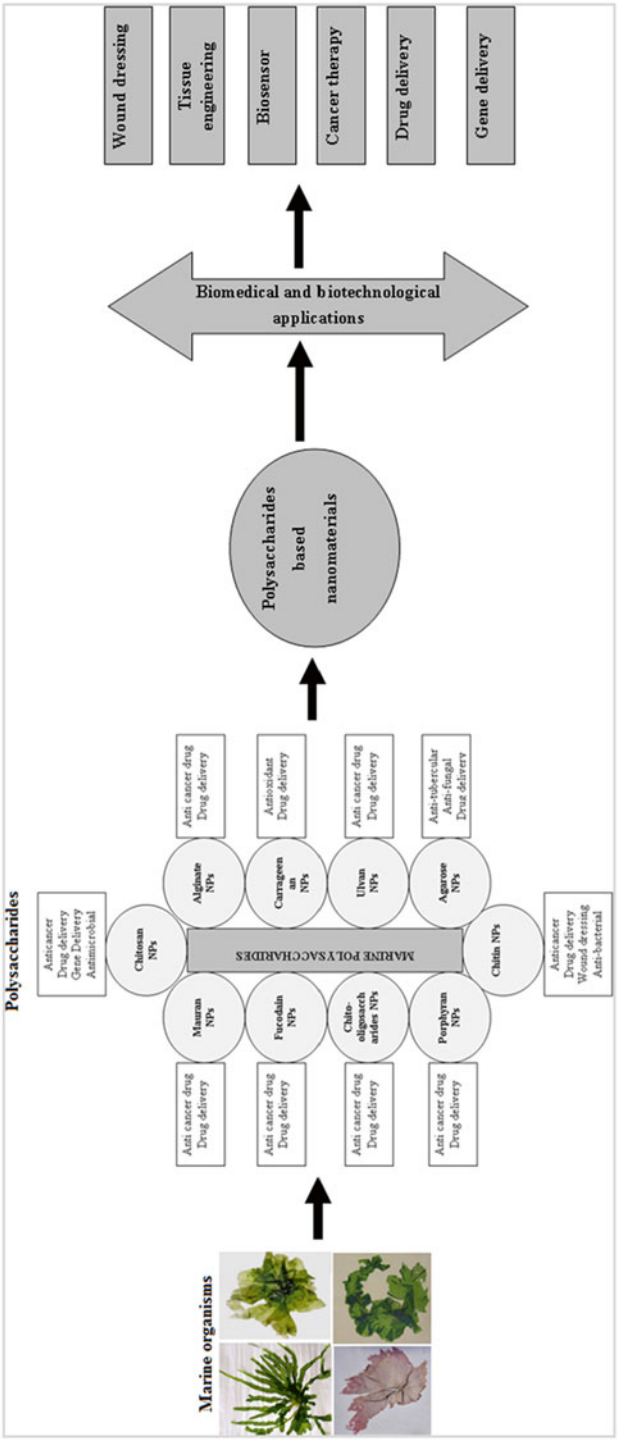


Fig. 5.3 Various types of sulfated polysaccharides and their applications in nanobiotechnology

5.2.2 Marine Crustaceans Derived Polysaccharides

5.2.2.1 Chitin

Among all the polysaccharides chitin is reported as one of the most abundant natural polymers and chiefly found in the exoskeletons of marine crustaceans and cell walls of marine fungi [5]. Shrimp, crab, and lobster shells are the major sources chitin and form the existing waste products of the seafood industry. According to present research this polymers can be easily modified in to chitosan and other forms whereas both unmodified as well as modified forms can be easily processed into microparticles, nanoparticles, nanofibers, scaffolds, sponge forms, beads, gels, and membranes. There maximum utilization in biomedical field is based on excellent low toxicity, high biodegradability and biocompatibility [16]. Such properties endow various biomedical applications such as wound dressing, targeted drug delivery, tissue engineering and gene delivery, and offer significant applications in nanotechnology [17].

5.2.2.2 Chitosan and Chitooligosaccharides

Modification of chitin helps in improving its various properties such as improvement in molecular weight, biocompatibility and toxicity profile. Chitosan, a naturally occurring polysaccharides isolated by the N-deacetylation of chitin has now become the most highlighted polymer in chemical, nutraceutical and pharmaceutical industries [18]. One more modification which has been now highlighted for its excellent properties is chitooligosaccharides obtained by the depolymerization products of chitin or chitosan by enzymatic and acidic hydrolysis methods. These methods significantly affect the molecular weight and ease of control and safety. Additionally such modification methods impart excellent properties such as high water solubility nonotoxicty, good biocompatibility, excellent biodegradability and low cost [19]. Chitosan and chitooligosaccharides are now recently considered for its great promising application in biomedical science, such as hypocholesterolemic effects [20], wound healing [21], drug delivery [22], tissue engineering [23], antitumor effects [24], and antimicrobial activity [25].

5.2.2.3 Marine Microorganisms

Among the potential class of marine based polysaccharides microbial polysaccharides especially

EPS are abundantly present in various marine sources such as fungi [26], bacteria [27], actinobacteria [28], and cyanobacteria [29]. Currently, EPS presents various interesting applications in cosmeceutical, nutraceutical and pharmaceutical industries. Additionally EPS also plays an important role in wastewater treatment and detergent applications [30]. All marine based microbial polysaccharide offers an increasing attention for biological activities such as antitumor, antiviral, anti-

inflammatory properties [31]. According to previous reports extremophilic bacterial polysaccharide, mauran (MR), explored as novel biocompatible and stable biomaterial and therefore becomes more favorable for its utilization in nanotechnology, pharmaceutics and biomedical field [1]. Previous findings also suggested the role of Streptomyces sp. based polysaccharides in the production of polysaccharide-based bioflocculant for the green synthesis of silver nanoparticles [32]. These NPs can be treated as choice for the advancement in novel bactericidal bio-nanomaterials especially for several biotechnological applications and wastewater treatment.

5.3 Nanomaterials Derived from Marine Sources

Owing to high biodegradability, good biocompatibility, nontoxic nature, low cost and other features, marine polysaccharide-based nanomaterials are considered as most suitable novel carriers in nanotechnology science [33]. Because of their unique physicochemical properties these polysaccharides have attracted considerable attention for imaging and therapeutic agents. Some special features such as its abundance, hydrophilic, biocompatible, biodegradable, inexpensive, nontoxic, safe, hydrophilic and biocompatible nature they are of particular importance in the area of nanotechnology and have a promising future as biomaterials. Recent research on polysaccharides based nanomaterials offers various biomedical application such as drug delivery, antimicrobial activity, tissue engineering, gene delivery, cancer therapy, and wound dressing [34–36].

5.3.1 Nano Scaffolds Derived from Fucoidan

Brown seaweeds derived sulfated polysaccharide known as Fucoidan (1) is an excellent drug candidate for pharmaceutical applications. Similarly fucan sulfates were obtained from marine invertebrates are having excellent pharmaceutical applications [37]. Fucoidan in the presence of formamide, pyridine and acetic anhydride yields acetylated nanoparticles. Whole process is conducted at room temperature for 24 h (Fig. 5.4, Table 5.3). Fucoidan is considered as an excellent candidate for various biological applications such antiproliferative properties, immunomodulating properties [47], anticoagulant [48], antiviral [47], antiangiogenic, antitumor, anti-inflammatory, [49], antioxidant [50]. Previous findings suggested that compounds such as fucoidans are now considered as the novel bioactive agents especially for nanotechnology and biomedical applications [38]. Recent research has investigated the role of fucoidans in the biosynthesis of metalnanoparticles, cancer treatment and drug delivery. Synthesis and characterization of fucoidan-coated poly (isobutylcyanoacrylate) nanoparticles was reported by Lia et al. [51]. Polymerization and redox radical emulsion polymerization were used to prepare of isobutylcyanoacrylate using fucoidan as a novel coating biomaterial. These nanoparticles exhibit potential in vitro cytotoxic effect against different fibroblast cell lines. Nanoparticles

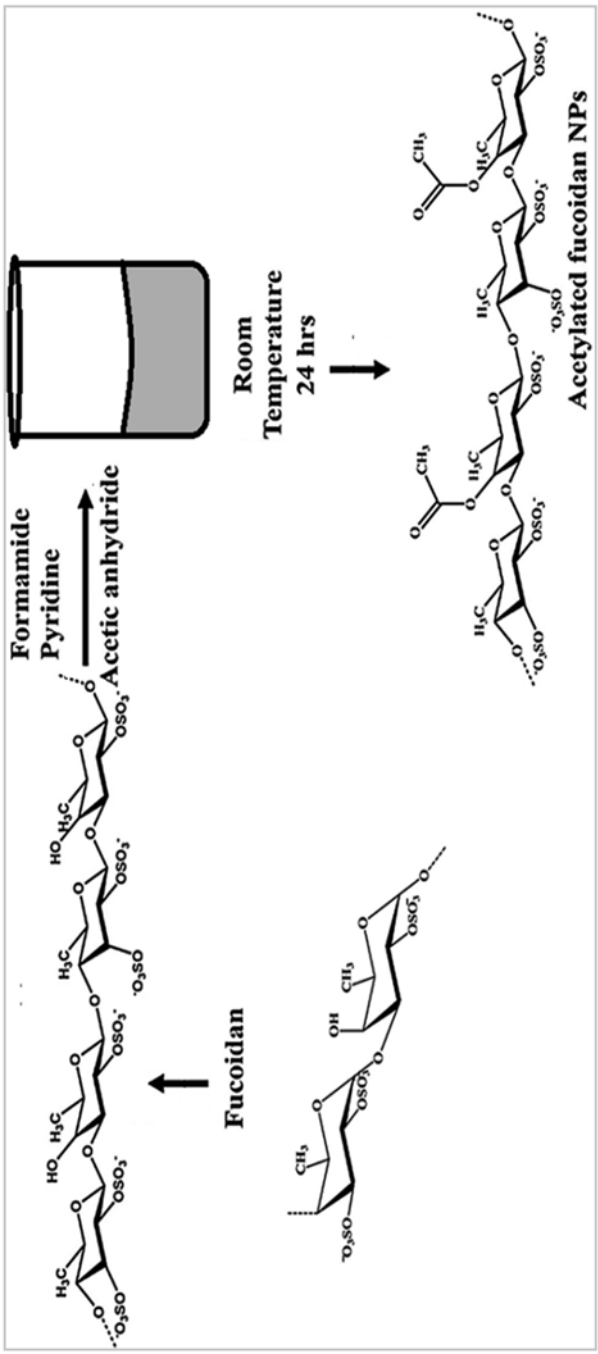


Fig. 5.4 Synthesis of acetylated fucoidan NPs from natural fucoidan

Table 5.3 Various advancement and applications of fucodain in nanoparicle drug delivery [38–46]

Polysaccharides	Modifications	Nanoaprticles	Drug delivery applications
Fucodain	Oversulphated, acetylated and benzoylated fucoidan Phosphorylated and aminated derivatives offucoidan Radical degradation Polyethylene terephthalate (PET) polymer surface attachment Fucosidase,enzyme degradation and microvate associated degradation Degraded fucoidans were coupled with several hydrophobic groups (oligofucose-dodecylaniline combination)	Cytotoxicity and fucoidan-coated nanoparticles Stromal cell-derived factor-1 released from chitosan/ tripolyphosphate/ fucoidan nanoparticles Carboxymethylated-curdlan and fucoidan Green synthesis of silver nanoparticles Chitosan/Fucoidan pH Sensitive Nanoparticles Fucoidan-Stabilized Gold Nanoparticles	Suppressive effect of fucoidan on an attachment of Cryptosporidium parvum Fucoidan cures infection with both antimony-susceptible and -resistant strains of Leishmania donovani Growth-inhibitory effect of a fucoidan On Plasmodium parasites Asexual growth inhibiton of Babesia bovis

prepared by anionic emulsion polymerization nanoparticles showed IC_{50} at 2 g/mL. As far as the sources are concerned fucoidans are derived from various sources two of marine algae such as *Cladosiphon okamuranus* and Kjellamaniellacrassifolia. Recent research showed its potential application in green synthesis of gold nanoparticles [52]. Previous findings on production of silver nanoparticles using carboxymethylated curdlan or fucoidan as reducing and stabilizing agents has opened gateway for the synthesis of metallic nanoparticles [39]. C. okamuranus derived fucoidan encapsulated in nanoparticles using liposomes as nanocarriers showed potential in vitro anticancer activity against osteosarcoma [53]. It was observed that hydrophobically modified fucoidan (synthesized by the acetylation of fucoidan) was required to prepare the chemotherapeutic agent loaded nanoparticles e.g. acetylated fucoidan nanoparticles was used to encapsulate Doxorubicin.

5.3.2 Alginate Nanoparticles

Owing to excellent properties such as low cost, low toxicity, biocompatibility and mild gelation, alginate 'a natural polysaccharide' derived from brown seaweeds has been widely investigated and used for biomedical applications [54]. Previous findings have explored various functionalization and modification steps for alginate to yield modified alginate with improved physic-chemical and biological properties (Fig. 5.5). Current research on alginate-based nanoparticles offers various

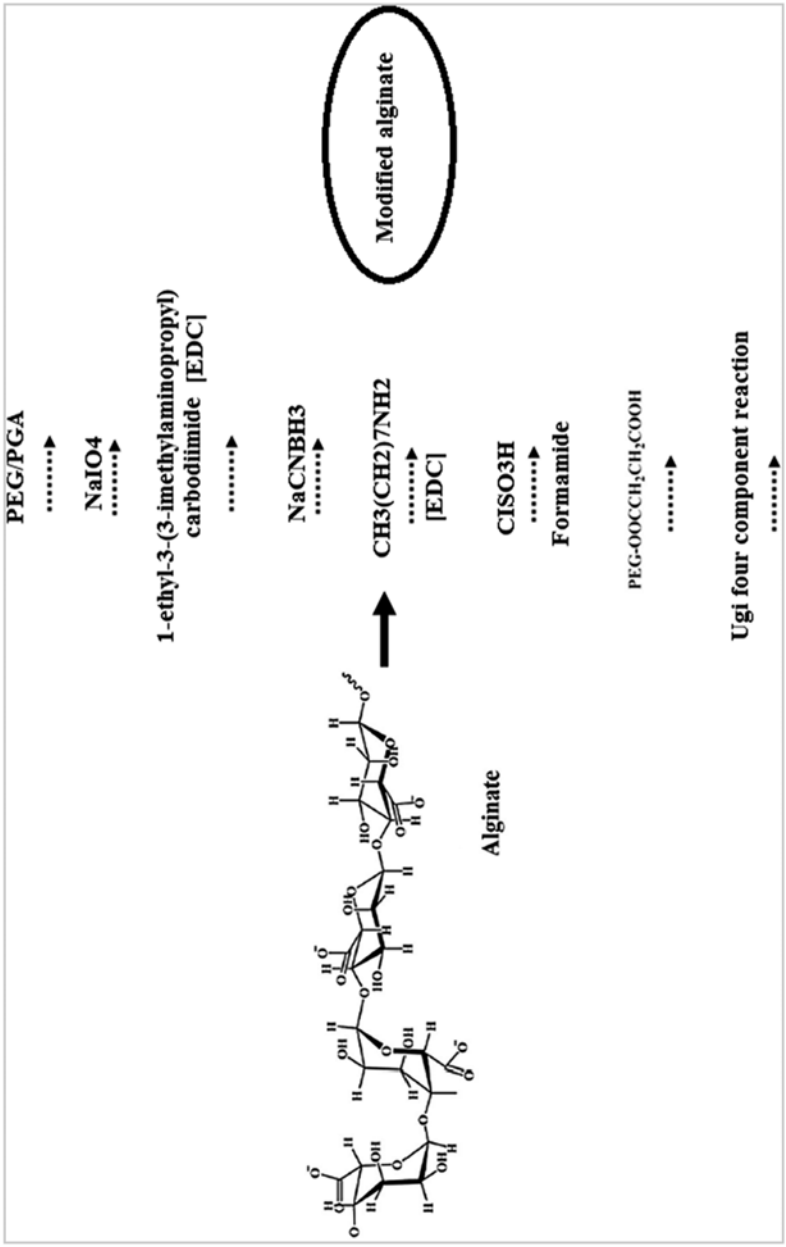


Fig. 5.5 Various functionalization and modification steps recently explored to yield modified alginate

applications in insulin delivery [55] and antifungal and antitubercular drugs [56]. Alginate based nanoparticles have raised greater interest in the medical field e.g. [55] insulin-loaded nanoparticles using alginate ionotropic pre-gelation followed by CS polyelectrolyte complexation. This has proven the elastic nature of alginate to hold and shield drug by polyelectrolyte complexation with chitosan. During this study it was observed that insulin-loaded nanoparticles using alginate showed loading capacity of 14.3 %. Similarly loading of insulin in alginate–dextran nanospheres through nanoemulsion dispersion resulted in increase in encapsulation efficiency up to 82.5 %. Various methods like irradiation method was also reported for the preparation of gold nanoparticles using alginate as a stabilizer. Utilization of effective methods such as irradiation method for the preparation of gold nanoparticles using alginate as astabilizer was also reported. During this study it was observed that irradiation technique is suitable for the production of alginate-stabilized gold nanoparticles with controllable size and high purity. This technique yields alginate-stabilized gold nanoparticles which are spherical in nature having particle size ranging from 5 to 40 nm [57]. Recently hydrothermal synthesis of silver nanoparticles using sodium alginate as a reducing and stabilizing agent was studied by Yang and Pan [58]. During this study it was observed that incubation time and temperature of the reaction played an important role under suitable effective sodium alginate concentrations and Ag^{+} precursor in the development of silver nanoparticles with desirable shapes. So generally triggering the temperature and incubation time of reaction were favorable for the formation of nanoplates whereas low temperature and short incubation time of reaction were shown to result in the formation of nanospheres. In one report the role of alginate in DOX-loaded glycyrrhetinic acid-modified alginate nanoparticles was suggested by determining liver-targeting efficiency, and antitumor activity. It was observed that nanoparticles showed strong liver-targeting efficiency, reduced cardiac toxicity and improved antitumor activity of DOX against liver tumors [59, 60]. One of the major causes of therapeutic failure of antituberculosis medicines is patient non-compliance. This happens due to the multidrug administration for at least 6 months. Delivery systems such as nano formulations are more suitable for co-bacterial infections (such as tuberculosis) [61]. An alginate-encapsulated anti-tubercular drugs such as isoniazid, rifampicin, pyrazinamide, and ethambutol was studied by Ahmad et al. [62]. During their oral administered to mice it was observed that all the encapsulated drugs in nano forms showed better antitubercular effects. Additionally these nanoparticles showed high encapsulation efficiency with average particle size. Various nanoparticle applications and its recent modification/functionalization of alginate are mentioned in Table 5.4.

5.3.3 Carrageenan Based Nanoparticles

Red algae such as Kappaphycus sp. and Eucheuma sp are the chief source of carrageenan. This natural polymer is having D-galactose and anhydro-galactose units joined by glycosidic linkages and ester sulfate groups. Carrageenan on the basis of

Table 5.4 Alginate modifications and its recent nanparticulate forms [57, 63–124]

Polymer	Modification	Nano applications	Drug delivery applications
Aliginate	• Modification of hydroxyl groups of alginate include oxidation, reductive-amination, sulfation, copolymerization and coupling of cyclodextrin units • Methods used for modification of carboxyl groups include esterification, use of the Ugi reaction, and amidation. • Surface modification of sphalerite with sodium alginate • Degradation by epimerases • Ionotropic gelation of sodium alginate using N,N′-methylenebisacrylamide and carboxy-methylcellulose • Covalent modification of alginate with polyethylene glycol–conjugated anthracene molecules has the potential to both stabilize the alginate and act as a photosensitive crosslinker • Chemoselective cross-linking of alginate with thiol-terminated peptides for tissue engineering applications • Modification of alginate through partial crosslinking with a matrix metalloproteinase (MMP) • By simple covalent modifications • Novel crosslinking reagent (alginate dialdehyde) for biological tissue fixation • Thermo-sensitive alginate-based injectable hydrogel for tissue engineering • Alginate dialdehyde for biological tissue fixation • Modification of alginate by grafting of N-vinyl-2-pyrrolidone • The effects of peptide-based modification of alginate • Modification of alginate through the grafting of 2-acrylamidoglycolic acid • Effect of gamma radiation on the physico-chemical properties of alginate • Hydrophobically modified alginate • Modification of alginate degradation properties using orthosilicic acid • Calcium alginate • Sodium alginate sulfates • Modification of PVA–alginate as a suitable immobilization matrix	• Alginate/Chitosan nanoparticles • Insulin-loaded alginate nanoparticles • Chitosan/alginate nanoparticles encapsulating antisense oligonucleotides • Alginate–AgNPs composite sponge • Paraquat-loaded alginate/chitosan nanoparticles • Chitosan or N-trimethyl chitosan and a cisplatin–alginate complex NPs • Superparamagnetic iron oxide nanoparticles stabilized by alginate • Thiolated alginate-albumin nanoparticles • Silica/alginate nanoparticles as Hybrid Magnetic Carriers • Magnetite nanoparticles, • Alginate–quaternary ammonium NPs • Alginate stabilized gold nanoparticles, • Silica/alginate nanoparticles as Hybrid Magnetic Carriers • Lipid nanoparticles into calcium alginate beads • Chitosan–sodium alginate microcapsules containing ZnS nanoparticles • Maghemite nanoparticles for Pb(II) removal in aqueous solution • Inhalable alginate nanoparticles • Superparamagnetic iron oxide nanoparticles stabilized by alginate • Layer by layer chitosan/alginate coatings on poly(lactide-co-glycolide) nanoparticles • Barium alginate caged Fe3O4@C18 magnetic nanoparticles • Alginate/Fe@Fe3O4 core/shell structured nanoparticles • Multifunctional alginate microspheres • Curcumin in alginate-chitosan-pluronic composite nanoparticles	Aliginate based leishmani vaccine Alginate-Capped Amphotericin B Lipid Nanoconstructs Against Visceral Leishmaniasis Acyclovir-loaded alginate mucoadhesive microspheres

extraction procedures and resources is further classified in to three types kappa, iota, and lambda (Fig. 5.6). Major difference between these types is that they all having sulphur group which is differing in the substitution degree. From gelling point of view, kappa and iota show high gelling efficiency whereas delta carrageenan is a non gelling polysaccharide [125]. Among these kappa carrageenan is rigid and firm whereas iota carrageenan elastic and soft in nature [126]. Using these all types Daniel-da-Silva et al. [127] studied the biosynthesis of magnetite nanoparticles and examined for their particle size morphology and chemical stability. Carrageenan has various applications in nanotechnological, biological and pharmaceutical field (Table 5.5). Additionally carrageenan also exhibit various food and non-food applications. Six types of sulfated polysaccharides from marine brown and red seaweeds was isolated and investigated for their respective antioxidant activities by DeSouza et al. [128]. It was observed that carrageenan and fucoidan exhibit strong antioxidant activity. In an another study inhibitory effects of delta carrageenan and a mixture of sulfated polysaccharides derived from red seaweeds against feline herpesvirus-1 under in-vitro was investigated [129]. Delta-carrageenan showed IC_{50} 5 μg/mL against feline herpes virus -1. Carrageenan and chitosan nanoparticles were investigated by Grenha et al. [130]. These nanoparticles were produced by hydrophilic conditions using very mild protocol and preventing the use of organic solvents and other intensive chemical conditions. This protocol yields suitable nanoparticles that can present sustained and controlled form of drug delivery system and can be treated as excellent candidates for biomedical applications. Additionally it has been studied that these nanoparticles were proved for their low toxicity against fibroblast cell lines as well as superior biocompatibility and high

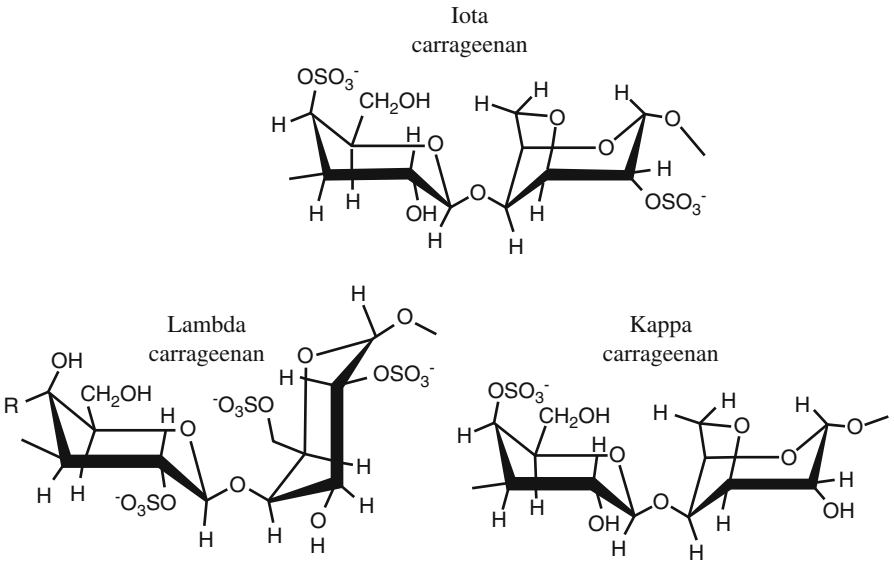


Fig. 5.6 Types of carrageenan

Table 5.5 Marine organisms derived sulfated polysaccharides with nanotechnological, biological and pharmaceutical applications

Type of SPS	Nanotechnology application	Biological and pharmaceutical applications
Carrageenans	Capping agent for biomineralizing metal oxides Composite gels Composite nanoparticles Drug encapsulation and delivery Gelling agent Hydrogels Microbeads for controlled release Stabilizing agent Stabilizing micelles Thickening agent	Anti-coagulant activity Anti-oxidant activity Anti-viral activity Free radical scavenging activity Microbicidal activity Prevention of sexually transmitted diseases Vaginal gel formulation
Fucoidans	Stabilizing agent Fucospheres or microspheres	Anti-angiogenic activity Anti-coagulant activity Anti-inflammatory activity Anti-oxidant activity Anti-proliferative activity Anti-tumor activity Anti-viral activity Immunomodulating property Treating dermal burns in rabbit
Ulvan	Nanofibrous scaffolds	Resist attack of necrotic pathogens in plants Antifungal activity

safety. CS, carrageenan, and cross linking agent tripolyphosphate based nanoparticles was investigated by Rodrigues et al. [131] for their stability smaller size and strong positive surface. In this study prepared nanoparticles were used for purpose in mucosal delivery of macromolecules. Metallic nanoparticles for gastrointestinal release using modified kappa-carrageenan was investigated to study the effect of genipin cross-linking and it was proved that metallic nanoparticles seem significantly improve gastrointestinaltract-controlled drug delivery [132]. Salgueiro et al. [133] studied the influence of introducing spherical and rod-shaped gold nanoparticles in the microstructure and thermomechanical properties of delta-carrageenan hydrogels. Moreover he has also investigated the effect of these nanoparticles in the release kinetics and mechanism of methylene blue from kappa-carrageenan nanocomposites. It was observed that hydrogel nanocomposites demonstrated enhanced viscoelastic properties in contrast with neat kappa-carrageenan, at the time they used with either with gold nanospheres and gold nanorods.

5.3.4 Agarose Nanoparticles

3,6 anhydro galactose based natural polymer known as Agarose (Fig. 5.7) derived from red seaweeds, *Gracilaria sp*. and *Gelidium sp*. This 3,6 anhydro galactose based natural polymer is a linear polysaccharide made up of repeating units of

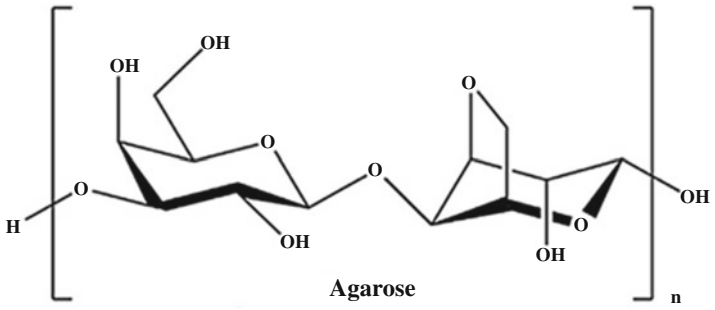


Fig. 5.7 Structure of agarose

agarobiose, which is a disaccharide made up of D-galactose. Agarose is having various applications in biotechnology biochemistry and molecular biology field especially in different types of electrophoresis techniques (for the separation of nucleic acids). This red seaweed derived polysaccharide usually used for its gel-forming property to create semiconductor and metal nanoparticles. It was observed that this type of nanoparticle exhibit strong antibacterial activity against *Escherichia coli*. It has been also discovered that the agarose composite films can be rapidly transformed to carbon metal composites by carbonizing the films in nitrogen atmosphere [134]. Previous finding suggested the application of agarose-stabilized gold nanoparticles for the detection of micromolar concentrations of DNA nucleosides via surface-enhanced Raman spectroscopic detection [135]. Results suggested that agarose-stabilized goldnanoparticles yield higher surface-enhanced Raman spectroscopic detection for DNA nucleosides, which is used for on-chip biosensing applications.

5.3.5 Porphyran Based Nanoparticles

Porphyran (Fig. 5.8) 3,6-anhydro galactose is a natural sulphated polysaccharides obtained from marine red seaweed, *Porphyra vietnamensis* [136]. Porphyran is a hot-water-soluble fraction of the cell wall having the similar characteristics like agar. It is the major constituent (40–50 %) of the marine *P. vietnamensis* and has nutritional value. It's a anionic disaccharide units consisting of 3-linked D-galactosyl residues alternating with 4-linked 3,6-anhydro-L-galactose and 6-sulfate residues. Porphyran can be extracted from various species of *Porphyra* and having some important pharmaceutical properties reported in various structural and functional studies.

Bhatia et al 2009 described biological properties emphasizing the role of porphyran in pharmaceutical world [137–143]. Bhatia et al (2010) demonstrated the structure based gelling and emulsifying properties of porphyran. Molecular weight based antioxidant property of porphyran was described by Bhatia et al. (2011) [137–143]. Further molecular weight dependent potential immunomodalation

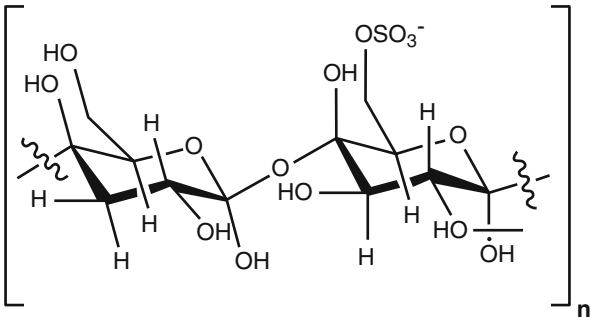


Fig. 5.8 Structure of Porphyran

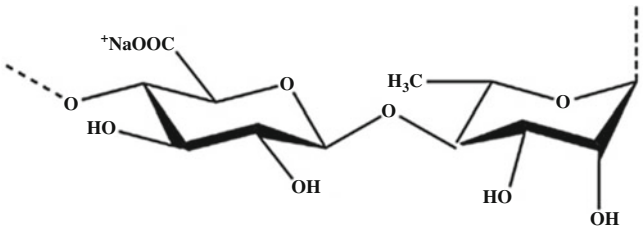
effect of porphyran was investigated by Bhatia et al. (2013). Porphyran molecular weight was modified and immunomodulation effects of modified and natural sample were investigated [137–143]. It was observed that alkali treated sample showed better immunomdulation effects then natural one. Bhatia et al. (2013) has examined the role of porphyran in development of oral amphotericin B loaded nanoparticles to reduce its toxicity and other associated problems [137–143]. In this study Amphotericin B was packed between two oppositely charged ions (chitosan and porphyran) by polyelectrolyte complexation technique with TPP as a crosslinking agent. Formulation was optimized using three-factor three-level (33) central composite design. High concentration of POR in NPs was confirmed by sulfated polysaccharide assay [137–143]. Degradation and dissolution studies suggested the stability of NPs over wide pH range. Hemolytic toxicity data suggested the safety of prepared formulation. In vivo and in vitro antifungal activity data suggested the high antifungal potential of optimized formulation when compared with standard drug and marketed formulations. Hence, these experimental oral NPs may represent an interesting carrier system for the delivery of AmB. Bhatia et al. (2015) investigated the factors influencing the molecular weight of porphyran and its associated antifungal activity. During this study various extraction methodologies have been employed to derive porphyran from high tide and low tide samples of *P. vietnamensis*. Results suggested that *P. vietnamensis* collected during low tide yields high percentage of porphyran with relatively low molecular weight and high sulfate content than the high tide sample. Among various extraction methodologies alkali modified POR yield slow molecular weight polysaccharide but surprisingly with high sulfate content which have shown improved physico-chemical and antifungal properties than chitosan without any toxicological affects. Bhatia et al. (2015) has established the relationship between structural features and pharmaceutical properties of porphyran [137–143]. This polysaccharide exhibit molecular weight dependent activity as highlighted in reports on the anticancer and antioxidant activities of porphyran [144]. Biosynthesis of gold nanoparticles using a porphyran and subsequent loading of Doxorubicin was investigated [145]. Toxicological data of porphyran-reduced gold nanoparticles was performed on normal monkey kidney cell line, which showed a non-toxic nature of nanoparticles [146].

5.3.6 *Nanofibers of Ulvan*

Ulvan (Fig. 5.9) is obtained from the cell walls of marine green algae (Ulvales, Chloro-phyta). It's a complex anionic sulfated polysaccharide contains sulfated, xylose, rhamnose, glucuronic, and iduronicacids. Ulvan is abundantly present in green algae especially Ulva rigida and have a low cost of production. These polysaccharides are still under-exploited and have been investigated as an antitumor, anticoagulant, antioxidant, and immune modulator [15]. Current utilization of ulvan in nanotechnology is especially towards preparation of nanofibers with the special interest in the biomedical engineering field because of their potential applications in tissue engineering, drug delivery and wound dressing. Due to some physicochemical and biological properties ulvan becomes good candidate for nanofiber production and has been successfully explored into nanobiotechnology for presenting novel promising biomaterials in biomedical applications, including drug delivery systems, wound dressing, and tissue engineering. Earlier report suggested that spinnability of *U. rigida* based polysaccharide can be used for the fabrication of nanofibers which imply that spinnability plays an important role in improving the properties of ulvan [147].

5.3.7 *Mauran Based Nanoparticles*

Just like other sulphated polysaccharides, mauran is *Halomonas maura* (halophilic bacterium) derived sulfated polysaccharide with high sulfate, phosphate, and uronic acid content. Moreover it has been also reported that mauran constitute mannose, glucose, galactose, and glucuronic acid. Recent research has explored the utilization of mauran for the biosynthesis of metal nanoparticles and their well known viscoelastic properties. Previous finding suggested the role of sulfated polysaccharide-based nanoparticles as a good biocompatible material for bioimaging, drug delivery and anticancer activity [1]. Additionally thixotropic and pseudoplastic properties of mauran make it a supreme molecule for material science applications [148].



Ulvan

Fig. 5.9 Structure of Ulvan

5.3.8 Chitin and Its Nanoparticles

Chitin (Fig. 5.10) is one of the abundantly present biopolymer in nature [149], isolated from the various marine sources crab, shrimp, and lobster shells and their by-product in the seafood industry. Million tons of chitin per annum generated as waste by the seafood industry [150]. There are several methods involved in the production of chitin such as enzymatic methods, hydrolytic methods using boiling HCl and methods applied using chitin whiskers. Various applications of chitin in nanoscience are mentioned in Table 5.6.

5.3.9 Chitosan Based Nanoparticles

Chitosan is a naturally occurring linear polysaccharide which is composed of glucosamine and N-acetylglucosamine units via β-(1 → 4) linkages. These linkages are randomly or block-spread all over the polymer chain. Arrangement or distribution of these linkages is dependent on the extraction procedures to derive chitosan from chitin. Degree of deacetylation is known as the parameter that define molar ratio of glucosamine to N-acetyl glucosamine. Degree of deacetylation significantly determines the physicochemical properties and industrial applications of chitosan [126]. Once the chitosan get deactylated it can be easily dissolved in an acidic medium and develop into the only sulfated polysaccharide that possesses a

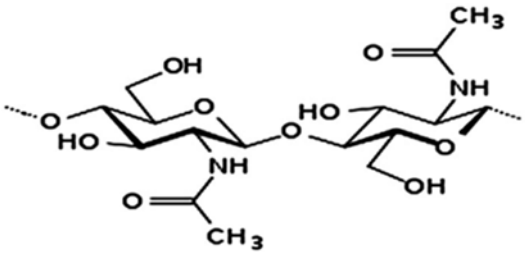


Fig. 5.10 Structure features of chitin and chitosan

Table 5.6 Chitin and its nano-applications

Type of chitin	Method	NPs	Applications	Uses/benefits	References
Chitin	Ion cross linking agent	Chitin NPs	Reinforcing fillers in polymeric matrices		[151]
Carboxy-methyl chitin	–	CMC NPs	Wound-healing dressings, exhibits good bio-compatibility	Nontoxic, water-soluble anionic derivative	[152]
Carboxymethyl chitin	Cross-linking approach with $FeCl_3$ & $CaCl_2$	CMC NPs	Controlled drug delivery applications, in vitro cyto-toxicactivity against mouse L929 cell lines, NPs showed strong antibacterial activity against Staphylococcus.	Nontoxic, water-soluble anionic derivative	[153]
Amorphous chitin	Ionic cross-linking approach using pentasodium tripolyphosphate	Paclitaxel loaded amorphous chitin nanoparticles	For colon cancer drug delivery	Hemo-compatible & in vitro drug release exhibited a sustained release, enhanced efficacy	[154]
Amorphous chitin	Ionic cross-linking	Rifampicin-loaded amorphous chitin	Intracellular delivery of rifampicin inside polymorphonuclear leukocytes, anti-bacterial activity	Sustained drug delivery could reduce dosing frequency, lower toxicity, enable long-term treatment, & prevent potential side effects related to the free drug	[155]

high density of positive charges. This positive charge is due to the protonation of amino groups on its backbone. In addition to its unique features chitosan has been reported to have various other essential properties such as good biocompatibility and biodegradability and non-toxicity [156]. There are different protocol reported for the preparation of various derivatives of chitosan (Figs. 5.11 and 5.12). Currently chitosan has offered significant applications (Table 5.7) in nanotechnological area especially in biomedical sector such as drug delivery [168], nutrition [169], and tissue engineering [170].

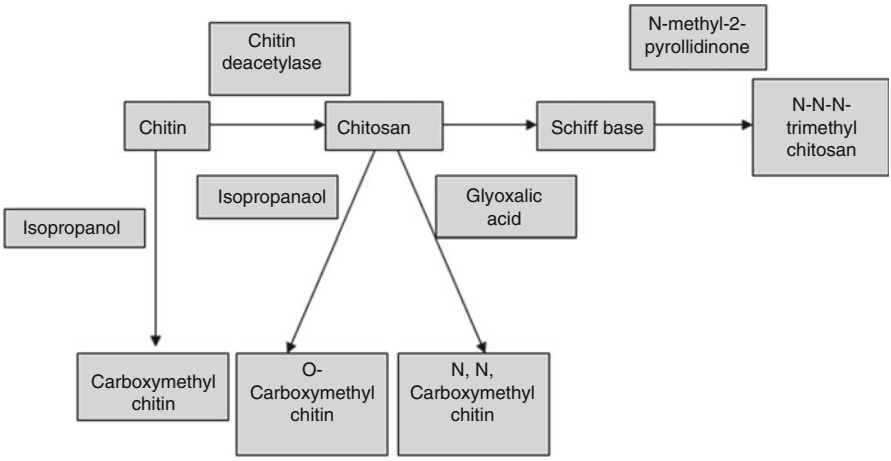


Fig. 5.11 Steps involved in the chemical modification of chitin/chitosan

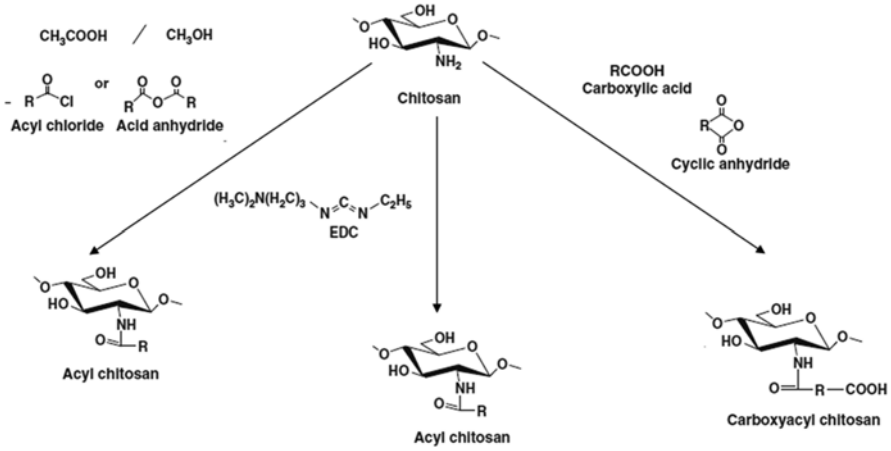


Fig. 5.12 Protocol for the preparation of various chitosan derivatives

Table 5.7 Applications of chitosan nanoparticles

NPs type	Method	Model drug	Application	References
CS- and CS-gold nanoconjugates	Ionic gelation method	Salmon leutinizing hormone releasing hormone	Showed considerable reproductive efficacy in female fish (Cyprinus carpio)	[157]
CS-dextran sulfate NPs	Ionic gelation method	Doxorubicin	Anticancer activity (increase cytotoxicity activity)	[158]
Dextran-DOX encapsulated in CS nanoparticles	Reverse micro-emulsion	Doxorubicin	Improved antitumor activity in murine tumor models	[159]
CS NPs		Immuno-suppressive peptide cyclosporin A	Targeted delivery of drugs to the ocular mucosa,	[160]
CS NPs cross-linked- glutaraldehyde	AOT/n hexane reversemicellar system	–	Particle size of CS NPs was mainly influenced by the degree of cross-linking	[161]
CS NPs Cu-loaded CS NPs		Copper	Anti-antibacterial activity against pathogenic microorganisms	[162]
CS–TPP-NPs	polyanion-initiated elation	gene or protein macromolecules	Delivery of gene or protein macromolecules	[163]
CS NPs	Ionic gelation method	siRNA	Good vehicles for siRNA delivery	[164]
CS–TPP-NPs	Ionic gelation method	siRNA	CS–TPP-NPs proved as good vehicles for siRNA delivery then CS NPs	[165]
Hydrophobically modified glycol chitosan NPs		Camptothecin	Substantial enhancement of antitumor activity	[165]
CS and silver-loaded CS NPs		Silver	Antimicrobial textile applications, silver-loaded CS NPs exhibited synergistic antibacterial activity	[166]
CS NPs	Blank		Hemolytic activity	[167]

Table 5.8 Various applications of COS nanoparticles

Nanoparticles (NPs)	Applications/advantages	References
Hydrophobically modified amphiphilic COS derivatives for self-assembled polymeric NPs	Drug delivery in cancer therapy by improving the solubility of insoluble drugs, drug targeting, and absorption	[172]
Iron oxide loaded magnetic COS NPs	Colloidally stable in water and buffer & attractive for biomedical applications	[173]
CS oligosaccharide-stabilized ferri-magnetic iron oxide nanocubes	Efficient heat nano-mediator for cancer hyperthermia & exhibited strong antitumor activity without toxicity.	[174]
Multidentate dithiolanelipoic acid and phosphorylcholine conjugated CS oligosaccharide derivative to stabilize gold NPs	Used for bio-logical various applications	[175]
COS NPs polyelectrolyte complexes	Showed strong inhibition of the proliferation of HeLa & B16 melanoma cells.	[176]
COS-based multidentate NPs	Ultrastable, cytotoxicity & biocompatible	[177]

5.3.10 Chitooligosaccharide Based Nanoparticles

Chitooligosaccharide is low molecular weight polymer obtained by depolymerization of CS and offers various superior features such as biocompatiblity, water-solublity, biodegradablity and nontoxicity in nature. Chitosan has various applications in biomedical and pharmaceutical sectors and also exhibit unique biological activities such as immune-enhancing, antimicrobial, and antitumor activities. Recently this oligosaccharide is exploited for its polymer-drug conjugate applications. This is because of its accessibility for coupling with the primary amino groups and hydroxyl groups of each polymer subunit. Further the cationic nature of COS allows ionic crosslinking [171]. Different applications of COS are mentioned in Table 5.8.

5.4 Marine Polysaccharide-Based Nanomaterials and Its Biomedical and Biotechnological Applications

Marine polysaccharides especially algal polysaccharides based nanomaterials are considered as nanomedicine offering high possibilities for diagnosis and therapeutical applications. Current researches on these nanomaterails have attracted attention of all the researchers in the field of biotechnological and biomedical science [178]. Current researchers are working on the structural features of these polysaccharides to synthesize more potential derivatives that are suitable for various applications. Recent innovations in polymeric sciences lead to the production of potential lower molecular weight oligosaccharide derivatives which have shown to possess a

variety of biomedical applications. We have traced some of the important nanobiotechnological applications of these biopolymer based nanomaterials in the field of antimicrobial activity, drug delivery, gene delivery, tissue engineering, cancer therapy, wound dressing biosensors, and water treatment.

5.4.1 Biomedical Applications of Marine Polysaccharides

5.4.1.1 Antimicrobial Activity

Marine organism has their potential antimicrobial activities since they live in such a dampish environment where moisture promotes the growth of microorganisms. Such type of organisms are usually found on intertidal zone. Antimicrobial therapy has been evolved in recent years with development of more resistance of pathogenic microorganisms against different types of antimicrobial agents. Prevailing resistance of many infectious microorganisms contributes a serious problem in clinical practice, therefore limits the development of novel drugs to fight against them [179]. Explorations of those substances which can prevent the development of resistant pathogenic species of microorganisms are more preferred nowadays. Potential effects of certain inorganic agents have been recently explored and it was found that these candidates can act effectively against resistant strains of microorganisms [180]. Among these candidates, silver compounds and their derivatives are extensively studied for antimicrobial activity. Moreover recent research is exploring these compounds and their derivatives in form of nanoparticles using marine polysaccharides as biopolymer, to enhance their antimicrobial potential more effectively [181, 182]. A report suggested the role of agar as biopolymer (derived from the red alga *Gracilaria dura*) in the synthesis of silver nanoparticles and nanocomposite material [182]. It was found that these silver loaded NPs showed potential antibacterial effect with 99.9 % reduction of bacteria over the control value. These types of nanocomposites may considered as effective antibacterial activity and may offer various applications in food preservation and wound dressing. Previous finding on green synthesis of silver nanoparticles using marine polysaccharide derived from red algae, *P. vietnamensis* suggested the dose-dependent effect of biosynthesized silver nanoparticles. This report has explored the effective anti-bacterial activity against Gram-negative bacteria compared with Gram-positive bacteria [183].

5.4.1.2 Marine Based Nanomaterials and Its Drug Delivery Applications

Development in nano-biotechnology allows the medicines to be administered in a more convenient and safe way. This can be achieved by the development of more efficient and advance drug delivery systems to diagnose, cure or to treat any disorder. Current research is more emphasizing on the safe and targeted delivery of many bioactive compounds for cancer treatment. Innovations in biomedical sciences are paying attention towards nanomaterials for reducing dosing frequency, their

Table 5.9 Applications of algal based NPs in drug delivery system (DDS)

Nanoparticles (NPs)	Applications in DDS	References
CS NPs	As novel drug delivery system for the ocular mucosa	[185]
CS NPs	Topical ocular route drug delivery system	[186]
DOX-loadedgold NPs using porphyran	Strong cytotoxicity on LN-229 cell line	[187]
Berberine-loaded CS/Fucodain–taurine complex NPs	Oral delivery of berberine (Ber).	[187]

toxicity, and avoiding potential side effects however they do not recognize that delivery systems themselves may impose risks to the patient [184]. Various applications of algal based polysaccharides are mentioned in Table 5.9.

5.4.1.3 Genetic Transformation

Developments in biotechnology endow different alternatives to treat the disease e.g. gene therapy can be utilized for correcting genetic disorders by use of genes itself. Genetic transformation can be successfully achieved by plasmid DNA. In this process plasmid DNA which carries gene of interest is introduced into the target cells. The introduced plasmid DNA should get transcribed and further the genetic information should finally be translated into the respective protein. There are number of obstacles to be overcome by the gene delivery device during this genetic transformation [171]. According to current research nucleic acids are being functionally utilized for both vaccination and therapeutic gene expression and chitosan nanoparticles have been suggested as promising non viral gene carriers. Chitosan-alginate NPs (core-shell structured) were fabricated using water-in-oil reverse microemulsion template and utilized to encapsulate a plasmid DNA for gene delivery through the cell endocytosis pathway [188]. Nevertheless it has been already reported that chitosan-DNA NPs can be easily fabricated by complex coacervation between the positively charged amine groups on CS and negatively charged phosphate groups on DNA [189]. Previous finding suggested that CS nanoparticles can potentially provide protection to encapsulated plasmid DNA from *nuclease* attack. This was established by evaluating degradation in the presence of *DNase* I. Further the incorporation of the plasmids with incubated nanoparticles was investigated by *galactosidase* assay. Plasmid DNA based model present as combination of both supercoiled and open circular forms. Utilization of si-RNA as a significant therapeutic agent for the management of several diseases is inadequate due to its rapid degradation and low intracellular organization in vitro and in vivo. Recent report suggested the role of chitosan–polyguluronate NPs in delivering siRNA to HEK 293 FTand HeLa cells [190]. To transport siRNA into cells, chitosan–polyguluronate NPs have a great promise and exhibit low cytotoxicity cells [190].

5.4.1.4 Algal Polymers and Its Applications in Tissue Engineering

Algal polymers has their various applications in tissue engineering e.g. in the development of bio-artificial implants and/or promote modification in tissues with the objective of repairing, maintaining, replacing, or enhancing tissue or organ function. With the aid of bio-artificial constructs consisting living cells and biomaterials tissue engineering offers various applications in science and technology. According to report investigated by Noh et al. [191], cytocompatibility of etecrospinned chitin nanofibers for tissue engineering applications was established by cell attachment and spreading of normal human keratinocytes and fibroblasts. Similarly carboxymethyl chitin (CMC)/poly (vinyl alcohol) (PVA) blend was prepared by using electrospinning technique [192]. During this study it was observed that CMC/PVA scaffold supports cell adhesion/attachment and proliferation, and therefore, these scaffolds are useful for tissue engineering applications. CS–gelatin/nanophase hydroxyapatite composite scaffolds was developed by CS and gelatin with nanophasehydroxyapatite [193], exhibited well swelling characteristic, which could be modified by altering the quantity of chitosan and gelatin. Nanocomposite scaffolds showed superior response on MG-63 cells in terms og improved cell attachment, higher proliferation, and spreading than CS–gelatin scaffold.

5.4.1.5 For Delivery of Anticancer Drugs

Cancer is a terrible human disease which when happens affects the immune system of whole body. One of the significant properties of nanomaterials is that they are highly preferable for parenteral injection of aqueous insoluble drugs. This property can be utilized for drug targeting applications because their particle sizes are less than 1000 nm. Potential anticancer drug like doxorubicin was incorporated in to nano-materials using methoxy poly (ethylene glycol)-grafted carboxymethyl chitosan nanoparticles to investigate antitumor activity. Possible interaction between these two ingredients was due to the presence of positive amine groups which lead to the formation of nanoparticles. These nanomaterials were tested against DOX-resistant C-6 glioma. It has been observed that these nanoparticles exhibited higher cytotoxicity to DOX alone [194]. In another finding it was reported that chitosan nanoparticles were utilized as carrier for the mitotic inhibitor paclitaxel. These nanoparticles were fabricated by a solvent evaporation and emulsification cross-linking method. It was observed that paclitaxel-loaded CS nanoparticles had higher cell toxicity than individual paclitaxel. Additionally confocal microscopy investigation confirmed strong cellular uptake efficiency [195]. Potential chemotherapeutic agent, doxorubicin was incorporated in to fucodain acetate to form nanoparticles. These nanoparticles were tested for immunotherapy and chemotherapy in cancer treatment. According to observation acetate nanoparticles showed important function in immunomodulation and drug efflux pump inhibition [196].

5.4.1.6 Treatment of Infection and Wounds

According to current research antibacterial therapy-resistant pathogens is the most critical setback that requires more development in antimicrobial therapeutic agents in form of their formulation, delivery and physico-chemical properties. Advancement in this field lead to the development of newly designed wound dressing which has offered a main step forward for the treatment of infection and wounds. Current science is focusing on various strategies to develop novel therapies antibacterial agents to treat wounds infected with antibacterial treatment-resistant pathogens. Among the metallic nanoparticles silver nanoparticles are now become more effective bactericidal agents therefore exhibit various biomedical applications ranging from silver-based dressing to silver-coated therapeutic devices [197]. Various investigations are available on the utilization of chitosan scaffolds and membranes to treat patients with deep burns, wounds, etc. Electrospunned collagen-chitosan complex nanofibers were prepared by Chen et al. [198], showed positive effect on wound healing. Chitin scaffolds were utilized by Madhumathi et al. [21] for silver nanoparticles preparation. These nanoparticles showed effective wound-healing applications by showing antibacterial activity against pathogenic bacteria, with good blood-clotting ability. These outcomes proved that chitin/nanosilver composite scaffolds could be useful for wound-healing applications.

5.4.2 Role of Marine Based Polysaccharides for Biotechnological Applications

5.4.2.1 Biosensor Technology

Recent innovations in biosensor technology exploring its beneficial properties as analytical tools such as low cost, simple, portable, and laboratory-based well established method as well as allows miniaturization [199]. Several reports are available on chitosan nanofibrous membrane and its recent application in enzyme immobilization. Due to the favorable properties such as good biocompatibility, high surface, and large porosity of chitosan nanofibrous membrane has recently explored as better substrate for enzyme immobilization. The concentration of chitosan nanofibrous membrane that was utilized to immobilize lipase was 63.6 mg/g. Further the reported activity retention of the immobilized lipase was 49.8 % less than the optimum condition.

Developed chitosan based system can be used for biosensors [200]. According to previous investigation electrochemical tyrosinase biosensor showed good repeatability and stability. This electrochemical tyrosinase biosensor was used for determining phenolic compounds on the basic of the use of a glassy carbon electrode modified with tyrosinase-Fe_3O_4 magnetic nanoparticles-chitosan nanobiocomposite film. Such chitosan based novel tyrosinase biosensor offers wonderful applications for eco-friendly, fast and simple methods of phenolic contaminants in

ecological samples [201]. Amperometric biosensor was developed by Chauhan et al. [202] for the determination of glutathione by covalently immobilizing a glutathione oxidase onto the surface of gold-coated magnetic nanoparticles-modified Pt electrode. It was observed that glutathione oxidase/chitosan/gold-coated magnetic nanoparticles were an outstanding candidate for the production of extremely responsive glutathione biosensor.

5.4.2.2 Waste Water Management

Current innovations in polymeric sciences lead to its exploration in waste water management. Owing to the presence of reactive amino groups chitosan is considered as potential candidate in waste water management. Chitosan and chitin both have been widely investigated for the removal of toxic elements such as heavy metal ions from waste water [203]. Chitosan bead-immobilized algae system with the association of *Scenedesmus* sp. was investigated for removing phosphate and nitrate from water and it was observed that this system was proved to be more efficient conventional free cell system [204]. Since waste contains large amount pathogenic bacteria, fungi, viruses, therefore efficient antimicrobial system is required to reduce chances of infections or any other disease. For these purpose silver nanoparticles using polysaccharide-based bioflocculant was developed by Manivasagan et al. [32]. These nanoparticles were synthesized using polysaccharide-based bioflocculant by *Streptomyces* sp. MBRC-91. Fabricated silver nanoparticles showed potential antibacterial activity in sewage water. Therefore these types of researches can make a new opportunity in the waste water management.

5.5 Marine Polysaccharide-Based Nanomaterials and Its Patents

There are almost fifty patents are existing on marine polysaccharides-based nanomaterials and their applications. Among these polysaccharides only few claimed that nanocomposite materials based on metallic nanoparticles were stabilized with branched polysaccharides. More specifically chitosan derivatives such as alditolic or aldonic monosaccharide and oligosaccharide and their products was obtainable with these polysaccharide sin the presence or absence of reducing agents claimed to be stabilized metallic nanoparticles in their matrix. In these studies polysaccharides applications were explored for antimicrobial activities and molecular biosensors by exploring their features associated with the nanometric dimensions and the presence of biological signals on polymeric chains [205]. Claimed protocol suggested that these polysaccharides based nanocomposite materials are present in the form of 3-dimensional structure. This structure is formed by a polymeric matrix consisting of a polysaccharidic composition of neutral or anionic polysaccharides and branched cationic polysaccharides. It has been claimed that metallic nanoparticles are homogeneously dispersed and stabilized in this 3-D matrix [206].

References

1. Nitta SK, Numata K. Biopolymer-based nanoparticles for drug/gene delivery and tissue engineering. Int J Mol Sci. 2013;14(1):1629–54.
2. Dutta J, Tripathi S, Dutta PK. Progress in antimicrobial activities of chitin, chitosan and its oligosaccharides: a systematic study needs for food applications. Food Sci Technol Int. 2012;18(1):3–34.
3. Vu B, Chen M, Crawford RJ, Ivanova EP. Bacterial extracellular polysaccharides involved in biofilm formation. Molecules. 2009;14:2535–54. doi:10.3390/molecules14072535.
4. Manivasagan P, Oh J. Marine polysaccharide-based nanomaterials as a novel source of nanobiotechnological applications. Int J Biol Macromol. 2016;82:315–27. doi:10.1016/j.ijbiomac.2015.10.081. Epub 2015 Oct 30.
5. Jayakumar R, Menon D, Manzoor K, Nair SV, Tamura H. Biomedical applications of chitin and chitosan based nanomaterials-a short review. Carbohydr Polym. 2010;82(2):227–32.
6. Jesus Raposo MF, Morais AMB, Morais RMSC. Marine polysaccharides from algae with potential biomedical applications. Mar Drugs. 2015;13:2967–3028. doi:10.3390/md13052967.
7. Jayakumar R, Prabaharan M, Nair SV, Tamura H. Novel chitin and chitosan nanofibers in biomedical applications. Biotechnol Adv. 2010;28(1):142–50.
8. Toida T, Amornrut C, Linhardt RJ. Structure and bioactivity of sulfated polysaccharides. Trends Glycosci Glycotechnol. 2003;15:29–46.
9. Wu XZ. Effects of sulfated polysaccharides on tumor biology. West Indian Med J. 2006;55(4):270–3.
10. Parish CR, Freeman C, Brown KJ, Francis DJ, Cowden WB. Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity. Cancer Res. 1999;593:433–3441.
11. Costa L, Fidelis G, Cordeiro S, Oliveira R, Sabry D, Câmara R, Nobre L, Costa M, Almeida-Lima J, Farias E. Biological activities of sulfated polysaccharides from tropical seaweeds. Biomed Pharmacother. 2010;64:21–8.
12. Gustafson S. The influence of sulfated polysaccharides on the circulating levels of hyaluronan. Glycobiology. 1997;7:1209–14.
13. Renn D. Biotechnology and the red seaweed polysaccharide industry: status, needs and prospects. Trends Biotechnol. 1997;15:9–14.
14. Luscher-Mattli M. Polyanions-a lost chance in the fight against HIV and other virus diseases? Antivir Chem Chemother. 2000;11:249–59.
15. Lahaye M, Robic A. Structure and functional properties of ulvan, a polysaccharide from green seaweeds. Biomacromolecules. 2007;8:1765–74.
16. Pillai C, Paul W, Sharma CP. Chitin and chitosan polymers: chemistry, solubility and fiber formation. Prog Polym Sci. 2009;34:641–78.
17. Ehrlich H, Steck E, Ilan M, Maldonado M, Muricy G, Bavestrello G, Kljajic Z, Carballo J, Schiaparelli S, Ereskovsky A. Three-dimensional chitin-based scaffolds from Verongida sponges (Demospongiae: Porifera). Part II: biomimetic potential and applications. Int J Biol Macromol. 2010;47:141–5.
18. Xia W, Liu P, Zhang J, Chen J. Biological activities of chitosan and chitooligosaccharides. Food Hydrocolloid. 2011;25:170–9.
19. Lin SB, Lin YC, Chen HH. Low molecular weight chitosan prepared with the aid of cellulase, lysozyme and chitinase: characterisation and antibacterial activity. Food Chem. 2009;116:47–53.
20. Liao FH, Shieh MJ, Chang NC, Chien YW. Chitosan supplementation lowers serum lipids and maintains normal calcium, magnesium, and iron status in hyperlipidemic patients. Nutr Res. 2007;27:146–51.
21. Madhumathi K, Sudheesh Kumar PT, Abhilash S, Sreeja V, Tamura H, Manzoor K, Nair SV, Jayakumar R. Development of novel chitin/nanosilver composite scaffolds for wound dressing applications. J Mater Sci Mater Med. 2010;21(2):807–13.

22. Jayakumar R, Nwe N, Tokura S, Tamura H. Sulfated chitin and chitosan as novel biomaterials. Int J Biol Macromol. 2007;40:175–81.
23. Madhumathi K, Binulal N, Nagahama H, Tamura H, Shalumon K, Selvamurugan N, Nair S, Jayakumar R. Preparation and characterization of novel beta-chitin-hydroxyapatite composite membranes for tissue engineering applications. Int J Biol Macromol. 2009;44:1–5.
24. Xia W. Physiological activities of chitosan and its application in functional foods. J Chin Inst Food Sci Technol. 2003;3:77–81.
25. Zhao X, Xia W. Antimicrobial activities of chitosan and application in food preservation. Chin Food Res Dev. 2006;27:157–60.
26. Sun C, Wang JW, Fang L, Gao XD, Tan RX. Free radical scavenging and antioxidant activities of EPS2, an exopolysaccharide produced by a marine filamentous fungus Keissleriella sp. YS 4108. Life Sci. 2004;75:1063–73.
27. Manivasagan P, Kim SK. Extracellular polysaccharides produced by marine bacteria. Adv Food Nutr Res. 2014;72:79–94.
28. Manivasagan P, Sivasankar P, Venkatesan J, Senthilkumar K, Sivakumar K, Kim SK. Marine actinobacterial metabolites: current status and future perspectives. Int J Biol Macromol. 2013;59:29–38.
29. Chi Z, Su C, Lu W. A new exopolysaccharide produced by marine Cyanothece sp. Bioresour Technol. 2007;98:1329–32.
30. Mishra A, Jha B. Isolation and characterization of extracellular polymeric substances from micro-algae Dunaliellasalina under salt stress. Bioresour Technol. 2009;100:3382–6.
31. Zhang ML, Zhang PCK, Cheung VEC. Molecular weight and anti-tumor activity of the water-soluble polysaccharides isolated by hot water and ultrasonic treatment from the sclerotia and mycelia of Pleurotus tuber-regium. Carbohydr Polym. 2004;56:123–8.
32. Manivasagan P, Kang KH, Kim DG, Kim SK. Production of polysaccharide-based bioflocculant for the synthesis of silver nanoparticles by Streptomyces sp. Int J Biol Macromol. 2015;77:159–67.
33. Lee JW, Park JH, Robinson JR. Bioadhesive-based dosage forms: the next generation. J Pharm Sci. 2000;89:850–66.
34. Sinha V, Kumria R. Polysaccharides in colon-specific drug delivery. Int J Pharm. 2001;224:19–38.
35. Vandamme TF, Lenourry A, Charrueau C, Chaumeil J. The use of polysaccharides to target drugs to the colon. Carbohydr Polym. 2002;48:219–31.
36. Lemarchand C, Gref R, Couvreur P. Polysaccharide-decorated nanoparticles. Eur J Pharm Biopharm. 2004;58:327–41.
37. Berteau O, Mulloy B. Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide. Glycobiology. 2003;13:29–40.
38. Li B, Lu F, Wei X, Zhao R. Fucoidan: structure and bioactivity. Molecules. 2008;13:1671–95.
39. Leung TCY, Wong CK, Xie Y. Green synthesis of silver nanoparticles using biopolymers, carboxymethylated-curdlan and fucoidan. Mater Chem Phys. 2010;121:402–5.
40. Lira MCB, Santos-Magalhães NS, Nicolas V, Marsaud V, Silva MPC, Ponchel G, Vauthier C. Cytotoxicity and cellular uptake of newly synthesizedfucoidan-coated nanoparticles. Eur J Pharm Biopharm. 2011;79(1):162–70.
41. Huang YC, Liu TJ. Mobilization of mesenchymal stem cells by stromal cell-derived factor-1 released from chitosan/tripolyphosphate/fucoidan nanoparticles. Acta Biomater. 2012;8(3):1048–56.
42. Shibata H, Nagaoka M, Takagi IK, Hashimoto S, Aiyama R, Yokokura T. Effect of oligofucose derivatives on acetic acid-induced gastric ulcer in rats. Biomed Mater Eng. 2001;11(1):55–61.
43. Wang J, Liu L, Zhang Q, Zhang Z, Qi H, Li P. Synthesized oversulphated, acetylated and benzoylated derivatives of fucoidan extracted from *Laminaria japonica* and their potential antioxidant activity in vitro. Food Chem. 2009;114(4):1285–90.

44. Wang J, Zhang Q, Zhang Z, Zhang J, Li P. Synthesized phosphorylated and aminated derivatives offucoidan and their potential antioxidant activity in vitro. Int J Biol Macromol. 2009;44(2):170–4.
45. Hou Y, Wang J, Jin W, Zhang H, Zhang Q. Degradation of Laminaria japonica fucoidan by hydrogen peroxide and antioxidant activities of the degradation products of different molecular weights. Carbohydr Polym. 2012;87(1):153–9.
46. Hahn T, Lang S, Ulber R, Muffler K. Novel procedures for the extraction of fucoidan from brown algae. Process Biochem. 2012;47(12):1691–8.
47. Hayashi K, Nakano T, Hashimoto M, Kanekiyo K, Hayashi T. Defensive effects of a fucoidan from brown alga Undaria pinnatifida against herpes simplex virus infection. Int Immunopharmacol. 2008;8:109–16.
48. Becker CF, Guimarães JA, Mourão PA, Verli H. Conformation of sulfated galactan and sulfated fucan in aqueous solutions. Implications to their anticoagulant activities. J Mol Graph Model. 2007;26:391–9.
49. Cumashi A, et al. A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. Glycobiology. 2007;175:41–552.
50. Wang J, Zhang Q, Zhang Z, Li Z. Antioxidant activity of sulfated polysaccharide fractions extracted from Laminaria japonica. Int J Biol Macromol. 2008;42:127–32.
51. Lira M, Santos-Magalhães N, Nicolas V, Marsaud V, Silva M, Ponchel G, Vauthier C. Cytotoxicity and cellular uptake of newly synthesized fucoidan-coated nanoparticles. Biopharm Eur J Pharm. 2011;79:162–70.
52. Lirdprapamongkol K, Warisnoicharoen W, Soisuwan S, Svasti J. Eco-friendly synthesis of fucoidan-stabilized gold nanoparticles. Am J Appl Sci. 2010;7:1038–42.
53. Kimura R, Rokkaku T, Takeda S, Senba M, Mori N. Cytotoxic effects of fucoidan nanoparticles against osteosarcoma. Mar Drugs. 2013;11:4267–78.
54. Rajaonarivony M, Vauthier C, Couarraze G, Puisieux F, Couvreur P. Development of a new drug carrier made from alginate. J Pharm Sci. 1993;82:912–7.
55. Sarmento B, Ribeiro A, Veiga F, Ferreira D, Neufeld R. Insulin-loaded nanoparticles are prepared by alginate ionotropic pre-gelation followed by chitosan polyelectrolyte complexation. J Nanosci Nanotechnol. 2007;7:2833–41.
56. Ahmad Z, Sharma S, Khuller GK. Chemotherapeutic evaluation of alginate nanoparticle-encapsulated azole antifungal and antitubercular drugs against murine tuberculosisNanomed. Nanotechnol. 2007;3:239–43.
57. Anh NT, Van Phu D, Duy NN, Du BD, Hien NQ. Synthesis of alginate stabilized gold nanoparticles by γ-irradiation with controllable size using different Au3+ concentration and seed particles enlargement. Radiat Phys Chem. 2010;79:405–8.
58. Yang J, Pan J. Hydrothermal synthesis of silver nanoparticles by sodium alginate and their applications in surface-enhanced Raman scattering and catalysis. Acta Mater. 2012;60:4753–8.
59. Zhang C, et al. Doxorubicin-loaded glycyrrhetinic acid-modified alginate nanoparticles for liver tumor chemotherapy. Biomaterials. 2012;33:2187–96.
60. Guo H, Lai Q, Wang W, Wu Y, Zhang C, Liu Y, Yuan Z. Functional alginate nanoparticles for efficient intracellular release of doxorubicin and hepatoma carcinoma cell targeting therapy. Int J Pharm. 2013;451:1–11.
61. Pandey R, Khuller G. Nanotechnology base drug delivery system(s) for the management of tuberculosis. Indian J Exp Biol. 2006;44:357–66.
62. Ahmad Z, Pandey R, Sharma S, Khuller GK. Pharmacokinetic and pharmacodynamic behaviour of antitubercular drugs encapsulated in alginate nanoparticles at two doses. Int J Antimicrob Agents. 2006;27:409–16.
63. Das RK, Kasoju N, Bora U. Encapsulation of curcumin in alginate-chitosan-pluronic composite nanoparticles for delivery to cancer cells. Nanomed Nanotechnol Biol Med. 2010;6(1):153–60.

64. Muñiz ME, Iglesias I, Teijón JM, Blanco MD. Enhanced preclinical efficacy of tamoxifen developed as alginate–cysteine/disulfide bond reduced albumin nanoparticles. Int J Pharm. 2012;436(1–2):574–81.
65. Strasdat B, Bunjes H. Incorporation of lipid nanoparticles into calcium alginate beads and characterization of the encapsulated particles by differential scanning calorimetry. Food Hydrocolloids. 2013;30(2):567–75.
66. Li Z, Chen P, Xu X, Ye X, Wang J. Preparation of chitosan–sodium alginate icrocapsules containing ZnS nanoparticles and its effect on the drug release. Mater Sci Eng C. 2009;29(7):2250–3.
67. Idrisa A, Ismaila NSM, Hassana N, Misrana E, Ngomsik AF. Synthesis of magnetic alginate beads based on maghemite nanoparticles for Pb(II) removal in aqueous solution. J Ind Eng Chem. 2012;18(5):1582–9.
68. Morales MA, Finotelli PV, Coaquira JAH, Rocha-Leão MHM, Diaz-Aguila C, Baggio-Saitovitch EM, Rossi AM. In situ synthesis and magnetic studies of iron oxide nanoparticles in calcium-alginate matrix for biomedical applications. Mater Sci Eng C. 2008;28(2):253–7.
69. Ma HL, Xu YF, Qi XR, Maitani Y, Nagai T. Superparamagnetic iron oxide nanoparticles stabilized by alginate: pharmacokinetics, tissue distribution, and applications in detecting liver cancers. Int J Pharm. 2008;354(1–2):217–26.
70. Finotelli PV, Morales MA, Rocha-Leão MH, Baggio-Saitovitch EM, Rossi AM. Magnetic studies of iron(III) nanoparticles in alginate polymer for drug delivery applications. Mate Sci Eng C. 2004;24(5):625–9.
71. Zhou J, Romero G, Rojas E, Ma L, Moya S, Gao C. Layer by layer chitosan/alginate coatings on poly(lactide-co-glycolide) nanoparticles for antifouling protection and Folic acid binding to achieve selective cell targeting. J Colloid Interface Sci. 2010;345(2):241–7.
72. Zhang S, Niu H, Cai Y, Shi Y. Barium alginate caged Fe3O4@C18 magnetic nanoparticles for the pre-concentration of polycyclic aromatic hydrocarbons and phthalate esters from environmental water samples. Anal Chim Acta. 2010;665(2):167–75.
73. Zahoor A, Sharma S, Khuller GK. Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis. Int J Antimicrob Agents. 2005;26(4):298–303.
74. Niu H, Dizhang MZ, Cai Y. Fast defluorination and removal of norfloxacin by alginate/Fe@ Fe3O4 core/shell structured nanoparticles. J Hazard Mater. 2012;227–228:195–203.
75. Joshi A, Solanki S, Chaudhari R, Bahadur D, Aslam M, Srivastava R. Multifunctional alginate microspheres for biosensing, drug delivery and magnetic resonance imaging. Acta Biomater. 2011;7(11):3955–63.
76. Gazori T, Khoshayand RM, Azizi E, Yazdizade P, Nomani A, Haririan I. Evaluation of alginate/chitosan nanoparticles as antisense delivery vector: formulation, optimization and in vitro characterization. Carbohydr Polym. 2009;77(3):599–606.
77. Sarmento B, Ferreira D, Veiga F, Ribeiro A. Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies. Carbohydr Polym. 2006;66(1):1–7.
78. Gazori T, Haririan I, Fouladdel S, Namazi A, Nomani A, Azizi E. Inhibition of EGFR expression with chitosan/alginate nanoparticles encapsulating antisense oligonucleotides in T47D cell line using RT-PCR and immunocytochemistry. Carbohydr Polym. 2010;80(4):1042–7.
79. Seo SY, Lee GH, Lee SG, Jung SY, Lim JO, Choi JH. Alginate-based composite sponge containing silver nanoparticles synthesized in situ. Carbohydr Polym. 2012;90(1):109–15.
80. Silva MS, Cocenza DS, Grillo R, Silva NFM, Tonello PS, Oliveira LC, et al. Paraquat-loaded alginate/chitosan nanoparticles: preparation, characterization and soil sorption studies. J Hazard Mater. 2011;190(1–3):366–74.
81. Cafaggi S, Russo E, Stefani R, Leardi R, Caviglioli G, Parodi B, Bignardi G, Totero DD, Aiello C, Viale M. Preparation and evaluation of nanoparticles made of chitosan or N-trimethyl chitosan and a cisplatin–alginate complex. J Control Release. 2007;121(1–2):110–23.
82. Ma H, Qi X, Maitani Y, Nagai T. Preparation and characterization of superparamagnetic iron oxide nanoparticles stabilized by alginate. Int J Pharm. 2007;333(1–2):177–86.

83. Martínez A, Iglesias I, Lozano R, Teijón JM, Blanco MD. Synthesis and characterization of thiolated alginate-albumin nanoparticles stabilized by disulfide bonds. Evaluation as drug delivery systems. Carbohydr Polym. 2011;83(3):1311–21.
84. Boissière M, Allouche J, Chanéac C, Brayner R, Devoisselle JM, Livage J, Coradin T. Potentialities of silica/alginate nanoparticles as Hybrid Magnetic Carriers. Int J Pharm. 2007;344(1–2):128–34.
85. Paul W, Sharma CP. Synthesis and characterization of alginate coated zinc calcium phosphate nanoparticles for intestinal delivery of insulin. Process Biochem. 2012;47(5):882–6.
86. Kim HW, Kim BR, Rhee YH. Imparting durable antimicrobial properties to cotton fabrics using alginate–quaternary ammonium complex nanoparticles. Carbohydr Polym. 2010;79(4):1057–62.
87. Xu XQ, Shen H, Xu JR, Xie MQ, Li XJ. The colloidal stability and core-shell structure of magnetite nanoparticles coated with alginate. Appl Surf Sci. 2006;253(4):2158–64.
88. Yang JS, Xie YJ, He W. Research progress on chemical modification of alginate: a review. Carbohydr Polym. 2011;84(1):33–9.
89. Wingender J, Winkler UK. A novel biological function of alginate in Pseudomonas aeruginosa and its mucoid mutants: stimulation of exolipase. FEMS Microbiol Lett. 1984;21(1):63–9.
90. Peng P, Xie H, Lu L. Surface modification of sphalerite with sodium alginate. Colloids Surf A Physicochem Eng Asp. 2006;274(1–3):150–3.
91. Pawar SN, Edgar KJ. Alginate derivatization: a review of chemistry, properties and applications. Biomaterials. 2012;33(11):3279–305.
92. Cheng Y, Lu L, Zhang W, Shi J, Cao Y. Reinforced low density alginate-based aerogels: preparation, hydrophobic modification and characterization. Carbohydr Polym. 2012;88(3):1093–9.
93. Wells LA, Sheardown H. Photosensitive controlled release with polyethylene glycol–anthracene modified. Eur J Pharm Biopharm. 2011;79(2):304–13.
94. Bubenikova S, Stancu IC, Kalinovska L, Schacht E, Lippens E, Declercq H, Cornelissen M, Santin M, Amblard M, Martinez J. Chemoselective cross-linking of alginate with thiol-terminated peptides for tissue engineering applications. Carbohydr Polym. 2012;88(4):1239–50.
95. Fonseca KB, Bidarra SJ, Oliveira MJ, Granja PL, Barrias CC. Molecularly designed alginate hydrogels susceptible to local proteolysis as three-dimensional cellular microenvironments. Acta Biomater. 2011;7(4):1674–82.
96. Xu Y, Li L, Yu X, Gu Z, Zhang X. Feasibility study of a novel crosslinking reagent (alginate dialdehyde) for biological tissue fixation. Carbohydr Polym. 2012;87(2):1589–95.
97. Tan R, She Z, Wang M, Fang Z, Liu Y, Feng Q. Thermo-sensitive alginate-based injectable hydrogel for tissue engineering. Carbohydr Polym. 2012;87(2):1515–21.
98. Xu Y, Li L, Wang H, Yu X, Gu Z, Huang C, Peng H. In vitro cytocompatibility evaluation of alginate dialdehyde for biological tissue fixation. Carbohydr Polym. 2012.
99. Yadav M, Mishra DK, Sand A, Behari K. Modification of alginate through the grafting of 2-acrylamidoglycolic acid and study of physicochemical properties in terms of swelling capacity, metal ion sorption, flocculation and biodegradability. Carbohydr Polym. 2011;84(1):83–9.
100. Huq T, Khan A, Dussault D, Salmieri S, Khan RA, Lacroix M. Effect of gamma radiation on the physico-chemical properties of alginate-based films and beads. Radiat Phys Chem. 2012;81(8):945–8.
101. Gomez CG, Chambat G, Heyraud A, Villar M, Auzély-Velty R. Synthesis and characterization of a β-CD-alginate conjugate. Polymer. 2006;47(26):8509–16.
102. Yang JS, Jiang B, He W, Xia YM. Hydrophobically modified alginate for emulsion of oil in water. Carbohydr Polym. 2012;87(2):1503–6.
103. Birdi G, Bridson RH, Smith AM, Bohari SPM, Grover LM. Modification of alginate degradation properties using orthosilicic acid. J Mech Behav Biomed Mater. 2012;6:181–7.
104. Oddo L, Masci G, Meo CD, Capitani D, Mannina L, Lamanna R, et al. Novel thermosensitive calcium alginate microspheres: physico-chemical characterization and delivery properties. Acta Biomater. 2010;6(9):3657–64.

105. Choudhary S, Bhatia SR. Rheology and nanostructure of hydrophobically modified alginate (HMA) gels and solutions. Carbohydr Polym. 2012;87(1):524–30.
106. Kong HJ, Smith MK, Mooney DJ. Designing alginate hydrogels to maintain viability of immobilized cells. Biomaterials. 2003;24(22):4023–9.
107. Zain NAM, Suhaimi MS, Idris A. Development and modification of PVA–alginate as a suitable immobilization matrix. Process Biochem. 2011;46(11):2122–9.
108. Bardajee GR, Hooshyar Z, Rostami I. Hydrophilic alginate based multidentate biopolymers for surface modification of CdS quantum dots. Colloids Surf B Biointerfaces. 2011;88(1):202–7.
109. Tafaghodi M, Eskandari M, Khamesipour A, Jaafari MR. Exp Parasitol. Alginate microspheres encapsulated with autoclaved Leishmania major (ALM) and CpG-ODN induced partial protection and enhanced immune response against murine model of leishmaniasis. Exp Parasitol. 2011;129(2):107–14.
110. Haji E, Shariatifar N, Tafaghodi M, Ofogh-e-Danesh Salari Z. Evaluation of immune response against cutaneous leishmaniasis induced by alginate microspheres encapsulated with autoclaved *Leishmania major* (ALM), Quillaja saponin or CpG-ODN adjuvants. Horizon Med Sci. 2008;13(4):44–50.
111. Shadab, Ahuja A, Khar RK, Baboota S, Chuttani K, Mishra AK, Ali J. Gastroretentive drug delivery system of acyclovir-loaded alginate mucoadhesive microspheres: formulation and evaluation. Drug Deliv. 2011;18(4):255–64.
112. Girish GK, Kansal S, Misra P, Dube A, Mishra PR. Uptake of biodegradable Gel-assisted LBL Nanomatrix by Leishmania donovani-infected macrophages. AAPS PharmSciTech. 2009;10(4):1343–7.
113. Babu GD, Chandra SR, Devi AS, Reddy BVV. Formulation and evaluation of novel effervescent metronidazole floating tablets. Int J Res Pharmaceut Biomed Sci. 2011; 2(4).
114. Coppi G, Sala N, Bondi M, Sergi S, Iannuccelli V. Ex-vivo evaluation of alginate microparticles for Polymyxin B oral administration. J Drug Target. 2006;14(9):599–606.
115. Coppi G, Iannuccelli V, Sala N, Bondi M. Alginate microparticles for Polymyxin B Peyer's patches uptake: microparticles for antibiotic oral administration. J Microencapsul. 2004;21(8):829–39.
116. Coppi G, Bondi M, Coppi A, Rossi T, Sergi S, Iannuccelli V. Toxicity and gut associated lymphoid tissue translocation of polymyxin B orally administered by alginate/chitosan microparticles in rats. J Pharm Pharmacol. 2008;60(1):21–6.
117. Tafaghodi M, Abolghasem S, Tabasi S, Payan M. Alginate microsphere as a delivery system and adjuvant for autoclaved *Leishmania major* and Quillaja Saponin: preparation and characterization. Iranian J Pharm Sci. 2007;3(2):61–8.
118. Wang FQ, Li P, Zhang JP, Wang AQ, Wei Q. pH-sensitive magnetic alginate-chitosan beads for albendazole delivery. Pharm Dev Technol. 2011;16(3):228–36.
119. Singodia D, Khare P, Dube A, Talegaonkar S, Khar RK, Mishra PR. Development and performance evaluation of alginate-capped amphotericin B lipid nanoconstructs against visceral leishmaniasis. J Biomed Nanotechnol. 2011;7(1):123–4.
120. Mandal TK, Bostanian LA, Graves RA, Chapman SR, Idodo TU. Porous biodegradable microparticles for delivery of pentamidine. Eur J Pharm Biopharm. 2001;52(1):91–6.
121. Buranapanitkit B, Oungbho K, Ingviya N. The efficacy of hydroxyapatite composite impregnated with amphotericin. Clin Orthop Relat Res. 2005;437:236–41.
122. Hori Y, Winans AM, Irvinea DJ. Modular injectable matrices based on alginate solution/microsphere mixtures that Gel in situ and Co-deliver immunomodulatory factors. Acta Biomater. 2009;5(4):969–82.
123. Patel RP, Dadhani B, Ladani R, Baria AH, Patel J. Formulation, evaluation and optimization of stomach specific in situ gel of clarithromycin and metronidazole benzoate. Int J Drug Deliv. 2010;2:141–53.
124. Gupta GK, Kansal S, Misra P, Dube A, Mishra PR. Uptake of biodegradable Gel-assisted LBL nanomatrix by leishmania donovani-infected macrophages. AAPS PharmSciTech. 2009;10(4):1343–7.

125. Shchipunov YA. Sol-gel-derived biomaterials of silica and carrageenans. J Colloid Interface Sci. 2003;268:68–76.
126. Luo Y, Wang Q. Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. Int J Biol Macromol. 2014;64:353–67.
127. Daniel-da-Silva AL, Trindade T, Goodfellow BJ, Costa BF, Correia RN, Gil AM. In situ synthesis of magnetite nanoparticles in carrageenan gels. Biomacromolecules. 2007;8:2350–7.
128. De Souza MCR, Marques CT, Dore CMG, da Silva FRF, Rocha HAO, Leite EL. Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. J Appl Phycol. 2007;19:153–60.
129. Stiles J, Guptill-Yoran L, Moore GE, Pogranichniy RM. Effects of lambda-carrageenan on in vitro replication of feline herpesvirus and on experimentally induced herpetic conjunctivitis in cats. Invest Ophthalmol Vis Sci. 2008;49:1496–501.
130. Grenha A, Gomes ME, Rodrigues M, Santo VE, Mano JF, Neves NM, Reis RL. Development of new chitosan/carrageenan nanoparticles for drug delivery applications. J Biomed Mater Res A. 2010;92:1265–72.
131. Rodrigues S, da Costa AMR, Grenha A. Chitosan/carrageenan nanoparticles: effect of cross-linking with tripolyphosphate and charge ratios. Carbohydr Polym. 2012;89:282–9.
132. Hezaveh H, Muhamad II. The effect of nanoparticles on gastrointestinal release from modified k-carrageenan nanocomposite hydrogels. Carbohydr Polym. 2012;89:138–45.
133. Salgueiro AM, Daniel-da-Silva AL, Fateixa S, Trindade T. k-Carrageenan hydrogel nanocomposites with release behavior mediated by morphological distinct Au nanofillers. Carbohydr Polym. 2013;91:100–9.
134. Datta K, Srinivasan B, Balaram H, Eswaramoorthy M. Synthesis of agarose-metal/semiconductor nanoparticles having superior bacteriocidal activity and their simple conversion to metal-carbon composites. J Chem Sci. 2008;120:579–86.
135. Kattumuri V, Chandrasekhar M, Guha S, Raghuraman K, Katti KV, Ghosh K, Patel R, Agarose-stabilized gold nanoparticles for surface enhanced Raman spectroscopic detection of DNA nucleosides. Appl Phys Lett. 2006;88:153114–153114-3.
136. Sahoo D, Baweja P, Kushwah N. Developmental studies in Porphyra vietnamensis: a high-temperature resistant species from the Indian coast. J Appl Phycol. 2006;18:279–86.
137. Bhatia S, et al. Novel algal polysaccharides from marine source: Porphyran. Pharmacogn Rev. 2009;2(4):271–6.
138. Bhatia S, et al. Immuno-modulation effect of sulphated polysaccharide (porphyran) from *Porphyra vietnamensis*. Int J Biol Macromol. 2013;57:50–6.
139. Bhatia S, et al. Significance of algal polymer in designing amphotericin B nanoparticles. Sci World J. 2014, 564573. doi:10.1155/2014/564573
140. Bhatia S, et al. Investigation of the factors influencing the molecular weight of porphyran and its associated antifungal activity. Bioact Carbohydr Dietary Fibre. 2015;5(2):153–68.
141. Bhatia S, et al. Structural characterization and pharmaceutical properties of Porphyran. Asian J Pharm. 2015;9(2).
142. Bhatia S, et al. Factors affecting the gelling and emulsifying property of natural polymer. Syst Rev Pharm. 2010;1(1):86.
143. Bhatia S, et al. Anti-oxidant potential of Indian porphyra. Pharmacol Online. 2011;1:248–57.
144. Kwon MJ, Nam TJ. Porphyran induces apoptosis related signal pathway in AGS gastric cancer cell lines. Life Sci. 2006;79:1956–62.
145. Venkatpurwar V, Shiras A, Pokharkar V. Porphyran capped gold nanoparticles as a novel carrier for delivery of anticancer drug: In vitro cytotoxicity study. Int J Pharm. 2011;409:314–20.
146. Venkatpurwar V, Mali V, Bodhankar S, Pokharkar V. In vitro cytotoxicity and in vivo subacute oral toxicity assessment of porphyran reduced gold nanoparticles. Toxicol Environ Chem. 2012;94:1357–67.

147. Toskas G, Hund RD, Laourine E, Cherif C, Smyrniotopoulos V, Roussis V. Anofibers based on polysaccharides from the green seaweed Ulva rigida. Carbohydr Polym. 2011;84:1093–102.
148. Raveendran S, Poulose AC, Yoshida Y, Maekawa T, Kumar DS. Bacterial exopolysaccharide based nanoparticles for sustained drug delivery, cancer chemotherapy and bioimaging. Carbohydr Polym. 2013;91:22–32.
149. Wibowo S, Velazquez G, Savant V, Torres JA. Surimi wash water treatment for protein recovery: effect of chitosan–alginate complex concentration and treatment time on protein adsorption. Bioresour Technol. 2005;96:665–71.
150. Hejazi R, Amiji M. Chitosan-based gastrointestinal delivery systems. J Control Release. 2003;89:151–65.
151. Chang PR, Jian R, Yu J, Ma X. Starch-based composites reinforced with novel chitin nanoparticles. Carbohydr Polym. 2010;80:420–5.
152. Song Y, Onishi H, Nagai T. Pharmacokinetic characteristics and antitumor activity of the N-succinyl-chitosan-mitomycin C conjugate and the carboxymethyl-chitin-mitomycin C conjugate. Biol Pharm Bull. 1993;16:48–54.
153. Dev A, Mohan JC, Sreeja V, Tamura H, Patzke G, Hussain F, Weyeneth S, Nair S, Jayakumar R. Novel carboxymethyl chitin nanoparticles for cancer drug delivery applications. Carbohydr Polym. 2010;79:1073–9.
154. Gnanadhas DP, Thomas MB, Elango M, Raichur AM, Chakravortty D. Chitosan-dextran sulphate nanocapsule drug delivery system as an effective therapeutic against intraphagosomal pathogen Salmonella. J Antimicrob Chemother. 2013;68:2576–86.
155. Smitha K, Nisha N, Maya S, Biswas R, Jayakumar R. Delivery of rifampicin-chitin nanoparticles into the intracellular compartment of polymorphonuclear leukocytes. Int J Biol Macromol. 2015;74:36–43.
156. Kean T, Thanou M. Biodegradation, biodistribution and toxicity of chitosan. Adv Drug Deliv Rev. 2010;62:3–11.
157. Rather MA, Sharma R, Gupta S, Ferosekhan S, Ramya V, Jadhao SB. Chitosan-nanoconjugated hormone nanoparticles for sustained surge of gonadotropins and enhanced reproductive output in female fish. Plosone. 2013;8:1–10.
158. Janes KA, Fresneau MP, Marazuela A, Fabra A, Alonso MAJ. Chitosan nanoparticles as delivery systems for doxorubicin. J Control Release. 2001;73:255–67.
159. Mitra S, Gaur U, Ghosh P, Maitra A. Tumour targeted delivery of encapsulated dextran-doxorubicin conjugate using chitosan nanoparticles as carrier. J Control Release. 2001;74:317–23.
160. Campos AMD, Sánchez AM, Alonso AJ. Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A. Int J Pharm. 2001;224:159–68.
161. Banerjee T, Mitra S, Singh AK, Sharma RK, Maitra A. Preparation, characterization and biodistribution of ultrafine chitosan nanoparticles. Int J Pharm. 2002;243:93–105.
162. Qi L, Xu Z, Jiang X, Hu C, Zou X. Preparation and antibacterial activity of chitosan nanoparticles. Carbohydr Res. 2004;339:2693–700.
163. Gan Q, Wang T, Cochrane C, McCarron P. Modulation of surface charge, particle size and morphological properties of chitosan-TPP nanoparticles intended for gene delivery. Colloid Surf B. 2005;44:65–73.
164. Katas H, Alpar HO. Development and characterisation of chitosan nanoparticles for siRNA delivery. J Control Release. 2006;115:216–25.
165. Min KH, Park K, Kim YS, Bae SM, Lee S, Jo HG, Park RW, Kim IS, Jeong SY, Kim K. Hydrophobically modified glycol chitosan nanoparticles-encapsulated camptothecin enhance the drug stability and tumor targeting in cancer therapy. J Control Release. 2008;127:208–18.
166. Ali SW, Rajendran S, Joshi M. Synthesis and characterization of chitosan and silver loaded chitosan nanoparticles for bioactive polyester. Carbohydr Polym. 2011;83:438–46.

167. Lima JMD, Sarmento RR, Souza JRD, Brayner FA, Feitosa APS, Padilha R, Alves LC, Porto IJ, Batista RFBD, Oliveira JED. Evaluation of hemagglutination activity of chitosan nanoparticles using human erythrocytes Padlock. BioMed Res Int. 2015;1–6
168. Bhattarai N, Gunn J, Zhang M. Chitosan-based hydrogels for controlled, localized drug delivery. Adv Drug Deliv Rev. 2010;62:83–99.
169. Luo Y, Wang TT, Teng Z, Chen P, Sun J, Wang Q. Encapsulation of indole-3-carbinol and 3, 3′-diindolylmethane in zein/carboxymethyl chitosan nanoparticles with controlled release property and improved stability. Food Chem. 2013;139:224–30.
170. Thein-Han W, Saikhun J, Pholpramoo C, Misra R, Kitiyanant Y. Chitosan–gelatin scaffolds for tissue engineering: physico-chemical properties and biological response of buffalo embryonic stem cells and transfectant of GFP–buffalo embryonic stem cells. Acta Biomater. 2009;5:3453–66.
171. Agnihotri SA, Mallikarjuna NN, Aminabhavi TM. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J Control Release. 2004;5–28.
172. Wei XH, Niu YP, Xu YY, Du YZ, Hu FQ, Yuan H. Salicylic acid-grafted chitosan oligosaccharide nanoparticle for paclitaxel delivery. J Bioact Compat Polym. 2010;25:319–35.
173. López-Cruz A, Barrera C, Calero-DdelC VL, Rinaldi C. Water dispersible iron oxide nanoparticles coated with covalently linked chitosan. J Mater Chem. 2009;19:6870–6.
174. Bae KH, Park M, Do MJ, Lee N, Ryu JH, Kim GW, Kim C, Park TG, Hyeon T. Chitosan oligosaccharide-stabilized ferrimagnetic iron oxide nanocubes for magnetically modulated cancer hyperthermia. ACS Nano. 2012;6:5266–73.
175. Liu X, Huang H, Liu G, Zhou W, Chen Y, Jin Q. Multidentate zwitterionic chitosan oligosaccharide modified gold nanoparticles: stability, biocompatibility and cell interactions. J Nanoscale. 2013;5:3982–91.
176. Lin YS, Wu MF, Takamori Y, Okamoto Y, Minami S. In vivomodulatory effects of chitooligosaccharide nanoparticles on mouse serum cytokines and splenocytes. J Exp Nanosci. 2014;9:860–70.
177. Lu C, Park MK, Lu C, Lee YH, Chai KY. A mussel-inspired chitooligosaccharide based multidentate ligand for highly stabilized nanoparticles. J Mater Chem B. 2015;3:3730–7.
178. Wang W, Wang SX, Guan HS. The antiviral activities and mechanisms of marine polysaccharides: an overview. Mar Drugs. 2012;10:2795–816.
179. Mohanty S, Mishra S, Jena P, Jacob B, Sarkar B, Sonawane A. An investigation on the antibacterial, cytotoxic, and antibiofilm efficacy of starch-stabilized silver nanoparticles. Nanomed Nanotechnol. 2012;8:916–24.
180. Allaker R. The use of nanoparticles to control oral biofilm formation. J Dent Res. 2010;89:1175–86.
181. Kora AJ, Sashidhar R, Arunachalam J. Gum kondagogu (*Cochlospermum Gossypium*): a template for the green synthesis and stabilization of silver nanoparticles with antibacterial application. Carbohydr Polym. 2010;82:670–9.
182. Shukla MK, Singh RP, Reddy C, Jha B. Synthesis and characterization of agar-based silver nanoparticles and nanocomposite film with antibacterial applications. Bioresour Technol. 2012;107:295–300.
183. Venkatpurwar V, Pokharkar V. Green synthesis of silver nanoparticles by Chrysanthemum morifolium Ramat. extract and their application in clinical ultrasound gel. Mater Lett. 2011;65:999–1002.
184. Jong WHD, Borm PJ. Drug delivery and nanoparticles: applications and hazards. Int J Nanomed. 2008;3:133–49.
185. Salamanca AED, Diebold Y, Calonge M, García-Vazquez C, Callejo S, Vila A, Alonso MJ. Chitosan nanoparticles as a potential drug delivery system for the ocular surface: toxicity, uptake mechanism and In Vivo tolerance. Invest Ophthalmol Vis Sci. 2006;47:1416–25.
186. Fuente MDL, Raviña M, Paolicelli P, Sanchez A, Seijo B, Alonso MJ. Chitosan-based nanostructures: a delivery platform for ocular therapeutics. Adv Drug Deliv Rev. 2010;62:100–17.
187. Wu SJ, Don TM, Lin CW, Mi FL. Delivery of berberine using chitosan/fucoidan-taurine conjugate nanoparticles for treatment of defective intestinal epithelial tight junction barrier. Mar Drugs. 2014;12:5677–97.

188. You JO, Liu YC, Peng CA. Efficient gene transfection using chitosan-alginate core-shell nanoparticles. Int J Nanomed. 2006;1:173–80.
189. Bozkir A, Saka OM. Chitosan nanoparticles for plasmid DNA delivery: effect of chitosan molecular structure on formulation and release characteristics. Drug Deliv. 2004;11:107–12.
190. Lee DW, Yun KS, Ban HS, Choe W, Lee SK, Lee KY. Preparation and characterization of chitosan/polyguluronate nanoparticles for siRNA delivery. J Control Release. 2009;139:146–52.
191. Noh HK, Lee SW, Kim JM, Oh JE, Kim KH, Chung CP, Choi SC, Park WH, Min BM. Electrospinning of chitin nanofibers: degradation behavior and cellular response to normal human keratinocytes and fibroblasts. Biomaterials. 2006;27:3934–44.
192. Shalumon K, Binulal N, Selvamurugan N, Nair S, Menon D, Furuike T, Tamura H, Jayakumar R. Electrospinning of carboxymethyl chitin/poly (vinyl alcohol) nanofibrous scaffolds for tissue engineering applications. Carbohydr Polym. 2009;77:863–9.
193. Peter M, Ganesh N, Selvamurugan N, Nair S, Furuike T, Tamura H, Jayakumar R. Preparation and characterization of chitosan–gelatin/nanohydroxyapatite composite scaffolds for tissue engineering applications. Carbohydr Polym. 2010;80:687–94.
194. Jeong YI, Jin SG, Kim IY, Pei J, Wen M, Jung TY, Moon KS, Jung S. Doxorubicin-incorporated nanoparticles composed of poly(ethylene glycol)-grafted carboxymethyl chitosan and antitumor activity against glioma cells in vitro. Colloid Surf B. 2010;79:149–55.
195. Li F, Li J, Wen X, Zhou S, Tong X, Su P, Li H, Shi D. Anti-tumor activity of paclitaxel-loaded chitosan nanoparticles: An In Vitro study. Mater Sci Eng C. 2009;29:2392–7.
196. Lee KW, Jeong D, Na K. Doxorubicin loading fucoidan acetate nanoparticles for immune and chemotherapy in cancer treatment. Carbohydr Polym. 2013;94:850–6.
197. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 2009;27:76–83.
198. Chen Z, Mo X, He C, Wang H. Intermolecular interactions in electrospun collagen–chitosan complex nanofibers. Carbohydr Polym. 2008;72:410–8.
199. Teles F, Fonseca L. Applications of polymers for biomolecule immobilization in electrochemical biosensors. Mater Sci Eng C. 2008;28:1530–43.
200. Huang XJ, Ge D, Xu ZK. Preparation and characterization of stable chitosan nanofibrous membrane for lipase immobilization. Eur Polym J. 2007;43:3710–8.
201. Wang S, Tan Y, Zhao D, Liu G. Amperometric tyrosinase biosensor based on Fe3O4 nanoparticles–chitosan nanocomposite. Bioelectron. 2008;23:1781–7.
202. Chauhan N, Narang J, Pundir C. An amperometric glutathione biosensor based on chitosan-iron coated gold nanoparticles modified. Int J Biol Macromol. 2012;51:879–86.
203. Gandhi MR, Viswanathan N, Meenakshi S. Preparation and application of alumina/chitosan biocomposite. Int J Biol Macromol. 2010;47:146–54.
204. Fierro S, Pilar Sanchez-Saavedra M, Copalcua C. Nitrate and phosphate removal by chitosan immobilized. Bioresour Scenedesmus Technol. 2008;99:1274–9.
205. Donati I, Marsich E, Travan A, Paoletti S. Nanocomposite materials based on metallic nanoparticles stabilized with branched polysaccharides. US 2011/0129536 A1. 2009.
206. Donati I, Marsich E, Travan A, Paoletti S. Three-dimensional nanocomposite materials consisting of a polysaccharidic matrix and metallic nanoparticles, preparation and use thereof in. US 2011/0123589 A1. 2009.