Practical Medicinal Chemistry

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PRACTICAL MEDICINAL CHEMISTRY

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PREFACE

This book *Practical Medicinal Chemistry* is intended for use in undergraduate pharmacy course on medicinal chemistry where there is a need to appreciate the rationales behind the synthesis of drugs. It provides a suitable background for graduates in chemistry who are just entering the pharmaceutical industry. In lecture, they will learn the principles and theories that, to date, best explain the observations that have accumulated. The problem is that, it is easy to forget that these theories apply to the real world. The laboratory experience is by design your opportunity to see these principles and theories in practice. This practical manual has been written not only to enhance students' understanding of chemistry, but also to capture data and take observations. The emphasis in this book is on principles, which are appropriately illustrated by groups of drugs in current use. This approach should provide the newly qualified graduates with an understanding of new developments as they take place in future years.

The students of pharmacy will find this book helpful in understanding the basic principles involved in the synthesis of organic compounds and in analyzing the drug samples. The titrimetric analysis in this book covers all the basic aspects for undergraduate level students. The book clearly picturizes the schemes and the reactions involved in the synthetic procedure and the analytical technique. Any suggestions for future improvement of the book are most welcome and will be highly appreciated.

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INTRODUCTION

SAFETY IN A CHEMISTRY LABORATORY

A well-designed, well-equipped and strategically located **chemical laboratory** is really a wonderful place for a research chemist where one may transform one's conceptualized theoretical novel ideas into sharply evident reality in the shape of useful **'target-drug-molecule'.** The on-going quest for newer **drugs** is an eternal endeavour across the globe to improve the quality of life of human beings irrespective of their caste and creed. Nevertheless, a chemistry laboratory should not be regarded as a 'dangerous place' to carry out planned experimental procedures, in spite of the several potential hazards that may be directly or indirectly associated with them, provided that one strictly observes and maintains certain basic fundamental important precautions amalgamated with unusual alertness, extraordinary presence of mind and superb common sense. It is, of course, an usual practice to have a chemical laboratory directly under the command and supervision of a senior cadre laboratory technical personnel who should be consulted, as and when required, for his expert opinion and advice. It is, however, pertinent to mention here that **two** vital universal truths and norms, namely: first, exercise of utmost care; and secondly, adoption of strict safe-working procedures, should be the prime responsibility of each and every individual working in a chemistry laboratory. No compromise, whatsoever, must be made with regard to even an iota of doubt as to the safety of a proposed experimental procedure yet to be undertaken. Liberal consultation, advice from senior research personnels, academic supervisors should be sought freely and frankly without the slightest hesitation in one's mind. Genuinely speaking, everybody should not only adopt but also execute an extremely high sense of responsible attitude towards their work. There is absolutely no scope of any sort of hurried behaviour, short-cut procedures, thoughtless or ignorant line-of-action that may end-up with an accident and most probable harm caused to themselves and others too. They must be fully aware of what is going on elsewhere or around them in the same laboratory setup; and be fully conversant of the possible hazards taking place either ensuing from their own experiments or arising from others. It has been observed beyond any reasonable doubt that most of the unfortunate accidents in a chemical laboratory invariably occurs on account of such glaring facts, namely: to achieve results in the quickest possible time-frame, to ignore knowingly certain already familiar and prohibited short-cut method(s), and lastly to work half-heartedly and carelessly in a laboratory. Therefore, one must abide by the **Golden Rules** to maintain

and create the safest environment in a chemical laboratory, such as: to work carefully, methodically, painstakingly, thoughtfully, diligently and above all whole-heartedly. In short, it may be summarized that an unplanned event causing damage or injury to oneself, otherwise termed as an 'accident', in a **chemical laboratory** can be avoided to a bearminimum-level, if not cent-per-cent, by adopting all safety norms and procedures besides working with a 'cool mind' and a 'smile' on the face.

A 'research chemist' must ensure that he/she is not subjected to any sort of risk or danger against his/her personal safety, at any cost, while working in a **chemical laboratory.**

1. Protective Coat

Each and every person working in a chemical laboratory should put on a full-length and fullsleeve protective coat, preferably white, because any type of stains and inadvertent spillages are more apparently visible and detected vividly.

2. Protection for Eyes

The human eye is probably the most vital sense-organ, and obviously the most delicate due to its fragility. Therefore, the protection for eyes is of top-priority with regard to several possible eye-hazards, namely: exposure to the dust of fine chemicals, fumes or vapours, sudden splashing of liquid chemicals (hot or cold) and even from splinters of glass wares that get exploded while performing an experiment. In order to avoid such untoward and unpredictable possible hazards in a chemical laboratory the use of a pair of **safety glasses** should be mandatory. There are a plethora of superb quality, pretested, certified, light-weight spectacles and goggles abundantly available from various reputed laboratory suppliers. These eye protective guards do provide in routine use the necessary required good coverage of the eyes and also the upper face. Of course, there are several models and designs that are quite suitable for use upon the prescription glasses.

Nevertheless, **prescription safety glasses**, that are made-to-order, are readily available through specialized sources only, and though a little more expensive, should be used exclusively for the full-time laboratory researcher or staff. It has been observed that the contact lenses do provide certain extent of protection against possible mechanical damage to the eye; however, the wearing of protective goggles is still very much essential and almost a must. It is pertinent to mention here that either the usage of **close-fitting-safety spectacles** or, preferably, **a vison covering the entire face** may provide a much enhanced level of protection in the event of chemical splashing or spraying of corrosive or toxic hot liquids or gases.

Importantly, while carrying out experiments that are either suspected to be explosive or hazardous in nature, additional protection afforded by **safety-screens** is vehemently recommended.

Fume-Cupboards. All experiments involving toxic solvents and reagents should be carried out in an efficient fume-cupboard provided with a heavy-duty chemical protected exhaust system.

Disposable Plastic Gloves. Good quality disposable plastic gloves must be used profusely while handling both corrosive and poisonous chemicals.

3. Conduct in a Chemistry Laboratory

The overall conduct in a **'chemical laboratory'** should be associated with dignity, discipline, maturity, poised behaviour, cool temperament, charged with excellent presence of mind and above all a soft-spoken pleasant disposition. It is, however, absolutely necessary to invoke a high degree of self-discipline with regard to the following cardinal aspects, namely:

- Over-hurried activity
- Smoking
- Eating and drinking
- Irresponsible behaviour (or practical jokes)
- Shouting and screaming.

Over-hurried activity particularly in a **chemical laboratory** may tantamount to serious mishaps thereby causing both intensive and extensive damage/injury to oneself, others and also the laboratory as such. Smoking is strictly prohibited in a **chemical laboratory for** obvious reasons that invariably the organic solvent or their fumes are **highly inflammable**. Eating and drinking in a **chemical laboratory** should be forbidden so as to avoid the possible risk of ingestion of toxic substances either directly or indirectly. Irresponsible behaviour (or practical jokes) must not be allowed while working in a **chemical laboratory** so as to maintain both santity and a congeneal atmosphere amongst the colleagues of either sex. Shouting and screaming may be avoided, as far as possible to distract someone's concentration or attention unduly that may perhaps cause personal distress or pain totally uncalled for.

4. Neatness and Cleanliness

It is a well-known common addage that—'next to godliness is cleanliness'. A chemical laboratory must maintain a high degree of neatness and cleanliness that may indirectly contribute as a major factor in laboratory safety. Passageways either around the working benches or in-between them should not be made untidy by litter rather these are to be thrown into a metallic-covereddustbin kept in one corner of the laboratory. The top of the working bench always be kept neat and tidy and avoid scattering with apparatus not-in-use. All such apparatus should be stored in the cup-board beneath the bench. Likewise, all dirty apparatus should be dipped in either a solution of a detergent or a cleansing-mixture in a plastic bowl a little away from the working area that may be cleaned and kept away for future usage as and when required.

Note. All solid and filter paper waste should not be thrown in the sink.

It is the prime responsibility of a 'good chemist' to meticulously and scrupulously clean and subsequently drying of all used glasswares. For highly moisture-sensitive compounds the glasswares need to be rinsed with acetone, twice at least, dried in an oven and brought to ambient temperature in a desicator. It is indeed advisable to clean-up the used reaction flasks and other apparatus immediately after their usage so as to avoid tedious cleansing process later on. It is pertinent to mention here that there exists not a single known **universal cleansing mixture**. Therefore, based on the nature of the deposit and amount of the deposit a chemist must undertake the process of cleaning accordingly in a systematic manner rather than adopting a haphazard style. The various usual standard cleansing processes are stated below in a sequential manner; namely:

1. For basic residues. Dilute sulphuric acid or hydrochloric acid may dissolve the basic residues completely.
2. For acidic residues. Dilute sodium hydroxide solution is probably the commonest and the best cleansing agent for most acidic residues.

Note: In (1) and (2) above cases the washings of basic and acidic aqueous solutions may be washed down the drain thoroughly with plenty of fresh water so that the drainage pipes are duly flushed out of the corrosive substances.

3. For organic solvent miscible residues. In instances where the stubborn residues that are miscible only in comparatively cheaper solvents, may be used profusely and should be collected in the 'residues' bottle and **not down the sink**. The combined residual organic solvent may be distilled off to recover the 'good' solvent and reject the heavily contaminated material appropriately.
4. Fro gross deposits. The cheapest, best, and simplest means to get rid of gross deposits may be accomplished by employing commercial household washing powder containing an abrassive component that does not necessarily scratch the glass surfaces at all, such as: 'Rin', 'Vim', 'Ajax' etc. The washing powder could be applied either directly into the apparatus previously moistened with water or using a test-tube cleaning brush that has been soaked into the powder; the surface of the glass is subsequently scrubbed gently followed by vigorously until the

sticking dirst has been removed entirely. Ultimately, the glass apparatus is washed and rinsed thoroughly with 'soft' tapwater.

Note: In the event when washing with a mixture of washing powder and water fails to give an entirely satisfactory results, the powder may be mixed with a polar organic solvent, for instance: acetone or iso-propanol.

Importantly, in case the above cited **four** cleansing methods do not offer hundred per cent satisfaction one may attempt any **one** of the following **three** vigorous and stringent '**alternative**' cleansing solutions, namely:

a. **Trisodium Phosphate Solution [Na_3PO_4 ; 15% (w/v)]. A** warm (30–40°C) solution of trisodium phosphate which has been mixed with a small quantum of an abrasive powder e.g., pumice powder. However, this particular reagent is not suitable for the cleansing of either tarry residues or sticky/gummy materials.
b. **Decon 90.** It is an extremely effective surface-active-agent, which is asserted to be practically able to take care of all laboratory cleansing operations. Besides, it also bears other remarkable characteristic features of the present day consumer acceptability requirements, namely: 100% biodegradable, almost non-toxic, phosphate-free, and totally rinsable. It has been widely recommended for the removal of various obstinate deposits, such as: tars, polymeric residues, greases and silicone oils.
c. **'Chromic Acid' Cleaning Mixture.** It is considered to be one of the commonest, tried and tested cleansing mixture most abundantly employed in practically all **chemical laboratories** across the globe.

Preparation. The 'chromic-acid' cleansing mixture may be prepared conveniently from the following ingredients:

(i) Sodium dichromate: 5 g
(ii) Water: 5 ml
(iii) Sulphuric acid (36 N): 100 ml.

First of all, 5 g of sodium dichromate are dissolved in 5 ml of water in a 250 ml pyrex glass beaker to which 100 ml of concentrated sulphuric acid are added in small lots at intervals with frequent stirring with a clean glass rod. Being an **exothermic reaction** the temperature will rise to 70–80°C initially, which may be allowed to fall down to 40°C over a span of time. The cooled cleansing mixture may be transferred to a clean, dry and labelled glass-stoppered bottle. The glass apparatus to be cleaned must be rinsed with water to get rid of the water soluble organic matter as far as possible along with the possible reducing agents, if any. Subsequently, the water is drained off from the apparatus to its maximum extent ; and the 'chromic acid' cleaning mixture is introduced into it in a quantity just sufficient to smear the solid residue adequately, while the main quantum of the cleaning mixture returned to the stock bottle. The cleaning mixture treated apparatus is allowed to stand for about 15–20 minutes, with occasional swirling of the apparatus to stretch out the liquid onto the surface of the solid residue, the former is rinsed thoroughly with running tap water an finally with distilled water.

Note: It is advisable not to attempt any other 'chemical treatment' whatsoever due to the possible ensuing explosion hazards.

Ultrasonic* bath. The use of ultrasonic energy to clean objects, including **medical** and **surgical instruments** is a very common practice in a hospital environment. Importantly, such sophisticated techniques have also been exploited from a highly sensitive sterile-zone of an **'operation theatre'** in a hospital to the **'chemical laboratory'** for the benefit of 'research chemists' as well. The ultimate and final removal of 'trace residues' from previously treated and cleaned glass apparatus may be accomplished by **ultrasonic bath** having various capacities ranging from 2.7 to 85 litres, and the tank fluid in **Decon 90**.

***Ultrasonic**. Pertaining to sounds of frequencies above approximately 20,000 cycles per second, which are inaudible to the human ear.

Note: It is important to **warn** here that all apparatus essentially loaded with gross impurities must **not** be cleaned in these high-tech baths for obvious reasons because the 'tank fluid' shall become profusely contaminated thereby minimising its overall efficiency to a significant extent.

Advantage. One of the major and most crucial functional utilities of **ultrasonic baths** is their excellent and remarkable ability to loosen difficult and rather stubborn ground-glass joints when these get 'fused' on account of degraded chemical contaminants or a prolonged neglet by an user.

Drying of cleaned laboratory glasswares. There are, the fact, two different sizes of glass apparatus one invariably comes across in a **chemical laboratory,** for instance:

a. small; and
b. large and bulky.

a. **Small Apparatus.** These are thoroughly cleaned and rinsed with distilled water and kept in an electrically heated oven, preferably having an inside chamber and trays made up of stainless steel, previously maintained at 100–120°C for a duration of 60 minutes.
b. **Large and Bulky Apparatus.** There are quite a few really large and bulky apparatus which fail to enter an oven for drying or sometimes needed soon after washing for urgent experimental operations. Therefore, other viable, effective and convenient means of drying such large and bulky apparatus have been devised duly, such as:
   (i) In case, the apparatus is wet with water, the latter is removed to the maximum extent and subsequently rinsed with small quantity of either acetone or industrial spirit.

Note: For the sake of economising on solvents the aqueous acetone or industrial spirit are collected separately and stored in labelled 5 litre HDPE bottles for future recovery by distillation are re-cycled usage.

(ii) The final drying is afforded by the help of Hot-Air-Blower* (supplied by Gallenkamp).

5. After-Hours Working

Dedicated and diligent 'research chemist' may have to work late in the evening or in the night to complete the on-going reactions that invariably requires close supervision or monitoring. In such instances, it is absolutely necessary and a must that at least two persons should be physically present in a **chemical laboratory** particularly in after-hours working. Personal harmonious understanding amongst the chemists working in a laboratory is equally important and vital whereby one may look after simple operations, such as refluxing, evaporations on a water- bath, digestion, distillation, column chromatography, soxhlet extraction and the like. In such instances, clear written instructions must be communicated so that the other chemist can stop the experiment when it is either over or in an emergency.

6. Guidelines for Accident or Injury

Each and every individual working in a **chemical laboratory** must be fully aware about the location of the fire escapes and exits; and also ensure that there is no obstacle or restrictions ***Hot-Air-Blower**. A sturdy, heavy duty power-driven blower that functions on a simple principle i.e., it draws air through a filter, passes it through a heater, and forces it upwards through pointing tubes that hold the apparatus. to them. It is also important that all chemists of either gender must know the exact positions of the '**Fire Extinguishers**'*, fire-blankets, and drench showers, and should make sure how they are made operational. (**Caution**: The checking of such equipment(s) should be carried out periodically and duly certified by the appropriate authorities). Each chemical laboratory must-clearly display such available facilities at strategically located positions, namely: first-aid equipment, nearest telephone, emergency medical team(s), hospital(s), and fire brigade(s), so that in the event of an accident and immediate action is feasible. Besides, all these gospel truths one should always exercise the utmost presence of mind in any accident big or small.

Burning Chemicals and Clothing. Accidental fire from highly inflammable organic solvents is observed to be one of the most common and equally dangerous fire hazards in a chemical laboratory. In

case the fire is exclusively limited to a small vessel, such as: beaker or china-dish or flask then cover it instantly with an asbestos-wire-gauze so as to cut off the air containing oxygen to the burning solvent. Because, most of the inflammable organic solvents are actually having lesser density than water; therefore, **water should never be employed to extinguish fire**. However, ordinary bucket-of-sand is invariably useful for small fire incidents; and for comparatively larger fire cases a fire-extinguisher should be put into action. Of course, for fires beyond reasonable control, first the fire alarm must be triggered, and **immediately** the **fire-brigade** summoned without a second thought. In such circumstances when one's clothes catch fire due to the splash of burning organic solvents, the victim should be immediately made to roll over on the ground to extinguish the fire or he/she must be covered instantly with a **fire-blanket**. (**Note:** Any type of fire-extinguisher must not be used on a person). **Minor Injuries**. Minor injuries on palm or fingers on either hands are usually inflicted due to sharp broken edges of laboratory glass tubings or glasswares. The exposed or cut should be thoroughly flushed under a running cold-water tap, excess water removed, applied with an antibiotic cream, and covered with a suitable bandage. In the event, when one receives a deep and serious cut, an immediate medical assistance must be sought for adequate specialized attention, such as: stitching (under local anaesthetic conditions), medication with an antiseptic cream, pain-killing tablets, and lastly an **anti-tetanus** toxoid injection**. Likewise, minor burns caused either by hot equipment or corrosive chemicals, e.g., caustic, concentrated mineral acids, liquid bromine and the like, are observed to be a routine laboratory hazards. Simply flush out the excessive chemicals from the affected area with cold running water or sometimes even ice-cold water, and subsequently ask for due medical assistance.

7. Storage of Chemicals/Reagents in a Chemical Laboratory

All 'research chemists' are required to use various types of chemicals and reagents as cautiously and carefully as possible, and subsequently return them to their properly designated cupboards, **Fire Extinguisher.** A device for discharging liquid chemicals or foam to extinguish a fire. **Tetanus**. An acute infectious disease of the central nervous system caused by an exotoxin of the tetanus bacillus, Clostridium tetani. shelves or chemical stores soonafter their use. It is pertinent to state here that chemicals, in general, should never be allowed to accumulate either in fume cupboards or on working benches so as to avoid possible uncalled for inconveniences that may ultimately lead to possible accidents or spillages. Importantly, the following standard norms and regulations with regard to the storage of chemicals/reagents in a chemical laboratory should be observed rigidly and strictly:

(i) Bulky containers and bottles of dangerous and highly inflammable and corrosive chemicals must be returned to the main chemical store immediately which is governed exclusively by specific regulations for safe storage.

(ii) Each specific chemical laboratory is under strict regulations with regard to the storage of solvents, and that too in a specially designed fire-proof steel cabinet fitted with a vapour-seal door. Furthermore, such an area should be duly assigned and adequately equipped for the safe issue of toxic, corrosive and flammable solvents and reagents.

(iii) Transportation of innocuous or dangerous chemicals stored in properly capped Winchester bottles for a short distance must be **duly supported both at the base and at the neck, and never at only one of these critical places**. However, for longer distances the specially designed movable safety carriers that are commonly available must always be used.

(iv) Hazard code or hazard symbol should be positively imprinted on a container into which the chemical or reagent has been transferred from a bulk container. Besides, the **'label'** must essentially bear such informations as: nature of the contents, risk and safety summaries stating clearly the possible danger linked with the contents.

(v) **Proper Labelling of Reagents and Chemicals.** In a chemical laboratory all usable reagent bottles and chemicals must be labelled clearly and explicitely either with computerized labels, typed labels or neat hand-written labels. In such instances where the containers have lost their

labels, their contents must be identified positively and relabelled accordingly; should there be an iota of doubt, the material must be disposed of immediately and safely. It has been found frequently that the gummed labels peel off rapidly; hence, it is always preferable to seal them to the bottle or container with a good quality adhesive tape. As there are good many chemicals that are found to deteriorate with age; therefore, it is always better to inscribe on the label itself indicating the exact date of its manufacture.

8. Toxicity and Hazards of Chemicals/Reagents

A human being handles chemicals directly or indirectly, in one form or the other, whether it is in the **chemical laboratory** or in the house or contracted from a contaminated atmosphere. Invariably, a large number of chemicals are not only hazardous in nature but also toxic potentially. Toxicity usually refers to the inherrent property of a substance to cause injury on reaching either in an organism or a susceptible site. Innumerable chemical substances that one normally happens to come across in a laboratory may produce undesirable harmful effects by inhalation, ingestion or absorption through the skin. In the light of the above stark naked reality about the wide spectrum of chemical substances known till date one must handle them with utmost care and precaution so as to avoid any possible threat to one's health in particular and one's life in general.

Units for Expressing Concentration:

Concentration is a general measurement unit stating the amount of solute present in a known amount of solution

$$\text{Concentration} = \frac{\text{Amount of Solute}}{\text{Amount of Solution}}$$

Althtough the terms "solute" and "solution" are often associated with liquid samples, they can be extended to gas-phase and solid-phase samples as well. The actual units for reporting concentration depend on how the amounts of solute and solution are measured. The following table lists the most common units of concentration.

Common Units for Reporting Concentration

Name and symbols	Units
molarity (M)	*moles solute/liters solution*
formality (F)	*number Formula wt solute/liters solution*
normality (N)	*number Equivalent wt solute/liters solution*
molality (m)	*moles solute/kg solvent*
weight % (% w/w)	*g solute/100 g solution*
volume % (% v/v)	*ml solute/100 ml solution*
weight-to-volume % (% w/v)	*g solute/100 ml solution*
parts per million(ppm)	*g solute/10^6 g solution*
parts per billion (ppb)	*g solute/10^9 g solution*

Molarity and Formality: Both molarity and formality express concentration as moles of solute per liter of solution. There is, however, a subtle difference between molarity and formality. **Molarity** is the concentration of a particular chemical species in solution. **Formality,** on the other hand, is a substance's total concentration in solution without regard to its specific chemical form. There is no difference between a substance's molarity and formality if it dissolves without dissociating into ions. The

molar concentration of a solution of glucose, for example, is the same as its formality. For substances that ionize in solution, such as NaCl, molarity and formality are different. For example, dissolving 0.1 mol of NaCl in 1 L of water gives a solution containing 0.1 mol of Na and 0.1 mol of Cl^-. The molarity of NaCl, therefore, is zero since there is essentially no undissociated NaCl in solution. The solution, instead, is 0.1 M in Na and 0.1 M in Cl^-. The formality of NaCl, however, is 0.1 F because it represents the total amount of NaCl in solution. The rigorous definition of molarity, for better or worse, is largely ignored in the current literature, as it is in this text. When we state that a solution is 0.1 M NaCl we understand it to consist of Na and Cl^- ions. The unit of formality is used only when it provides a clearer description of solution chemistry. Molar concentrations are used so frequently that a symbolic notation is often used to simplify its expression in equations and writing. The use of square brackets around a species indicates that we are referring to that species' molar concentration. Thus, [Na] is read as the "molar concentration of sodium ions."

Normality

Normality is an older unit of concentration that, although once commonly used, is frequently ignored in today's laboratories. Normality is still used in some handbooks of analytical methods, and, for this reason, it is helpful to understand its meaning. For example, normality is the concentration unit used in Standard Methods for the Examination of Water and Wastewater,1 a commonly used source of analytical methods for environmental laboratories. **Normality** makes use of the chemical equivalent, which is the amount of one chemical species reacting stoichiometrically with another chemical species. Note that this definition makes an equivalent, and thus normality, a function of the chemical reaction in which the species participates. Although a solution of H_2SO_4 has a fixed molarity, its normality depends on how it reacts. The number of **equivalents,** n, is based on a reaction unit, which is that part of a chemical species involved in a reaction. Normality is the number of **equivalent weights** (EW) per unit volume and, like formality, is independent of speciation. An equivalent weight is defined as the ratio of a chemical species' **formula weight** (FW) to the number of its equivalents(EW = FW/n).
Consequently, the following simple relationship exists between normality and molarity.

$$N = n * M$$

Molality: Molality is used in thermodynamic calculations where a temperature independent unit of concentration is needed. Molarity, formality and normality are based on the volume of solution in which the solute is dissolved. Since density is a temperature dependent property a solution's volume, and thus its molar, formal and normal concentrations, will change as a function of its temperature. By using the solvent's mass in place of its volume, the resulting concentration becomes independent of temperature.

Weight, Volume, and Weight-to-Volume Ratios

Weight percent (% w/w), **volume percent** (% v/v) and **weight-to-volume percent:** (% w/v) express concentration as units of solute per 100 units of sample. A solution in which a solute has a concentration of 23% w/v contains 23 g of solute per 100 ml of solution.

Parts per million (ppm) and **parts per billio**n (ppb) are mass ratios of grams of solute to one million or one billion grams of sample, respectively. For example, a steel that is 450 ppm in Mn contains 450 µg of Mn for every gram of steel. If we approximate the density of an aqueous solution as 1.00 g/ml, then solution concentrations can be expressed in parts per million or parts per billion using the following relationships. For gases a part per million usually is a volume ratio. Thus, a helium concentration of 6.3 ppm means that one liter of air contains 6.3 µL of He.

Basic Equipment and Instrumentation

Measurements are made using appropriate equipment or instruments. The array of equipment and instrumentation used in analytical chemistry is impressive, ranging from the simple and inexpensive, to the complex and costly. With two exceptions, we will postpone the discussion of equipment and instrumentation to those chapters where they are used. The instrumentation used to measure mass and much of the equipment used to measure volume are important to all analytical techniques and are therefore discussed in this section.

Instrumentation for Measuring Mass

An object's mass is measured using a balance. The most common type of balance is an electronic balance in which the balance pan is placed over an electromagnet. The sample to be weighed is placed on the sample pan, displacing the pan downward by a force equal to the product of the sample's mass and the acceleration due to gravity. The balance detects this downward movement and generates a counterbalancing force using an electromagnet. The current needed to produce this force is proportional to the object's mass. A typical electronic balance has a capacity of 100–200 g and can measure mass to the nearest ±0.01 to ±1 mg. Another type of balance is the single-pan, unequal arm balance. In this mechanical balance the balance pan and a set of removable standard weights on one side of a beam are balanced against a fixed counterweight on the beam's other side. The beam itself is balanced on a fulcrum consisting of a sharp knife edge. Adding a sample to the balance pan tilts the beam away from its balance point. Selected standard weights are then removed until the beam is brought back into balance. The combined mass of the removed weights equals the sample's mass. The capacities and measurement limits of these balances are comparable to an electronic balance,adding a sample moves the balance pan down, allowing more light to reach the detector. The control circuitry directs the electromagnetic servomotor to generate an opposing force, raising the sample up until the original intensity of light at the detectoris restored.

The mass of a sample is determined by difference. If the material being weighed is not moisture-sensitive, a clean and dry container is placed on the balance. The mass of this container is called the tare. Most balances allow the tare to be automatically djusted to read a mass of zero. The sample is then transferred to the container, the new mass is easured and the sample's mass determined by subtracting the tare. Samples that absorb moisture from the air are weighed differently. The sample is placed in a covered weighing bottle and their combined mass is determined. A portion of the sample is removed, and the weighing bottle and remaining sample are reweighed. The difference between the two masses gives the mass of the transferred sample. Several important precautions help to minimize errors in measuring an object's mass. Balances should be placed on heavy surfaces to minimize the effect of vibrations in the surrounding environment and should be maintained in a level position. Analytical balances are sensitive enough that they can measure the mass of a fingerprint. For this reason, materials placed on a balance should normally be handled using tongs or laboratory tissues. Volatile liquid samples should be weighed in a covered container to avoid the loss of sample by evaporation. Air currents can significantly affect a sample's mass. To avoid air currents, the balance's glass doors should

be closed, or the balance's wind shield should be in place. A sample that is cooler or warmer than the surrounding air will create convective air currents that adversely affect the measurement of its mass. Finally, samples dried in an oven should be stored in a desiccator to prevent them from reabsorbing moisture from the atmosphere.

Equipment for Measuring Volume

Analytical chemists use a variety of glassware to measure volume, several examples of which are shown in. The type of glassware used depends on how exact the volume needs to be. Beakers, dropping pipets, and graduated cylinders are used to measure volumes approximately, typically with errors of several percent. Pipets and volumetric flasks provide a more accurate means for measuring volume. When filled to its calibration mark, a **volumetric flask** is designed to contain a specified volume of solution at a stated temperature, usually 20ºC. The actual volume contained by the volumetric flask is usually within 0.03–0.2% of the stated value. Volumetric flasks containing less than 100 ml generally measure volumes to the hundredth of a milliliter, whereas larger volumetric flasks measure volumes to the tenth of a milliliter. For example, a 10-ml volumetric flask contains 10.00 ml, but a 250 ml volumetric flask holds 250.0 ml (this is important when keeping track of significant figures). Because a volumetric flask contains a solution, it is useful in preparing solutions with exact concentrations. The reagent is transferred to the volumetric flask, and enough solvent is added to dissolve the reagent. After the reagent is dissolved, additional solvent is added in several portions, mixing the solution after each addition. The final adjustment of volume to the flask's calibration mark is made using a dropping pipet. To complete the mixing process, the volumetric flask should be inverted at least ten times.

A **pipet** is used to deliver a specified volume of solution. Several different styles of pipets are available. Transfer pipets provide the most accurate means for delivering a known volume of solution; their volume error is similar to that from an equivalent volumetric flask. A 250 ml transfer pipet, for instance, will deliver 250.0 ml. To fill a transfer pipet, suction from a rubber bulb is used to pull the liquid up past the calibration mark (never use your mouth to suck a solution into a pipet). After replacing the bulb with your finger, the liquid's level is adjusted to the calibration mark, and the outside of the pipet is wiped dry. The pipet's contents are allowed to drain into the receiving container with the tip of the pipet touching the container walls. A small portion of the liquid remains in the pipet's tip and should not be blown out. Measuring pipets are used to deliver variable volumes, but with less accuracy than transfer pipets. With some measuringpipets, delivery of the calibrated volume requires that any solution remaining in the tip be blown out. Digital pipets and syringes can be used to deliver volumes as small as a microliter.

Three important precautions are needed when working with pipets and volumetric flasks. First, the volume delivered by a pipet or contained by a volumetric flask assumes that the glassware is clean. Dirt and grease on the inner glass surface prevents liquids from draining evenly, leaving droplets of the liquid on the container's walls. For a pipet this means that the delivered volume is less than the calibrated volume, whereas drops of liquid above the calibration mark mean that a volumetric flask contains more than its calibrated volume. Commercially available cleaning solutions can be used to clean pipets and volumetric flasks. Second, when filling a pipet or volumetric flask, set the liquid's level exactly at the calibration mark. The liquid's top surface is curved into a **meniscus**, the bottom of which should be exactly even with the glassware's calibration mark . The meniscus should be adjusted with the calibration mark at eye level to avoid parallax errors. If your eye level is above the calibration mark the pipet or volumetric flask will be overfilled. The pipet or volumetric flask will be underfilled if your eye level is below the calibration mark. Finally, before using a pipet or volumetric flask you should rinse it with

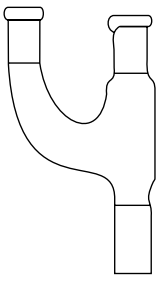
several small portions of the solution whose volume is being measured. This ensures that any residual liquid remaining in the pipet or volumetric flask is removed.

Equipment for Drying Samples

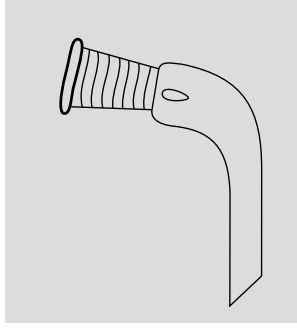
Many materials need to be dried prior to their analysis to remove residual moisture. Depending on the material, heating to a temperature of 110–140°C is usually sufficient. Other materials need to be heated to much higher temperatures to initiate thermal decomposition. Both processes can be accomplished using a laboratory oven capable of providing the required temperature. Commercial laboratory ovens are used when the maximum desired temperature is 160–325°C (depending on the model). Some ovens include the ability to circulate heated air, allowing for a more efficient removal of moisture and shorter drying times. Other ovens provide a tight seal for the door, allowing the oven to be evacuated. In some situations a conventional laboratory oven can be replaced with a microwave oven. Higher temperatures, up to 1700°C, can be achieved using a muffle furnace. After drying or decomposing a sample, it should be cooled to room temperature in a desiccator to avoid the re adsorption of moisture. **A desiccator** is a closed container that isolates the sample from the atmosphere. A drying agent, called a **desiccant,** is placed in the bottom of the container. Typical desiccants include calcium chloride and silica gel.

A perforated plate sits above the desiccant, providing a shelf for storing samples. Some desiccators are equipped with stopcocks that allow them to be evacuated.

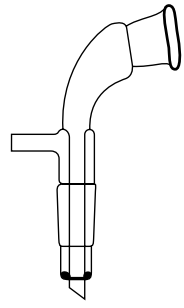
Adaptors: These are used normally to facilitate the delivery of distillate from condenser to the receiver. Vacuum can also be applied to adaptors if needed.



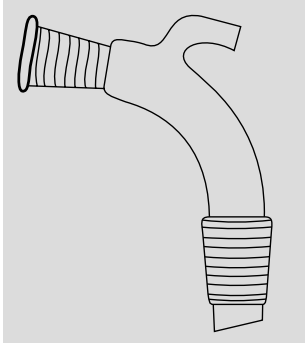
Double necked adapter



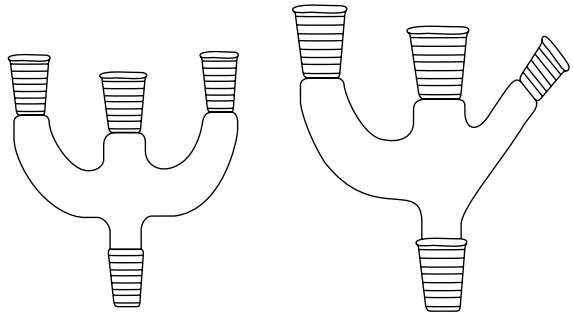
One joint receiver adapter



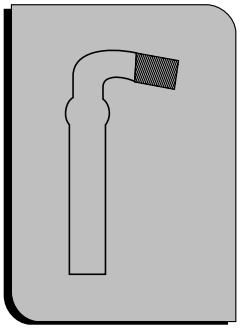
Two joint receiver adapter for vacuum



Two joint receiver adapter



Triple necked adapter



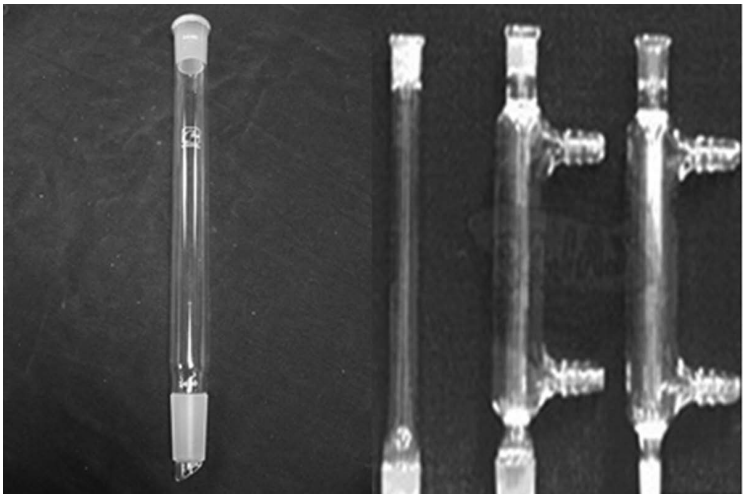
Calcium chloride guard tube

Guard tube: It is filled with anhydrous calcium chloride keeping the cotton plug at both the ends at the bent, it is widely used to protect the substance or assemblies of apparatus from moisture by attaching it to the apparatus.

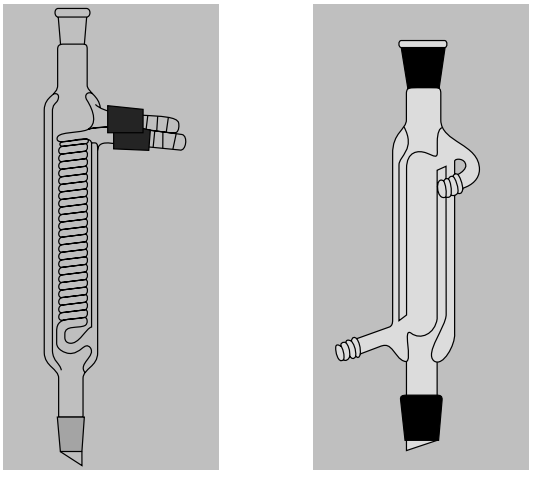
Condensors: The condensers are used for refluxing and ordinary distillation when the mixture of liquids have boiling point close to each other. The distillation is carried out using condensers. These provide large surface area for up going vapors reach ultimately into condensers and less volatile vapors are condensed on a large scale surface of the column and are returned to distillation flask

There are two types of condensers

1. Water condensers
2. Air condensers



Air condensers

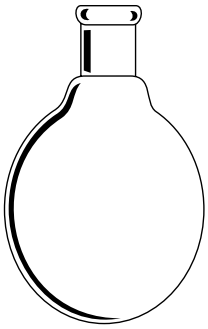


Double surface condensers

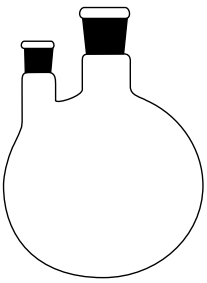
Flasks: There are common types of flasks used for a variety of purpose. They are employed for refluxing and distillation, Erlenmeyer flask used for titration.

Flasks are of following types:

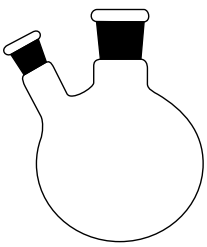
1. Round bottom flask.
2. Flat bottom flask
3. Volumetric flask
4. Long neck flask
5. Conical flask
6. Iodine flask



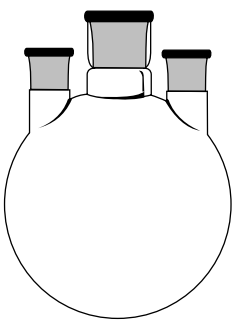
Round Bottom Flask single Necked



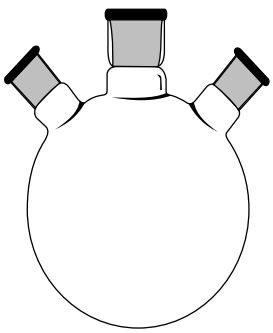
Round Bottom Flask (Two Necked)
Side Neck parallel



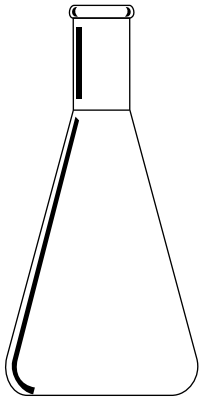
Round Bottom Flask (Two Necked)
Side Neck inclined.



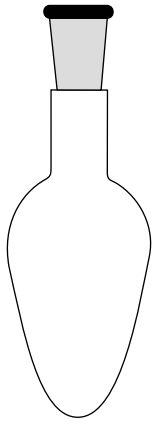
Round bottom flask (Three necked)
Side neck parellel



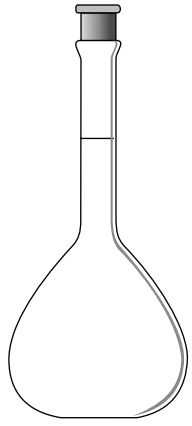
Round bottom flask (Three necked)
side neck inclined



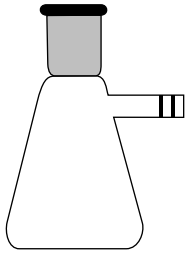
Concal/ Erlenmeyer flask



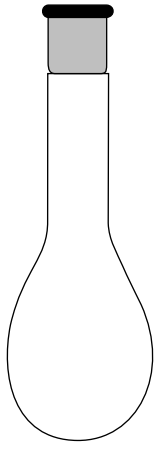
Pear shaped Flask



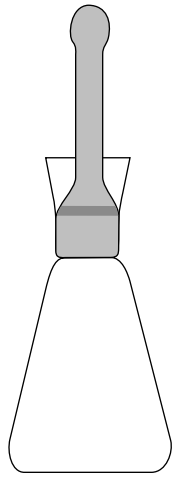
Volumetric flask



Conical flask with side tubulation



Kjeldal flask



Iodine flask

Beaker: It is a cylindrical glass ware vessel with flat bottom. A small spout provides to make the liquid flow without spilling. Beakers are of different capacities from 100–1000 ml are available volumetric solution are often taken in to beaker. The volume of beaker is noted on surface of it.

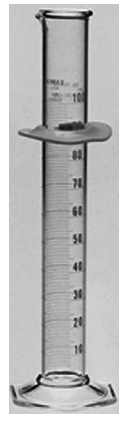
Beaker

Measuring cylinder: It is a cylindrical tube made up of thick glass and is marked in ml. They are available in various capacity, commonly employed measuring cylinder are 10 ml, 50 ml, 100 ml, 250 ml etc. They are used to measure definite volume of liquids.

Funnels: Funnels are used extensively in the synthesis for filtration of products.

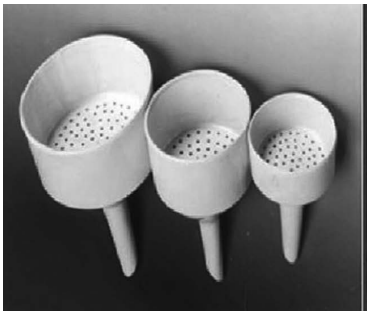
Types of funnels:

1. Ordinary funnel



Measuring cylinder

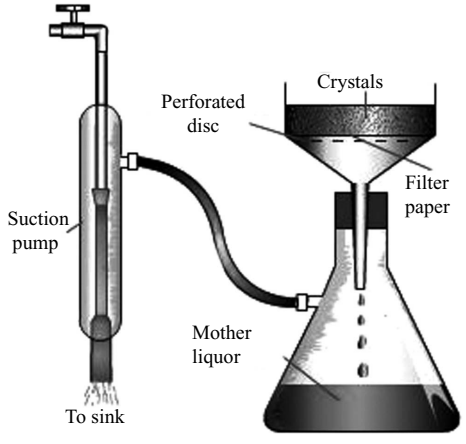
2. Buchner funnel
3. Separating funnel



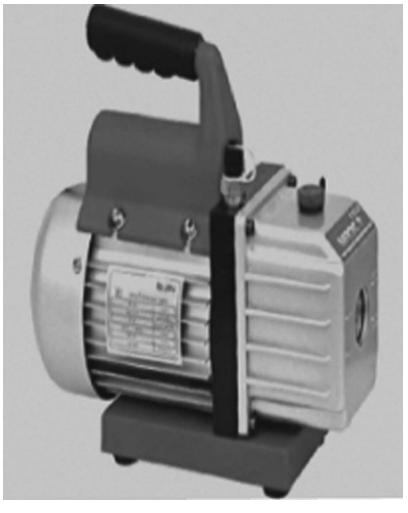
Buchner funnels

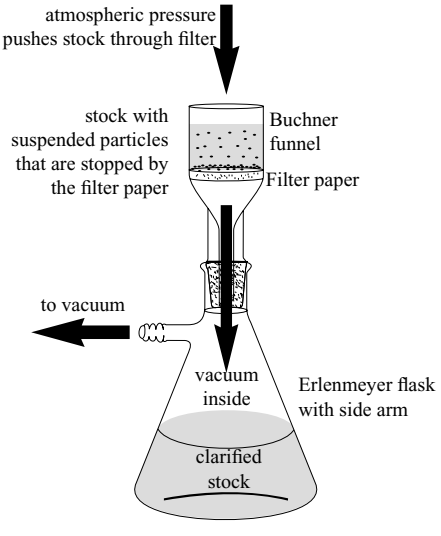
Sinterd glass funnels

Seperating funnel



Filtration by suction pump





Filtration by vacuum pump

Vacuum or Suction Filtration

The pure crystals generated by recrystallization can be collected by vacuum filtration. In this process a Buchner funnel is in a filter flask with a rubber adapter fitting between the two. (A Hirsch funnel can be used for samples smaller than a gram. A circle of filter paper is placed inside the funnel. The paper should be big enough to cover the holes in the funnel but small enough to lay flat. The filter flask should be connected to a source of vacuum with thick wall tubing (if the tubing collapses under vacuum you've got the wrong tubing!). A trap should be placed between the filter flask and the source of vacuum if there is any chance of vapor or liquid being drawn into the vacuum line. A clamp and ring stand can be used to secure the flask so that it will not fall over.

Once the vacuum filtration setup is assembled add a small amount of the solvent or supernatant to moisten the filter paper. Turn on the vacuum to seal the paper against the funnel and pour the material to be filter into the funnel evenly. Make sure the filter flask is never more than half full. If this occurs turn off the vacuum and pour out the filtrate into another flask. This filtrate can also be used to rinse any remaining crystals into the funnel. After removing the filtrate, the cold wash liquid can be poured into the funnel with the vacuum off. After 1–2 minutes turn on the vacuum and leave it on for several minutes to dry the crystals.

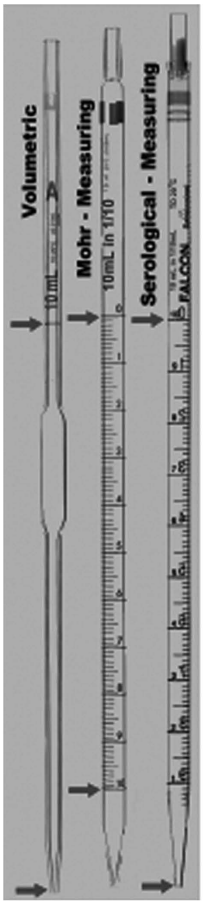
Desicators: It is a covered glass container designed for storage of compounds in a dry atmosphere. It usually contains drying agents in lower part and separated by means of circular porcelain plate having holes. Drying agents used in alimentary work are anhydrous $CaCl_2$, silica gel, activated aluminum. It should be noted that a substance can't be dried by desiccators whose vapor pressure is greater than that of substance. They can be classified in two types

1. Ordinary desiccators
2. Vacuum desiccators

Ordinary desiccator

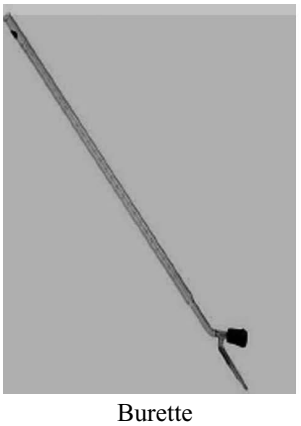
Vacuum desiccator

Pippete: It is a glass tube which in flattened at the centre to bear volume of liquid as marked on it or it may be cylindrical with graduation.

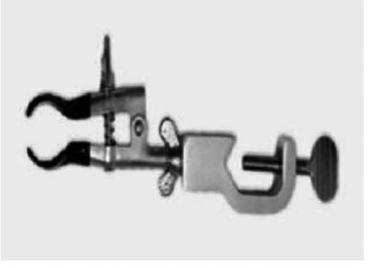


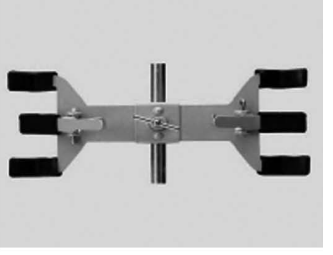
Volumetric Pipettes Dropping pipette

Burette: It is a long graduated with a stop cork or pinch cork at one end. Burette is made of glass or poly vinyl chloride, available in different volume each ml of volume of liquid can be read on the graduated surface of burette one can transfer or measure desired volume of liquid using burette.

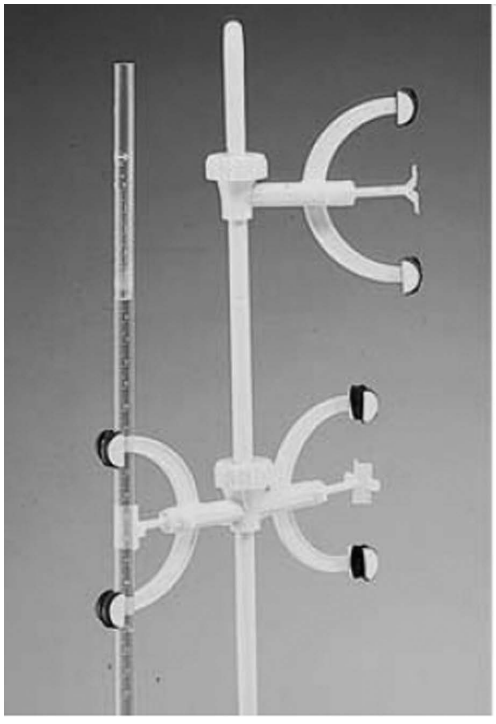


Burette

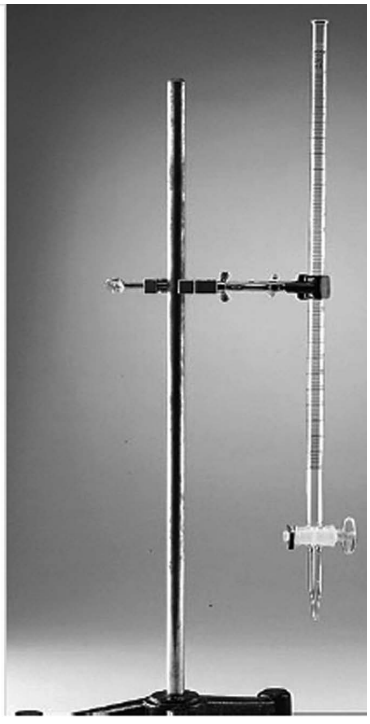




Clamps

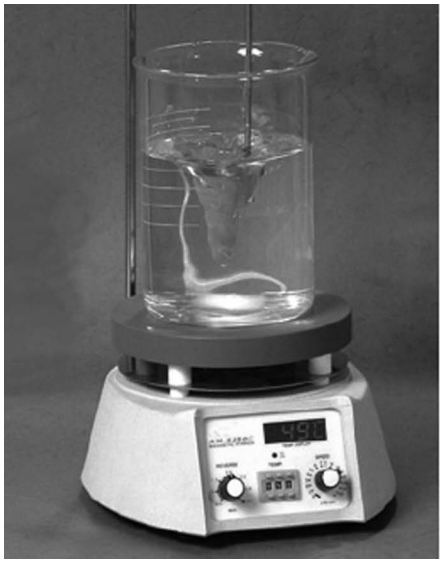


Burette stand with double clamp

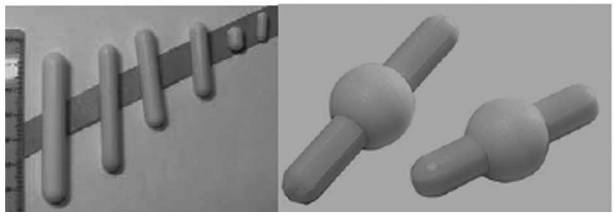


Burette stand with single clamp

Magnetic stirrer: A magnetic stirrer is useful for a small quantities and non-viscous reactive mixtures. The stirring is achieved by magnetic stirrer bar, which is added to a reaction mixture.



Magnetic Stirrer



Magnetic beads

Water bath: It is a source of heating for temperature around 100ºC which consists of a series of concentric rings to accommodate different sizes of flasks and beakers.

Water bath circular type

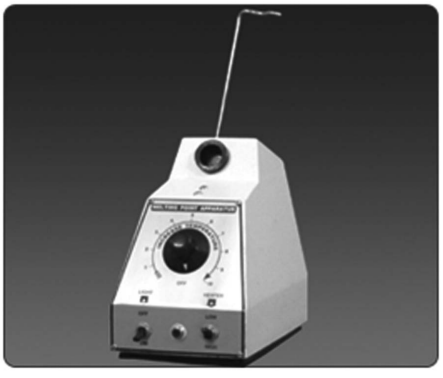
Water bath rectangular type with five holes

Heating mantle: It is a method of electrical heating,the rate of heating is controlled by variable voltage transformer

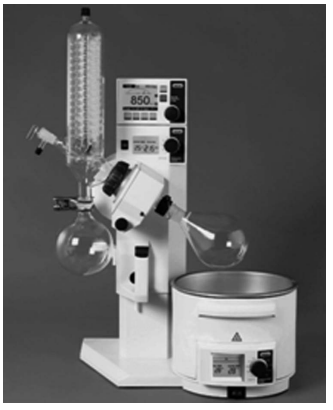
Heating mantle

Multiple Heating mantle

Melting point apparatus: It is used to determine the melting point of various organic compounds. The sample is placed into the sealed capillary tube and placed into the small hole provided. Thermometer is placed in the hole provided with the support of steel rod.

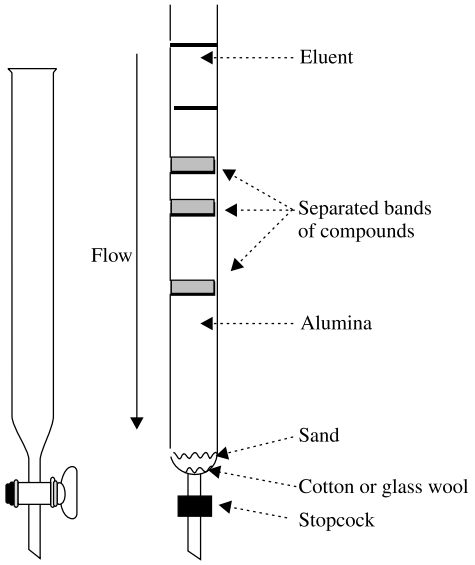


Rotary film evaporator: It is employed for the removal of solvents from the solution of organic compound under redused pressure and at low temperature by using a vaccuum pump.



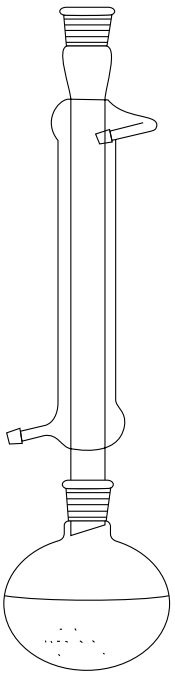
Rotary film evaporator

Column: It is a long narrow glass tube used for separation of mixture into its components.

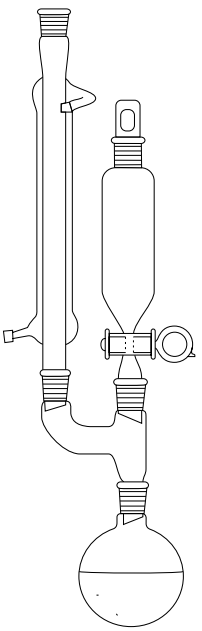


Columns with stop cock

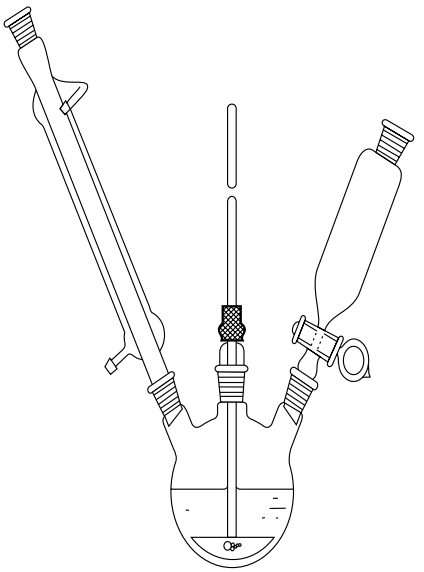
Apparatus used for different types of reactions:



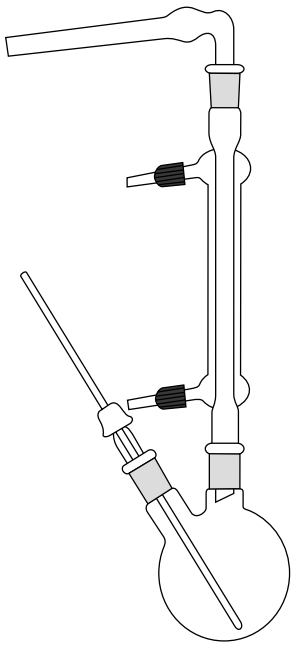
Assembly for heating a reaction mixture under reflux



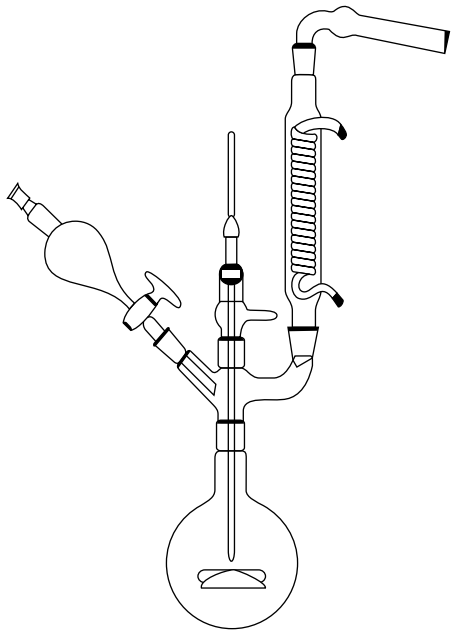
Assembly for heating a reaction mixture under reflux with addition of liquid



Assembly for heating a reaction mixture under reflux with the addition of liquid and with stirring



Apparatus for reaction under reflux with a guard tube



Apparatus for reaction under reflux with a guard tube, with addition of liquid and with stirring.

Distillation

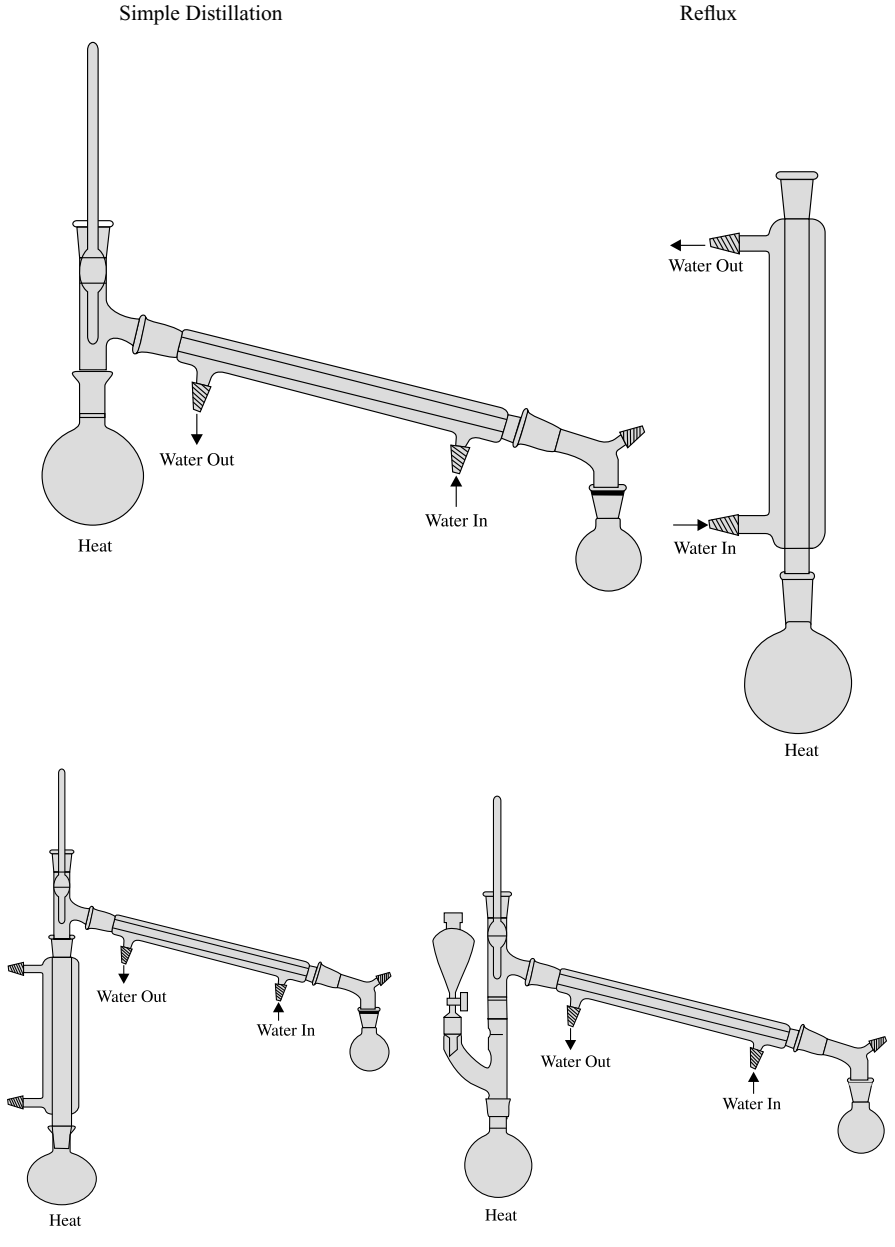
When pure water is heated in the distillation apparatus shown in below figure, there is an increased tendency for molecules to escape from the surface of the liquid. Thus, the vapor pressure of the liquid increases until it becomes equal to the atmospheric pressure and the liquid begins to boil. Continued heating supplies the heat of vaporization necessary for further conversion of liquid to gas. Thus, vapors rise, warm the still head, and begin flowing into the condenser, which is cooled by water. Vapors passing through the condenser are therefore cooled to give a liquid condensate, the **distillate**, which can be collected, in a receiving flask.

Distillation should be done steadily and at such a rate that the thermometer bulb always carries a drop of condensate and is bathed in a flow of vapor. Liquid and vapor are then in equilibrium around the bulb, and the temperature registered is the true boiling point of the liquid. If excessive heat is applied, the vapor becomes superheated, the drop disappears, the liquid-vapor equilibrium is upset, and the temperature rises above the boiling point.

Since all of the heat being supplied isn't immediately dissipated by vaporization, some superheating of the liquid may occur. A thermometer immersed in the boiling liquid would therefore record a temperature a little above the boiling point, but a thermometer in the vapor space shown in Figure l records the true boiling point, even if the liquid is superheated or if it contains a nonvolatile solvent. For example, when a solution of sugar in water is distilled, the boiling point recorded on a thermometer in the vapor phase is 100 degrees (at 760 mm) throughout the distillation. Whereas the temperature of the boiling liquid is initially somewhat above 100 degrees and continues to rise as the sugar solution becomes more concentrated. The vapor pressure of the sugar solution is dependent upon the number of water and sugar molecules present in a given volume. Hence, the vapor pressure at any given temperature decreases with increasing concentration of nonvolatile sugar molecules and decreasing

concentration of water, and a higher temperature is required for boiling. However, sugar molecules do not leave the solution, and the drop clinging to the thermometer bulb is pure water in equilibrium with pure water vapor.

When a distillation is done in a system open to air, the boiling point is the temperature at which the pressure of the boiling liquid equals that of the atmosphere. The prevailing barometric pressure should be noted and allowance should be made



Note: Be sure to support or clamp all round bottom flasks and grease all joints. Make sure all joints are sealed and that cooling water goes in the lower entrance of the condenser and exits the upper entrance. Secure the exit hose in a cup sink to prevent floods in the lab. In Figures 1–4 all the condensers are water cooled except the vertical one in figure 3, the fractionating column. For appreciable deviations from the normal pressure of 760 mm by reference to a table like Table I, distillation can also be done under a vacuum created by an oil or water pump with substantial reduction of boiling point.

Preparing Solutions

Preparing a solution of known concentration is perhaps the most common activity in any analytical lab. The method for measuring out the solute and solvent depend on the desired concentration units, and how exact the solution's concentration needs to be known. Pipets and volumetric flasks are used when a solution's concentration must be exact; graduated cylinders, beakers, and reagent bottles suffice when concentrations need only be approximate. Two methods for preparing solutions are described in this section.

Preparing Stock Solutions

A **stock solution** is prepared by weighing out an appropriate portion of a pure solid or by measuring out an appropriate volume of a pure liquid and diluting to a known volume. Exactly how this is done depends on the required concentration units. For example, to prepare a solution with a desired molarity you would weigh out an appropriate mass of the reagent, dissolve it in a portion of solvent, and bring to the desired volume. To prepare a solution where the solute's concentration is given as a volume percent, you would measure out an appropriate volume of solute and add sufficient solvent to obtain the desired total volume.

stock solution: A solution of known concentration from which other solutions are prepared.

Volumetric reagents and solutions: Volumetric solutions, also known as standard solutions, are solutions of reagents of known concentrations intended primarily for use in quantitative determinations. Concentrations are usually expressed in terms of molarity (M).

Molar Solutions: A molar solution contains 1 g molecule of the reagent in 1000 ml of the solution. Thus, each litre of a molar solution of sodium nitrite contains 69.0 g of $NaNO_2$ and each litre of a molar solution of disodium edetate contains 372.2 g of $C_{10}H_{14}N_2Na_2O_8$, $2H_2O$. Solutions containing one-tenth of a gram-molecule of the reagent in 1000 ml are designated as 'tenth-molar' or 0.1 M; other molarities are similarly indicated.

Blank determinations: Where it is directed that "any necessary correction" be made by a blank determination, the determination should be done using the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under examination but omitting the substance under examination. In a blank titration, the assay is carried out, then repeated without any sample being present. This appears, at first sight, to be a perfect waste of time, but determinations of this type allow the analyst to measure any changes that occur to the reagent during the course of the assay. If the procedure involves heating and subsequent cooling of the sample (e.g. to allow the sample to dissolve), some of the volumetric reagent may be lost either by evaporation or mechanically due to splashing or bubbling. The blank determination must be identical to the test determination in every way except, of course, that there is no sample in the blank. This means that heating times, dilutions, etc. must all be duplicated exactly.

Back titrations: In the example above, a reaction was chosen that was quick to carry out and was quantitative, i.e. it went to completion. In many pharmaceutical analyses this is not the case and a back titration has to be carried out. Back titrations are often combined with blank titrations, particularly if there is some loss of reagent during the assay (e.g. as a result of splashing or vigorous boiling) or the concentration of a volumetric reagent changes during the assay. A back titration involves addition of a known excess of reagent to the sample (this drives the reaction to completion) and titration of the

unreacted excess of reagent with a suitable titrant. The volume thatreacted with the sample is determined by simple subtraction. For example, if 50.0 ml of reagent were added to the sample and the back titre was 30.0 ml then, clearly, 20.0 ml of reagent has reacted with the sample.

Primary standards: These are materials which, after drying under the specified conditions, are recommended for use as primary standards in the standardisation of volumetric solutions. The following are recommended for use as primary standards.

Benzoic acid: Sublime benzoic Acid in an appropriate apparatus and store in a tightly- closed container.

Potassium Dichromate: Heat potassium dichromate to 140–150°in an oven, cool in a desiccator and powder in a glass mortar.

Potassium Hydrogen Phthalate: Recrystallise potassium hydrogen phthalate from boiling water, collect the crystals at a temperature above 35° and dry to constant weight at 110°. Store in a tightly-closed container.

Sodium Carbonate, Anhydrous: Filter at room temperature a saturated solution of sodium carbonate. Introduce slowly into the filtrate a stream of carbon dioxide, with constant cooling and stirring. After about 2 hours, collect the precipitate on a sintered glass filter. Wash the filter with ice-cold water saturated with carbon dioxide. After drying at 100–105°, heat to constant weight at 270–300°, stirring from time to time. Store in a tightly-closed container.

Sodium Chloride: To 1 volume of a saturated solution of sodium chloride add 2 volumes of hydrochloric acid. Collect the crystals formed and wash with hydrochloric acid. Remove the hydrochloric acid by heating on a water-bath and dry the crystals to constant weight at 300°. Store protected from moisture.

Preparation and Standardisation of Volumetric Solutions: It is not always possible nor is it essential, to prepare volumetric solutions of a desired theoretical molarity. A solution of approximately the desired molarity is prepared and standardised by titration against a solution of a primary standard. The molarity factor so obtained is used in all calculations, where such standardised solutions are employed. As the strength of a standard solution may change upon standing, the molarity factor should be redetermined frequently. Volumetric solutions should not differ from the prescribed strength by more than 10 per cent and the molarity should be determined with a precision of 0.2 per cent. When solutions of a reagent are used in several molarities, the details of the preparation and standardisation are usually given for the most commonly used strength. Stronger or weaker solutions are prepared and standardised using proportionate amounts of the reagent or by making an exact dilution of a stronger solution. Volumetric solutions prepared by dilution should be restandardised either as directed for the stronger solution or by comparison with another volumetric solution having a know ratio to the stronger solution. The water used in preparing volumetric solutions complies with the requirements of the monograph on Purified Water, unless otherwise specified. When used for the preparation of unstable solutions such as potassium permanganate or sodium thiosulphate, it should be freshly boiled and cooled. When a solution is to be used in an assay in which the endpoint is determined by an electrochemical process (e.g. potentiometrically), the solution must be standardised in the same way.

Hydrochloric Acid, 1 M: Dilute 85 ml of hydrochloric acid with water to produce 1000 ml. Standardise the solution in the following manner.

Weigh accurately about 1.5 g of anhydrous sodium carbonate, previously heated at about 270º for 1 hour. Dissolve it in 100 ml of water and add 0.1 ml of methyl red solution. Add the acid slowly from a burette, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool and continue the titration. Heat again to boiling and titrate further as necessary until the faint pink colour is no longer affected by continued boiling.

1 ml of 1 M hydrochloric acid is equivalent to 0.05299 g of Na_2CO_3.

Hydrochloric Acid, 0.5 M Methanolic: Take 40 ml of water in a 1000 ml volumetric flask and slowly add 43 ml of hydrochloric acid. Cool and add methanol to volume. Standardise the solution in the following manner.

Weigh accurately about 800 mg of anhydrous sodium carbonate, previously heated at about 270º for 1 hour, and proceed as directed under 1 M hydrochloric acid.

Iodine, 0.05 M: Dissolve about 14 g of iodine in a solution of 36 g of potassium iodide in 100 ml of water, add three drops of hydrochloric acid and dilute with water to 1000 ml. Standardise the solution in the following manner.

Weigh accurately about 0.15 g of arsenic trioxide, previously dried at 105º for 1 hour, and dissolve in 20 ml of 1 M sodium hydroxide by warming, if necessary. Dilute with 40 ml of water, add 0.1 ml of methyl orange solution and add dropwise dilute hydrochloric acid until the yellow colour is changed to pink. Add 2 g of sodium carbonate, dilute with 50 ml of water and add 3 ml of starch solution. Titrate with the iodine solution until a permanent blue colour is produced.

1 ml of 0.05 M iodine is equivalent to 0.004946 g of As_2O_3.

Store in amber-coloured, glass stoppered bottles.

Nitric Acid, 1 M: Dilute 63 ml of nitric acid with sufficient water to produce 1000 ml. Standardise the solution in the following manner.

Dissolve 2 g of anhydrous sodium carbonate in 50 ml of water and titrate with the nitric acid solution using methyl orange solution as indicator until the solution becomes reddish yellow. Boil for 2 minutes, cool and continue the titration until the reddish yellow colour is restored.

1 ml of 1 M nitric acid is equivalent to 0.053 g of Na_2CO_3.

Perchloric Acid, 0.1 M: Mix 8.5 ml of perchloric acid with 500 ml of anhydrous glacial acetic acid and 25 ml of acetic anhydride, cool and add anhydrous glacial acetic acid to produce 1000 ml. Allow the prepared solution to stand for 1 day for the excess acetic anhydride to be combined and carry out the determination of water. If the water content exceeds 0.05 per cent , add more acetic anhydride. If the solution contains no titratable water, add sufficient water to obtain a content of water between 0.02 per cent and 0.05 percent. Allow the solution to stand for 1 day and again titrate the water content. The solution so obtained should contain between 0.02 per cent and 0.05 per cent of water. Standardise the solution in the following manner.

Weigh accurately about 0.35 g of potassium hydrogen phthalate, previously powdered lightly and dried at 120º for 2 hours and dissolve it in 50 ml of anhydrous glacial acetic acid. Add 0.1 ml of crystal violet solution and titrate with the perchloric acid solution until the violet colour changes to emerald-green. Peform a blank determination and make any necessary correction.

1 ml of 0.1 M perchloric acid is equivalent to 0.02042 g of $C_8H_5KO_4$.

Other strengths of perchloric acid should be prepared by diluting 0.1 M perchloric acid appropriately with anhydrous glacial acetic acid. In the tests and assays of the Pharmacopoeia, this solution is specified as "0.1 M perchloric acid". Thus the solution in anhydrous glacial acetic acid is to be used unless the words "in dioxan" are stated.

Potassium Hydrogen Phthalate, 0.05 M: Dissolve 10.21 g of potassium hydrogen phthalate in about 800 ml of anhydrous glacial acetic acid, heat on a water-bath until completely dissolved, protected from humidity, cool to 20° and add sufficient anhydrous glacial acetic acid to produce 1000 ml.

Potassium Hydroxide, 0.1 M: Dissolve about 6 g of potassium hydroxide in sufficient carbon dioxide free water to produce 1000 ml. Standardise the solution in the following manner. Titrate 20.0 ml of the solution with 0.1 M hydrochloric acid using 0.5 ml of phenolphthalein solution as indicator. 1 ml of 0.1 M hydrochloric acid is equivalent to 0.005611 g of KOH.

Sodium Hydroxide, 1 M: Dissolve 42 g of sodium hydroxide in sufficient carbon dioxide-free water to produce 1000 ml. Standardise the solution in the following manner.

Weigh accurately about 5 g of potassium hydrogen phthalate, previously powdered and dried at 120° for 2 hours, and dissolve in 75 ml of carbon dioxide-free water. Add 0.1 ml of phenolphthalein solution and titrate with the sodium hydroxide solution until a permanent pink colour is produced. 1 ml of 1 M sodium hydroxide is equivalent to 0.2042 g of $C_8H_5KO_4$.

Store in bottles with well-fitted suitable stoppers which prevent access to atmospheric carbon dioxide. Volumetric solutions of sodium hydroxide must be restandardise frequently. Solutions of lower concentrations are prepared by quantitatively diluting accurately measured volumes of 0.1 M sodium hydroxide with sufficient carbon dioxide-free water to give the desired concentration.

Sodium Hydroxide, 0.1 M Ethanolic: Dissolve 4.2 g of sodium hydroxide in 5 ml of water and add sufficient aldehyde-free ethanol to produce 1000 ml. Allow the solution to stand in a tightly-stoppered bottle for 24 hours. Then quickly decant the clear supernatant liquid into a suitable, tightly-closed container. Standardise the solution in the following manner. Weigh accurately about 0.6 g of benzoic acid, dissolve in a mixture of 30 ml of ethanol (95 per cent) and 6 ml of water and titrate with the ethanolic sodium hydroxide solution, using 0.2 ml of thymolphthalein solution as indicator. 1 ml of 0.1 M ethanolic sodium hydroxide is equivalent to 0.01221 g of $C_7H_6O_2$. Store protected from light and moisture.

Sodium Methoxide, 0.1 M: Cool 150 ml of anhydrous methanol in ice water and add, in small portions, about 2.5 g of freshly cut sodium. When the metal has dissolved, add sufficient toluene, previously dried over sodium wire, to produce 1000 ml. Standardise the solution in the following manner immediately before use. Weigh accurately about 0.4 g of benzoic acid, dissolve in 80 ml of dimethylformamide, add 0.15 ml of thymolphthalein solution and titrate with sodium methoxide solution to a blue end-point. Protect the solution from atmospheric carbon dioxide throughout the titration. Perform a blank determination and make any necessary correction. 1 ml of 0.1 M sodium methoxide is equivalent to 0.01221 g of $C_7H_6O_2$.

Store protected from carbon dioxide and moisture.

Sodium Nitrite, 0.1 M: Dissolve 7.5 g of sodium nitrite in sufficient water to produce 1000 ml. Standardise the solution in the following manner. Dissolve 0.3 g of sulphanilic acid in 50 ml of 2 M hydrochloric acid, add 3 g of potassium bromide, cool in ice and titrate with the sodium nitrite solution determining the end-point potentiometrically. 1 ml of 0.1 M sodium nitrite is equivalent to 0.01732 g of $C_6H_7NO_3S$.

Sodium Thiosulphate, 0.1 M: Dissolve 25 g of sodium thiosulphate and 0.2 g of sodium carbonate in carbon dioxide-free water and dilute to 1000 ml with the same solvent. Standardise the solution in the following manner.

Dissolve 0.200 g of potassium bromate, weighed accurately, in sufficient water to produce 250.0 ml. To 50.0 ml of this solution add 2 g of potassium iodide and 3 ml of 2 M hydrochloric acid and titrate with the sodium thiosulphate solution using starch solution, added towards the end of the titration, as indicator until the blue colour is discharged. 1 ml of 0.1 M sodium thiosulphate is equivalent to 0.002784 g of $KBrO_3$. Restandardise the solution frequently.

Sulphuric Acid, 0.5 M: Add slowly, with stirring, 30 ml of sulphuric acid to about 1000 ml of water, allow to cool 25° and standardise against anhydrous sodium carbonate as described under 1 M hydrochloric acid. 1 ml of 0.5 M sulphuric acid is equivalent to 0.05299 g of Na_2CO_3.

Sulphuric Acid, 0.25 M Ethanolic: Add slowly, with stirring, 13.9 ml of sulphuric acid to a sufficient quantity of ethanol to produce 1000 ml. Cool and standardise against anhydrous sodium carbonate as described under 0.5 M methanolic hydrochloric acid.

Tetrabutylammonium Hydroxide, 0.1 M: Dissolve 40 g of tetrabutylammonium iodide in 90 ml of dehydrated methanol in a glass-stoppered flask. Place in an ice-bath, add 20 g of powdered silver oxide, insert the stopper and agitate vigorously for 1 hour. Centrifuge a few ml, and test the supernatant liquid for iodides (2.3.1). If the test is positive, add an additional 2 g of silver oxide and continue to stand for 30 minutes with intermittent agitation. When all of the iodide has reacted, filter through fine sintered-glass filter. Rinse the flask and filter with three quantities, each of 50 ml, of anhydrous toluene. Add the washings of the filtrate and dilute to 1000 ml with anhydrous toluene. Flush the solution for 10 minutes with dry, carbon dioxide-free nitrogen. Store protected from carbon dioxide and moisture,

and discard after 60 days. Alternatively, prepare the solution by diluting a suitable volume of commercially available tetrabutylammonium hydroxide solution in methanol with a mixture of four volumes of anhydrous toluene and 1 volume of dehydrated methanol. Standardise the solution in the following manner immediately before use.

Weigh accurately about 0.4 g of benzoic acid, dissolve in 80 ml of dimethylformamide, add a few drops of a 1 per cent w/v solution of thymol blue in dimethylformamide and titrate with the tetrabutylammonium hydroxide solution to a blue endpoint. Protect the solution from atmospheric carbon dioxide throughout the titration. Perform a blank determination and make any necessary correction.

1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.01221 g of $C_7H_6O_2$.

Sulphuric Acid: H_2SO_4 = 98.07

Where no molarity is indicated, use analytical reagent grade of commerce containing about 98 per cent w/w of sulphuric acid and about 18 M in strength. Colourless, corrosive oily liquid; evolves much heat when added to water; wt. per ml, about 1.84 g.

Sulphuric Acid × M: Solutions of any molarity × M may be prepared by carefully adding 54 × ml of sulphuric acid to an equal volume of water and diluting to 1000 ml with water.

Sulphuric Acid × per cent: Mix × ml of sulphuric acid carefully with water, cool and adjust the volume to 100 ml to produce the specified percentage v/v of sulphuric acid.

Sulphuric Acid, Dilute: Contains approximately 10 per cent w/w of H_2SO_4. Dilute 57 ml of sulphuric acid to 1000 ml with water.

Nitric Acid: HNO_3 = 63.01.

Clear, Colourless, fuming liquid; corrosive; about 16 M in strength; wt. per ml, about 1.42 g; contains about 70 per cent w/w of HNO_3. Store protected from light.

Nitric Acid, XM: Solutions of any molarity XM may be prepared by diluting 63x ml of nitric acid to 1000 ml with water.

Nitric Acid, Dilute: Contains approximately 10 per cent w/w of HNO_3. Dilute 106 ml of nitric acid to 1000 ml with water.

Nitric Acid, Fuming: HNO_3 = 63.01

Analytical reagent grade of commerce. Clear, almost colourless to yellow, fuming liquid; corrosive; about 22.5 M in strength; wt. per ml, about 1.5 g; contains about 95 per cent w/w of HNO_3. Store protected from light.

Hydrochloric Acid, x M: Solutions of any molarity xM may be prepared by diluting 85x ml of hydrochloric acid to 1000 ml with water. Store in containers of polyethylene or other non-reacting material at a temperature not exceeding 30º.

Iodine, xM: Solutions of any molarity xM may be prepared in the following manner. Dissolve 400x g of potassium iodide in the minimum amount of water, add 260x g of iodine, allow to dissolve and add sufficient water to produce 1000 ml. Weaker solutions may be prepared using proportionately lesser amounts of reagents or by appropriate dilution.

Iodine Solution: Dissolve 2.0 g of iodine and 3 g of potassium iodide in water to produce 100 ml.

2

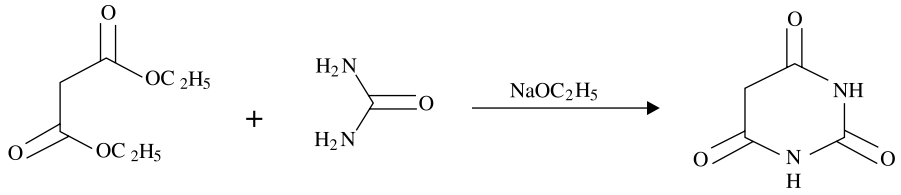
SYNTHESIS OF SOME OFFICIAL MEDICINAL COMPOUNDS

Experiment No.1

Synthesis of Barbituric Acid from Diethyl Malonate

Aim: To synthesise and submit barbituric acid from diethyl malonate.
Apparatus required: Round Bottom flask, Reflux Condensor, oil bath, Guard tube($CaCl_2$)
Chemicals required : Diethylmalonate, urea, con Hcl, Alcohol, Na metal

Reaction involved



Diethy Malonate Urea Barbituric acid

Principle
Condensation of diethyl Malonate with urea in the presence of sodium ethoxide gives barbituric acid. Barbituric acid shows acidic property due to lactam – lactim and keto-enol tautomerism. All the four hydrogen's are involved in tautomerism. Active methyline group at p[position] five undergoes substitution to enhance potency.

Procedure

Synthesis of Barbituric Acid from Diethyl Malonate: In a 250 ml RBF, fitted with a double surface reflux condenser, place 1.15 gm of clean sodium. Add 25 ml of absolute ethanol in one portion, if the reaction is vigorous immerse the flask momentarily in ice. When all the sodium has reacted, add 8 gm of diethyl Malonate, followed by a solution of 3 gm of dry urea in 25 ml of hot absolute ethanol. Shake

the mixture well, fit a calcium chloride guard–tube to the top of the condenser and reflux the mixture for 7 hours in an oil bath heated to 110°C, A white solid separates. Treat the reaction mixture with 45 ml of hot water and then with conc.HCl, with stirring, until the solution is acidic. Filter the solid at the pump, wash it with 2.5 ml of cold water, drain well and then dry at 100°C for 4 hours. The yield of Barbituric acid is weighed and reported. Determine its melting point.

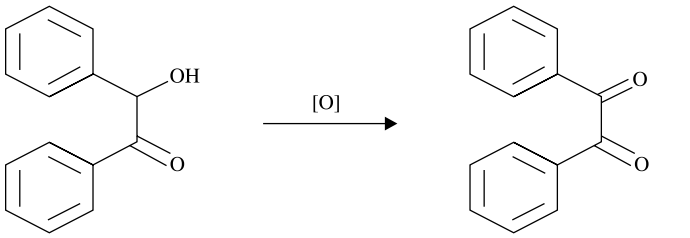
Report

Barbituric acid was synthesized from diethyl malonate and submitted.
The melting point was found to be…… .

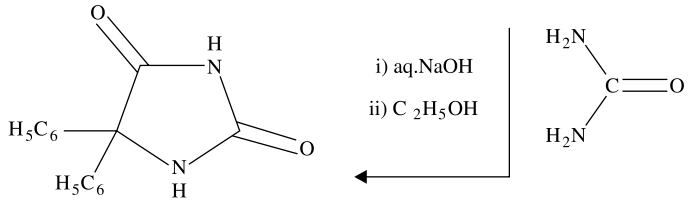
Experiment No.2

Synthesis of Phenytoin from Benzoin

Aim: To Synthesis and submit Phenytoin from Benzoin.
Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle
Chemicals required: Benzoin, Conc.HNO_3, Urea, NaOH (30%), Ethanol (70%), Conc. Hcl
Reaction involved



2-hydroxy-1,2-diphenylethanone 1,2-diphenylethane-1,2-dione



Diphenyl hydantoin

Principle

Urea may be recognized as a structural unit in 5, 5-diphenyl hydantoin and it constitutes one of the reagents in the synthesis along with benzil. The base catalyzed reaction proceeds via an intermediate heterocyclic pinacole which on acidification yields the required hydantoin as a result of pinacolic rearrangement. Pinacol-pinacolone rearrangement mechanism involves the conversion of pinacol-pinacolone under acidic conditions with a loss of water from 1, 2diole accompanied by a 1, 2 nucleophilic shift of an alkyl, aryl or hydride group.

Procedure

Synthesis of Benzil from Benzoin

Step –I

Place 20 gm of crude benzoin and 100 ml of conc.$HNO_3$with occasional shaking until the evolution of oxides of nitrogen has ceased (about 1.5 hrs). Pour the reaction mixture into 300–400 ml of cold water

contained in a beaker, stir well until the oil crystallizes completely as a yellow solid. Filter the crude benzil at pump, and wash it thoroughly with water to remove the nitric acid. Recrystalize from ethanol or rectified spirit the yield is around 19 gms.

Step –II

Synthesis of Phenytoin from Benzil

Place 5.3 gm of benzil, 3.0 gm of urea, and 15 ml of 30% aqueous NaOH solution and 75 ml of ethanol in a 100 ml RBF. Attach a reflux condenser and boil under reflux using an electric heating mantle for at least 2 hrs. Cool to room temperature, pour the reaction product into 125 ml of water and mix thoroughly. Allow to stand for 15 min and then filter under suction to remove an insoluble by product. Render the filteratre strongly acidic with conc. HCl, cool in ice water and immediately filter off the precipitated product under suction. Recrystalize at least once from industrial spirit. Report the melting point and the yield of the product.

Report

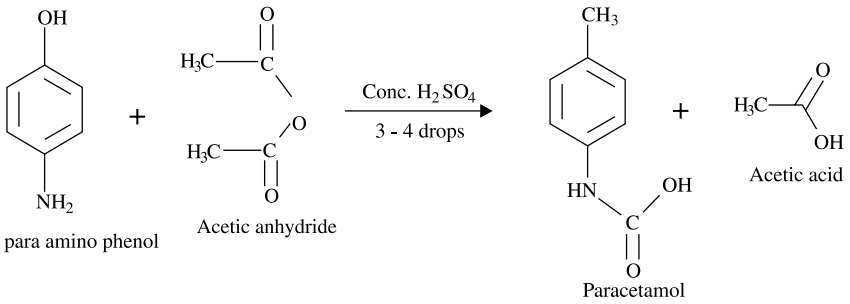
Phenytoin was synthesized from diethyl malonate and submitted.
The melting point was found to be…… .

Experiment No.3

Synthesis of Paracetamol from *P*- Amino Phenol

Aim: To Synthesis and submit Paracetamol from *p*- amino phenol.
Apparatus required: Beaker, conical flask. Magnetic stirrer.
Chemicals required: Para-aminophenol, acetic anhydride, concentrated sulphuric acid

Reaction involved



Principle

The synthesis of paracetamol is an acetylation reaction involving acetylation of free primary amino group to acetamido group. Acetic anhydride undergoes rearrangement to give an active acetylating species and acetic acid. Acetylation of para-amino phenol involves two steps, first step is fast and the second step is slow and is the rate determining step and involves the addition of nucleophile. Primary amino groups are acetylated readily and hence it is possible to acetylate this group easily in compounds containing both an amino group and a hydroxyl group.

Procedure

Synthesis of paracetamol from *para*- Aminophenol

Weigh 6 g of *para*-aminophenol and transfer to a 100 ml thoroughly cleaned and dried conical flask. To this add 6.5 ml of acetic anhydride and 3–4 drops of concentrated sulphuric acid cautiously. The contents of the flask may be mixed thoroughly. Warm the mixture on a water bath previously maintained

at 60°C for about 20–25 minutes with constant stirring. Allow the contents of the flask to attain room temperature, and pour it directly into a beaker having 100 ml of cold water (with a few chips of crushed ice) and stir it vigorously. The crude product obtained in (4) is filtered onto a Büchner funnel using suction, wash it with plenty of cold water, drain well and dry the product either between the folds of filter paper and air-dry it or dry it in an electric oven maintained at 100°C. The yield of crude paracetamol (169–170.5°C) is approximately 6.8 g. Recrystalisation is performed by dissolving the crude product in 70% (v/v) ethanol and warm it to 60°C; add 2 g of powdered animal charcoal (decolorizing carbon). Filter and concentrate the filtrate over a water-bath. Allow it to cool and large monoclinic crystals will separate out. Determine its yield and melting point.

Report

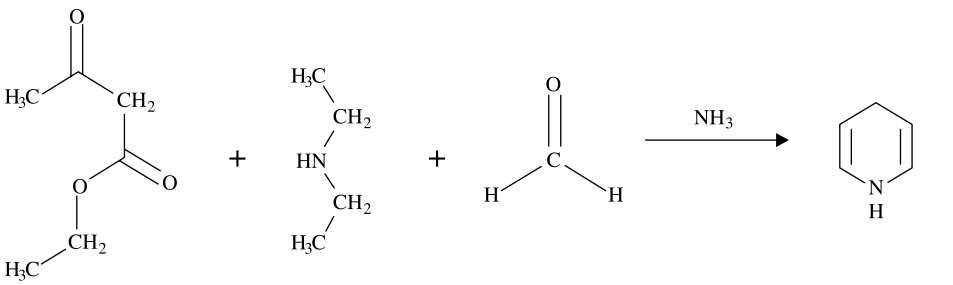
Paracetamol was synthesized from diethyl malonate and submitted.
The melting point was found to be…… .

Experiment No.4

Synthesis of 1, 4–Dihydro Pyridine from Ethyl Acetoacetate

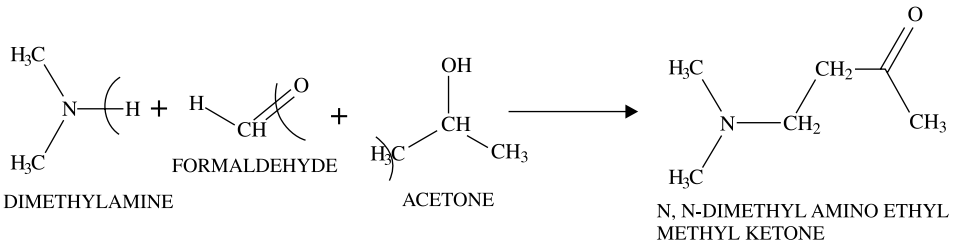
Aim: To Synthesis and submit 1, 4–dihydro pyridine from ethyl acetoacetate.
Apparatus required: Beaker, conical flask.
Chemicals required: Ethyl aceto acetate , formaldehyde solution, ethanol.
Principle
In the present reaction ethyl aceto acetate, formaldehyde and ammonia condenses to form 1, 4–dihydro pyridine. This is a mannich reaction where an alkyl group containing active hydrogen, aldehyde and an amine condenses to form mannich base. Diethylamine is used as a catalyst.

Reaction involved



Ethylacetoacetate Diethylamine Formaldehyde 1,4 - dihydropyridine

Mannich reaction: The reaction of compounds having an active hydrogen atom with non-enolizable aldehydes and ammonia or primary or secondary amines to give rise to the formation of aminomethylated product exclusively is commonly known as the **Mannich Reaction;** and the product is invariably termed as the **Mannich Base,** as depicted below: **Explanation.** The active H-atom of the methyl function in acetone, the H-atom of the secondary amine (dimethy amine) and the O-atom of the aldehyde (formaldehyde) gets eliminated as one mole of water. Thus, the resulting aminomethylated product essentially possesses an additional methylene (—CH_2—) moiety. In other words, in all Mannich reactions the carbon-chain shall be increased by **one** due to the —CH_2— methylene function forming a part of the Mannich Base. In general, the Mannich bases are scantly water soluble; therefore, they are mostly employed as their respective hydrochlorides which are fairly water soluble.



Procedure: Synthesis of 1, 4- dihydro pyridine from ethyl Acetoacetate

Cool 52 gms of ethyl aceto acetate to 0ºC and add 15 ml of 40% aq formaldehyde solution followed by addition of few drops of diethyl amine as a catalyst. Keep the reaction mixture at 0ºC for 6 hrs and then at room temperature for 40 hrs. Seperate the lower organic layer, extract the aq phase with ether and dry the combined organic fractions over anhydrous calcium chloride. Remove the anhydrous ether at reduced pressure and transfer the residue together with an equal volume of ethanol to a stout reagent bottle cooled in an ice bath. Pass a steady stream of ammonia gas in to the solution held at 0ºC for 1 hr, close the bottle with a bung securely attached with a wire and set the bottle and contents aside at room temperature for 40 hrs. Filter the resulting yellow solution to remove a small quantity of almost color less material and heat the filtrate on a boiling water bath in an evaporating dish until most of the ethanol has been removed, and then cool and crystallize the residue from about 400 ml of rectified spirit.

Report: 1, 4 dihydro pyridine was synthesized from ethyl acetoacetate and submitted.
The melting point was found to be 181–183ºC.

Experiment No.5

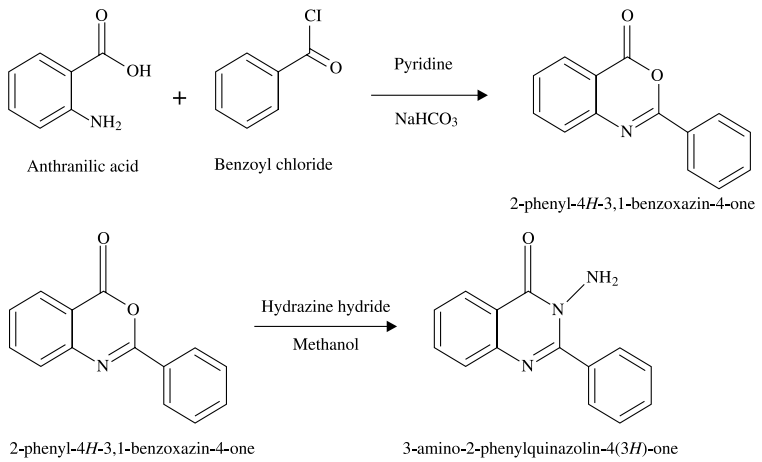
Synthesis of Quinazolinone from Anthranilic Acid

Aim: To Synthesis and submit Quinazolinone from anthranilic acid.

Principle

Anthranilic acid is condensed with benzoyl chloride to form condensed product benzoxazinone,which on reaction with hydrazine hydride loses one molecule of water to form the final product. Pyridine is used as a solvent and sodium bicarbonate is used to maintain the pH of the reaction mixture. In step 2 methanol is used as solvent.

Reaction involved



Procedure: Synthesis of Quinazolinone from Anthranilic Acid

Step 1: Synthesis of benzoxazinone: To a solution of anthranilic acid (0.1 mol) dissolved in pyridine, benzoyl chloride (0.2 mol) was added.The reaction mixture is stirred continuously for 30min, followed by addition of 5% sodium bicarbonate. The solid obtained is recrystalized from ethanol for two times and dried.

Step 2: Synthesis of quinazolinone: A mixture of benzoxazinone and hydrazine hydrate in ethanol was refluxed for 3 hrs and the resulting solution was poured into the ice. A white precipitate is obtained which is recrystalized from ethanol for two times and dried.

Report

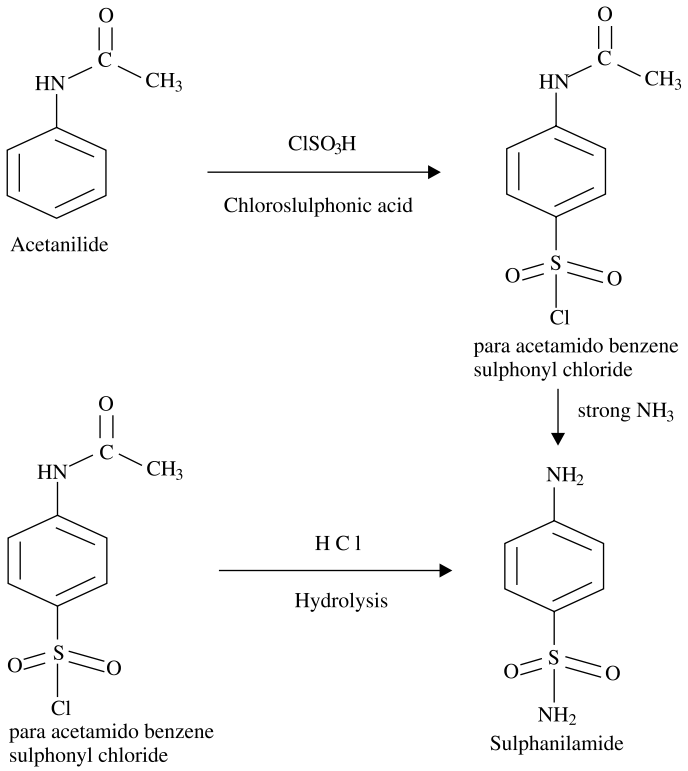
Quinazolinone was synthesized from anthranilic acid and submitted.
The melting point was found to be…… .

Experiment No.6

Synthesis of Sulfanilamide from Acetanilide

Aim: To Synthesis and submit Sulfanilamide from acetanilide.
Apparatus required: Beaker, conical flask., RBF
Chemicals required : Chloro Sulfonic Acid, Acetanilide, Ammonia, Sulfuric Acid

Reaction Involved



Principle

Chloro Sulfonation of Acetanilide with excess of ChloroSulfonic Acid gives P-Acetamido Benzo Sulfonyl Chloride. This is an essential intermediate in this synthesis. A simplest Sulfonamide, P-Amino Benzene Sulfonamide is attained by converting P–Acetamido Benzene Sulfonyl Chloride into Amide with Aq. Ammonia, & then selectively removing Acetyl group by boiling wih aq. HCl, Sulfonamide will be obtained.

Synthesis of P-Acetamidobenzene Sulfonyl Chloride

Chemicals-chlorosulfonic acid-8 ml, acetanilide-3 gm,

Procedure

Take 8 ml of Chloro Sulfonic Acid in a RBF & add 3 gm of Acetanilide with boiling & stirring. The RBF is fitted to a water condenser and heated to 50–60ºC for 15–20 min on water bath. Cool to room temp., and pour these contents into crushed ice. P-Acetanilide Benzene Sulfonyl Chloride separates out, filter and recrystallize from alcohol.

Synthesis of P-Acetamido Benzene Sulfonamide

Chemicals- p-acetamido benzene sulfonyl chloride, ammonia-3.5ml.

Procedure

Take P–Acetamido Benzene Sulfonyl Chloride in a dried conical flask & 6N Ammonia, mix thoroughly. A smooth paste is obtained and heats it at 70ºC for 20 min. with constant stirring. Cool to room temperature & acidify with dil. Sulfuric Acid. Filter the precipitate and wash with water and recrystallize with water.

Synthesis of p-amino benzene sulphonamide (sulfanilamide)

Chemicals – p–acetamido benzene sulfanilamide-2gm, HCl-3ml

Procedure

Take 2 gm of P-Acetamido Benzene Sulfanilamide, 3 ml HCl & 5 ml water in a RBF & boil gently on Reflux condenser for 1 hr & add 6 ml water again & transfer it into 250 ml Beaker. Add powdered Sodium Carbonate in small qty., Sulfanilamide separates out. Filter & recrystallize from water.

Report

Sulfanilamide was synthesized from acetanilide and submitted.
The melting point was found to be…… .

Experiment No.7

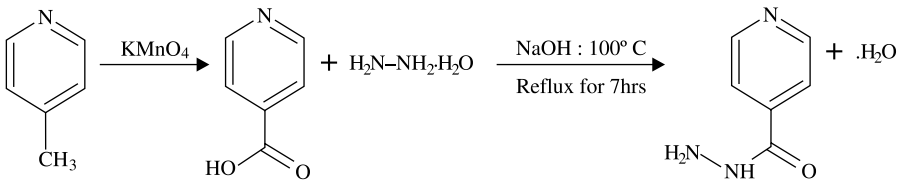
Synthesis of Isoniazid from Gamma–Picoline

Aim: To Synthesis and submit Isoniazid from γ- picoline
Apparatus required: Beaker, conical flask., RBF, measuring cylinder.
Chemicals required: 4-methyl pyridine, NaOH, hydrazine hydrate, $KMnO_4$.
Principle

First of all the 4-methylpyridine undergoes oxidation whereby the methyl function at the C-4 position gets converted to a carboxylic moiety to form 4-pyridine carboxylic acid. Now, this resulting product on being treated with hydrazine hydrate, in the presence of NaOH and subjected to vigorous reflux

for a long duration, gives rise to the formation of isoniazid with the elimination of a mole of water as indicated above.

Reaction involved



4 - Methyl pyridine 4 - pyridine carboxylic acid Isoniazid

Procedure

Synthesis of Isoniazid from γ-*picoline*

Step1: Place 10 gms of 4-methyl pyridine in 100 ml of water in a three neck Round Bottom Flask (RBF) equipped with a thermometer, sealed stirrer unit and a reflux condenser and heat to 70ºC on a water bath. Add 45 gms of $KMnO_4$ in 10 equal portions through the condenser over a period of 3–4 hrs. Maintain the temperature at 70C for the first five additions and 85 – 95ºC for the last five additions. Make each successive addition of $KMnO_4$ only after the preceding amount is decolorized and wash it down with 2.0–2.5 ml of water. After the last charge of $KMnO_4$ is decolorized raise the temperature to 95C, filter the hot reaction mixture with suction and wash the manganese di oxide cake on the filter with 20 ml portions of hot water, allow each portion to soak into the cake without application of vacuum and finally suck dry before adding fresh water. Evaporate the combined filtrate and washings to about 150 ml and add conc. HCl until the PH reaches 3.6, Isonicotinic acid precipitates out. Heat to 90 – 95 C and allow the mixture to crystallize slowly. Collect the crude Isonicotinic acid by suction filtration, wash well with water and dry at 100ºC. Concentrate the mother liquor to about half the original volume and so obtain a second crop of acid. The first crop of acid yields 64% and the second crops weighs 5% with a melting point of 311ºC.

Step2: 24.6 g (0.2 mol) of Isonicotinic acid is reacted with 10 g (0.2 mol) hydrazine hydrate in the presence of 0.016 g (0.04 mol) sodium hydroxide at 100°C under reflux on a heating mantle for a duration of 7–8 hours at a stretch. (2) The resulting mixture was filtered in Büchner funnel under suction and the clear filtrate was evaporated to dryness on an electric water-bath carefully. (3) The m.p of the crudue product was 170–171°C.

Reference

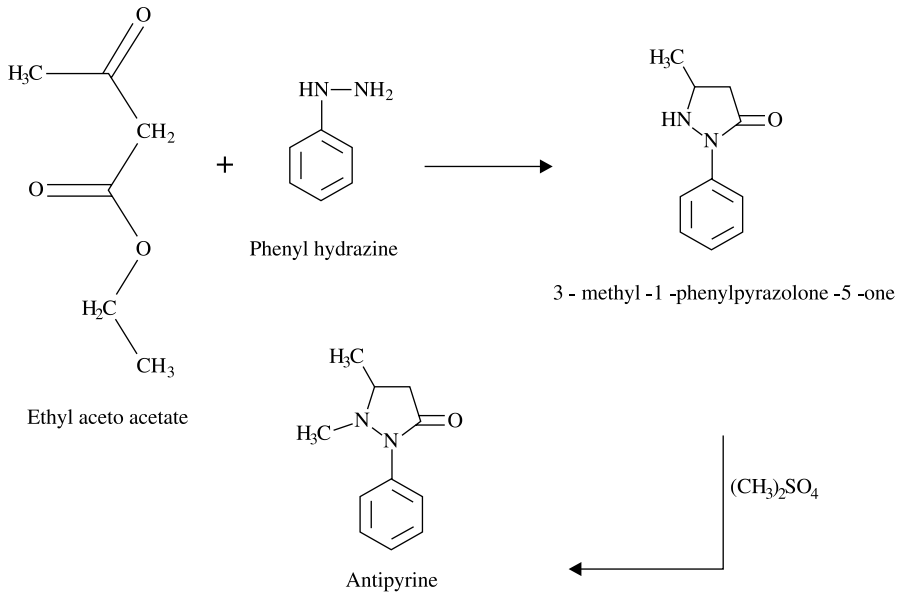
Report: Isoniazid was synthesized from γ-*picoline* and submitted.
The melting point was found to be…… .

Experiment No.8

Synthesis of Antipyrine from Ethylacetoacetate

Aim: To Synthesis and submit Antipyrine from ethylacetoacetate.
Apparatus: Round Bottom flask, Reflux Condensor,beaker, funnel,heting mantle
Chemicals required: Ethyl acetoacetate, phenylhydrazine.NaOH

Reaction involved



Principle

Anti pyrine is a derivative of pyrazole. Generally pyrazoles are synthesized from di carbonic compounds by reaction with hydrazine. In this synthesis ethylacetoacetate is used along with hydrazine. Ethylacetoacetate is heated with an equal quantity of phenyl hydrazine to form corresponding phenyl hydrazone. On further heating ring formation occurs with loss of ethanol. Resultant compound is methyl phenyl pyrazolone which is obtained as colorless crystals. This pyrazolone is having an active proton on N–group at 2nd position so this portion is easily replaced by methyl group by using Dimethyl Sulphide as methylating agent in presence of strong base. In the first step unreacted substances are removed by using non polar solvents like ether.

Procedure: Preparation of 3–methyl–1–phenyl pyrazol–5–one

Mix together 5.0 g of ethylacetoacetate and 4.0 gms of phenyl hydrazine in a large evaporating dish. Heat the mixture on a boiling water bath in the fume cupboard for about 2 hours. And stir time to time with a glass rod. Allow the heavy reddish syrup to cool some what, add about 10 ml of ether and stir the mixture vigorously. The syrup, which is insoluble in ether, will solidify within 15 minutes. Filter the solid at pump and wash it thoroughly with ether to remove colored impurities recrystalize with hot water or from mixture of equal volumes of ethanol and water. The yield of methyl phenylpyrazolone is m.p is 127ºC

Preparation of 2, 3 dimethyl–1–phenyl pyrazol–5–one (Antipyrine)

Place a solution of 10 gms of NaOH in water and also a solution of 1–phenyl–3–methyl–5–pyrazoline in 20 ml of methanol. Warm the mixture on waterbath and add 36 gm (27 ml) of dimethyl sulphide. Reflux the mixture for 1 hour and allow cooling with continuous stirring. Distill off methanol. Add hot water to residue. Filter from impurities. Extract Antipyrine with benzene and evaporate solvent. Recrystalize the product from hot water, by adding little decolorizing carbon.

Report: Antipyrine was synthesized from ethylacetoacetate and submitted.
The melting point was found to be…… .

Experiment No.9

Synthesis of Benzocaine from PABA

Aim: To synthesis and submit benzocaine from PABA.

Chemicals Required

For Step-I. Sodium dichromate dihydrate: 20 g; Conc. Sulphuric Acid (36 N): 25 ml; p–Nitrotoluene: 6.8 g; NaOH [10% (w/v)]: 30 ml; Decolourizing Carbon: 1.5 g; Conc. Hydrochloric acid (12 N): 20 ml.

For Step-II. p-Nitrobenzoic acid: 3.4 g; Absolute Ethanol: 30 ml; Conc. Sulphuric acid (36 N): 5 ml; NaOH [10% (w/v)]: 50 ml.

For Step-III. Calcium chloride: 1 g; Ethanol (95%): 55 ml; Ethyl-p-nitobenzoate: 2.5 g; Zine dust pure: 25 g; Solvent Ether: 100 ml; Sodium chloride: 100 g; n-Pentane; 50 ml.

Principle: Benzocaine is ester of ehtyl para amino benzoate, synthesized by direct esterification of PABA with ethanol in the presence of hydrochloric acid or sulphuric acid. Mineral acids speed up the process by protonating the carbonyl oxygen and thus renders carbonyl carbon is more susceptible to nucleophilic attack. It acts as local anaesthetic by blocking the generation of nerve impulse.

Theory: The synthesis of Benzocaine starting from p-nitrotoluene is usually accomplished by means of **three** sequential reactions i.e., Eq. (a) through Eq. (c) as given above. Eq. (a) shows the oxidation of p–nitrotoluene by sodium dichromate in an acidic medium (with H_2SO_4) to yield p–nitro benzoic acid (I) whereby the methyl function in the starting material gets oxidized to the corresponding carboxylic moiety due to the evolution of 3–moles of nascent oxygen as given in Eq. (a) (ii).

Eq. (b) depicts the esterification of (I) with ethanol in the presence of sulphuric acid whereby the corresponding ester i.e., ethyl-p-nitrobenzoate (II) is formed with the abstraction of one mole of water.

Eq. (c) illustrates the reduction of (II) in the presence of Zn, calcium chloride and dilute acetic acid, whereby the nitro group at the para-position gets reduced to amino function; and the desired product i.e., Benzocaine (III) is obtained. It is, however, pertinent to mention here that the aforesaid three reactions, namely: (i) **oxidation**; (ii) **esterification**; and (iii) **reduction** must be carried out in the **same sequence strictly**, otherwise one may not get the desired product.

Case-I: A situation where reduction of the nitro function precedes oxidation.

In this particular instance an altogether new compound para-toluidine shall be formed which upon oxidation with sodium dichromate and sulphuric acid shall undergo **aromatic ring oxidation** instead, because 'anilines' with strong oxidizing agents, e.g., dichromate usually gives similar products.

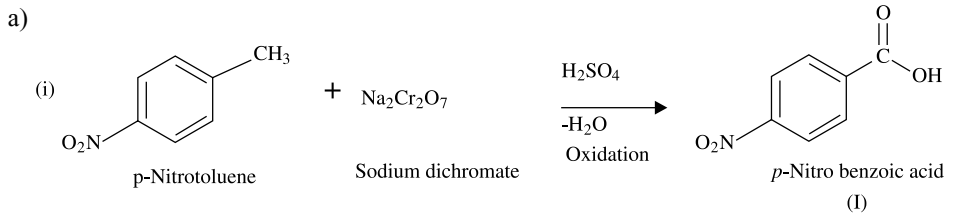
Case-II. A similar situation wherein reduction of the nitro function precedes esterification.

In this specific case the initial reaction involving the oxidation of p-nitrotoluene gives rise to the formation of p-nitrobenzoic acid which on further reduction with tin and HCl yields p-aminobenzoic acid (PABA); and PABA being soluble in both acid and base is rather difficult to isolate. Moreover, PABA may be isolated only under precisely neutral conditions and that too after removal of the metal ions which eventually form complexes with it. Therefore, it is always advisible to employ the previously cited reaction sequence rigidly viz., oxidation-esterification-reduction, in order to circumvent these

aforesaid difficulties. Further, the commercial production of benzocaine is usually carried out by **catalytic hydrogenation** in place of **using zinc dust**.

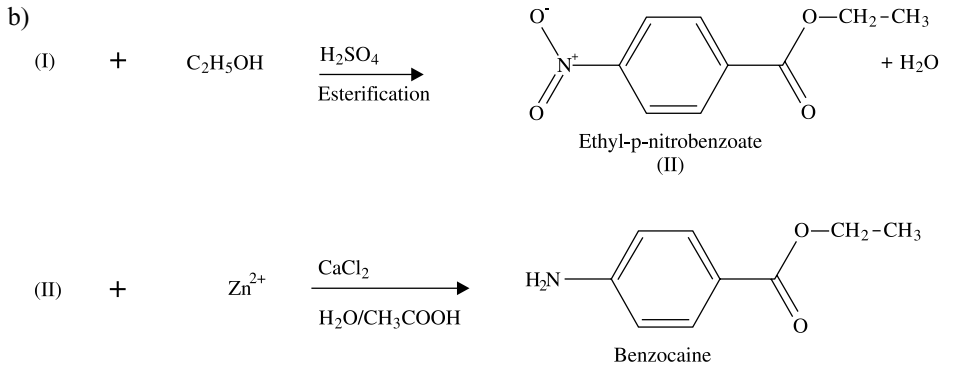
Reaction involved

a)



(ii) $Na_2Cr_2O_7 + 4H_2SO_4 \longrightarrow Na_2SO_4 + Cr_2(SO_4)_3 + 4H_2O + 3(O)$ Nescent Oxygen

b)



Procedure: The synthesis of 'benzocaine' is accomplished in three different steps as given under

Step-I. Oxidation of p-Nitrotoluene

1. Dissolve 20 g (0.67 mol) of sodium dichromate dihydrate in 50 ml of water into a 250 ml round bottom flask. Slowly and carefully add 25 ml of concentrated sulphuric acid with frequent stirring into the above chromic acid solution (an exothermic reaction).
2. Allow the reaction mixture to cool down to less than 50ºC, and then add 6.8 g (0.05 mol) of p-nitrotoluene. Now add a few boiling chips (or stones) into the reaction flask, attach the Claisen head to the round bottom flask and place the thermometer adapter on the central connection of the Claisen head. Insert a thermometer (preferably 0–360ºC) through the adapter right into the reaction solution and attach a double surface reflux condenser to the side connection of the Claisen head.
3. Heat the reaction mixture gently to 75ºC when an **exothermic reaction** could be seen by a sudden and rapid increase in the reaction temperature. Remove the source of heat for a while till the temperature starts falling and then replace the heat source once again. Reflux the contents for 60 minutes, allow it to cool for 15 minutes and pour it out 100 g of crushed ice in a 250 ml conical flask (i.e., Erlenmeyer flask).
4. Collect the solid precipitate in a Büchner funnel under suction, and wash the residue with two 30 ml portion of water.
5. Transfer the solid residue into a 250 ml beaker, add 30 ml of water, and 30 ml of 10% aqueous NaOH solution to affect dissolution of p-nitrobenzoic acid. Warm the resulting mixture on a steam bath for 10 minutes to permit coagulation of residual chromium salts as their insoluble

hydroxides and then filter by suction. Add 1.5 g of decolourizing carbon to the resulting filtered solution, heat the contents for 10 minutes; and filter the mixture by gravity through a coarse filter paper.

6. Prepare separately an aqueous acidic solution by adding 20 ml of concentrated HCl (12N) to 30 g of crushed ice in a 250 ml beaker. Now slowly and with constant stirring, pour the basic charcoal-decolourized solution (Step-5 above) into the aqueous acidic solution. At the end ensure that the pH of the resulting solution is strongly acidic (test with litmus paper).
7. The resulting precipitate is filtered in a Büchner funnel under suction, wash the precipitate with 10 ml portions of water.

The yield of the crude p-nitobenzoic acid is 6.2 g having mp 240–241ºC. The crude product may be further recrystallized from ethanol to get 5.8 g of the pure product mp 241–242ºC.

Step-II. Esterification of p-Nitrobenzoic Acid

1. Transfer 30 ml of absolute ethanol to 3.4 g (0.02 mol) of p-nitrobenzoic acid in a 100 ml round bottom flask. Place a few anti-bumping chips into the flask, and attach a reflux condenser for heating under reflux.
2. Add 5 ml of concentrated sulphuric acid to the reaction mixture through the condenser in small lots at intervals. Reflux the mixture for about 60 minutes until all the solid p-nitrobenzoic acid gets dissolved.
3. Cool the reaction mixture to room temperature and pour the contents into a mixture of 50 ml of 10% aqueous NaOH solution and nearly 50 g of crushed ice.
4. Filter the precipitate in Büchner funnel under suction and wash with a thin spray of cold water.
5. The yield of the crude product is 2.95 g having mp ranging between 54.5–55ºC. The crude product may be recrystallized from a minimum volume of ethanol-water (1 : 1) to obtain 2.75 g of pure product mp 55–56ºC.

Step-III. Reduction of Ethyl p-Nitrobenzoate

1. Transfer 1 g of calcium chloride in 12 ml of water placed in a 100 ml beaker; and mix this solution with 55 ml of 95% (v/v) ethanol.
2. Pour the resulting solution into a 250 ml round bottom flask that contains 2.5 g (0.013 mol) of ethyl p-nitrobenzoate (Step-II), add to it 25 g of Zn-dust, and attach to it a reflux condenser.
3. Reflux the reaction mixture for 2 hours gently and at a stretch and then cool to room temperature.
4. Separate the unreacted Zn-dust from the aqueous ethanolic solution in Büchner funnel under suction, and wash the filtered solid with two 25 ml portions of solvent ether.
5. Extract the filtrate with 150 ml of water previously saturated with NaCl. Wash the aqueous layer twice with 25 ml portions of solvent ether. Combine all the ethereal layers together (including one obtained in (4) above; and wash it with two successive portions each of 40 ml of water.
6. Dry the resulting ethereal solution over anhydrous Mg SO4, filter, and subsequently distil the ether on a steam bath to a final volume of 10 to 15 ml. Transfer the ethereal residue to an Erlenmeyer flask and add to it 20 ml of pentane to precipitate the desired product benzocaine. The yield of the crude benzocaine is 1.58 g having mp 88–89.5°C.

Precautions

1. The mild reduction of ethyl p-nitrobenzoate is required which is accomplished with Zn-dust and HCl obtained by the interaction of $CaCl_2$ and water.
2. The ethereal layer needs to be dried as far as possible with anhydrous $MgSO_4$ before distilling off the excess of ether on a water-bath.

3. n-Pentane should be used carefully to separate out the precipitate of benzocaine from the concentrated ethereal fraction.

Recrystallization: The crude product is recrystallized from a minimum quantity of a mixture of ether and pentane (1 : 1) and the yield of the pure product is 1.40 g mp 89–90°C.
Physical Parameters: It is obtained as rhombohedra crystals from ether, mp 88–90°C, and fairly stable in air.1 g Dissolves in about 2.5 L water, 5 ml ethanol, 2 ml $CHCl_3$, 4 ml ether, and in 30 to 50 ml of expressed almond oil or olive oil. It is also found to be soluble in dilute acids and its dissociation constant pKa is 2.5. Following is the 1H-NMR spectrum of benzocaine recorded in $CDCl_3$ solution

Uses

1. It is usually employed as an ointment to relieve pain associated with ulcers, wounds, burns, and mucous surfaces.
2. It is also used as a lubricant and anaesthetic on intra tracheal catheters, pharyngeal and nasal airways, nasogastric and endoscopic tubes.
3. It is included in proprietary creams, lozenges, ointments, powders, sprays and suppositories to relievepain from damaged skin surfaces and inflamed mucous membranes.

Report

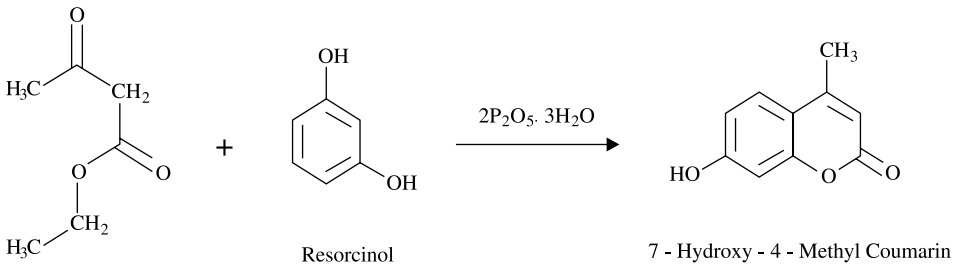
Benzocaine was synthesized from PABA and submitted.
The melting point was found to be…… .

Experiment No.10

Synthesis of 7-Hydroxy-4-Methyl Coumarin from Resorcinol

Aim: To Synthesis and submit 4-hydroxy coumarin from resorcinol.
Apparatus: Round Bottom flask, Reflux Condensor,beaker, funnel,Heating mantle
Chemicals required: Polyphosphoric acid, resorcinol, ethyl acetoacetate

Reaction involved



Ethylacetoacetate Resorcinol 7 - Hydroxy - 4 - Methyl Coumarin

Principle

The preparation of 7-hydroxy- 4-methyl coumarin is an example of Beckmann's rearrangement which consists of interaction of resorcinol with beta keto ester in the presence of acidic reagents (H_2SO_4, PCl_5, SO_3, $C_6H_5SO_2Cl_2$, SO_2Cl, PCl_5). The possible mechanism is transfer of proton from the acidic catalyst to the carbonyl group of beta keto ester. This results in a reduction of electron density from the carbonyl carbon.

Procedure

Synthesis of 7-hydroxy- 4-methyl coumarin from resorcinol

Add 160 gm of polyphosphoric acid to a solution of 1.1 gm of resorcinol in 1.3 gm of ethyl acetoacetate. Stir the mixture and heat at 75–80°C for 20 minutes, and then pour into ice-water. Collect the pale yellow solid by suction filtration, wash with a little cold water and dry at 60ºC. the yield of crude 7-hydroxy- 4-methyl coumarin is 97%. Recrystalize from dilute ethanol to yield colorless compound. Determine its melting point and %yield.

Report

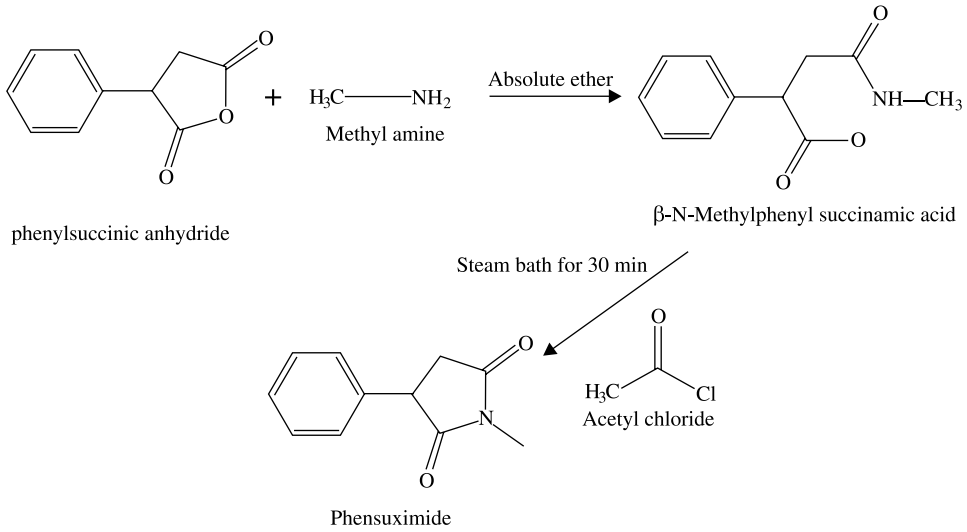
7-hydroxy- 4-methyl coumarin was synthesized from resorcinol and submitted.
The melting point was found to be…… .

Experiment.No:11

Synthesis of Phensuximide

Aim: To Synthesis and submit Phensuximide
Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle
Chemicals Required. Phenylsuccinic anhydride: 10 g; Absolute Ether: 350 ml; Dry Methylamine: q.s.; Ethanol: q.s.; β-N-Methylphenyl succinamic acid: 9 g; Acetyl chloride: 200 ml; Anhydrous $MgSO_4$: q.s.

Chemical Structure



Theory The synthesis of Phensuximide proceeds usually in two steps, namely:

(i) Preparation of β-N-Methylphenyl succinamic acid, and
(ii) Preparation of N-Methyl-α-phenyl succinimide (i.e., Phenusximide).

Eqn. (a). Illustrates the reaction between phenylsuccinic anhydride and methyl amine in the presence of absolute dry ether to give rise to the formation of (3-N-methylphenylsuccinamic acid (I).

Eqn. (b). Shows the interaction of compound (I) with acetyl chloride at an elevated temperature where upon closure of ring takes place to yield phensuximide.

Procedure. The synthesis of 'phensuximide' is carried out in two steps, namely

Step-I. Preparation of (β-N-Methylphenyl succinamic Acid)

1. 10 g (0.164 mol) Phenylsuccinic anhydride is dissolved in 250 ml absolute (dry) ether and the solution is treated with dry methyl amine until a precipitate ceases to form. After allowing it to stand for a duration of 30 minutes, the ether is decanted off carefully ; and the residue is washed with 40 ml of distilled water by decantation.
2. The resulting mixture is filtered and the precipitate washed with 10 ml of water (Crop-1). The filtrate is acidified with dilute HCl carefully to obtain a white precipitate (Crop-2). After drying it weighs approximately 8 g (mp 136°–140ºC). The two precipitates are combined and recrystallized from aqueous ethanol to yield β-Nmethylphenyl succinamic acid (I) that melts at 158º–160ºC.

Setp II. Preparation of N-Methyl-α-phenylsuccinimide (i.e., Phensuximide)

1. 9 g Compound (I), obtained from Step I, and 200 ml redistilled acetyl chloride are heated together on a steam-bath for 30 minutes with frequent swirling of the contents.
2. The excess of acetyl chloride is duly removed by distillation under vacuo; and 50 ml of distilled water is added to the rather thick-residue.
3. After allowing the hydrolysis of the excess acetyl chloride the water is decanted off carefully; and the yellow residue is dissolved in 75 ml of dry ether.
4. The resulting yellow-coloured solution is treated with charcoal (activated) twice; and subsequently dried over anhydrous magnesium sulphate.
5. When partial evaporation of ether is affected, a white solid precipitates out, which is nothing but phensuximide weighing 4.2 g having mp 71º–73ºC.

Precautions

1. In Step I, dry methyl amine gas is required to be passed through the reaction mixture slowily till the formation of further precipitates ceases completely. This step should preferably be carried out in an efficient fuming cupboard.
2. In Step II, the excess of acetyl chloride need to be removed by distillation, while the residual acetyl chloride must be hydrolyzed with water and decanted of before proceeding ahead for the recovery of phensuximide.

Physical parameters. Phensuximide may be obtained as fine crystals from hot 95% ethanol having mp ranging between 71–73ºC. It is found to be slightly soluble in water (about 4.2 mg mL–1 at 25ºC); readily soluble in ethanol and methanol. The aqueous solutions are observed to be fairly stable at pH 2.8; however, hydrolysis invariably sets in under more alkaline conditions.

Uses

1. It is mostly used as an antiepileptic agent.
2. It may also be used for myoclonic seizures.
3. It is also employed in the treatment of absence (petitmal) seizures.

Report : Phensuximide was prepared and submitted.

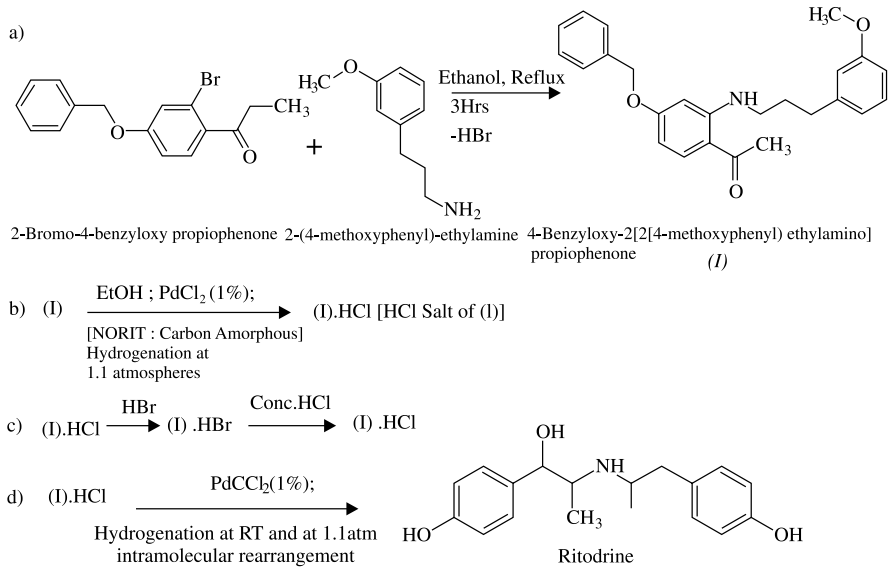
Experiment No:12

Synthesis of Ritodrine

Aim: To Synthesis and submit ritodrine.
Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle
Chemicals required: 2-Bromo-4′-benzyloxy propiophenone: 44 g; 2-(4-Methoxyphenyl) ethylamine: 44 g; Ethanol: 750 ml; Hydrochloric Acid (2 M): q.s.; 4′-Benzyloxy-2[2[4-methoxyphenyl) ethylamino] propiophenone (I): 12 g; Palladium chloride [1% (w/v)]: 50 ml; Norit: 6 g; Hydrobromic acid

(48%): 30 ml; Conc. Hydrochloric Acid (12 M): q.s.; Potassium chloride [1% (w/v)]: 8 ml; Dilute Ammonia solution: q.s.; Ether: q.s.

Chemical structure



Theory: The synthesis of 'ritodrine' is accomplished in four steps, namely:

Eqn. (a) shows the reaction between 2-bromo-4′-benzyloxypropiophenone and 2-(4-methoxyphenyl)-ethylamine in the presence of ethanol to yield an intermediate 4′-benzyloxy-2[2[4-methoxy-phenyl) ethylamino] propiophenone (I).

Eqn. (b) depicts the conversion of compound (I) into its corresponding hydrochloride salt by treatment with palladium chloride (1%) and hydrogenation at 1.1 atmospheres, (I) HCl.

Eqn. (c) shows the purification mode of (I) HCl through (I) HBr to (I) HCl again.

Eqn. (d) illustrates the final important step whereby the purified (I) HCl salt undergoes 'intramolecular rearrangement' in the presence of $PdCl_2$ (1%) and hydrogenation at roomtemperature at 1.1 atmospheres to result into the formation of one mole of ritodrine.

Procedure: The various steps involved in the synthesis are enumerated below in a sequential manner

1. A solution of 44 g (0.144 mol) 2-bromo-4′-benzyloxy propiophenone and 44 g (0.3 mol) 2-(4-methoxyphenyl) ethylamine in 270 ml ethanol was refluxed for a duration of 3 hours on a heating mantle. The excess of ehanol was distilled off under vacuo, and the resulting concentrate was mixed with ether. The ensuing crystallizate was removed by suction in a Büchner funnel ; and the filtrate was adequately mixed with an excess of 2 M.HCl. Thus, the corresponding hydrochloride salt of (I) crystallized out slowly. The resulting crude product was recrystallized from dilute alcohol with an yield of 25.5 g and mp ranging between 217º–218ºC, [(I).HCl].
2. 12 g of (I).HCl obtained from step (1) was dissolved in a mixture of 300 ml ethanol and 90 ml water in a 2 L round bottom flask. To the resulting solution were added 42 ml 1% $PdCl_2$ solution and 3.9 g Norit (Carbon amorphous). The solution was duly hydrogenated at room temperature and at a pressure of 1.1 atmospheres until approximately 760 ml hydrogen had been taken up. The catalyst was removed by filtration and the solvent present in the filtered solution was evaporated entirely under reduced pressure.
3. The resulting residue, which consisted of the hydrochloride of (I), was mixed with 30 ml of a 48% HBr solution and the mixture was boiled until no methyl bromide developed any more,

which was the case after nearly 45 minutes. The reaction mixture was stored in the refrigerator (0–10ºC) overnight, after which the hydrobromide of (I) crystallized out [Eqn. (c)]. It was subsequently sucked off and reconverted into its HCl salt by again dissolving the resulting substance in water, discolouring the solution with a little Norit, and then adding an equal volume of conc. HCl (12 M). Thus, the HCl salt of (I) got crystallized. The yield of the product was 9.6 g, mp 136–138ºC. After this product had been recrystallized once again it was reduced to the amino alcohol.

4. For this purpose, a solution of 3.2 g of the HCl in 160 ml DW was provided with 0.5 g of Norit and 8 ml 1% $PdCl_2$; and the mixture was hydrogenated at room temperature and at a pressure of 1.1 atmospheres until no hydrogen was taken up any more. The catalyst was now removed by filtration, after which the filtrate was concentrated in vacuo. To the concentrated solution of the reduced product was then added an excess of dilute ammonia, as a result of which the base of the desired product, ritodrine, precipitated as a hard mass. After the mixture had been kept in the refrigerator for 6–8 hours, the product was sucked off, washed with water and dried in vacuo. The final product of ritodrine was obtained as a resinous mass upto 2.3 g, mp 88–90ºC.

Precautions

1. In general, most of the evaporations of solvents etc., must be carried out at reduced pressure so as to avoid any possible deterioration of the final product.
2. Norit i.e., an amorphous carbon should only be used as a decolourising agent in this synthises.

Physical Parameters: Ritodrine is a base and obtained as a resinous mass having mp ranging between 88–90°C.

Uses

1. It is used just like salbutamol i.e., as a bronchodilator.
2. It decreases uterine contractions and is often employed to arrest premature labour i.e., as a 'tocolytic'.

Report : Ritodrine was prepared and submitted.

Experiment No:13

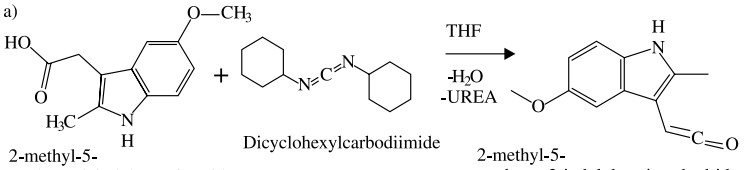
Synthesis of Indomethacin

Aim: To Synthesis and submit indomethacin.

Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle

Chemicals Required: Dicyclohexylcarbodiimide: 10 g; 2-Methyl-5-methoxy-3-indolylacetic acid: 22 g; Tetrahydrofuran (THF): 200 ml; Skellysolve B: q.s.; t-Butyl alcohol: 25 ml; Zinc chloride (fused): 0.3 g; Ether: q.s.; Saturated Sodium Bicarbonate (aqueous): q.s.; Aqueous saturated salt solution: q.s.; Dry Dimethylformamide (DMF): 450 ml; Sodium hydride (50% susp.): 4.9 g; para-Chlorobenzoyl chloride: 15 g; Acetic acid [5% (v/v)]: 1 L; Benzene: q.s.;
Anhydrous Magnesium Sulphate: q.s.; Activated charcoal powder: q.s.; Methanol q.s.; Powdered Porous plate: 1 g; Acetic Acid: q.s.; Dilute HCl (2 M): q.s.; Aqueous Ethanol: q.s.

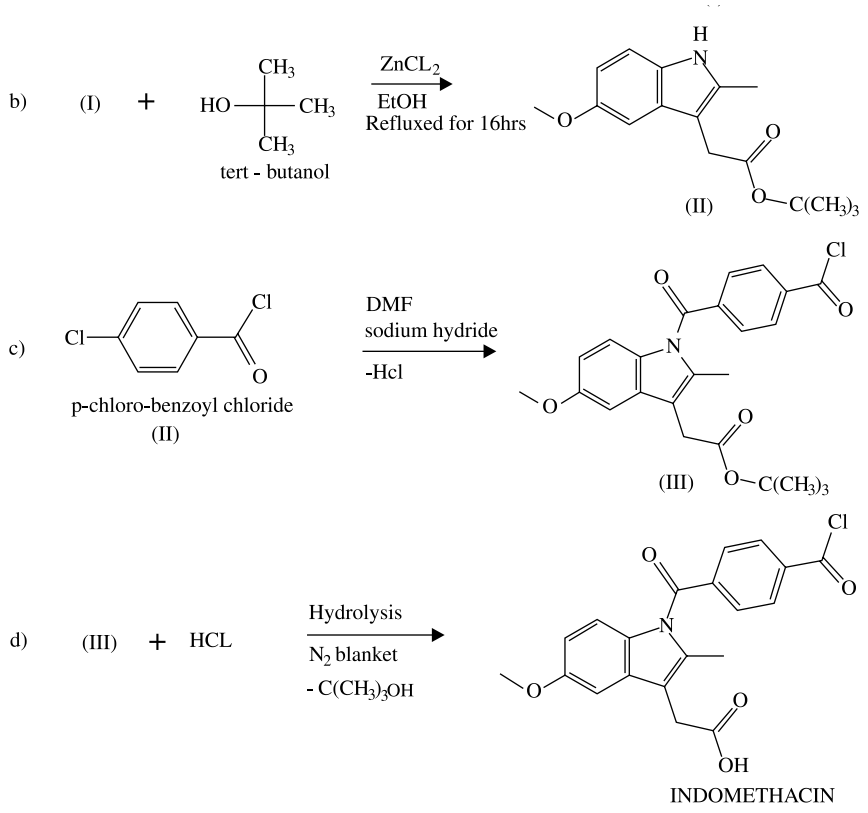
Chemical Structure



2-methyl-5-methoxy-3-indole acetic acid

Dicyclohexylcarbodiimide

2-methyl-5-methoxy-3-indolylacetic anhydride (I)



In Eqn. (a): dichlorohexylcarbodiimide undergoes an interaction with 2-methyl-5-methoxy-3-indole acetic acid in the presence of tetrahydrofuran (THF) when 2-methyl-5-methoxy-3-indolylacetic anhydride (I) is obtained with the elimination of a mole of urea.

In Eqn. (b): compound (I) is made to reflux with tert-butanol in the presence of fused $ZnCl_2$ for 16 hours at a stretch when the corresponding ester tert-butyl-2-methyl-5-methoxy-3- indolyl acetate (II) is obtained.

In Eqn. (c): compound (II) is treated with p-chloro-benzoyl chloride in the presence of dry dimethyl formamide and sodium hydride when the corresponding n-substituted ester i.e., tert-butyl-1-p-chlorobenzoyl-2-methyl-5-methoxy-3-indolyl-acetate (III) is formed with the elimination of one mole of HCl.

Finally, in Eqn. (d) the resulting product (III) is subjected to hydrolysis in an acidic medium under a blanket of N2, when the desired product indomethacin is obtained with the elimination of tert-butanol.

Procedure. The different steps adopted in the synthesis of indomethacin are enumerated below sequentially

Step I. Preparation of 2-Methyl-5-methoxy-3-indolylacetic anhydrides

1. Dissolve 10 g (0.49 mol) dicyclohexylcarbodiimide in a solution of 2-methyl-5-methoxy-3-indolyl acetic acid (22 g; 0.10 mol) in 200 ml of tetrahydro furan (THF); and the solution is maintained at room temperature 25 ± 2°C for at least 2 hours.
2. The precipitated urea is removed in a Büchner funnel under suction; and the resulting filtrate is evaporated in vacuo to a small residue and subsequently flushed with Skellysolve B.
3. The residual oily anhydride (I) is used without any purification in the next step.

Step-II. Preparation of tert-Butyl-2-methyl-5-methoxy-3-indolylacetate

1. The whole of the anhydride obtained from Step-I, is added carefully to 25 ml tertbutyl alcohol and 0.3 g fused zinc chloride. The resulting solution is refluxed for 16 hours at a stretch; and the excess of unreacted t-butyl alcohol is removed under reduced pressure.
2. The residue, thus obtained, is dissolved in ether, washed several times with saturated bicarbonate, water and saturated salt solution.
3. After drying over anhydrous $MgSO_4$, the resulting solution is treated with charcoal, evaporated, and flushed several times with Skellysove B for complete removal of alcohol.
4. The residual oily ester (18 g; 93%) is used without any purification whatsoever in Step-III.

Step-III. Preparation of tert-Butyl-1-p-chlorobenzoyl-2-methyl-5-methoxy-3- indolylacetate

1. A stirred solution of ester (II) (18 g; 0.065 mol) in dry dimethylformamide (DMF) (450 ml) is eventually cooled down to 4ºC in an ice-bath, and sodium hydride (4.9 g, 0.098 mol, 50% suspension) is added in small lots at intervals.
2. After a duration of 15–20 minutes, 15 g (0.085 mol) para-chlorobenzoyl chloride is added dropwise over a span of 10–15 minutes, and the mixture is stirred for 9 hours continuously without replenishing the ice-bath.
3. The resulting mixture is then poured into 1 L to 5% (v/v) acetic acid, extracted successively with a mixture of ether and benzene, washed thoroughly with water, bicarbonate, saturated salt, dried over $MgSO_4$, treated with charcoal, and evaporated to a residue that partly crystallizes.
4. The residue is shaken with ether, filtered and the filtrate is carefully evaporated to a residue (17 g) that solidifies after being refregerated overnight.
5. The entire crude product is boiled gently with 300 ml Skellysolve B, cooled to room temperature, decanted from certain 'gummy material', treated with activated charcoal, concentrated to 100 ml, and allowed to crystallize. The product, thus obtained (10 g) is recrystallized from 50 ml of methanol ; and yields 4.5 g of analytically pure material having mp 103–104°C.

Step IV. 1-para-Chlorobenzoyl-2-methyl-5-methoxy-3-indolylacetic acid

1. A mixture of 4.5 g ester (III) and 0.45 g powdered porous plate is heated in an oil-bath maintained at 210°C, with continuous magnetic stirring, under a blanket of N2 for a duration of 2 hours. **No intensification of colour** (pale yellow) **takes place during this period.**
2. The resulting product is cooled under N2, dissolved in benzene and ether, filtered, and extracted with bicarbonate.
3. The aqueous solution is filtered with suction to get rid of ether, neutralized with acetic acid carefully, and then acidified weakly with dilute HCl.
4. The yield of the crude product is 2.2 g having mp ranging between 149–150.5ºC.

Precautions

1. It is a multi-step synthesis; and, therefore, each step (I through IV) has to be followed rigidly and meticulously.
2. All reagents must be of maximum purity so as to achieve pure product at each step; and, hence, the final product should also be in the purest form.

Recrystallization. The entire curde product (2.2 g) is recrystallized from aqueous ethanol and subsequently dried under vacuo at a temperature not exceeding 65°C. The yield of the pure product is 2.0 g having mp 151°C.

Physical Parameters. The crystals of indomethacin usually exhibits **polymorphism*** having mp for one form ~ 155ºC and the other ~ 162ºC. It has uv_{max} (ethanol): 230, 260, 319 nm; pKa 4.5. It is found

to be soluble in ethanol, acetone, caster oil ; almost insoluble in water. It is quite stable in neutral or slightly acidic media, and found to be decomposed by strong alkali.

Uses

1. It is a potent non-steroidal anti-inflammatory drug (NSAID).
2. It is used in musculoskeletal and joint disorders including ankylosing spondylitis, osteoarthritis, rheumatoid arthritis and acute gouty arthritis.
3. It is employed in peri-articular disorders e.g., bursitis**, and tendinitis.
4. It is also used in pain, inflammation and oedema orthopaedic procedures.
5. It is used in mild to moderate pain in dysmenorrhoea.
6. It is employed as an adjunct to opioids in the control and management of post-operative pain.

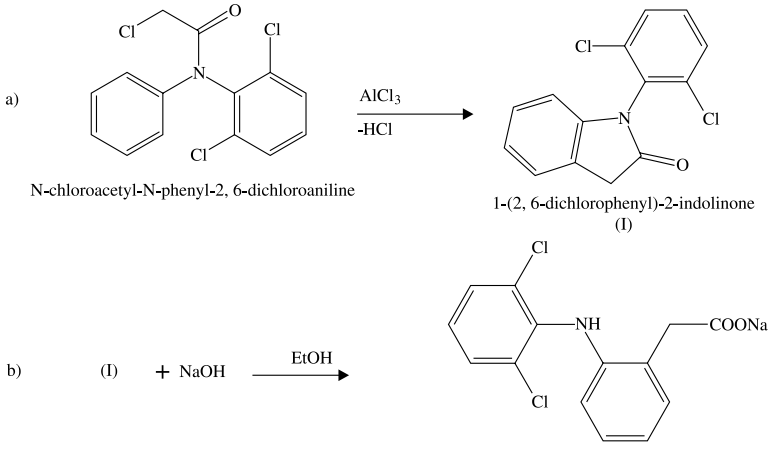
Report : Indomethacin was prepared and submitted.

Experiment No:14

Synthesis of Diclofenac Sodium

Aim: To synthesis and submit diclofenac sodium.
Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle
Chemicals Required: N-Chloroacetyl-N-phenyl-2, 6-dichloroaniline: 16 g; Anhydrous Aluminium Chloride: 16 g; Chloroform: 200 ml; 1-(2, 6-Dichlorophenyl)-2-indolinone: 18.6 g; Ethanol: 66 ml; NaOH (2N) solution: 66 ml.

Chemical Structure



N-chloroacetyl-N-phenyl-2, 6-dichloroaniline

1-(2, 6-dichlorophenyl)-2-indolinone
(I)

2-[(2, 6-Dichlorophenyl) amino] benzeneacetic acid monosodium salt

Theory

Eq. (a) shows the interaction between N-chloroacetyl-N-phenyl-2, 6-dichloroaniline (I) and anhydrous aluminium chloride whereupon the indolin ring closure occurs to yield 1-(2, 6-dichlorophenyl)-2-indolinone (II) with the elimination of a mole of HCl.
Eq. (b) illustrates the formation of the corresponding sodium salt of diclofenac by treatment of (II) with NaOH in the presence of ethanol when the indolinone ring ruptures as shown with dotted lines to obtain the desired product i.e., dichlofenac sodium.

Procedure

The various steps involved are as follows

1. 16 g each of N-chloroacetyl-N-phenyl-2, 6-dichloroaniline and anhydrous aluminium chloride are thoroughly mixed together and heated gently for a duration of 2 hours at 160°C in a 150 ml round bottom flask.
2. The resulting melt thus obtained is allowed to cool and poured onto in a thin stream into a 500 ml beaker containing 200 g of crushed ice with constant stirring. The coil which gets separated is dissolved in 200 ml of chloroform. The chloroform layer is subsequently washed with 40 ml of DW ; and dried over sodium sulphate anhydrous and concentrated under 11 torr. The residue thus obtained is distilled and allowed to cool. The intermediate, 1-(2, 6-dichlorophenyl)-2-indolinone (II) is obtained as a solid product mp 126–127ºC.
3. A solution of 18.6 g 1-(2, 6-dichlorophenyl)-2-indolinone (II) is made in 66 ml ethanol and 66 ml 2 N NaOH solution into a 250 ml round bottom flask fitted with a reflux condenser for a duration of 4 hours. The resulting solution is allowed to cool at 0–5°C in a refrigerator for at least 4 hours. The crude crystals thus obtained is filtered in a Büchner funnel, washed with a little spray of chilled water, dried in the oven; 23.2 g having mp 281–283ºC.

Precautions

1. The compound (I) and $AlCl_3$ must be heated gently upto 150°C for 2 hours, cooled to ambient temperature and poured onto crushed ice with stirring to obtain product (II).
2. The product (II) must be treated with dilute NaOH solution and refluxed cautiously for 4 hours before allowing it to be chilled at 0–5ºC for another similar span.

Recrystallization. The crude product obtained above (23.2 g) is dissolved in minimum amount of water, a few grammes of activated decolourizing carbon may be obtained, filtered and cooled to obtain 22.5 g of recrystallized product mp ranging between 283–285ºC.

Physical parameters: The crystals obtained from water has mp 283–285ºC. It exhibits uv_{max} (methanol) 283 nm. It has solubility at 25ºC (mg. ml–1); deionized water (ph 5.2) > 9; methanol > 24; acetone 6; acetonitrile < 1; cyclohexane < 1; HCl (pH 1.1) < 1; phosphate buffer (ph 7.2)6. It has dissociation constant pKa 4; and partition coefficient (n-octanol/aqueous buffer): 13.4.

Uses:

1. It is a non-steroidal antiinflammatory drug (NSAID) and used mainly as its sodium salt for the relief of pain and inflammation in various conditions, such as : musculoskeletal and joint disorders viz., rheumatoid, arthritis, osteoarthritis; and ankylosing spondolytis; peri-articular disorders, for instance: bursitis* and tendenitis**; soft-tissue disorders, such as: sprains and strains; and other painful conditions, namely: renal colic, acute gout, dysmenorrhoea, and following certain surgical procedures.
2. It is mostly employed as a broad-based antiinflammatory agent.

Note. The corresponding 'potassium salt' i.e., dicolfenac potassium is recommended for patients having hypertension indications (i.e., to avoid sodium ions).

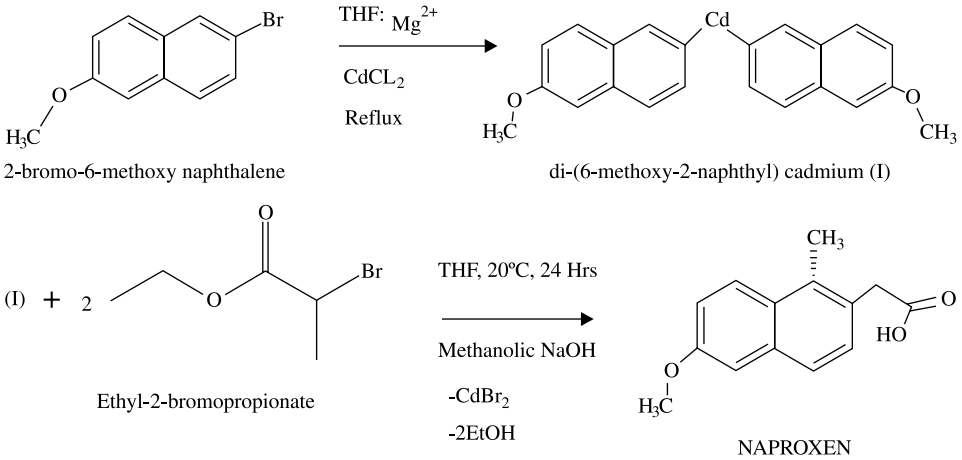
Report : Diclofenac sodium was prepared and submitted.

Experiment No:15

Synthesis of Naproxen

Aim: To Synthesis and submit naproxen.
Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle
Chemicals Required: 2-Bromo-6-methoxynaphthalene: 24 g; Tetrahydrofuran (THF): 450 ml; Magnesium turnings (Fresh): 2.5 g; Cadmium chloride: 20 g; Ethyl-2-bromopropionate: 18 g; Methanolic NaOH solution [5% (w/v)]: 200 ml; Ether: q.s.; Acetone: Hexane (1 : 1): q.s.

Chemical Structure



Theory

Eqn. (a) represents the interaction between 2-bromo-6-methoxy naphthalene and cadmium chloride in the presence of fresh magnesium turnings and tetrahydrofuran (THF) followed by reflux to give rise to the formation of one mole of di-(6-methoxy-2-naphthyl) cadmium (I).

Eqn. (b) shows the interaction between (I) and two moles of ethyl-2-bromopropionate in the presence of THF, at 20°C for 24 hours followed by hydrolysis in the presence of methanolic NaOH to yield the desired product naproxen with the elimination of one mole of $CdBr_2$ and two moles of ethanol.

Procedure

The different steps followed sequentially are as stated below* :

1. A solution of 24 g 2-bromo-6-methoxynaphthalene in 300 ml THF is poured gradually to 2.5 g fresh magnesium turnings and 100 ml THF at reflux temperature (~ 66°C).
2. Once the addition is complete, 20 g cadmium chloride is added; and the resultant mixture is refluxed for 10 minutes to yield a solution of di-(6-methoxy-2-naphthyl) cadmium (I).**
3. A solution of 18 g ethyl-2-bromopropionate in 20 ml THF is now added to the previously cooled reaction mixture obtained in step (2). After allowing to keep the resulting mixture at 20°C for a duration of 24 hours, the product is subjected to hydrolysis by adding carefully 200 ml of methanolic NaOH solution, followed by heating to reflux for 60 minutes.
4. The resulting mixture is then diluted with excess of sulphuric acid (1 N) to acidic condition; and extracted with ether successively. The ethereal layer is separated, evaporated to dryness.
5. The residue is recrystallized from a mixture of acetone and hexane (1 : 1) to give rise to the ultimate yield of the desired product, naproxen, to the extent of 16.66 g, mp 152–154°C.

Precautions

1. Step (3) is very crucial in the synthesis of 'naproxen' and each step must be followed rigidly.
2. In step (4) the bulk of the ether from the combined ethereal extract must be removed in a thin-film rotary evaporator carefully.

Physical Parameters. Naproxen is obtained as bitter crystals from acetone hexane having mp 152–154°C. It has specific optical rotation [α]D + 66° (in chloroform). It is found to be soluble in 25 parts

ethanol (96%); 20 parts methanol ; 15 parts chloroform ; 40 parts ether ; and almost insoluble in water. It has apparent pKa 4.15.

Uses

1. It is indicated for relief of symptoms of rheumatiod arthritis, both of acute flares and long-term management of the disease.
2. It is used to relieve mild-to-moderate postoperative pain as well as postpartum pain, primary dysmenorrhea, orthopedic pain, headache, and visceral pain associated with cancer.
3. Its analgesic actions are fairly comparable with those of aspirin or indomethacin.

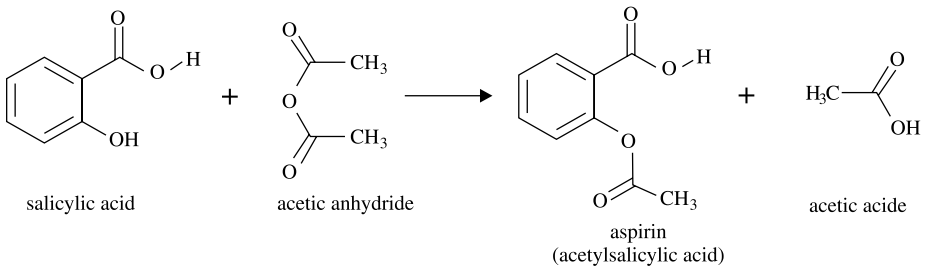
Report : Naproxen was prepared and submitted.

Experiment:No:16

Synthesis of Aspirin

Aim: To Synthesis and submit aspirin.
Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle
Chemicals required: (i) Salicylic acid: 6 g; (ii) Acetic anhydride: 10 ml; and (iii) Glacial acetic acid: 10 ml.

Chemical



Structure

Aspirin is prepared from salicylic acid, acetic anhydride and glacial acetic acid.
Theory: Salicylic acid interacts with acetic anhydride in the presence of glacial acetic acid whereby the cleavage in acetic anhydride takes place with the formation of aspirin and a mole of acetic acid. The glacial acetic acid helps in the generation of excess acetate ion which carries the reaction in the forward direction. The acetic acid obtained as a product of reaction is reused in the reaction itself.
Procedure: The following steps may be adopted in a sequential manner:

1. Prepare an admixture of 10 ml each of acetic anhydride and glacial acetic acid in a 100 ml clean and dry beaker.
2. Now, add this mixture carefully to 6 g salicylic acid previously weighed and placed in a 100 ml round bottom flask; and fit the same with a reflux condenser.
3. Boil the reaction mixture on an electric heating mantle for a duration of 35–45 minutes.
4. Pour the hot resulting mixture directly into 100 ml cold water, contained in a 500 ml beaker in one lot; and stir the contents vigorously with a clean glass rod when the shining tiny crystals of aspirin separate out.
5. Filter off the crude aspirin in a Büchner funnel fitted with an air-suction device and wash the residue with sufficient cold water, drain well and finally remove the excess of water by pressing it between the folds of filter paper and spread it in the air to allow it dry completely. However, it may also be dried expeditiously by drying it in an electric oven maintained at 100°C for about an hour. The yield of crude aspirin (mp 133.5–135°C) is approximately 7.5 g.

Precautions

1. All glass apparatus to be used in the synthesis must be perfectly dried in an oven.
2. Gentle refluxing should be done to complete the acetylation of salicylic acid.

Recrystallizatoin: Recrystallize the crude product from a mixture of acetic acid and water.
Physical Parameters: Aspirin is obtained as monoclinic tablets or needlelike crystals, mp 135°C (rapid heating) ; the melt gets solidified at 118°C ; uv_{max} (0.1 NH_2SO_4) : 229 nm (E1 cm 1% 484) ; $CHCl_3$: 277 nm (E1 cm 1% 68). It is usually odourless, but in moist air it gets hydrolyzed slowly into salicylic acid and acetic acid, and overall acquires the odour of acetic acid. It is fairly stable in dry-air, 1 g dissolves in 300 ml water at 25°C, in 100 ml of water at 37°C, in 5 ml ethanol, 17 ml chloroform and 10–15 ml solvent ether.

Uses

1. It is used for the relief of minor aches and mild to moderate pain.
2. It is recommended for arthritis and related arthritic conditions.
3. It is also indicated for myocardial infarction prophylaxis.
4. It is employed to reduce the risk of transient ischemic attacks in men.

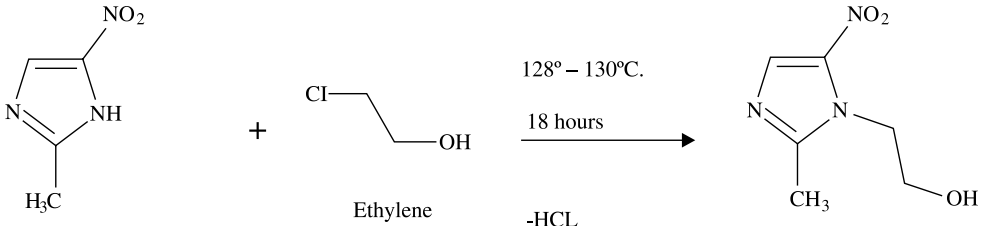
Report : Aspirin was prepared and submitted.

Experiment No:17

Synthesis of Metronidazole

Aim: To Synthesis and submit metronidazole.
Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle
Chemicals Required: 2-Methyl-5-nitroimidazole: 25.4 g; Ethylene chlorohydrin: 160 g; Sodium Hydroxide: 20 ml; Chloroform: 200 ml; Ethyl acetate: 90 ml.

Reaction:



Theory: The reaction between 2-methyl-5-nitroimidazole and Ethylene chlorohydrin at an elevated temperatures ranging from 128–130°C for a period of 18 hours results into the formation of metronidazole with the elimination of one mole of HCl.

Procedure:

The various steps involved in the synthesis are as follows :

1. 25.4 g (0.256 mol) 2-Methyl-5-nitroimidazole is heated with ethylene chlorohydrins (160 g; 2 mol) for a period of 18 hours at 128–130°C.
2. The unreacted and excess of chlorohydrin (~ 133 g) is now distilled under reduced pressure (30 mmHg).
3. The resulting product (residue) is subsequently treated with 60 ml water (DW) and filtered. The filtrate is made alkaline by the addition of sodium hydroxide solution (d = 1.33; 20 ml).
4. The alkaline solution, thus obtained, is successively extracted with chloroform (200 ml). The combined layer of chloroform is evaporated under vacuo to obtain ~ 15.5 g of a pasty mass.

5. The pasty mass is recrystallized form 90 ml ethyl acetate in the presence of a small quantum of activated powdered charcoal. The pure creamy white crystalline powder of metronidazole weighing 4.8 g, mp 158°– 160°C is obtained.

Precautions

1. In the very first step the two reactants must be heated for 18 hours at a stretch between 128°–130°C.
2. The excess of unreacted ethylene chlorohydrin should be removed under vacuo (30 mm Hg) so that the decomposition of the final product is avoided to the maximum extent.
3. The residue is taken up in water and made alkaline with a calculated amount of NaOH solution carefully.

Physical Parameters: Metronidazole is obtained as cream-coloured crystals having mp 158–160°C. Its solubility at 20°C (g/100 ml): water 1.0; ethanol 0.5; ether < 0.05; and chloroform < 0.05. It is found to be sparingly soluble in dimethyl formamide (DMF) and soluble in diluted acids. The pH of a saturated aqueous solution stands at 5.8.

Uses

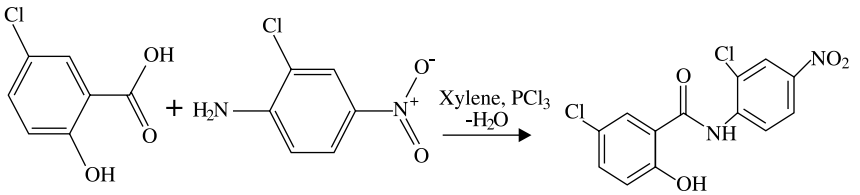
1. Metronidazole long has been the drug of choice for the treatment of trichomoniasis and more recently in combination with idoquinol for the treatment of symptomatic amebiasis.
2. It is also the drug of choice for the treatment of Dracunculus (guinea worm).
3. It is the alternative drug to treat giardiasis, balantidiasis, blastocystitis, and infections by Entameba polecki.
4. It is used widely for the treatment and prophylaxis of infections caused by anaerobic bacteria.
5. It is a drug of choice against GI strains of Bacteroides fragilis ; and vaginal infections caused by Gardnerella vaginalis.
6. It has been used successfully in the treatment of antibiotic-associated psedomembranous colitis.
7. It is also useful in **Crohn's disease*.**

Report : Metronidazole was prepared and submitted.

Experiment No:18

Synthesis of Niclosamide

Aim: To Synthesis and submit niclosamide.
Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle
Chemicals Required: 5-Chlorosalicylic acid: 17.25 g; 2-Chloro-4-nitroaniline: 20.9 g; Xylene: 250 ml; Phosphorous trichloride (PCl_3): 5 g; Ethanol: q.s.
Reaction:



5-Chlorosalicylic acid 2-Chloro-4-nitroaniline Niclosamide

Theory: The interaction between 5-chlorosalicylic acid and 2-chloro-4-nitro aniline in the presence of xylene, phosphorous tri-chloride and heating results into the formation of niclosamide with the elimination of one mole of water.

Procedure

The various steps involved in the synthesis of niclosamide are as stated below :

1. 17.25 g (0.1 mol) 5-Chlorosalicylic acid and 20.9 g (0.12 mol) 2-chloro-4-nitroaniline are dissolved carefully in 250 ml pure xylene in a 500 ml round bottom flask fitted with a double surface condenser.
2. The reaction mixture is boiled on a heating mantle and are introduced in small lots at intervals 5 g pure PCl_3 from the top end of the condenser. Heating is continued for two further hours.
3. The reaction mixture is allowed to cool down when the crude crystals of niclosamide start separating out. Filter the crude product in a Büchner funnel under suction. The crude product is recrystallized from ethanol to yield 26.5 g having mp 233°C.

Precautions

1. Both xylene and phosphorous trichloride should be freshly distilled and under perfectly anhydrous conditions to yield better product and maximum yield.
2. The crude product may be recrystallized from a minimum quantity of ethanol.

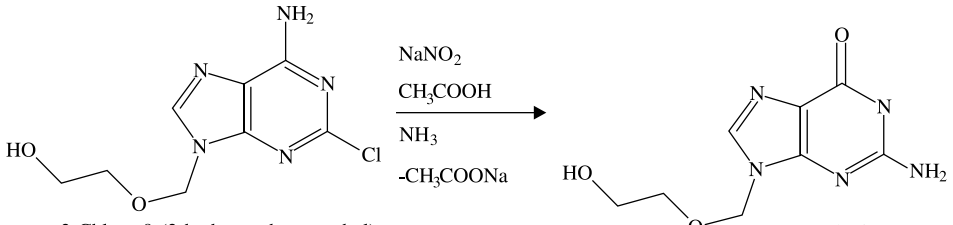
Physical Parameters. Niclosamide is obtained as pale yellow crystals having mp 225–230°C. It is found to be practically insoluble in water ; and sparingly soluble in ethanol, ether and chloroform.
Uses: It is a potent anthelminthic especially effective against the cestodes* that infect humans.

Report : Niclosamide was prepared and submitted.

Experiment No:19

Synthesis of Acyclovir

Aim: To Synthesis and submit acyclovir.
Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle
Chemicals Required: Sodium nitrite: 4.85 g; 2-Chloro-9-(2-hydroxyethoxymethyl) adenine: 2.5 g; Glacial Acetic Acid: 50 ml; Ammonia gas: q.s.; Ethanol: q.s.;
Reaction:



2-Chloro-9-(2-hydroxyethoxymethyl) adenine — Acyclovir

Theory: The substituted adenine i.e., 2-chloro-9-(2-hydroxyethoxy-methyl) adenine is treated with pure sodium nitrite in glacial acetic acid and ammonia gas is passed through the reaction mixture for a stipulated period when the amino function gets rearranged from C-6 to C-2 together with a carbonyl moiety at C-6. Besides, there is a shift of double bond between positions from 2–3 and 5–6 to 2–3 and 4–5.

Procedure

The steps followed are as follows:

1. Solid sodium nitrite (4.85 g) was added at an ambient temperature (RT**) with constant stirring over a span of 60 minutes, in small lots at intervals, into a solution of 2.5 g of 2-chloro-9-(2-hydroxyethoxymethyl) adenine in 50 ml of glacial aceitic acid in a 250 ml round bottomed flask

fitted with a mechanical stirrer, an inlet for NH_3-gas and an air-condenser fitted with a $CaCl_2$-guard tube.
2. The reaction mixture was stirred for an additional 4 hours and 30 minutes in an atmosphere of ammonia gas.
3. The resulting white precipitate was removed by filtration in a Büchner funnel under suction, washed with a spray of cold acetic acid; and then triturated nicely with cold water to get rid of the sodium acetate present.
4. The white solid product was retained duly. The combined acetic acid filtrate and wash was carefully evaporated under reduced pressure at 40°C bath temperature, and the resulting residual oil triturated again with cold water. **RT = Room Temperature.
5. The resulting solid material was combined with the previously retained/isolated white solid and the combined solids dried and weighed. The yield of the crude product was 1.30 g having mp 250–251°C.

Precautions

1. The sodium nitrite must be added in small lots at intervals over a span of 60 minutes.
2. The reaction mixture should be stirred constantly for almost 4 1/2 hours in an atmosphere of ammonia gas to facilitate the intramolecular changes.
3. The crude product needs to be recrystallized either from ethanol methanol.

Recrystallization: The crude product is recrystallized from ethanol to obtain a pure product having mp 256.5–257°C and yield 1.25 g.

Physical Parameters. Acyclovir is obtained as colourless crystals from methanol mp 256-257ºC.

Uses

1. It is invariably employed in the treatment and prophylaxis of infections due to Herpes simplex* or Varicellazoster viruses.
2. It is used broadly as an antiviral agent.

Report : Acyclovir was prepared and submitted.

Experiment No:20

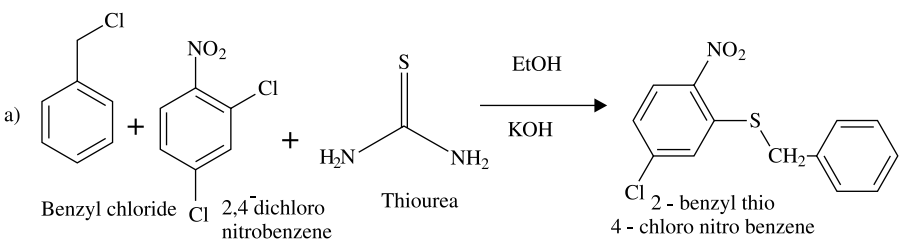
Synthesis of Diazoxide

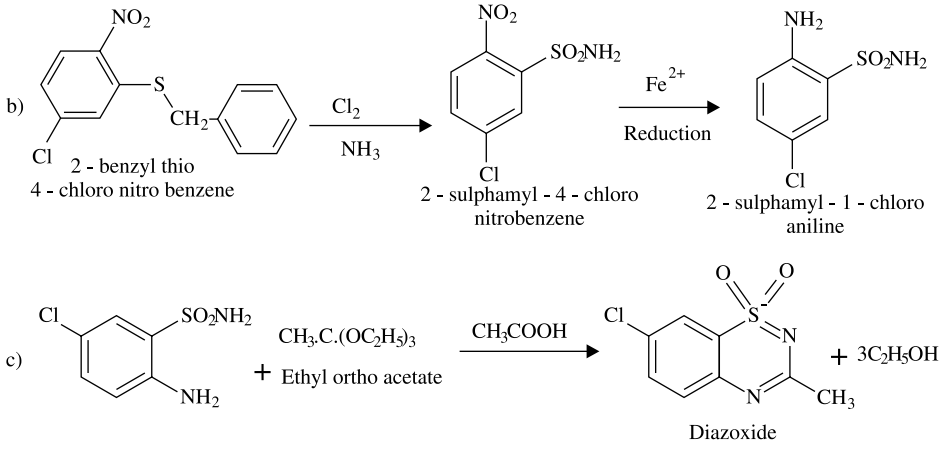
Aim: To Synthesis and submit diazoxide.

Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle

Chemicals Required. Benzyl chloride: 63 g; Thiourea: 38 g; Conc.Ammonia Soln.: 3 drops; 2, 4-Dichloronitrobenzene: 96 g; Ethanol: 200 ml; Ethanolic KOH Soln. (70 g in 500 ml EtOH): 500 ml; 2-Benzylthio-4-chloronitrobenzene: 5 g; Aq. Acetic Acid [33% (v/v)] 1 L; Chloroform: 1.5 L; Anhydrous Sodium Sulphate: q.s.; Liquid Ammonia: 400 ml; n- Hexane: q.s.; Methanol: q.s.; Ammonium chloride: 4.4 g; 2-Sulphamyl-4-chloro-nitrobenzene: 3 g; Iron Fillings: 4.4 g; 2-Sulphamyl-4-chloro-aniline: 6 g; Ethyl orthoacetate: 15 ml.

Reaction





Theory:

Eq. (a) shows the interaction of benzyl chloride, 2-4-dichloronitrobenzene and thiourea to result into the formation of 2-benzylthio-4-chloro-nitrobenzene (I).

Eq. (b) depicts how (I) on being treated with Cl_2-gas followed by ammonia produces the 2-sulphamyl-4-chloro-nitrobenzene (II) ; which upon reduction with iron filings yields the corresponding 2-Sulphamyl-4-chloroaniline (III).

Eq. (c) illustrates the interaction of (III) with ethyl ortho-acetate in the presence of acetic acid to obtain the desired compound, diazoxide, with the elimination of three moles of ethanol.

Procedure

The various steps involved are given below in a sequential manner:

1. Mix 63 g benzyl chloride, 38 g thiourea, 3 drops concentrated NH_4OH solution, and 250 ml [95% (v/v)] ethanol into a 2L round bottom flask fitted with a double-surface reflux condenser. Reflux the reaction mixture for 3 hours and allow it to cool.
2. Add to the resulting solution 96 g 2, 4-dichloro-nitrobenzene in 200 ml ethanol. Heat the mixture to reflux and then add drop-wise a solution of 500 ml ethanolic KOH solution. Continue the refluxing for another 2 hours, cool the contents, filter the solid product in a Büchner funnel under suction, wash with aqueous ethanol and dry between the folds of filter paper. The product thus obtained is 2-benzylthio-4-chloronitrobenzene (I).
3. Suspend 50 g of (I) obtained in step (2) in 1 L of 33% aqueous acetic acid. Pass pure Cl_2-gas through the suspension by means of gentle bubbling for a span of 2 hours, while strictly maintaining the temperature of the suspension at a low temperature ranging between 0–5°C.
4. Extract the resulting mixture at least thrice successively with 400 ml each of pure dry chloroform, combine the extracts, and wash the chloroform extract several times with DW. Now, dry the chloroform solution with anhydrous sodium sulphate and filter.
5. Evaporate the dried chloroform layer under reduced pressure to a residue, add to it 400 ml of liquid ammonia, stir well mechanically in a fuming cup-board; and allow the excess ammonia to evaporate completely. Triturate the residue with n-hexane to form a crystalline solid, continue trituration with water and subsequently filter the solid to yield sufficiently pure 2-sulphamyl-4-chloro-nitrobenzene (II).

[Note: The product (II) may be recrystallized from aqueous MeOH.]

6. Transfer to a 250 ml round bottom flask 4.4 g ammonium chloride, 18 ml methanol, 9 ml water, and 3 g of (II) obtained from step (5). Reflux the resulting mixture gently, while adding from the

top-end of the condenser 4.4 g iron fillings in small lots at intervals during a period of 90–100 minutes. Cool the mixture and filter the solid product at the pump. Recrystallize the crude product from minimum quantity of aqueous methanol to yield substantially pure 2-sulphamyl-4-chloroaniline (III).

7. Heat a mixture of 6 g (III) and 15 ml ethyl orthoacetate at 100–110°C for a period of 90–100 minutes. Cool, the contents to obtain the desired crude product, diazoxide, filter at the pump and drain well. The yield of the crude product is 5.32 g having mp 329–330.5°C.

Precautions

1. In step (3) the chlorine gas must be passed through the suspension slowly and strictly at a temperature between 0–5°C.
2. In step (5) the introduction of 400 ml of liquid ammonia into the chloroform evaporated residue obtained from step (4) must be done very cautiously in an efficient fuming cup-board.
3. In step (6) the addition of iron-fillings into the refluxing reaction mixture is to be carried out over a span of 90–100 minutes in small lots at intervals.

Recrystallization. The crude diazoxide is dissolved in minimum amount of aqueous ethanol (1 : 1) to obtain white crystalline mass 5.1 g having mp 329.5–330°C.

Physical Parameters. Diazoxide is obtained as crystals from dilute alcohol having mp 330–331°C. It has uv_{max} (methanol): 268 nm (ε11300). It is found to be soluble in ethanol and alkaline solutions; and practically insoluble in water.

Uses

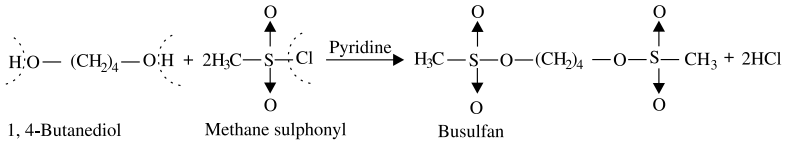
1. It is a direct acting peripheral vasodilator which reduces blood pressure (anti-hypertensive).
2. It also exhibits antidiuretic and hyperglycaemic effects.

Report : Diazoxide was prepared and submitted.

Experiment No:21

Synthesis of Busulfan

Aim: To Synthesis and submit busulphan.
Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle
Chemicals Required: 1, 4-Butanediol (redistilled): 3.6 g; Pyridine (redistilled): 10 ml; Methane sulphonyl chloride (redistilled): 9.6 g; Acetone: 50 ml; Ether: 50 ml.
Reaction



Chemical Structure

Theory: One mole of 1, 4-Butanediol reacts with two moles of methane sulphonyl chloride in the presence of pyridine to yield one mole of busulfan and two moles of HCl are eliminated.
Procedure: The various steps involved are as follows :

1. 3.6 g (0.04 mol) of redistilled 1, 4-butanediol was dissolved in 10 ml of redistilled pyridine* and the resulting solution was chilled in an ice-bath.
2. 9.6 g (0.08 mol) of redistilled methane sulphonyl chloride were added dropwise at such a regulated rate that the temperature was not permitted to go beyond 18 ± 2°C. After the completion of addition of methane sulphonyl chloride, the reaction mixture was allowed to stand at room

temperature for a duration of 30 minutes, during which material time the temperature was elevated to ~ 60°C of its own (exothermic reaction).

3. A thick precipitate of pyridine hydrochloride was formed. *Pyridine being basic in nature gets oxidized with atmospheric oxygen thereby retarding its purity and reactivity; hence, it should always be freshly redistilled before use in a reaction. The same holds good for **aniline**.
4. The mass was cooled in ice-water and was treated with 30 ml of ice-cold water. A mere agitation with a glass rod shall yield a white crystalline solid.
5. Filter of the white crystalline product in a Büchner funnel under vacuo, wash with a spray of iced water and allow to drain on the pump thoroughly. The yield of the crude product was 7.75 g and had a mp 100°C.

Precautions

1. Always make use of freshly redistilled 1, 4-Butanediol, Pyridine and Methane sulphonyl chloride in this reaction to obtain a pure product with better yield.
2. The addition of methane sulphonyl chloride must be carried out **only dropwise** taking care that the temperature of the reaction mixture must not exceed 20°C, in any case.
3. The pyridine hydrochloride is obtained as a thick precipitate, duly formed by the interaction of pyridine and HCl formed as a product of reaction. This has got to be removed and set apart.

Recrystallization: The crude product is recrystallized from a mixture of acetone and ether (1 : 1) to obtain beautiful small white needles with a yield of 7.50 g and mp 106-107°C.

Physical Parameters. It is obtained as crystals mp 114-118°C. It is found to be soluble in acetone at 25°C: 2.4 g/100 ml; in ethanol: 0.1 g/100 ml; almost insoluble in water, but will dissolve slowly as hydrolyses takes place.

Uses

1. It is approved for the palliative treatment of chronic granulocytic leukaemia*.
2. It is also quite effective in the treatment of polycythemia vera** and primary thrombocytocytosis.***

*A polymorphonuclear leukocyte (viz, neutrophil, esosinophill, or basophil).

**A chronic, life-shortening mycloproliferative disorder of unknown etiology involving all bone marrow elements ; characterized by an increase in RBC mass and homoglobin concentration.

***Primary dissolution of thrombocytes (i.e., platelet).

Report : Busulfan was prepared and submitted.

Experiment No:22

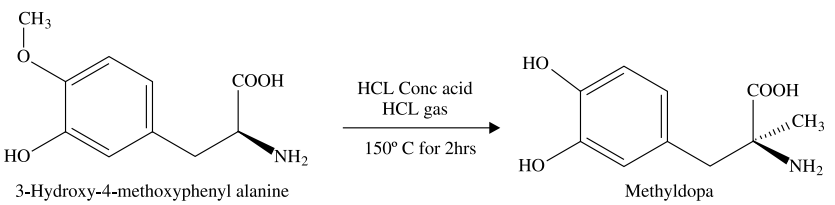
Synthesis of Methyldopa

Aim: To Synthesis and submit methyldopa.

Apparatus required : Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle

Chemicals Required. 3-Hydroxy-4-methoxyphenyl alanine : 2.5 g ; Concentrated Hydrchloric Acid (12 M) : 100 ml ; Ethanol [95% v/v] : q.s. ; Ammonium Hydroxide : q.s. ; Ether : q.s.

Reaction



3-Hydroxy-4-methoxyphenyl alanine Methyldopa

Theory: The interaction of 3-hydroxy-4-methoxy phenylanine with concentrated hydrochloric acid in the presence of HCl-gas and subsequent heating at 150°C for a period of 2 hours results into an intramolecular rearrangement yielding a racemic mixture of **methyldopa**.

Procedure. The procedural details consist of **two** parts:

(a) Preparation of dl-α-methyl-3, 4-dihydroxyphenylalanine.
(b) Separation of L-α-methyl-3, 4-dihydroxyphenylalamine from the 'racemate'.

Part A. Preparation of dl-α–Methyl-3, 4-dihydroxyphenylalanine**

1. 2.5 g 3-Hydroxy-4-methoxyphenyl alanine was dissolved in 100 ml conc. Hydrochloric acid. The resulting solution was duly saturated with hydrogen chloride (gas), and heated subsequently in a sealed tube at 150°C for a duration of 2 hours at a stretch. * Pseudohypertrophic muscular dystrophy marked by weakness and pseudohypertrophy of the affected musles.
2. The resulting 'dark' reaction mixture was concentrated to dryness under reduced pressure; and the excess of mineral acid removed by flushing several times with ethanol.
3. The dark residue, thus obtained, was dissolved in a minimum quantity of water. The pH of the clarified solution was adjusted to pH 6.5 with ammonium hydroxide carefully when fine crystals separated out which was filtered in a Büchner funnel under suction, washed with ethanol followed by solvent ether. The crystalline product which is a racemic mixture of methyl dopa weighed 2.24 g haivng mp ranging between 299.5–300°C with decomposition.

Part B. Separation of dl-α–Methyl-3, 4-dihydroxyphenyl-alanine*

1. 3.7 g Racemic α–methyl-3, 4-dihydroxyphenylalanine are slurried at 35°C in 10 ml of 1 N hydrochloric acid. The excess solids are filtered leaving a saturated solution containing 3.46 g racemic amino acid of which approximately 61% is present as the hydrochloride.
2. The resulting solution is subsequently 'seeded' at 35°C with 0.7 g hydrated L-α–methyl- 3, 4-dihydroxyphenylalanine (≡0.62 g anhydrous material). The mixture is then cooled to 20°C in 30 minutes and aged at 20°C for 60 minutes.
3. The separated material is isolated by filtration, washed twice with 10 ml of cold water and subsequently dried under vacuo. The yield of the product is 1.41 g L-α–methyl-3, 4-dihydroxyphenylalanine in the form of a sequihydrate of 100% purity**.

Physical Paramete: It is obtained as L-form sesquihydrate, crystals from water. It may also be obtained as minute anhydrous crystals from methanol. It is found to be considerably hygroscopic in nature; and gets decomposed at ~ 300°C. It shows uv_{max} 281 nm. It is found to be soluble in water at 25°C: ~ 10 mg. ml–1. The pH of a saturated solution (aqueous) is about 5.0. It is almost insoluble in the common organic solvents, but soluble in diluted mineral acids.

Uses

1. It is used in the management and control of hypertension (e.g., essential hypertension).
2. Its metabolite α-methylnorepinephrine shows potent α2–agonist activity.

Report : Methyldopa was prepared and submitted.

Experiment No:23

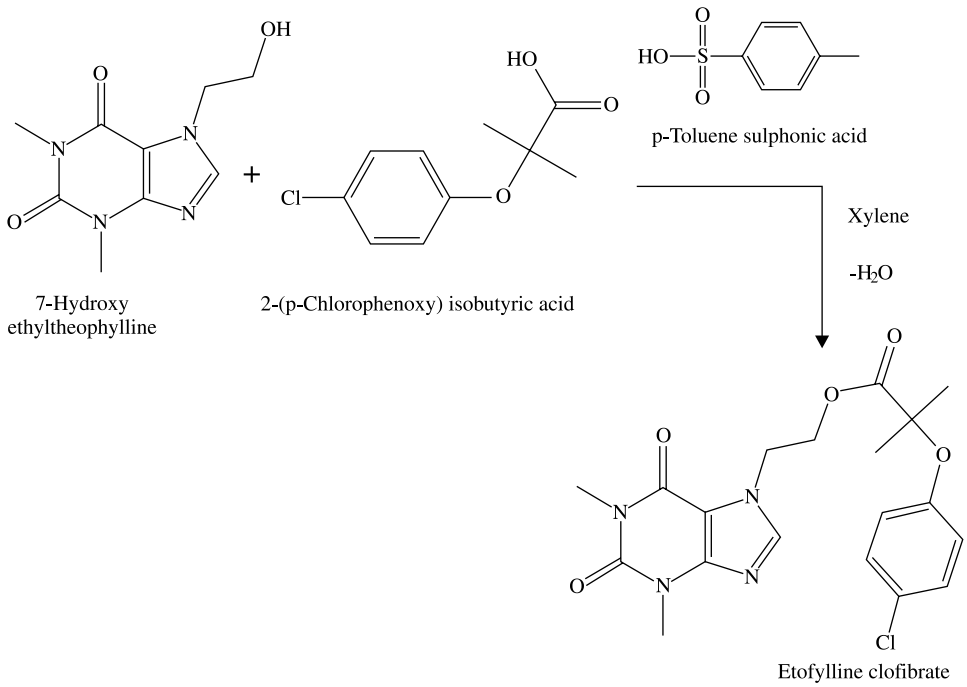
Synthesis of Etofylline Clofibrate

Aim: To Synthesis and submit etofylline clofibrate.

Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle

Chemicals Required: 2-(p-Chlorophenoxy) isobutyric acid = 10.73 g; 7-Hydroxy ethyltheophylline: 5.6 g; p-Toluene sulphonic acid = 0.15 g; Sodium bicarbonate solution (0.5 M): 50 ml; Isopropanol: 50 ml; Xylene: 25 ml.

Reaction



Theory: Etofylline interacts with clofibric acid in the presence of p-toluene sulphonic acid in a medium of xylene to give rise to the formation of etofylline clofibrate with the elimination of a mole of water. However, the presence of p-toluene sulphonic acid acts as a felicitator in the abstraction of a mole of water to obtain the corresponding desired ester.

Procedure

The various steps involved are as given below:

1. Transfer 10.73 g (0.005 mol) 2-(p-chlorophenoxy) isobutyric acid and 5.6 g (0.025 mol) 7-hydroxy ethyltheophylline were suspended together in 25 ml xylene in a 100 ml round bottom flask. The resulting mixture was heated together for almost 15 hours at a stretch in a water-separator following the addition of 0.15 g p-toluenesulphonic acid.
2. The resulting solution was shaken adequately with dilute sodium bicarbonate solution till it became alkaline to litmus paper, washed with water ; and the solvent was carefully evaporated in a rotary evaporator.
3. The solid residue thus obtained was filtered in a Büchner funnel under suction, washed with a little chilled water, drained and dried in between the folds of filter paper. The yield of the crude product is 6.1 g mp 130.5-131.5°C.

Precautions

1. The reaction mixture is heated together for 15 hours in a water separator, which can be accomplished by simply placing some **activated sieves*** in the reaction flask, so as to remove the small amount of water formed during the course of reaction
2. After completion of the reaction, the resulting mixture is carefully made alkaline to litmus paper with sodium bicarbonate solution (0.5 M).

3. The solvent i.e., Xylene should be removed either under rotary evaporator or under reduced pressure.

Physical parameters. Theofibrate is obtained as colourless crystals from ethanol mp 133-135°C. It is practically insoluble in water at pH 2-7.4 and in cold alcohols. It is, however, found to be soluble in acetone, chloroform and hot alcohols.

Uses

(1) It is used as a hypolipidaemic agent in conjunction with dietary modification.
(2) It is employed as antihyperlipoproteinemic.

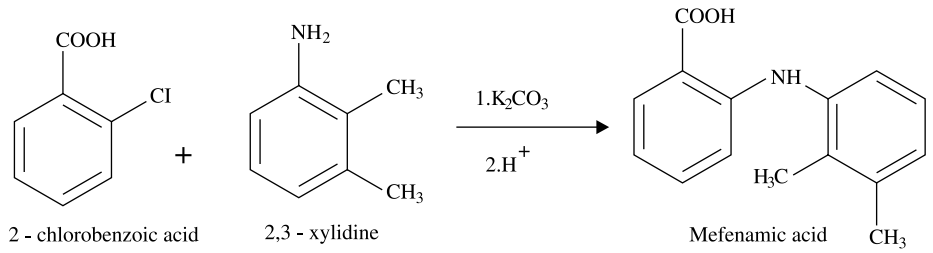
Report : Etofyline clofibrate was prepared and submitted.

Experiment No. 24

Synthesis of Mefenamic Acid

Aim: To Synthesis and submit mefenamic acid.
Apparatus required: Round Bottom flask, Reflux Condenser, beaker, funnel, Heating mantle
Chemicals required: *O*-chlorobenzoic acid, 2,3-xylidine, potassium carbonate.

Reaction involved



Principle: It may be prepared by the condensation of *O*-chlorobenzoic acid with 2, 3-xylidine in the presence of potassium carbonate to give the potassium salt of mefenamic acid, which on treatment with hydrochloric acid yields the official compound.The anthranilates have primarily antiinflammatory with some analgesic and antipyretic activity and are non-COX selective. The anthranilates are used as mild analgesics and occasionally to treat inflammatory disorders. Diclofenac is used for rheumatoid arthritis, osteoarthritis and post-operative pain and mefenamic acid as an analgesic for dysmennorhea.

Procedure: 1 mole of O-chlorobenzoic acid was refluxed with 1.2 mole of 2,3-xylidine and anhydrous potassium carbonate (8 g) for 7 h. The solid thus obtained was suspended in water. Finally mefenamic acid was precipitated with the help of dilute hydrochloric acid, dried and recrystalized from ethanol (95%).

Report : Mefenamic acid was synthesized and submitted.
The melting point was found to be…….

Experiment. No:25

Synthesis of Benzimidazole from Ortho–Phenylene Diamine

Aim: To prepare and calculate percent yield of benzimidazole.
Apparatus: Round bottom flask, condenser, water bath.

Chemicals required: O-phenylene diamine, formic acid, and 10%NaOH, charcoal, litmus paper
Principle: Synthesis of benzimidazole is carried out by Phillips Reaction. It involves both treating O-Phenylene diamine and formic acid. The excess of formic acid is neutralized by the addition of NaOH resulting in the precipitation of benzimidazole.

O-Phenylene Diamine Benzimidazole

Mechanism: Place 27gms of o-phenylene diamine in 250ml round bottom flask and add 17.5gms of 90% formic acid. Keep the mixture on water bath at 100°C for 2 hrs. Cool the mixture and add 10% NaOH solution slowly with constant stirring until the mixture is just alkaline to litmus. Filter thje solution and wash the residue with cold water and the product is recrystallised with hot water and charcoal. (Yield, 54.23%)

Report : Benzimidazole was prepared and submitted.

Experiment.No:26

Synthesis of P-Amino Salicylic Acid from P-Nitro Salicylic Acid

Aim: To prepare and submit p-amino salicylic acid.
Apparatus: Round bottom flask, Condenser, Water bath.
Chemicals required: P–Nitro Salicylic Acid, Granulated Tin, Conc. HCl, 20-40% NaOH, Ethanol, Ether
Principle: Primary aryl amines are generally prepared by the reduction of nitro compounds. When only small quantities are to be redused and cost is secondary consideration tin and hydrochloric acid may be employed. Theoretically 1.5 mol of tin is needed to reduse nitro group the metal being oxidized to tin(IV) state. When reduction is complete a complex amine chlorostanate may separate from which the amine is liberated by basification using enough alkali to dissolve the tin hydroxides formed.
Procedure: In a 50 ml RBF fitted with a reflux condenser place 1g of the p-nitro salicylic acid and 2 g of granulated tin. Measure out 10ml of Conc. HCL and add it in the three equal portions to the mixture; shake thoroughly after each addition. When the vigorous reaction subsides, heat under reflux on a water bath until the p-nitro salicylic acid has completely reacted (20–30min).Shake the reaction mixture time to time if the nitro compound appears to be very insoluble, add 5 ml of ethanol. Cool the reaction mixture and add 20–40% NaOH solution until the precipitate of tin hydroxide dissolves. Extract the resulting p-amino salicylic acid from the cooled solution with ether and remove the ether by distillation.

Report: p-amino salicylic acid was prepared and submitted.

Experiment.No:27

Synthesis of Dichloramine-T from Toluene P– Sulphonamide

Aim: To prepare and submit Dichloramine-T.
Apparatus: Round bottom flask, Condenser, Water bath.
chemicals required: Toluene-p-sulphonyl chloride: 5 g; Ammonium carbonate: 10 g; OR concentrated Ammonia solution (d 0.88): 15 ml.

Theory: Dichloramine-T may be prepared by the help of a two-step synthesis, namely

Step I. Preparation of Toluene-p-sulphonamide, and

Step II. Preparation of Dichloramine-T from toluene-p-sulphonamide.

Step I. Toluene-p-Sulphonamide

Chemical Structure

Theory

Toluene-p-sulphonyl chloride either on heating with ammonium carbonate or liquid ammonia replaces the chloro group with an amino moiety to result the formation of toluene- sulphonamide and a mole of HCl gets eliminated.

Procedure. In actual practice, there are two different procedures that are used for the synthesis of toluene-p-sulphonamide as given below:

Method–I. The various steps involved are as follows:

1. Grind together 5 g (0.0525 mol) of toluene-p-sulphonyl chloride, and 10 g of ammonium carbonate in a mortar until a fine uniform powder is accomplished.
2. Transfer the resulting mixture to an evaporating dish and heat the contents over a water-bath for a duration of 1–2 hours, and stir the mixture frequently with a clean stainless-steel spatula.
3. Allow the resulting mixture to attain room temperature and extract with a little cold water to remove the excess unreacted ammonium salts. The yield of the crude product (mp 136–137.5°C) is 4.1 g.

Precautions

1. The two main reactants must be intimately triturated to a fine powder so as to facilitate the conversion to the final desired product.
2. Constant heating over the water-bath of the mixture is very much important to ascertain completion of reaction.

Recrystallization. The crude product (8.3 g) may be recrystallized from boiling water (100–125 ml), and dry the colourless crystals at 100°C. The yield of pure product (mp 137.5–138°C) is 3.8 g.

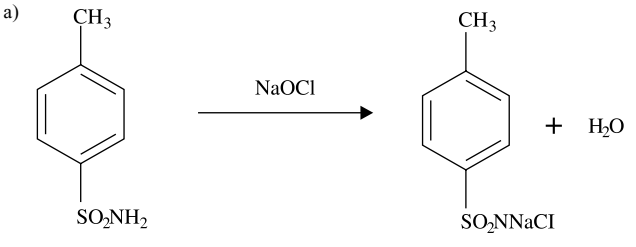
Method–II. An alternate equally effective and feasible method for the preparation of toluene-p-sulphonamide is as stated below :

1. Grind 5 g of toluene-p-sulphonyl chloride to a fine powder and add to it 15 ml of concentrated ammonia solution (d 0.88).
2. Heat the mixture to boiling in a **Fume Cupboard** and then cool.
3. Filter the crude product and recrystallize the toluene-p-sulphonamide from boiling water (add 0.5 g of decolourizing carbon, if required). The yield of pure product (mp 137.5–138°C) is nearly to that of theoretical yield (4.89 g).

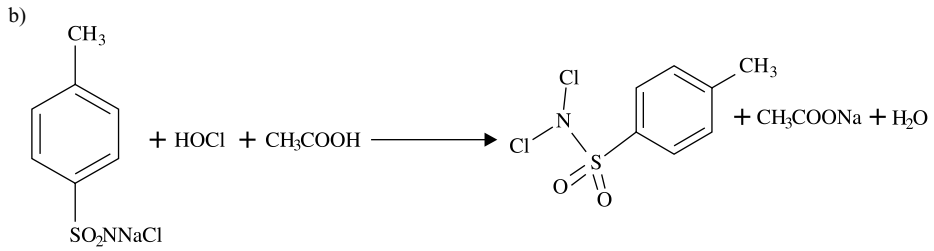
Step II. Dichloramine-T

Theory

a)



b)



Equation (a) Toluene-para-sulphonamide on dissolution in an excess of sodium hypochlorite solution gives rise to the formation of toluene-p-sulphon-chloro-sodio-amide (I)*, which being water-soluble does not ordinarily crystallise out unless and until very concentrated solutions are employed.

Equation (b) At this particular stage if a weak acid, e.g., acetic acid is added to the resulting solution of (I) above, the latter compound (i.e., I) readily interacts with the hypochlorus acid yielding the **Dichloramine-T** (or Toluene-p-sulphon-dichloro amide), which being aterinsoluble gets separated rapidly.

Chemicals required. Sodium hypochlorite solution (2 M)**: 80 ml; Toluene-p-sulphonamide: 5 g; Glacial acetic acid/water (1 : 1) : 50 ml.

Procedure

1. Dilute 80 ml of **freshly prepared** sodium hypochlorite solution (2 M) with 80 ml of water in a 250 ml beaker.
2. Add to the above solution 5 g of **finely powdered** toluene-p-sulphonamide with constant stirring so as to obtain a rapid clear solution.

   *Compound (I) has a close resemblance to sodium acet-bromoamide, [$CH_3CONNaBr$], which is an **INTERMEDIATE PRODUCT** in Hoffman's primary amine synthesis. ****Sodium Hypochlorite Solution (2 M).** 100 ml: Dissolve 10 g of NaOH in 20 ml water in a 250 ml beaker, cooling the solution, and then adding about 50 g of crushed ice. Now counterpoise the beaker on a rough set of scales, and pass in chlorine from a cylinder until an increase in weight of 72 g is achieved. Make up the volume of the solution to 100 ml and shake thoroughly. The solution should be kept in a cool, dark place, but even then it slowly decomposes.
3. Cool the resulting solution in ice-water, and initiate addition of 50 ml of a mixture containing equal volumes of glacial acetic acid and water, in small lots at intervals, with constant stirring until complete precipitation takes place.
4. Dichloramine-T separates at first as a **fine emulsion**, that readily forms brittle colourless crystals.
5. Crystals are filtered on the Büchner funnel with a suction, washed well with water, drained thoroughly, and dried without any lapse of time preferably in a desiccators or between the folds of filter paper. The yeild of crude product (mp 82–82.5°C) is approximately 6.5 g.

Precautions

1. Always make use of (2 M) sodium hypochlorite solution for the synthesis that has been prepared afresh.
2. Toluene-p-sulphonamide must be pulverised to fine powder before it is used in the reaction to get better yield.
3. The crude product must be dried either in a desiccator or between the folds of filter paper as quickly as possible to avoid possible decomposition. (**Sensitive Product**)

Recrystallization. The crude product may be recrystallized from minimum quantity of petroleum ether (60–80°C). It is obtained as needles (mp 82.5–83°C) upto 6.3 g.

Physical Parameters. It is obtained as prisms from a mixture of chloroform and petroleum ether (60–80°C) having mp 83°C. It has a strong odour of chlorine, and gets decomposed on exposure to air with loss of Cl_2 (mp 80°C). It is almost insoluble in water and decomposed by alcohol when warmed. 1 g Dissolves in about 1 ml benzene, 1 ml chloroform, 2.5 ml CCl_4; soluble in eucalyptol, chlorinated paraffin hydrocarbons, glacial acetic acid; and slightly soluble in petroleum ether. It contains 28–30% of active available chlorine.

Uses

1. A 1% (w/v) solution in chlorinated paraffin is employed for application of mucous membranes as a germicide; and a 5% (w/v) solution in the same solvent is invariably used in dressing wounds as an antibacterial agent.
2. As it is far less alkaline than Sodium Hypochlorite Solution NF, it finds its application as an antiseptic and disinfectant.

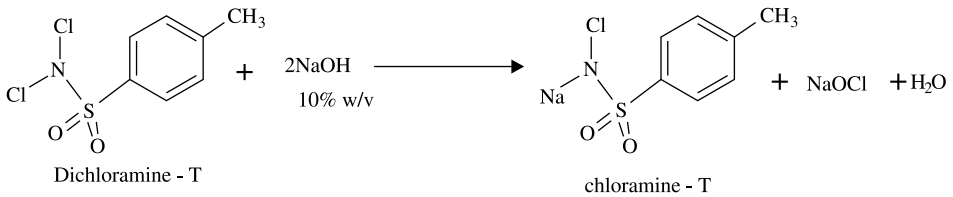
Report: Dichloramine–T was prepared and submitted.

Experiment.No:28

Synthesis of Chloramine-T

Aim: To prepare and submit Chloramine-T.
Apparatus: Round bottom flask, Condenser, Water bath.
Chemicals Required. Dichloramine-T: 6 g; Sodium Hydroxide Solution [10% (w/v)]: 40 ml.
Chloramine-T may be prepared by two methods, namely:
Method–I. From Dichloramine-T, and
Method–II. Direct from Toluene-p-Sulphonamide.

Theory (Method–I). From Dichloramine-T



Dichloramine - T chloramine - T

Dichloramine-T when heated with sufficient amount of 10% (w/v) sodium hydroxide solution it gives rise to the formation chloramine-T and a mole each of sodium hypochlorite and water.

Chemicals Required. Dichloramine-T: 6 g; Sodium Hydroxide Solution [10% (w/v)]: 40 ml.
Procedure

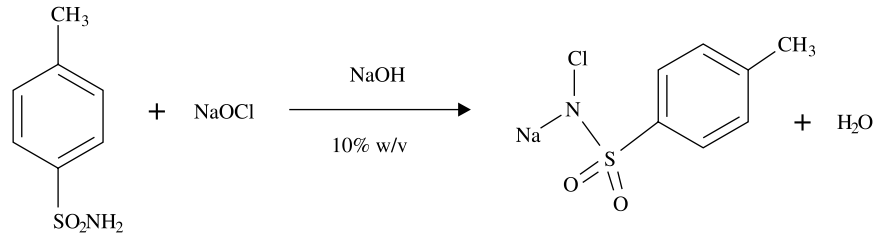
1. Heat 40 ml of sodium hydroxide solution in a 250 ml beaker over an asbestos-wire gauze gently until the solution is almost boiling.
2. To the above solution add 7 g of the crude product i.e., Dichloramine-T, in small lots at intervals with constant stirring.
3. When the addition is complete, cool the reaction mixture in ice-cold water, whereupon the desired product chloramine-T shall separate out as crystals readily.
4. Filter the crystalline product on the Büchner funnel with the suction and drain thoroughly. The yield of the sufficiently pure product is almost near to the theoretical yield. It may be dried with

drying-paper or in a $CaCl_2$—desiccator or in a vacuum (i.e., reduced pressure). The yield of the product is approximately 5.5 g which does not exhibit any definite mp.

Note

1. The product may be recrystallized, if desired, from a small quantity of hot water, and
2. The product is **NOT** dried over **sulphuric acid** in a desiccator as it loses water of crystallization rapidly.

Theory (Method–II). From Toluene-p-sulphonamide



The interaction between toluene-p-sulphonamide with freshly prepared sodium hypochlorite solution (2 M) in the presence of 10% NaOH solution results into the formation of chloramine-T, and a mole of H_2O gets eliminated.

Chemicals Required. Toluene-p-sulphonamide: 5 g; Freshly prepared 2 M Sodium Hypochlorite solution: 45 ml; Sodium Hydroxide solution [10% (w/v)]: 40 ml.

Procedure

1. First of all mix together 45 ml of 2 M sodium hypochlorite solution and 40 ml of 10% NaOH solution in a 250 ml conical flask.
2. Add to the above solution quickly 5 g of finely powdered toluene-p-sulphonamide and cork the flask securedly.
3. Shake the contents of the flask vigorously by holding the cork-in-position for 5–8 minutes, whereupon the toluene-p-sulphonamide shall undergo complete dissolution ; and at the same time a white crystalline chloramine-T would appear almost distinctly.
4. Warm the contents of the flask until a clear solution is obtained; so as to ensure removal of any unreacted dichloramine-T, and then cool.
5. Chloramine-T will start separating out on gradual cooling in the form of needles; while on **'sudden-chilling'** in the form of distinct characteristic leaflets.
6. Filter, drain and dry over $CaCl_2$ in a desiccator. The yield of the product is 6.3 g having no definite mp.

Physical Parameters. It is obtained as trihydrate prisms that lose water on drying. It gets decomposed gradually on being exposed to air. It is fairly soluble in water; practically insoluble in benzene, chloroform, ether; and gets decomposed by alcohol. It contains 11.5–13 per cent of active available chlorine.

Uses

1. It is mostly employed as an antiseptic and disinfectant but is less irritant in nature.
2. It is invariably applied to mucous membranes as a 0.1% aqueous solution.
3. It is also used to irrigate or dress wounds as a 1% (w/v) solution.

Report: Chloramine-T was prepared and submitted.

Experiment.No:29

Synthesis of Fluorescein

Aim: To prepare and submit Fluorescein.
Apparatus: Round bottom flask, Condenser, Water bath.
Chemicals required: Phthalic anhydride (powder): 5 g; Resorcinol: 7.5 g; Sulphuric Acid (Conc.) = 2–3 ml; Dilute NaOH solution = q.s.; Dilute HCl: q.s.;

Theory

It is a two-step preparation, namely:

(i) Preparation of Fluorescein, and
(ii) Bromination of Fluorescein to Eosin.

Step-I. Preparation of Fluorescein.

Theory

Resorcinol and phthalic anhydride interact in the presence of a strong dehydrating agent, such as: concentrated sulphuric acid to give a condensed product fluorescein with the elimination of two moles of water. Fluorescein exhibits keto-enol tautomerism and the two forms do exist as given above.

Procedure: The various steps involved are as given below:

1. Mix thoroughly 5 g of phthalic anhydride powder and 7.5 g of resorcinol in a dry 100-ml round bottom flask fitted with an air condenser.
2. Hold the flask in position in an oil-bath and commence heating slowly till the mixture starts melting.
3. Add 2–3 ml of concentrated sulphuric acid to the reaction mixture and continue heating it for 3–4 hours by maintaining the temperature of the oil-bath at 180 ± 3°C. During the course of heating the resulting mixture turns viscous and practically a semi-solid mass.
4. Discontinue the heating-process, allow the mass to attain ambient temperature; and dissolve the solidified product in dilute sodium hydroxide solution in 4–5 successive instalments of dilute NaOH solution (30–40 ml each).
5. After complete extraction of the solid mass from the flask, the resulting solution is neutralized carefully with dilute HCl with constant stirring when fluorescein gets precipitated apparently.
6. Cool the contents of the flask in an ice-bath and filter the crude fluorescein in a Büchner funnel with suction, wash with a little cold water, drain well and finally dry in an electric oven maintained at 100°C. The yield of crude product (mp 124–125°C) is 8.8 g.

Precautions

1. Both phthalic anhydride and resorcinol should be powdered individually before mixing and starting the reaction.
2. All glass apparatus must be perfectly dry so that concentrated sulphuric acid used in the reaction is fully utilized in the removal of two moles of water.
3. Extraction of the semi-solid mass with dilute NaOH solution is to be repeated till such time when almost every small bit of it undergoes dissolution.
4. Subsequent acidification with dilute HCl is to be carried out carefully to regenerate the fluorescein as a precipitate.

Recrystallization. Crude fluorescein may be recrystallized by dissolving a small portion of it again in dilute NaOH solution and reprecipitating it with dilute HCl solution. The pure fluorescein has mp 125–127°C.

Physical Parameters. It is obtained as a bright yellow powder, mp 125–127°C. It is found to be practically insoluble in water, but soluble in alkali carbonates, or hydroxides, alcohol and ether.

Report: Fluorescein was prepared and submitted.

Experiment.No:30

Synthesis of Eosin from Fluorescein

Aim: To prepare and submit Eosin.
Apparatus: Round bottom flask, Condenser, Water bath.
Chemicals required: Fluorescein: 5 g; Bromine: 3.7 ml (11.6 g); Rectified alcohol (95% v/v): 25 ml;

Theory

Fluorescein is dissolved in ethanol and the solution is chilled between 0–5°C in an icebath. Bromination of fluorescein is an exothermic reaction ; and when half of the requisite quantum of bromine is added the solution becomes clear in appearance due to the formation of dibromofluorescein which being soluble in ethanol. Further addition of bromine gives rise to the corresponding tetrabromoderivative (eosin)), which being insoluble in ethanol separates out.

Procedure

1. Suspend 5 g fluorescein in 25 ml rectified spirit (alcohol) in a 100-ml round bottom flask; and chill the contents of the flask in an ice-bath.
2. Add to the fluorescein solution 3.7 ml of bromine from a burette in small lots at intervals with constant vigorous shaking. It is an exothermic reaction and, therefore, the addition of Br_2 must be very slow and gradual.
3. When one-half of Br_2 has been added a clear solution is obtained.
4. Continue adding the remaining portion of Br_2 gradually with stirring, the appearance of the tetrabromo derivative (eosin) which being insoluble in rectified alcohol shall separate out instantly. Allow it to stand for 2 hours with occasional shaking.
5. Filter the product in a Büchner funnel, wash with a little alcohol and dry in an oven maintained at 100°C. The yield of eosin is 9.3 g.

Precautions

(i) The addition of bromine solution to fluorescein solution should be done very slowly with constant stirring, because the reaction is exothermic in nature.
(ii) After the complete addition of bromine the resulting mixture should be allowed to stand for 2 hours with occasional shaking so as to complete the bromination.

Physical Parameters. It is obtained as brownish-red powder, freely soluble in water and less in ethanol; and is insoluble in ether. The concentrated aqueous solution is deep brownish-red, the dilute (1 : 500) solution is yellowish-red with greenish fluorescence; and the alcoholic solution exhibits a strong green fluorescence.

Uses

(i) It is frequently employed in microbiological differential media.
(ii) It is also used as biological stain.
(iii) It has been duly approved by FDA* for use in drugs and cosmetics except for use in eye area.

Report: Eosin was prepared and submitted.

Experiment.No:31

Synthesis of Sulphacetamide from Sulphanilamide

Aim: To prepare and submit sulphacetamide.
Apparatus: Round bottom flask, Condenser, Water bath.

Chemicals required: sulphanilamide, acetic anhydride, water, dilute ethanol
Principle: Primary amines react readily upon warming with acetic anhydride to yield monoacetyl derivative. Acetylation of an aromatic primary or secondary amine may be readily achieved by using an acid chloride in the presence of base; however, acetylation is more usually effected with acetic anhydride rather than the more obnoxious acetyl chloride.

Procedure

1. Reflux gently in a test tube under a short air condenser 1gm of the sulphanilamide with 2.5 mols of acetic anhydride for 10–15 min.
2. Cool the reaction mixture and pour it into 20 ml of cold water.
3. Boil to decompose the excess of acetic anhydride.
4. When cold filter the residual insoluble acetyl derivative and wash with a little cold water.
5. Recrystalize from water or from dilute ethanol.

Report: sulphacetamide was prepared and submitted.

Experiment.No:32

Synthesis of Phenothiazine from Diphenylamine

Aim: To prepare and submit Phenothiazine.
Apparatus: Round bottom flask, Condenser, Water bath, conical flask, beaker.
Chemicals required: diphenylamine, sulphur,iodine.
Principle: Heating diphenylamine and sulfur with iodine catalyst is the classic synthesis of phenothiazine:

Procedure

1. Phenothiazine was synthesized by refluxing elementary sulphur with diphenylamine in the molar ratio of 2:1.3 respectively, for 1 h at 180°C in 1, 2-dichlorobenzene under an atmosphere of nitrogen, 1% (w/w) iodine being added as a catalyst.
2. The evolved hydrogen sulphide was trapped in an aqueous solution of $FeCl_3$ 1 ml.
3. The solvent was removed by distillation, the excess diphenylamine precipitated in anhydrous ethereal solution by dry HCl gas and the phenothiazine recrystallized twice from aqueous ethanol to give light yellow crystals, m.p. 181–183°C.

Uses

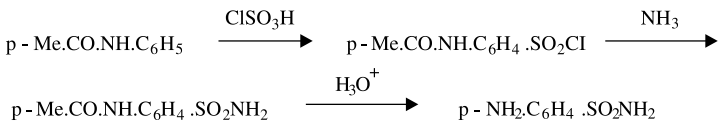
1. Phenothiazine is used in the management of psychotic conditions. It also controls excitement, aggression and agitation.
2. It has antiemetic, antipruritic, anti-histaminic and sedative properties.

Report: Phenothiazine was prepared and submitted.

Experiment.No:33

Synthesis of P-Aminobenzene Sulphonamide(Sulphanilamide)

Aim: To synthesize sulphanilamide.
Apparatus: Dropping funnel, reflux condenser, two necked flask, calcium chloride guard tube
Reaction



Step.1

p-acetamidobenzenesulphonyl chloride: Equip a 500 ml two necked flask with a droping funnel and a reflux condenser attach the top of the later to a device for the absorption of hydrogen chloride. Place 20 g (0.148 mol) of dry acetanilide in the flask and 50 ml (90 g, 0.77 mol) of a good grade chlorosulphonic acid in the dropping funnel and insert a calcium chloride guard tube in to the latter. Add the chlorosulphonic acid in small portions and shake the flask from time to time to ensure thorough mixing(1). When the addition has been made, heat the reaction mixture on a water bath for 1 Hr in order to complete the reaction. Allow to cool and pour the oily mixture in a thin stream with stirring into 300 g of crushed ice contained in a1lt beaker. Carryout this operation carefully in the fuming cupboard since the excess of chlorosulphonic acid reacts vigorously with the water.Rinse the flask with a little ice water and add the rinsings to the contents of the beaker. Breakup any lumps of any solid material and stir the mixture for several min in order to obtain an even suspension of the granular white solid. Filter of the p-acetamidobenzene sulphonyl chloride at the pump and wash it with a little cold water; press and drain well. Use the crude product (2) immediately in the next stage

Step.2

Preparation of p-acetamidobenzenesulphonamide: Transfer the crude p-acetamidobenzenesulphonyl chloride to the rinsed reaction flask, and add a mixture of 70 ml of Conc. ammonia solution and 70 ml of water. Mix the contents of the flask thoroughly, and heat the mixture with occasional swirling (fume cupboard) to just below the boiling point for about 15 min.The sulphonyl chloride will be converted into a pasty suspension of the corresponding sulphonamide. Cool the suspension in ice, and then add dilute sulphuric acid until the mixture is just acid to Congo red paper. Collect the product on a Buchner funnel, wash with a little cold water and drain as completely as possible. It is desirable but not essential, to dry the crude p-acetamidobenzenesulphonamide at 100°C: the yield is about 18 g. The material is sufficiently pure (3) for the next stage.

Step.3

Preparation of p-aminobenzenesulphonamide: Transfer the p-acetamidobenzenesulphonamide to a 500 ml flask, add 10 ml of Conc. HCl and 30 ml of water. Boil the mixture gently under reflux for 30–45 min. The solution when cooled to room temp, should deposit no solid amide; if a solid seperates, heat for further short period. Treat the cooled solution with 2 g of decolorizing carbon, heat the mixture to boiling and filter with suction through a hardened filter paper. Place the filtrate (a solution of sulphanilamide hydrochloride) in a liter beaker and cautiously add 16 g of solid sodium hydrogen carbonate in portions with stirring. After the evolution of gas has subsided, test the suspension with litmus paper and if still it is acid, add more sodium hydrogen carbonate until neutral. Cool in ice, filter of the sulphanilamide with suction nand dry. The yield is 15 g, m.p is 161–163°C. A pure product, m.p. 163–164, may be obtained by recrystallisation from water or from ethanol.

Report: sulphanilamide was prepared and submitted.

Experiment.No:34

Synthesis of Cinnamic Acid

Aim: To prepare and submit cinnamic acid.

Apparatus: Round bottom flask, Condenser, $CaCl_2$-guard tube, Water bath, conical flask, beaker.

Chemicals required: Pure redistilled Benzaldehyde: 10.5 g; Fused and powdered Potassium acetate: 6 g; Acetic Anhydride: 15 g; Sodium carbonate: 20 g; Conc. Hydrochloric Acid (12 N): q.s.; and Rectified Spirit: 50 ml.

Theory

The interaction between benzaldehyde (aromatic aldehyde) and acetic anhydride (an aliphatic anhydride capable of providing an 'active methylene' moiety) in the presence of a basic catalyst, such as : acetate ion and a hydronium ion yields an α, β-unsaturated carboxylic acid, cinnamic acid, and a mole of acetic acid.

Procedure

Following steps may be followed in a sequential order:

1. Transfer carefully 10.5 g (10 ml, 0.2 mol) of freshly distilled pure benzaldehyde, 15 g (14 ml, 0.29 mol) of acetic anhydride together with 6 g (0.122 mol) of freshly fused and finely powdered potassium acetate in an absolutely dry 250 ml round bottomed flask duly provided with $CaCl_2$-guard tube at its top-end.

[Note. Potassium acetate may be replaced with sodium acetate also, but in that case the reaction is appreciably slower and sluggish; and a further heating for 3-4 hours is required and mandatory.

2. Mix the contents of the RB-flask thoroughly and heat the reaction mixture in an oil bath maintained at 160°C for 60 minutes; and further at an elevated temperature of 170–180°C for almost 3 hours.
3. Pour the contents of the reaction flask while still hot (90–100°C) into about 50 ml of water contained in a 500 ml round-bottomed flask that has been duly fitted for steam-distillation operation; rinse the contents of the flask with a little hot water and pour it in the 500 ml RB-flask.
4. Now, make the resulting solution in the 500 ml RB-flask alkaline (to litmus paper) by adding gradually a saturated solution of Na_2CO_3 with vigorous shaking until a drop of the liquid withdrawn on the tip of a glass rod turns red litmus to a distinct blue.

[Note : NaOH cannot be used (instead of Na_2CO_3) for affecting alkalinity, because it may produce BENZOIC ACID by the Cannizarro Reaction from the unchanged/unreacted portion of Benzaldehyde.]

5. Subject the solution to steam-distillation meticulously until all the 'unreacted benzaldehyde' is removed and the distillate is absolutely clear. Cool the contents of the distillation flask and filter at the suction pump to get rid of most resinous unwanted by-products.
6. Carefully, render the filtrate to acidic pH by adding concentrated HCl gradually in small lots at intervals, and with vigorous continuous agitation until the evolution of CO_2 ceases completely.
7. Chill the resulting solution when cinnamic acid gets separated as almost colourless crystals, filter in the Buchner funnel, wash with a little cold water, drain well with an inverted glass stopper, and dry at 100°C. The yield of the crude product is 9.5 g having mp ranging between 131–132.5°C.

Precautions

1. All reagents, namely: benzaldehyde, acetic anhydride and potassium acetate must be of very good quality and absolutely dry so as to accomplish reasonably purer end product with maximum yield.
2. Make the reaction mixture distinctly alkaline prior to the removal of 'Benzaldehyde' (unreacted) by steam-distillation.
3. The resulting reaction mixture is cooled and acidified cautiously to litmus paper when the desired product i.e., cinnamic acid is knocked out in an acidic medium.

Recrystallization. The crude product may be recrystallized either from a mixture of water and rectified spirit (3 : 1) or from hot water. The yield of pure recrystalized product is 9.1 g, mp 132–133°C.

Physical Parameters. Cinnamic acid is obtained as monoclinic crystals having mp 133°C; d4 4 1.2475; bp 300°C; K at 25° = 3.5×10^{-5}; uv_{max} (ethanol): 273 nm. Its solubility profile is as follows: 1 g dissolves in 2L water at 25°C (more soluble in hot water); in 6 ml ethanol; 5 ml methanol; 12 ml chloroform; and almost freely soluble in benzene, ether, acetone, glacial acetic acid, carbon disulphide and oils. The alkali salts are observed to be soluble in water.

Uses

1. A few typical esters of cinnamic acid, for instance; chaulmoogryl and other derivatives are used in medicine exclusively.
2. The main use of cinnamic acid is in the manufacture of the methyl, ethyl and benzyl esters for the perfume industry.
3. The 'ethyl ester' is used importantly in preparing sophisticated glass lenses and prisms that form a vital component in designing the 'optics' in various analytical equipments for the Quality Assurance Laboratories in testing drug substances.

Report: cinnamic acid was prepared and submitted.

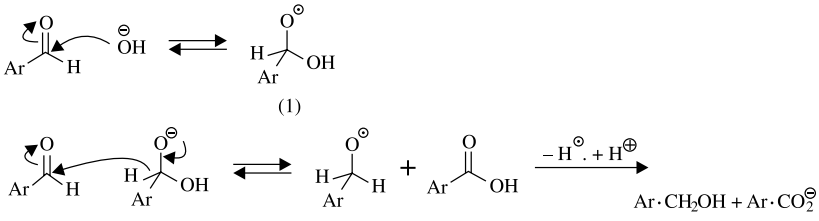
Experiment.No:35

Synthesis of Benzyl alcohol by Cannizzaro Reaction

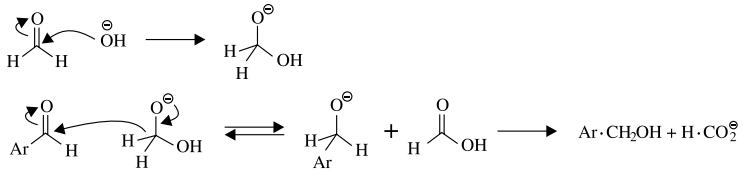
Aim: To prepare and submit benzyl alcohol.
Apparatus: Round bottom flask, Condenser, Water bath, conical flask, beaker.
Chemicals Required: Potassium hydroxide, benzaldehyde, sodium metabisulphite
Principle: Aromatic aldehydes (and other aldehydes in which α-hydrogen atoms are absent, e.g. formaldehyde and trimethylacetaldehyde) under the influence of strong aqueous or alcoholic alkali undergo simultaneous oxidation and reduction yielding the alcohol and corresponding carboxylate salt. Thus:

$$2Ar{\cdot}CHO \xrightarrow{KOH} Ar{\cdot}CH_2OH + Ar{\cdot}CO_2^{\ominus}K^{\oplus}$$

This dismutation or disproportionation reactionis known as the Cannizzro reaction. The mechanism of the reaction involves the production of the anion (1) which may transfer a hydride ion to a carbonyl carbon atom in another aldehyde molecule. The reaction sequence is completed by a proton transfer to yield the carboxylate anion and the alcohol.



The reaction is illustrated here by the conversion of benzaldehyde into benzoic acid and benzyl alcohol and by a similar conversion of furfural into furfuryl alcohol and furoric acid. A preparatively more useful form of this reaction is the crossed Cannizzaro reaction which ensues when a mixture of an aromatic aldehyde and formaldehyde is allowed to react under the influence of strong base (e.g. the preparation of p-methyl benzyl alcohol. A substantial proportion of the aromatic aldehyde is reduced to the corresponding alcohol while the formaldehyde is oxidised to formate. This is a reflection of the fact that nucleophilic attack of the hydroxide ion takes place preferentially at the more electrophilic carbonyl carbon atom in formaldehyde.



Procedure

Dissolve 29 g of potassium hydroxide in 27 ml of water contained in a beaker or conical flask and cool the solution to about 20°C in ice water. Pour the solution into a 250 ml reagent bottle, and add 32 g (30 ml, 0.3 mol) of pure benzaldehyde cork the bottle securely and shake the mixture vigorously until it has been converted into a thick emulsion. Allow the mixture to stand over night in the stopperd bottle. Add just sufficient water (about 105 ml) to dissolve the potassium benzoate. Pour the liquid into a seperatory funnel, rinse out the bottle with about 30 ml of ether and add this ether to the solution in the funnel. Shake the solution in order tothorouhly extract the benzyl alcohol with ether, separate the lower aqueous solution and carry out two further extractions each with about 25 ml of ether. Save the aqueous solution. Combine the ether extracts and distil the ether from a water bath until the volume is about 25 ml. Cool and shake the ether solution twice with 5 ml portions of saturated sodium metabisulphite solution in order to remove any benzaldehyde which may be present. Separate the ethereal solution, wash it with 10 ml of 10% sodium carbonate solution (to ensure complete removal of the bisulphaite), then with 10 ml of water, and dry with anhydrous potassium carbonate. Remove the ether on a water bath and distil the residual liquid from an air bath, replace the water condenser by an air condenser or empty the water completely or the condenser jacket. Collect the benzyl alcohol at 204–207°C, the pure compound boils at 205°C, the yield is 13 g.

Report: Benzyl alcohol was prepared and submitted.

Experiment.No:36

Synthesis of 1, 1, 1-Trichloro-2-Methyl-2-Propanol (Chlorobutanol)

Aim: To prepare and submit chlorobutanol.
Apparatus: Round bottom flask, Condenser, Water bath.
Chemicals required: Acetone, potassium hydroxide, chloroform.
Principle: Chlorobutanol is prepared by the addition of chloroform to acetone under the catalytic influence of powdered potassium hydroxide; It has a local anaesthetic potency to a mild degree and is used as an anaesthetic dusting powder. Chlorobutanol has also antibacterial and germicidal properties

Procedure: 45 ml Acetone, 5 ml chloroform and 1 gram powdered potassium hydroxide was mixed in a 250 ml flask, and the reaction mixture was stirred at -5°C for two hours. The resulting suspension was filtered, and the filtrate was freed from excess acetone by distillation. The yellowish oily residue was mixed with 50 ml of ice-cold water and chlorobutanol hemihydrate precipitated as a white crystalline material, which was filtered off and dried (preferably in a vacuum desiccator). Yield 71% of theory, melting point 78.4°C after recrystallization from water.

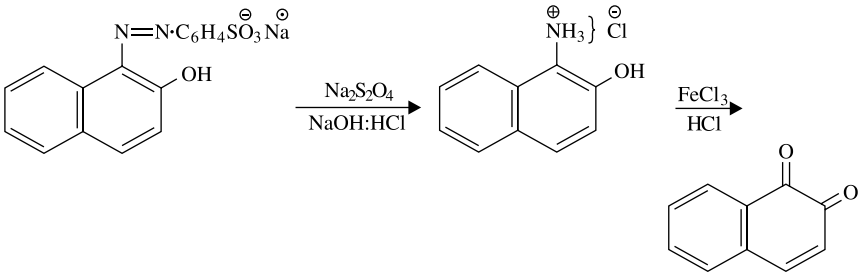
Uses and administration: Chlorobutanol is a widely used, very effective preservative in many pharmaceuticals and cosmetic products, e.g. injections, ointments, products for eyes, ears and nose, dental preparations, etc. It has antibacterial and antifungal properties. Chlorobutanol is typically used at a concentration of 0.5% where it lends long term stability to multi-ingredient formulations. Chlorbutol has antibacterial and antifungal properties and it is used at a concentration of 0.5% as a preservative in injections, eye drops and mouth washs as well as cosmetics.

Report: chlorobutanol was prepared and submitted.

Experiment.No:37

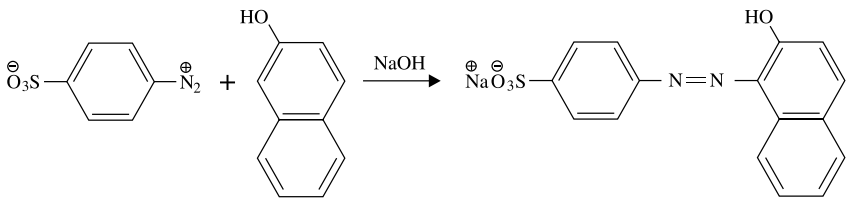
Synthesis of 1, 2-Naphthoquinone

Aim: To prepare and submit 1, 2-Naphthoquinone.
Apparatus: Round bottom flask, Condenser, Water bath.
Chemicals required: orange II, sodium dithionite, sulphanilic acid dehydrate, sodium chloride
Procedure



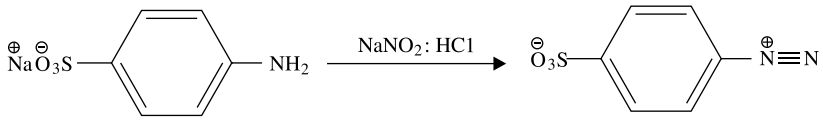
Place 20 g (0.057 mol) of orange II in a 600 ml beaker and dissolve it in 250 ml of water at 40–50°C. Add with stirring 24–25 g (0.114) of hydrated sodium dithionite; this discharges the color and yields a pink or cream colored finely divided precipitate of 1-amino-2–naphthol.Heat the mixture nearly to boiling until it commences to froth considerably then cool to 25°C in ice, filter on a Buchner funnel and wash with a little cold water. Transfer the precipitate to a beaker containing a solution of 0.25 g of tin (II) chloride in 5 ml of conc HCl diluted with 100 ml of water; upon stirring aminonaphthol dissolves and a small amount of insoluble matter remains. The function of the tin chloride is an antioxidant, preventing the readily oxidisable aminonaphthol HCl from undergoing appreciable change. Stir the solution for 5 min with 2 gm of decolorizing carbon, and filter at the pump. If crystalline material should separate at any stage dissolve it by warming and by the addition of a little water if necessary. Transfer the clear solution to a beaker, add 25 ml of conc. HCl and warm until the solid dissolves. Cool to 0°C filter the almost color less crystals of the amino naphthol HCl with suction and wash with 25 ml of dil HCl (1 : 4 by volume) From this point all operations must be carried out rapidly. In the mean time prepare the oxidizing solution by dissolving 30 g of crystallized iron chloride in a mixture of 10 ml of conc. HCl and 25 ml of water by heating, cool to room temperature by adding 30 gm of crushed ice and filter the solution at pump. Wash the crystalline 1-amino 2-naphthol hydrochloride into a 600 ml beaker with water, add 150 ml of water and a few drops of conc HCl and dissolve the precipitated solid by stirring and warming to about 35°C. If necessary filter rapidly by suction from a trace of residue, transfer to a 500 ml RBF add the iron (III) chloride solution all at once while shaking the flask vigorously. The quinone seperates rapidly as a voluminous micro crystalline yellow precipitate. Filter on a Buchner funnel and wash it thoroughly with water at 30ºC to remove all traces of acid. The yield of 1, 2 naphthoquinone is 7 g, m.p is 145–147°C.

Preparation of orange II: (β -naphthol orange)



Diazotize 10.5 g of sulphanilic acid dehydrate and keep the suspension of the diazonium compound in ice water until required. Dissolve 7.2 g (0.05 mol) of a good grade of 2-naphthol in 40 ml of cold 10% NaOH solution in a 600 ml beaker, cool to 5°C and pour in, with stirring the well mixed suspension of diazotized sulphanilic acid. Coupling takes place readily and the dye stuff separates' as a crystalline paste. Stir well and after 15 min heat the mixture until all the solid has dissolved. Add 20 g of sodium chloride (to decrease the solubility of the product further) and warm until this dissolves. Allow the solution to cool spontaneously in the air for 1 Hr, and then cool in ice until crystallization is complete. Collect the product on a Buchner funnel and apply gentle suction and dry at 80°C. The product weighs about 22 g and contains about 20% of NaCl; further purification is unnecessary for dyeing purposes. To obtain pure crystalline orange II, dissolve the crude substance in the minimum volume of boiling water ;allow to cool to about 80°C, add about twice the volume of rectified spirit and allow crystallization to proceed spontaneously. When cold filter at the pump, wash the pure dye stuff (it is a dehydrate) with a little ethanol and dry in the air. The yield is 14 g(80%)

Diazotization of sulphanilic acid dihydrate



In a 250 ml conical flask place 10.5 gm (0.05 mol) of sulphanilic acid dehydrate, 2.65 g of (0.025 mol) of anhydrous sodium carbonate and 100 ml of water, and warm until a clear solution is obtained. Cool the solution under the tap to 15°C and add a solution of 3.7 gm (0.059 mol) of sodium nitrite in 10 ml of water. Pour the resulting solution slowly and with stirring into a 600 ml beaker containing 10.5 ml of conc. HCl and 60 g of crushed ice. Test for the presence of free nitrous acid with potassium iodide starch paper after 15 min. Fine crystals of diazobenzene sulphonate will soon precipitate; do not filter these off as they will dissolve during the next stage of the preparation.
Report: 1, 2-Naphthoquinone was prepared and submitted.

Experiment.No: 38

Synthesis of 2, 3–Diphenylquinoxaline

Aim: To prepare and submit 2, 3-diphenylquinoxaline
Apparatus: Round bottom flask, Condenser, Water bath.
Chemicals required: Benzil, Rectified Spirit, O-Phenylenediamine, and Ethanol.
Procedure: To a warm solution of 2.1 g (0.01 mol) of benzil in 8 ml of rectified spirit add a solution of 1.1 g (0.01 mol) of o-phenylenediamine in 8 ml of rectified spirit. Warm in a water bath for 30 min add water until a slight cloudiness persists and allow to cool. Filter and recrystalize from aqueous ethanol to give 1.43 g (51%) of 2, 3-diphenyl-quinoxaline, m.p 125–126ºC

Report: 2, 3-diphenylquinoxaline was prepared and submitted.

Experiment.No:39

Synthesis of Benztriazole

Aim: To prepare and submit Benztriazole.
Requirements: Beaker, Funnel, Ice., etc.
Chemicals: O-Phenylene diamine, glacialaceticacid, water and activated charcoal.

Procedure

1. Dissolve 2.7 gm.of O-phenylene diamine in a mixture of 2.9 ml. of glacialaceticacid and 7.5 ml. of H_2O in a beaker and stirr, and then add 1.9 gm of $NaNO_2$ in 4 ml of water in one portion.
2. The reaction mixture becomes warm and within 2–3 min. reaches the temperature of 80ºC.
3. Then begine to cool, while the colour changes from deep red to pale brown. Continue stirring for 15 min. by then the temperature must have dropped to 35–40ºC.
4. Then throughly chill in an ice bath for 30 min. collect the pale brown solid which seperates out by filtration (solid) wash with cold water until free from acid Impurities.
5. Dissolve the solid in 45 ml. of boiling water, add charcoal and filter.
6. Allow the filterate to cool. Add a few crystals of benztrizole. Allow the mixture to attain room temperature and then throughly chill in ice and collect the benzotriazole.

Report: Benztriazole was prepared and submitted.

Experimnt.No: 40

Synthesis of 2, 4, 5-Tri Phenyl Imidazole

Aim: To prepare and submit 2, 4, 5-Triphenylimidazole.
Apparatus: RBF, rubber, condensor, and water.
Chemicals: Benzil, Ammonium acetate-1.27 gm., Benzaldehyde-20 ml Ethanol.
Principle: Benzil reacts with benzaldehyde in presence of ammonium acetate results in formation of 2-mercapto-4, 5-diphenyl imidazole.

Procedure

1. Reflux a solution of Benzil, Ammonium acetate and benzaldehyde in glacial acetic acid for 2 hours.
2. The reaction mixture is allowed to stand to attain room temperature.
3. To that resulting solution add 150 ml of water and was filterted.
4. The filtrate is neutralized with NH_4OH to give a solid pasty mass and filtered.
5. Then solid mass was washed with toluene.
6. Recrystallization is done with ethanol, To the product add 20 ml of amyl alcohol, and reflux for 30–40 min onto get the final product.

Report: 2-mercapto-4, 5-diphenyl imidazole was prepared and submitted.

3

ASSAY OF SOME OFFICIAL COMPOUNDS

Experiment No: 1

Assay of Sulphamethoxazole

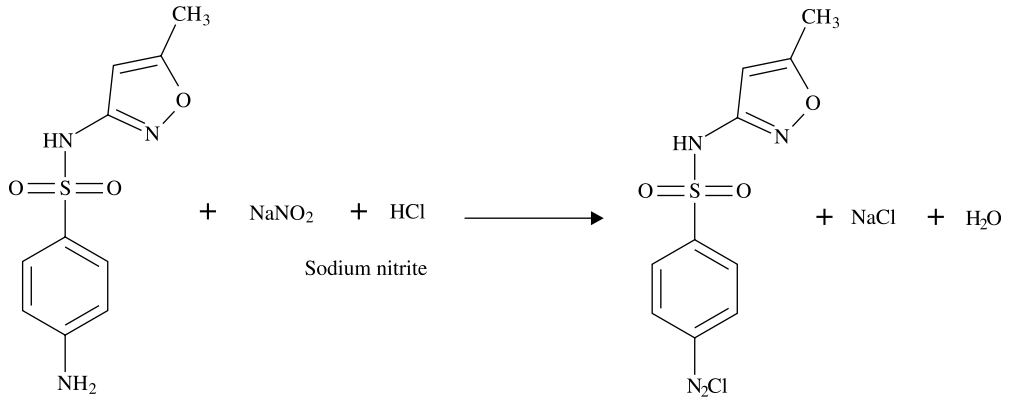
Aim: To carry out the assay of Sulphamethoxazole.

Chemical and apparatus required: Sulphamethoxazole, 2 N Hydrochloric acid, Potassium bromide, 0.1 M sodium nitrite, sulphanilic acid, sulphanilamide, starch iodide paper and ice cubes.

Apparatus – Potentiometer. A suitable open vessel of about 200 ml capacity is fitted with two similar clean platinum electrodes (platinum - calomel or platinum - platinum and a stirrer. The electrodes may be of platinum foil 0.5 cm square and should be placed 1.5 cm apart. They may be cleaned by immersing for a few seconds in boiling nitric acid containing a small amount of ferric chloride, followed by washing with water. The polarizing voltage may be obtained from a 1.5-volt dry cell and potentiometer or other convenient device which enables a small but definite voltage to be applied across the electrodes. The current flowing in the system is indicated by a series galvanometer which should have adequate sensitivity.

Principle: Sulphamethoxazole being a primary aromatic amine will undergo reaction with sodium nitrate in acidic condition (nitrous acid) to form a diazonium salt. Observation of end point depends on determination of small excess of nitrous acid which is determined by using the starch paper as an external indicator. The iodine librated reacts with starch to form a blue colour.

Limits: Sulphamethoxazole contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H11N_3O_3S$, calculated on the dried basis.



Sulphamethoxazole

Diazonium salt

Category: Antibacterial.
Dose: Initial dose, 2 g; subsequent doses, 1 g two or three times daily.
Description: White or almost white, crystalline powder; almost odorless.
Solubility: Freely soluble in acetone; sparingly soluble in ethanol (95%); slightly soluble in chloroform and in ether; practically insoluble in water. It dissolves in dilute solutions of sodium hydroxide.
Storage: Store in well-closed, light-resistant containers.

Preparation and Standardization of Standard Solutions

1. **Hydrochloric Acid, xM:** Solutions of any molarity xM may be prepared by diluting 85x ml of hydrochloric acid to 1000 ml with water. Store in containers of polyethylene or other non-reacting material at a temperature not exceeding 30º.
2. **Sodium Nitrite, 0.1 M:** Dissolve 7.5 g of sodium nitrite in sufficient water to produce 1000 ml. Standardize the solution in the following manner. Dissolve 0.3 g of sulphanilic acid in 50 ml of 2 M hydrochloric acid, add 3 g of potassium bromide cool in ice and titrate with the sodium nitrite solution determining the end-point potentiometrically. Each ml of 0.1 M sodium nitrite is equivalent to 0.01732 g of $C_6H_7NO_3S$.
3. **Standardization of 0.1 M Sodium Nitrite:** weigh accurately about 0.5 gm of sulphanilamide P.S., previously dried at 105ºC for three hours, and transfer to a beaker. Add 20 ml of hydrochloric acid and 50 ml of water, stir until dissolved, and cool to 15ºC. carry out the nitration titration. Each 0.01722 gm of sulphanilimide is equivalent to 1 ml of 0.1 M sodium nitrite.

Assay procedure

1. **Diazotization titrations method:** Weigh accurately about 0.5 g of Sulphamethoxazole sample and dissolve in 50 ml of 2M hydrochloric acid. Cool it in ice bath and add 2 gm of potassium iodide (KI). Dissolve and titrate slowly with 0.1 M sodium nitrite solution. The temperature should never go above 15ºC. continue titration until yellow-brown colour appear. The end point is determined by placing one small drop of solution is drawn from the conical flask with the help of glass rod on to a starch iodide paper. Appearance of blue colour is the end point. Each ml of 0.1 M $NaNO_2$ equivalent to 0.02528gm of $C_{10}H_{11}N_3O_3S$.
2. **Potentiometric method:** Weigh accurately about 0.2 g, dissolve in 50 ml of 2 M hydrochloric acid, and add 3 g of potassium bromide cool in ice and carry out the nitrite titration, Add 20 ml of hydrochloric acid and 50 ml of water, stir until dissolved, cool to about 15°. Immerse the electrodes in the solution and apply a voltage of about 50 mV across the electrodes when polarization of the electrodes takes place. Place the burette tip just below the surface of the solution to eliminate air oxidation of the sodium nitrite and stir the solution gently, maintain-

ing the temperature of about 15º. The titration may be carried out manually or by means of an automatic titrator. In the manual titration, add the titrant slowly and when the titration is within 1 ml of the end - point, add then in 0.1 ml portions, allowing not less than 1 minute between additions. (The galvanometerneedle deflects and then returns to approximately its original position until the end - point is reached). At the end - point, when a slight excess of sodium nitrite is present, the electrodes are depolarised, current flows and a permanent deflection of the needle is obtained. NOTE — It will be necessary to adjust the sensitivity of the galvanometer or the applied voltage before the titration is begun in order to obtain an adequate deflection at the end-point. Each ml of 0.1 M sodium nitrite is equivalent to 0.02533 g of $C_{10}H_{11}N_3O_3S$.

Report : The given sample contains _____ mg of sulphamethoxazole.

Experiment No: 2

Assay of Glibenclamide Tablets

Aim: To carry out the assay of **Glibenclamide Tablets** IP.

Chemical and apparatus required: Glibenclamide tablets, 0.1 M methanolic hydrochloric acid 200ml volumetric flask, UV spectrometer.

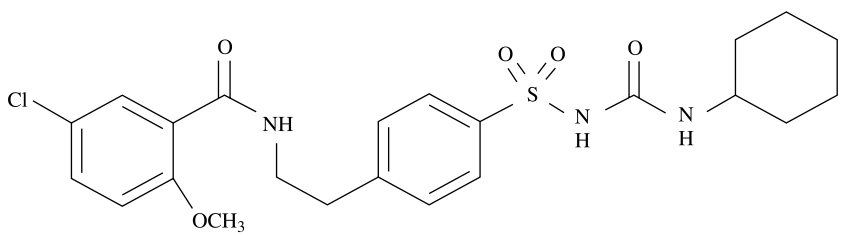
Principle: When radiation is passed through a layer of a solution containing an absorbing substance, part of the radiation is absorbed; the intensity of the radiation emerging from the solution is less than the intensity of the radiation entering it. The magnitude of the absorption is expressed in terms of the absorbance A defined by the expression

$$A = \log 10 (I_o/I),$$

Where I_o is the intensity of the radiation passing into the absorbing layer and I is the intensity of the radiation passing out of it. The absorbency depends on the concentration of the absorbing substance in the solution and the thickness of the absorbing layer taken for measurement. For convenience of reference and for ease in calculations, the absorbence of a 1-cm layer of a 1% w/v solution is adopted in this Pharmacopoeia for several substances unless otherwise indicated, and is evaluated by the expression

$$A (1\%, 1 cm) = A/cl,$$

Where c is the concentration of the absorbing substance expressed as percentage w/v and l is the thickness of the absorbing layer in cm. The value of A (1%, 1 cm) at a particular wavelength in a given solvent is a property of the absorbing substance.



Glibenclamide

$C_{23}H_{28}ClN_3O_5S$, Mol. Wt. 494.0 Glibenclamide is 1-{4-[2-(5-chloro-2 methoxybenzamido) ethyl} benzenesulphonyl}-3-cyclohexylurea. Glibenclamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{23}H_{28}ClN_3O_5S$, calculated on the dried basis.

Category: Hypoglycaemic.

Dose: 5 mg daily, adjusted according to response; maximum 15 mg daily, after food.

Description: White or almost white, crystalline powder.
Solubility: Sparingly soluble in dichloromethane; slightly soluble in ethanol (95%) and in methanol; practically insoluble in water and in ether. It dissolves in dilute solutions of alkali hydroxides.

Preparation and Standardization of Standard Solutions

1. **Hydrochloric Acid, xM Methanolic :** Solutions of any molarity xM may be prepared by diluting 85x ml of hydrochloric acid to 1000 ml with methanol.

Assay Procedure: Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 20 mg of Glibenclamide and shake with 40 ml of 0.1 M methanolic hydrochloric acid, heat gently and centrifuge. Repeat the extraction with three further quantities, each of 20 ml, of 0.1 M methanolic hydrochloric acid. To the combined extracts add sufficient 0.1 M methanolic hydrochloric acid to produce 200.0 ml and measure the absorbance of the resulting solution at the maximum at about 300 nm, using 0.1 M methanolic hydrochloric acid heated to the same degree as the blank.

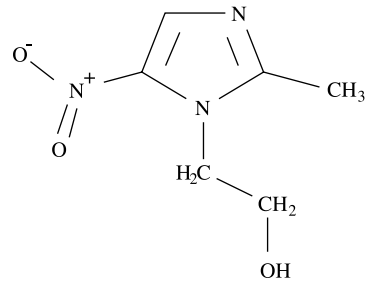
Calculate the content of $C_{23}H_{28}ClN_3O_5S$ taking 63 as the value of A (1%, 1 cm) at the maximum at about 300 nm.

Report : The given sample contains _____ mg of glibenclamide.

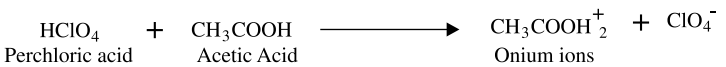
Experiment No: 3

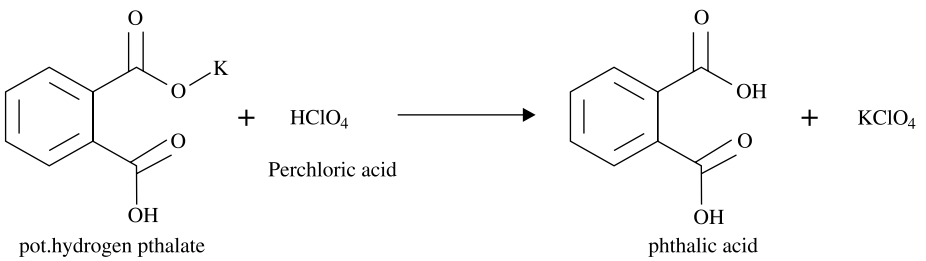
Assay of Metronidazole Tablets

Aim: To carry out the assay of **Metronidazole Tablets**.
Chemical and apparatus required: Metronidazole tablets, 0.1 N perchloric acid, anhydrous glacial acetic acid, brilliant green, potassium hydrogen phthalate and crystal violet.
Principle: Metronidazole tablet are assayed by non-aqueous titrations in which the tertiary amine group is titrated with perchloric acid using brilliant green as indicator. The amount of perchloric acid consumed in the reaction indicates the amount of Metronidazole in the sample.

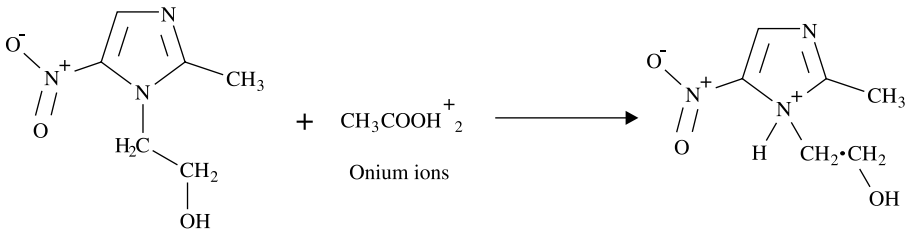


Metronidazole





pot.hydrogen pthalate phthalic acid



Metronidazole

Protonated Metronidazole

$C_6H_9N_3O_3$ Mol. Wt. 171.2 Metronidazole is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol.

Metronidazole contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_9N_3O_3$, calculated on the dried basis.

Description. A white or yellowish, crystalline powder.

Preparation and Standardization of Standard Solutions

1. Perchloric Acid, 0.1 M: Mix 8.5 ml of perchloric acid with 500 ml of anhydrous glacial acetic acid and 25 ml of acetic anhydride, cool and add anhydrous glacial acetic acid to produce 1000 ml. Allow the prepared solution to stand for 1 day for the excess acetic anhydride to be continued and carry out the determination of water, If the water content exceeds 0.05%, add more acetic anhydride. If the solution contains no titrable water, add sufficient water to obtain a content of water between 0.02% and 0.05%. Allow the solution to stand for 1 day and again titrate the water content. The solution so obtained should contain between 0.02% and 0.05% of water.
2. Standardization of 0.1 N Perchloric acid: Weigh accurately about 0.35 g of potassium hydrogen phthalate, previously powdered lightly and dried at 120º for 2 hours and dissolve it in 50 ml of anhydrous glacial acetic acid. Add 0.1 ml of crystal violet solution and titrate with the perchloric acid solution until the violet colour changes to emerald-green. Peform a blank determination and make any necessary correction.

   Each ml of 0.1 M perchloric acid is equivalent to 0.02042 g of $C_8H_5KO_4$.

Note: In the tests and assays of the Pharmacopoeia, this solution is specified as "0.1 N perchloric acid:. Thus the solution in anhydrous glacial acetic acid is to be used unless the words "in dioxan" are stated.

Assay Procedure: Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of Metronidazole, transfer to a sintered-glass crucible and extract with six quantities, each of 10 ml, of hot acetone. Cool, add to the combined extracts 50 ml of acetic anhydride. Titrate with 0.1 M perchloric acid, using 0.1 ml of a 1 per cent w/v solution of brilliant green in anhydrous glacial acetic acid as indicator to a yellowish-green end-point. Carry out a blank titration.

Each 1 ml of 0.1 M perchloric acid is equivalent to 0.01712 g of $C_6H_9N_3O_3$.

Report : The given sample contains _____ mg of metronidazole.

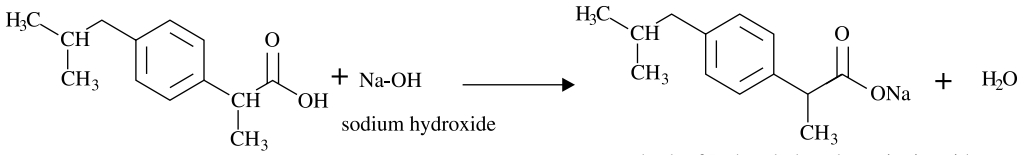
Experiment No: 4

Assay of Ibuprofen Tablets

Aim: To carry out the assay of **Ibuprofen Tablet** IP.

Chemical and apparatus required: Ibuprofen tablets, chloroform, Phenolphthalein Solution, and 0.1 M sodium hydroxide.

Principle: Ibuprofen is determined by neutralization titration in which free carboxylic group is titrated with sodium hydroxide Solution using phenolphthalein indicator. The amount of sodium hydroxide consumed in the reaction indicates the amount of ibuprofen present in the sample.



Isobutyl phenyl propionic acid
(Ibu profen)

sod.salt of Isobutyl phenyl propionic acid

$C_{13}H_{18}O_2$ Mol. Wt. 206.3 Ibuprofen is (RS)-2-(4-isobutylphenyl)propionic acid. Ibuprofen contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{13}H_{18}O_2$, calculated on the dried basis.
Description. A white or almost white, crystalline powder or colourless crystals; odour, slight.

Preparation and Standardization of Standard Solutions

1. **Sodium Hydroxide, xM:** Solutions of any molarity xM may be prepared by dissolving 40x g of Sodium Hydroxide in sufficient water to produce 1000 ml.
2. **Standardization of 0.1 M NaOH:** Weigh accurately about 5 gm of potassium hydrogen phthalate previously dried at 120ºC for two hours dissolve in 75 ml of corbondioxide free water. Add 0.1 ml of phenolphthalein solution and titrate with the sodium hydroxide until a permanent pink colour is produced.
   Each ml of 1M NaOH equivalent to 0.2042 g of potassium hydrogen phthalate.
   Each ml of 0. 1M NaOH equivalent to 0.02042 g of potassium hydrogen phthalate.
3. **Phenolphthalein Solution:** A 1.0 % w/v solution of phenolphthalein in ethanol (95%).

Assay Procedure: Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 0.5 g of Ibuprofen, extract with 60 ml of chloroform for 15 minutes and filter. Wash the residue with three quantities, each of 10 ml, of chloroform and gently evaporate the filtrate just to dryness in a current of air. Dissolve the residue in 100 ml of ethanol (95%), previously neutralized to phenolphthalein solution, and titrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator.

Each ml of 0.1 M sodium hydroxide is equivalent to 0.02063 g of $C_{13}H_{18}O_2$.

Report : The given sample contains _____ mg of ibuprofen.

Experiment No: 5

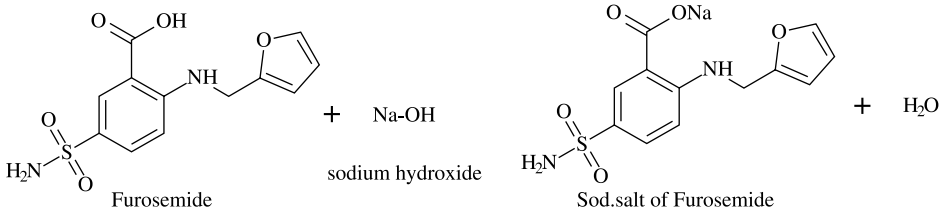
Assay of Frusemide Tablets

Aim: to carry out the assay of **Frusemide Tablets** IP

Chemical and apparatus required

Frusemide tablets, 0.1 M Sodium hydroxide, UV spectrophotometer.

Principle: It is assayed by aqueous acid – base titration between weak acid frusemide and strong alkali sodium hydroxide. In this assay protophilic solvent dimethyl formamide is used which enhances the acidity of frusemide so that it can be titrated with sodium hydroxide. To nullify the effect of acid impurities present in the solvent blank determination is carried out.



Furosemide

Sod.salt of Furosemide

$C_{12}H_{11}ClN_2O_5S$ Mol. Wt. 330.7 Frusemide is 4-chloro-N-furfuryl-5-sulphamoylanthranilic acid. Frusemide contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{12}H_{11}ClN_2O_5S$, calculated on the dried basis.

Description. A white or almost white, crystalline powder.

Preparation and Standardization of Standard Solutions

1. **Sodium Hydroxide, xM:** Solutions of any molarity xM may be prepared by dissolving 40x g of Sodium Hydroxide in sufficient water to produce 1000 ml.
2. **Standardization of 0.1 M NaOH:** Weigh accurately about 5 gm of potassium hydrogen phthalate previously dried at 120ºC for two hours dissolve in 75 ml of corbondioxide free water. Add 0.1 ml of phenolphthalein solution and titrate with the sodium hydroxide until a permanent pink colour is produced. Each ml of 0. 1 M NaOH equivalent to 0.02042 g of potassium hydrogen phthalate.

Assay Procedure

1. **Assay method by (Neutralization titration)**
   Weigh accurately about 0.5 gm and dissolve in 40ml of dimethyl formamide and titrate with 0.1 M sodium hydroxide using bromothymol blue as an indicator the end point shows the colour change from yellow to blue. Carry out an blank titration.
2. **Assay method by (UV- Spectrophotometer)**
   Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 0.1 g of Frusemide and shake with 150 ml of 0.1 M sodium hydroxide for 10 minutes. Add sufficient 0.1 M Sodium hydroxide to produce 250.0 ml and filter. Dilute 5.0 ml to 200.0 ml with 0.1 M sodium hydroxide and measure the absorbance of the resulting solution at the maximum at about 271 nm. Calculate the content of $C_{12}H_{11}ClN_2O_5S$ taking 580 as the value of A (1%, 1 cm) at the maximum at about 271 nm.

Report : The given sample contains _____ mg of frusemide.

Experiment No: 6

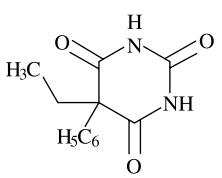
Assay of Isoniazid Tablets

Aim: To carry out the assay of **Isoniazid Tablets** IP

Chemical and apparatus required

Isoniazid tablets, 0.0167 M potassium bromated, methyl red, dimethylformamide, sodium carbonate, sodium thiosulphate, starch solution and UV spectrometer

Principle: The reaction involved in the titration is between isoniazid and potassium bromine, but solution of bromine is not stable. Therefore to the acidified solution of isonoazid a small amount of potassium bromide is added which is slowly titrated with potassium bromated. The reaction between $KBrO_3$, KBr, HCl, liberate bromine. This bromine oxidizes isoniazid. At the end point when HCl gets deflected, changes of colour from red to yellow.



Phenobarbitone

$C_6H_7N_3O$ Mol. Wt. 137.1 Isoniazid is isonicotinic acid hydrazide. Isoniazid contains not less than 98.0 per cent and not more than 101.0 per cent of $C_6H_7N_3O$, calculated on the dried basis.

Description. Colourless crystals or a white, crystalline powder; odourless.

Preparation and Standardization of Standard Solutions

1. **Potassium Bromate, 0.0167 M:** Dissolve 2.783 g of potassium bromate in sufficient water to produce 1000 ml.

2. **Sodium Thiosulphate, xM:** Solutions of any molarity xM may be prepared by dissolving 248x g of sodium thiosulphate and 2x g of sodium carbonate in sufficient carbon dioxidefree water to produce 1000 ml.
3. **Standardzation of Potasium Bromate:** Transfer an measured volume of about 30 ml of of potassium bromate into a glass stoppered flask, add 3ml of potassium iodide, following by 3 ml of hydrochloric acid. Allow to stand for five minutes, then titrate the librated iodine with standard sodium thiosulphate, add 3 ml of starch solution as a indicator towards the end point. Correct for a blank on the same quantities of the same reagents. Each ml of 0.1 N sodium thiosulphate is equvilent to 0.002784 gm of potassium bromate.

Assay procedure

1. **Assay method by (Volumetric analysis):** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 0.4 g of Isoniazid, dissolve as completely as possible in water, filter and wash the residue with sufficient water to produce 250.0 ml. To 50.0 ml of the resulting solution add 50 ml of water, 20 ml of hydrochloric acid and 0.2 g of potassium bromide and titrate slowly with continuous shaking with 0.0167 M potassium bromate using 0.05 ml of methyl red solution as indicator, until the red colour disappears. Each ml of 0.0167 M potassium bromate is equivalent to 0.003429 g of $C_6H_7N_3O$.
2. **Assay method by (UV-Spectroscopic method):** Carry out the following procedure protected from light.Weigh accurately about 80 mg, add 150 ml of dimethylformamide, swirl to dissolve and add sufficient water to produce 500.0 ml. Dilute 5.0 ml to 100.0 ml with water and mix. Measure the absorbance of the resulting solution at the maximum at about 367 nm. Calculate the content of $C_8H_7N_3O_5$ taking 750 as the specific absorbance at 367 nm.

Report : The given sample contains _____ mg of isoniazide.

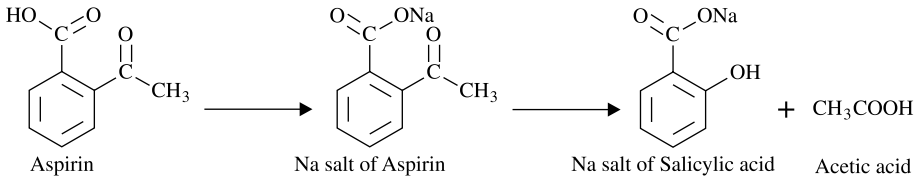
Experiment No: 7

Assay of Aspirin Tablets

Aim: To carry out the assay of **Aspirin Tablets** IP

Chemical and apparatus required: Aspirin tablets, 0.5 M sodium hydroxide, 0.5 M Hydrochloric acid, phenol red, 0.5 M sulphuric acid, reflux condenser, ether, 0.05 M bromine, and potassium iodide and 0.1 M sodium thiosulphate.

Reaction:



$C_9H_8O_4$ Mol. Wt. 180.2 Aspirin is 2-acetoxybenzoic acid. Aspirin contains not less than 99.5 per cent and not more than 100.5 per cent of $C_9H_8O_4$, calculated on the dried basis.

Description: Colourless crystals or a white, crystalline powder; odourless or almost odourless.

Preparation and Standardization of Standard Solutions

1. **Sodium Hydroxide, xM:** Solutions of any molarity xM may be prepared by dissolving 40x g of Sodium Hydroxide in sufficient water to produce 1000 ml.

2. **Hydrochloric Acid, xM Methanolic:** Solutions of any molarity xM may be prepared by diluting 85x ml of hydrochloric acid to 1000 ml with methanol.
3. **Sodium Thiosulphate, xM:** Solutions of any molarity xM may be prepared by dissolving 248 x g of sodium thiosulphate and 2x g of sodium carbonate in sufficient carbon dioxidefree water to produce 1000 ml.
4. **Standardization of Hydrochloric Acid:** Weigh accurately about 800 mg of anhydrous sodium carbonate, previously heated at about 270º for 1 hour Dissolve it in 100 ml of water and add 0.1 ml of methyl red solution. Add the acid slowly from a burette, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool and continue the titration. Heat again to boiling and titrate further as necessary until the faint pink colour is no longer affected by continued boiling. 1 ml of 1 M hydrochloric acid is equivalent to 0.05299 g of Na_2CO_3.
5. **Standardization of Sodium Thiosulphate:** Dissolve 0.200 g of potassium bromate, weighed accurately, in sufficient water to produce 250.0 ml. To 50.0 ml of this solution add 2 g of potassium iodide and 3 ml of 2 M hydrochloric acid and titrate with the sodium thiosulphate solution using starch solution, added towards the end of the titration, as indicator until the blue colour is discharged. Each 1 ml of 0.1 M sodium thiosulphate is equivalent to 0.002784 g of $KBrO_3$. Restandardise the solution frequently.

Assay Procedure (Acid-Base Titrations)
Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 0.5 g of Aspirin, add 30.0 ml of 0.5 M sodium hydroxide, boil gently for 10 minutes, cool and titrate the excess of alkali with 0.5 M hydrochloric acid using phenol red solution as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of sodium hydroxide required. Each ml of 0.5 M sodium hydroxide is equivalent to 0.04504 g of $C_9H_8O_4$.

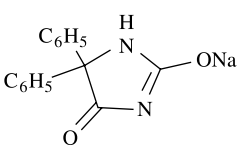
Assay Procedure (Iodimetric Titration) Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of Aspirin, dissolve in 10 ml of 0.5 M sulphuric acid and boil under a reflux condenser for 1 hour. Cool, transfer to a separating funnel with the aid of small quantities of water, and extract the liberated salicylic acid with four quantities, each of 20 ml, of ether. Wash the combined ether extracts with two quantities, each of 5 ml, of water, remove the ether in a current of air at a temperature not exceeding 30º, dissolve the residue in 20 ml of 0.5 M sodium hydroxide, and dilute to 200.0 ml with water. Transfer 50.0 ml to a stoppered flask, add 50.0 ml of 0.05 M bromine and 5 ml of hydrochloric acid, protect the mixture from light and shake repeatedly during 25 minutes. Add 20 ml of potassium iodide solution shake thoroughly and titrate with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Each 1 ml of 0.05 M bromine is equivalent to 0.003003 g of $C_9H_8O_4$.

Report : The given sample contains _____ mg of aspirin.

Experiment No: 8

Assay of Phenytoin Tablets

Aim: To carry out the assay of **Phenytoin Tablets** IP
Chemical and apparatus required: Tetrabutylammonium Iodide, silver oxide, silver oxide, anhydrous toluene, anhydrous toluene, carbon dioxide-free nitrogen, dimethylformamide
Principle: Phenytoin is assayed by non-aqueous titration. phenytoin being a weak acid cannot be titrated in an aqueous medium. Tetrabutyl ammonium hydroxide is used as titrant using thymol blue as indicator.



$C_{15}H_{11}N_2NaO_2$ Mol. Wt. 274.3 Phenytoin Sodium is 4-oxo-5,5-diphenyl-2-imidazolidin-2-olate Phenytoin Sodium contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{15}H_{11}N_2NaO_2$, calculated on the anhydrous basis.

Preparation and Standardization of Standard Solutions

1. **Tetrabutylammonium Hydroxide, 0.1 M:** Dissolve 40 g of tetrabutylammonium iodide in 90 ml of dehydrated methanol in a glass-stoppered flask. Place in an ice-bath, add 20 g of powdered silver oxide, insert the stopper and agitate vigorously for 1 hour. Centrifuge a few ml, and test the supernatant liquid for iodides. If the test is positive, add an additional 2 g of silver oxide and continue to stand for 30 minutes with intermittent agitation. When all of the iodide has reacted, filter through fine sintered-glass filter. Rinse the flask and filter with three quantities, each of 50 ml, of anhydrous toluene. Add the washings of the filtrate and dilute to 1000 ml with anhydrous toluene. Flush the solution for 10 minutes with dry, carbon dioxide-free nitrogen. Store protected from carbon dioxide and moisture, and discard after 60 days. Alternatively, prepare the solution by diluting a suitable volume of commercially available tetrabutylammonium hydroxide solution in methanol with a mixture of four volumes of anhydrous toluene and 1 volume of dehydrated methanol. Standardise the solution in the following manner immediately before use.
2. **Standardization of 0.1 M Tetrabutylammonium Hydroxide:** Weigh accurately about 0.4 g of benzoic acid, dissolve in 80 ml of dimethylformamide, add a few drops of a 1 per cent w/v solution of thymol blue in dimethylformamide and titrate with the tetrabutylammonium hydroxide solution to a blue endpoint. Protect the solution from atmospheric carbon dioxide throughout the titration. Perform a blank determination and make any necessary correction. 1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.01221 g of $C_7H_6O_2$.

Assay procedure: Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.25 g of Phenytoin Sodium, shake with 40 ml of 0.01 M sodium hydroxide for 5 minutes and add sufficient 0.01 M sodium hydroxide to produce 50.0 ml. Centrifuge, acidify 25.0 ml of the clear liquid with 10 ml of 0.1 M hydrochloric acid and extract successively with 50, 40, 25 and 25 ml of ether. Wash the combined extracts with 10 ml of water, evaporate to dryness and dry the residue at 105º. Dissolve in 50 ml of anhydrous pyridine and titrate with 0.1 M tetrabutylammonium hydroxide, using 0.3 per cent w/v solution of thymol blue in pyridine as indicator and taking care to prevent absorption of carbon dioxide from the atmosphere. Carry out a blank titration. 1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.02743 g of $C_{15}H_{11}N_2NaO_2$.

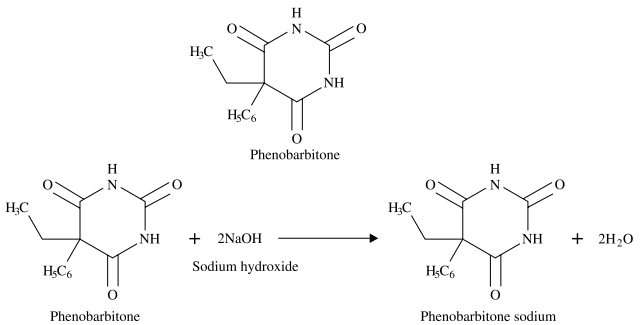
Report : The given sample contains _____ mg of phenytoin.

Experiment No: 9

Assay of Phenobarbitone Sodium Tablets

Aim: To carry out the assay of **Phenobarbitone Sodium Tablets** IP

Chemical and apparatus required: Sodium hydroxide, sufficient aldehyde-free ethanol, benzoic acid, thymolphthalein solution, Phenobarbitone Sodium, silver nitrate pyridine, and ether.



$C_{12}H_{11}N_2NaO_3$, Mol. Wt. 254.2 Phenobarbital Sodium; Soluble Phenobarbitone; Soluble Phenobarbital. Phenobarbitone Sodium contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{11}N_2NaO_3$, calculated on the dried basis.

Description. A white powder or crystalline granules or flaky crystals; hygroscopic.

Preparation and Standardization of Standard Solutions

1. **Sodium Hydroxide, 0.1 M Ethanolic:** Dissolve 4.2 g of sodium hydroxide in 5 ml of water and add sufficient aldehyde-free ethanol to produce 1000 ml. Allow the solution to stand in a tightly-stoppered bottle for 24 hours. Then quickly decant the clear supernatant liquid into a suitable, tightly-closed container.
2. **Sodium Hydroxide, 0.1 M Ethanolic:** Weigh accurately about 0.6 g of benzoic acid, dissolve in a mixture of 30 ml of ethanol (95 per cent) and 6 ml of water and titrate with the ethanolic sodium hydroxide solution, using 0.2 ml of thymolphthalein solution as indicator. 1 ml of 0.1 M ethanolic sodium hydroxide is equivalent to 0.01221g of $C_7H_6O_2$.

Assay procedure

1 **Assay method (Non-aqueous titration):** Weigh accurately about 0.1 gm, dissolve in 5ml of pyridine and add 0.25 ml of thymolpthalein solution and 10ml silver nitrate pyridine reagent and titrate with sodium hydroxide, 0.1 M ethanolic solution until a pure blue colour is obtained. A blank titration is to be performed.

2. **Assay method by gravymetric titrations:** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of Phenobarbitone Sodium, dissolve as completely as possible in 10 ml of a 2 per cent w/v solution of sodium hydroxide, saturate with sodium chloride, acidify with hydrochloric acid and extract with successive quantities, each of 15 ml, of ether until complete extraction is effected. Wash the combined extracts with two quantities, each of 2 ml, of water and extract the combined washings with 10 ml of ether. Add the ether to the main ether layer and dry the residue to constant weight at 105º. 1 g of the residue is equivalent to 1.095 g of $C_{12}H_{11}N_2NaO_3$.

Report : The given sample contains _____ mg of phenobarbitone sodium.

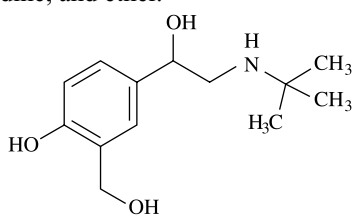
Experiment No: 10

Assay of Salbutamol Tablets

Aim: To carry out the assay of **Salbutamol Tablets** IP

Chemical and apparatus required: Sodium hydroxide, sufficient aldehyde-free ethanol, benzoic acid, thymolphthalein solution, Phenobarbitone Sodium, silver nitrate pyridine, and ether.

Principle: $C_{13}H_{21}NO_3$ Mol. Wt. 239.3 Salbutamol is (RS)-1-(4-hydroxy-3-hydroxymethylphenyl)-2- (tert-butylamino)ethanol. Salbutamol contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{13}H_{21}NO_3$, calculated on the dried basis.



Description. A white or almost white, crystalline powder.

Preparation and Standardization of Standard Solutions

1. **Perchloric Acid, 0.1 M:** Mix 8.5 ml of perchloric acid with 500 ml of anhydrous glacial acetic acid and 25 ml of acetic anhydride, cool and add anhydrous glacial acetic acid to produce 1000 ml. Allow the prepared solution to stand for 1 day for the excess acetic anhydride to be continued and carry out the determination of water, Appendix 3.24. If the water content exceeds 0.05 % , add more acetic anhydride. If the solution contains no titrable water, add sufficient water

to obtain a content of water between 0.02% and 0.05%. Allow the solution to stand for 1 day and again titrate the water content. The solution so obtained should contain between 0.02% and 0.05% of water

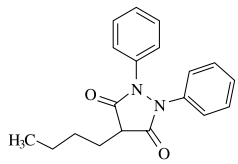
2. **Standardization of 0.1 N Perchloric Acid:** Weigh accurately about 0.35 g of potassium hydrogen phthalate, previously powdered lightly and dried at 120º for 2 hours and dissolve it in 50 ml of anhydrous glacial acetic acid. Add 0.1 ml of crystal violet solution and titrate with the perchloric acid solution until the violet colour changes to emerald-green. Peform a blank determination and make any necessary correction. Each ml of 0.1 M perchloric acid is equivalent to 0.02042 g of $C_8H_5KO_4$.

Assay procedure: Weigh accurately about 0.4 gm dissolve in 5 ml of anhydrous formic acid. Add 35 ml of anhydrous glacial acetic acid and carry out non-aqueous titration determination the end point potentiometrically. Perform a blank determination and make any necessary correction. Each ml of 0.1 M perchloric acid equivalent to 0.05767 gm of $(C_{13}H_{21}NO_3)_2 H_2SO_4$.

Report : The given sample contains _____ mg of salbutamol.

Experiment No.11

Assay of Phenyl Butazone Tablets



Aim: To carry out the assay of phenyl butazone tablets.

Chemical and apparatus required: Sodium hydroxide, potassium hydrogen phthalate, bromothymol blue, Phenylbutazone

Principle:

Preparation and Standardization of Standard Solutions

1. **Sodium Hydroxide, xM:** Solutions of any molarity xM may be prepared by dissolving 40x g of Sodium Hydroxide in sufficient water to produce 1000 ml.
2. **Standardization of 0.1 M NaOH:** Weigh accurately about 5gm of potassium hydrogen phthalate previously dried at 120º C for two hours dissolve in 75 ml of corbondioxide free water. Add 0.1 ml of phenolphthalein solution and titrate with the sodium hydroxide until a permanent pink colour is produced. Each ml of 0. 1 M NaOH equivalent to 0.02042 g of potassium hydrogen phthalate.

Assay procedure

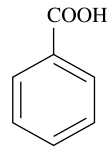
1. **Assay method (neutralization titration):** Weigh accurately about 0.5 gm dissolve in 25 ml of acetone and titrate with 0.1 N sodium hydroxide using 0.5 ml of bromothymol blue solution as indicator and continue the titeration until the blue colour persist for atleast 15 seconds. Perform a blank determination and make any necessary correction.
2. **Assay method (UV spectroscopy method):** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 0.5 g of Phenylbutazone and shake vigorously with 150 ml of 0.1 M sodium hydroxide for 45 minutes. Add sufficient 0.1 M sodium hydroxide to produce 250.0 ml, mix and filter, rejecting the first 20 ml of the filtrate. To 5.0 ml of the filtrate add 50 ml of water and 4 ml of hydrochloric acid and extract with three quantities, each of 30 ml, of ether. Combine the ether extracts and extract with three quantities, each of 30 ml, of 0.1 M sodium hydroxide. Combine the aqueous extracts, pass nitrogen through the solution to remove the residual ether and add sufficient 0.1 M sodium hydroxide to produce 100.0 ml and mix well. To 10.0 ml of this solution add sufficient 0.1 M sodium hydroxide to produce 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 264 nm, using 0.1 M sodium hydroxide as the blank. Calculate the content of $C_{19}H_{20}N_2O_2$ from the absorbance obtained by carrying out the Assay simultaneously on 0.5 g of phenylbutazone RS in place of

the substance being examined and from the declared content of $C_{19}H_{20}N_2O_2$ in phenylbutazone RS.

Report : The given sample contains _____ mg of phenylbutazone.

Experiment No.12

Assay of Compound Benzoic Acid Ointment



Aim: To carry out the assay of **Compound Benzoic Acid Ointment IP**

Chemical and apparatus required: Sodium hydroxide, phenolphthalein solution, sodium bicarbonate, 0.1 M bromine, hydrochloric acid, potassium iodide, 0.1 M sodium thiosulphate and starch solution.

$C_7H_6O_2$ Mol. Wt. 122.1 Benzoic Acid contains not less than 99.5 per cent and not more than 100.5 per cent of $C_7H_6O_2$, calculated on the anhydrous basis.

Description. Colourless, light crystals, scales or needles; odour, slight and characteristic.

Preparation and Standardization of Standard Solutions

1. **Sodium Hydroxide, xM:** Solutions of any molarity xM may be prepared by dissolving 40x g of Sodium Hydroxide in sufficient water to produce 1000 ml.
2. **Standardization of 0.1 M NaOH:** Weigh accurately about 5 gm of potassium hydrogen phthalate previously dried at 120ºC for two hours dissolve in 75 ml of corbondioxide free water. Add 0.1 ml of phenolphthalein solution and titrate with the sodium hydroxide until a permanent pink colour is produced. Each ml of 0. 1M NaOH equivalent to 0.02042 g of potassium hydrogen phthalate.
3. **Sodium Thiosulphate, x M:** Solutions of any molarity x M may be prepared by dissolving 248x g of sodium thiosulphate and 2x g of sodium carbonate in sufficient carbon dioxidefree water to produce 1000 ml.
4. **Standardization of Sodium Thiosulphate:** Dissolve 0.200 g of potassium bromate, weighed accurately, in sufficient water to produce 250.0 ml. To 50.0 ml of this solution add 2 g of potassium iodide and 3 ml of 2 M hydrochloric acid and titrate with the sodium thiosulphate solution using starch solution, added towards the end of the titration, as indicator until the blue colour is discharged. Each 1 ml of 0.1 M sodium thiosulphate is equivalent to 0.002784 g of $KBrO_3$. Restandardise the solution frequently.

Assay procedure

For benzoic acid: Weigh accurately about 2.5 g, dissolve with the aid of gentle heat, as completely as possible, in 50 ml of a mixture of equal volumes of ethanol (95 per cent) and ether, previously neutralized to phenolphthalein solution and titrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator. 1 ml of 0.1 M sodium hydroxide, after deducting 1 ml for each 0.01381 g of $C_7H_6O_3$ in the weight of the ointment taken (calculated from the result of the Assay for salicylic acid) is equivalent to 0.01221 g of $C_7H_6O_2$.

For salicylic acid: Weigh accurately about 2.5 g, dissolve with the aid of gentle heat, as completely as possible, in 50 ml of ether, and extract with 5 quantities, each of 10 ml, of a saturated solution of sodium bicarbonate, washing each extract with the same 50 ml of ether. Combine the aqueous extracts, cautiously add hydrochloric acid until the solution is distinctly acid to litmus paper and extract with 4 quantities, each of 25 ml, of ether; combine the extracts and evaporate the ether at a temperature below 40º. Dissolve the residue in 5 ml of 0.5 M sodium hydroxide, add 50.0 ml of 0.1 M bromine and 5 ml of hydrochloric acid, shake repeatedly during 15 minutes and allow to stand for 15 minutes. Add 10 ml of potassium iodide solution and titrate with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Repeat the operation without the substance under exami-

nation. The difference between the titrations represents the amount of bromine required. 1 ml of 0.1 M bromine is equivalent to 0.002302 g of $C_7H_6O_3$.

Report : The given sample contains _____ mg of compound benzoic acid.

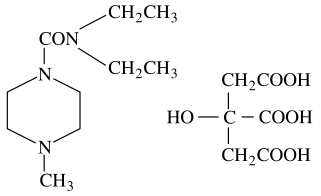
Experiment No.13

Assay of Diethylcarbamazine Citrate Tablets

Aim: To carry out the assay of **Diethylcarbamazine Citrate Tablets IP**

Chemical and apparatus required: Diethylcarbamazine Citrate tablets, sodium hydroxide, chloroform, phenolphthalein, 0.05 M sulphuric acid, phenolphthalein solution and bromocresol green.

Diethylcarbamazine Citrate Tablets, Diethylcarbamazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of diethylcarbamazine citrate, $C_{10}H_{21}N_3O,C_6H_8O_7$.



Preparation and Standardization of Standard Solutions:

1. **Sulphuric Acid, xM:** Solutions of any molarity xM may be prepared by carefully adding 54x ml of sulphuric acid to an equal volume of water and diluting to 1000 ml with water.
2. **Sodium Hydroxide, xM:** Solutions of any molarity xM may be prepared by dissolving 40x g of Sodium Hydroxide in sufficient water to produce 1000 ml.
3. **Standardization of 0.1 M NaOH:** Weigh accurately about 5 gm of potassium hydrogen phthalate previously dried at 120ºC for two hours dissolve in 75 ml of corbondioxide free water. Add 0.1 ml of phenolphthalein solution and titrate with the sodium hydroxide until a permanent pink colour is produced. Each ml of 0.1 M NaOH equivalent to 0.02042 g of potassium hydrogen phthalate

Assay procedure: Weigh 20 tablets and reduce to a fine powder. Weigh accurately a quantity of the powder equivalent to about 0.75 g of Diethylcarbamazine Citrate, dissolve as completely as possible in a mixture of 10 ml of water and 10 ml of 5 M sodium hydroxide and extract with four quantities, each of 20 ml, of chloroform, washing each extract with the same two quantities, each of 20 ml, of water, and a third quantity, if the second washing is alkaline to phenolphthalein solution. Extract the combined chloroform extracts in succession with 25.0 ml of 0.05 M sulphuric acid, 15 ml and 10 ml of water. Combine the aqueous extracts, warm to remove the chloroform, cool and titrate the excess of acid with 0.1 M sodium hydroxide using bromocresol green solution as indicator. Each ml of 0.05 M sulphuric acid is equivalent to 0.03914 g of $C_{10}H_{21}N_3O,C_6H_8O_7$.

Report : The given sample contains _____ mg of diethylcarbamazine citrate.

Experiment No.14

Assay of Diclofenac Sodium

Aim: To determine the amount of diclofenac present in given sample.

Apparatus required: Burette, Conical Flask, Beaker, Pipette etc.

Principle: Non-Aqueous Titration protect the solution and titrant from atmospheric carbon dioxide and moisture throughout the determination. Use the titrant, solvent and indicator specified in the individual monograph. The titrant is standardised using the same method, solvent and indicator as specified for the substance.

Method A

Dissolve the prescribed quantity of the substance being examined in a suitable volume of *anhydrous glacial acetic acid,* warming and cooling if necessary, or prepare a solution as directed in the monograph and determine the equivalence point potentiometrically using 0.1 M perchloric acid as titrant, unless otherwise specified in the monograph. Potentiometric titration may be carried out using a glass electrode and a standard reference electrode, e.g. calomel reference electrode containing saturated solution of *potassium chloride* in *water.* Potentiometric titrations may also be carried out by using a glass electrode and a saturated solution of potassium *chloride* in *water* has been replaced by a saturated solution of *potassium chloride* in *methanol.* It must be ensured that no leakage of salt-bridge solution occurs. Alternatively, a combined electrode may be used. The junction between the calomel electrode and the titration liquid should have a reasonably low electrical resistance and there should be minimum of transfer of liquid from one side to the other. The connections between the potentiometer and the electrode system must be made according to the manufacturer's instructions to avoid problems of instability.

When the temperature (t_2) of the titrant at the time of the assay is different from the temperature (t_1) of the tirant when it was standardised, multiply the volume of the titrant required by [1 + 0.0011 (t_1 -t_2) and calculate the result of the assay from the corrected volume.

Procedure

Assay: Weigh accurately about 0.2 g, dissolve in 50 ml of anhydrous glacial acetic acid and carry out the Method A for non-aqueous titration, determining the end-point potentiometrically. Perform a blank determination and make any necessary correction.

Equivalent Factor

Each ml of 0.1 M perchloric acid is equivalent to 0.03181 g of $C_{14}H_{10}C_{l2}NNaO_2$.

Report: The amount of diclofenac sodium present in the given sample found to be _____mg.

Experiment No.15

Analgin Tablets by Iodimetry

Aim: To determine the amount of analgin present in givenn tablets.
Apparatus required: Burette, Conical Flask, Beaker, Pipette etc.
Chemicals required: Iodine, ethanol, anal gin tablets
Principle: Iodine, 0.05 M: Dissolve about 14 g of iodine in a solution of 36 g of potassium iodide in 100 ml of water, add three drops
Procedure: Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 0.5 g of Analgin and transfer to a 50-ml volumetric flask. Add 10 ml of *water* and shake for 1 minute. Dilute to volume with *ethanol* (*95%)*, shake well and filter. Titrate 25.0 ml of the filtrate with 0.05 M iodine until a yellow colour stable for 30 seconds is produced.
Equivalent Factor: Each ml of *0.05 M iodine* is equivalent to 0.01757 g of $C_{13}H_{16}N_3NaO_4S,H_2O$.

Report : The amount of Ibuprofen present in the given tablets found to be_____mg.

Experiment No.16

Assay of Ephedrine Hydrochloride

Aim: To determine the amount of ephedrine present in givenn tablets.
Apparatus required: Burette, Conical Flask, Beaker, Pipette etc.
Chemicals required: Perchloric acid, glacial acetic acid, mercuric acetate, methyl orange, acetone.

Principle: Non-Aqueous Titration

Protect the solution and titrant from atmospheric carbon dioxide and moisture throughout the determination. Use the titrant, solvent and indicator specified in the individual monograph. The titrant is standardised using the same method, solvent and indicator as specified for the substance.

Method B

Dissolve the prescribed quantity of the substance being examined in a suitable volume of anhydrous *glacial acetic acid previously* neutralised using the indicator specified in the individual monograph, warming and cooling if necessary, or prepare a solution as directed in the monograph. When the substance is a hydrochloride or hydrobromide, add 15 ml of *mercuric acetate solution* unless otherwise directed in the monograph Titrate with 0.1 M Perchloric acid unless otherwise specified in the monograph to the full colourchange of indicator corresponding to the maximum absolute value of dE/dV (where E is the electromotive force and V is the volume of the titrant) in a potentionetric titration of the substance being examined. The indicator used for the titration is also used for neutralising the *mercuric acetate solution*.

When the temperature of the titrant at the time of the assay is different from the temperature of the titrant when it was standardised, the same correction factor as described under Method A is applied.

Perchloric Acid, 0.1 M: Mix 8.5 ml of perchloric acid with 500 ml of anhydrous glacial acetic acid and 25 ml of acetic anhydride, cool and add anhydrous glacial acetic acid to produce 1000 ml. Allow the prepared solution to stand for 1 day for the excess acetic anhydride to be continued and carry out the determination of water, Appendix 3.24. If the water content exceeds 0.05 % , add more acetic anhydride. If the solution contains no titrable water, add sufficient water to obtain a content of water between 0.02% and 0.05%. Allow the solution to stand for 1 day and again titrate the water content. The solution so obtained should contain between 0.02% and 0.05% of water.

Standardise the solution in the following manner.

Weigh accurately about 0.35 g of potassium hydrogen phthalate, previously powdered lightly and dried at 120º for 2 hours and dissolve it in 50 ml of anhydrous glacial acetic acid. Add 0.1 ml of crystal violet solution and titrate with the perchloric acid solution until the violet colour changes to emerald-green. Peform a blank determination and make any necessary correction. Each ml of 0.1 M perchloric acid is equivalent to 0.02042 g of $C_8H_5KO_4$. Other strengths of perchloric acid should be prepared by diluting 0.1 M perchloric acid appropriately with anhydrous glacial acetic acid.In the tests and assays of the Pharmacopoeia, this solution is specified as “0.1 N perchloric acid:. Thus the solution in anhydrous glacial acetic acid is to be used unless the words “in dioxan” are stated.

Mercuric Acetate Solution: A 5% w/v solution of mercuric acetate in glacial acetic acid.

Procedure: Weigh accurately about 0.17 g, dissolve in 10 ml of mercuric acetate solution, warming gently, add 50 ml of *acetone* and carry out Method B for *non-aqueous titration,* using 1 ml of a saturated solution of *methyl orange* in acetone as indicator, until a red colour is obtained. Perform a blank determination and make any necessary correction.

Equivalent factor: Each ml of 0.1 M perchloric acid is equivalent to 0.02017 g of $C_{10}H_{15}NO$,HCl.

Report: The amount of ephedrine present in the given tablets found to be _____mg.

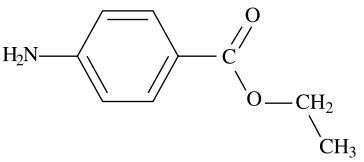
Experiment No.17

Assay of Benzocaine by Diazotization

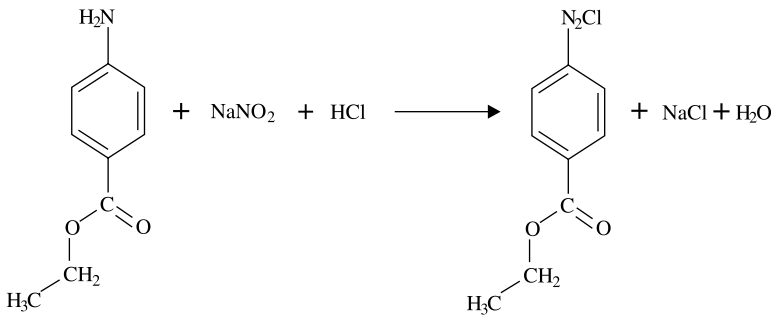
Aim: To estimate the amount of benzocaine present in the given sample.

Requirements: $NaNO_2$, Hydrochloric acid, Beaker, Volumetric flask, Distilled water, Analytical balance, Weight box, Burette, and Conical flask.

Principle: Assay of benzocaine involes diazotizatin titration. Under controlled experimental conditions the reaction is quantitative and can be used for the determination of most of the drugs containing a free primary amino group. Primary aromatic amines react with sodium nitrite in acid solution to form diazonium salts. The end point in this titration method is located virtually by using starch iodide paste as indicator. A small amount of iodide included in the titration mixture is converted to iodine by excess of nitrous acid, this is detected using starch indicator. Titration with nitrous acid is used in pharmacopoeial assays of the following: Benzocaine, procaine, dapsone, primaquine, suphacetamide, sulphadoxine, sulphamethizole.


Ethyl para amino benzoate(Benzocaine)

Reaction:



Procedure: Weigh accurately about 200 mg of benzocaine and dissolve it in 10 ml of water and 15 ml of HCl by heating. Cool the solution in an ice bath to about 10ºC and titrate with 0.1 M sodium nitrite until a blue colour is produced immediately, with starch paste as indicator.

Repeat the procedure omitting the sample for blank titration.

Equivalent Factor: Each ml of 0.1 N sodium nitrite is equivalent to 0.01652 g of Benzocaine.

Report: The given sample of contains_____ mg of benzocaine.

Experiment No.18

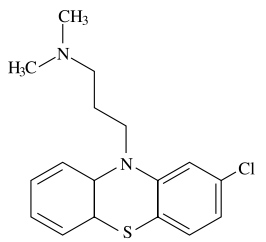
Assay of Chlorpromazine

Aim: To estimate the amount of chlorpromazine present in the sample.

Apparatus required: Measuring cylinder, Volumetric flask, Distilled water, Analytical balance, Weight box, Burette, and Conical flask.

Chemicals required: perchloric acid, glacial acetic acid, acetone, chlorpromazine tablets.

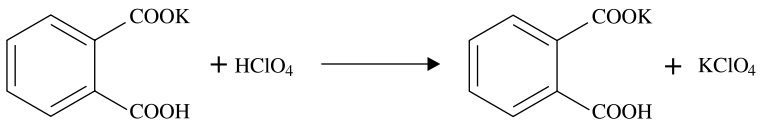
Principle: It is a non aqueous titration. Chlorpromazine hydrochloride is very weakly basic to react quantitatively with perchloric acid. Therefore mercuric acetate is added which replace the halide ion with an equivalent quantity of acetate ion,which is a strong base in acetic acid.


Chlorpromazine

Preparation of perchloric acid: Mix 8.5 ml of perchloric acid with 500 ml of anhydrous glacial acetic acid and 25 ml of acetic anhydride, cool and add anhydrous glacial acetic acid to produce 1000 ml.

Standardization of perchloric acid: Weigh accurately about 0.35 g of potassium hydrogen phthalate, previously powdered lightly and dried at 120º for 2 hours and dissolve it in 50 ml of anhydrous glacial acetic acid. Add 0.1 ml of crystal violet solution and titrate with the perchloric acid solution until the violet colour changes to emerald-green. Peform a blank determination and make any necessary correction.

Reaction



Procedure: Weigh accurately about 0.6 g, dissolve in 200 ml of acetone and add 15 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using a saturated solution of methyl orange in acetone as indicator. Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.03553 g of $C_{17}H_{19}ClN_2S$, HCl.

Mercuric Acetate Solution: A 5 per cent w/v solution of mercuric acetate in glacial acetic acid.

Report: The given sample contains _____mg of chlorpromazine.

Experiment No.19

Assay of Sulphadiazine

Aim: To estimate the amount of sulphadiazine present in the sample.

Apparatus

A suitable open vessel of about 200 ml capacity is fitted with two similar clean platinum electrodes (platinum - calomel or platinum-platinum and a stirrer. The electrodes may be of platinum foil 0.5 cm square and should be placed 1.5 cm apart. They may be cleaned by immersing for a few seconds in boiling nitric acid containing a small amount of ferric chloride, followed by washing with water.

The polarizing voltage may be obtained from a 1.5-volt dry cell and potentiometer or other convenient device which enables a small but definite voltage to be applied across the electrodes. The current flowing in the system is indicated by a series galvanometer which should have adequate sensitivity.

Assay: Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 0.5 g of Sulphadiazine and dissolve as completely as possible in a mixture of 50 ml of water and 10 ml of hydrochloric acid. Carry out the nitrite titration. Each ml of 0.1 M sodium nitrite is equivalent to 0.02503 g of $C_{10}H_{10}N_4O_2S$.

Nitrite Titration

The following method is suitable for the determination of most of the pharmacopoeial sulphonamide drugs and their preparations. It may also be used for other pahrmacopoeial drugs for which nitrite titration is recommended.

Method

Weigh accurately about 0.5 g in the case of a sulphonamide, or otherwise the quantity specified in the individual monograph, and transfer to the titration vessel. Add 20 ml of hydrochloric acid and 50 ml of water, stir until dissolved, cool to about 15º. Immerse the electrodes in the solution and apply a voltage of about 50 mV across the electrodes when polarization of the electrodes takes place. Place the burette tip just below the surface of the solution to eliminate air oxidation of the sodium nitrite and stir the solution gently, maintaining the temperature of about 15º. The titration may be carried out manually or by means of an automatic titrator. In the manual titration, add the titrant slowly and when the titration is within 1 ml of the end - point, add then in 0.1 ml portions, allowing not less than 1 minute between additions. (The galvanometerneedle deflects and then returns to approximately its original position until the end – point is reached). At the end - pint, when a slight excess of sodium nitrite is present, the electrodes are depolarised, current flows and a permanent deflection of the needle is obtained.

Note: It will be necessary to adjust the sensitivity of the galvanometer or the applied voltage before the titration is begun in order to obtain an adequate deflection at the end-point.

Report : The given sample contains _____ mg of sulphadiazine.

Experiment No.20

Assay of Chloroquine

Aim: To estimate the amount of chlorpromazine present in the sample.
Apparatus required: Measuring cylinder, Volumetric flask, Distilled water, Analytical balance, Weight box, Burette, and Conical flask.
Chemicals required: glacial acetic acid, perchloric acid
Assay: Weigh accurately about 0.5 g, dissolve in 50 ml of anhydrous glacial acetic acid and carry out Method A for non-aqueous titration, determining the end-point potentiometrically. Perform a blank determination and make any necessary correction. Each ml of 0.1 M perchloric acid is equivalent to 0.0418 g of $C_{18}H_{26}ClN_3$, H_2SO_4.
Perchloric Acid, 0.1 M: Mix 8.5 ml of perchloric acid with 500 ml of anhydrous glacial acetic acid and 25 ml of acetic anhydride, cool and add anhydrous glacial acetic acid to produce 1000 ml. Allow the prepared solution to stand for 1 day for the excess acetic anhydride to be continued and carry out the determination of water, Appendix 3.24. If the water content exceeds 0.05 % , add more acetic anhydride. If the solution contains no titrable water, add sufficient water to obtain a content of water between 0.02% and 0.05%. Allow the solution to stand for 1 day and again titrate the water content. The solution so obtained should contain between 0.02% and 0.05% of water. Standardise the solution in the following manner.

Weigh accurately about 0.35 g of potassium hydrogen phthalate, previously powdered lightly and dried at 120º for 2 hours and dissolve it in 50 ml of anhydrous glacial acetic acid. Add 0.1 ml of crystal violet solution and titrate with the perchloric acid solution until the violet colour changes to emerald-green. Peform a blank determination and make any necessary correction. Each ml of 0.1 M perchloric acid is equivalent to 0.02042 g of $C_8H_5KO_4$.

Other strengths of perchloric acid should be prepared by diluting 0.1 M perchloric acid appropriately with anhydrous glacial acetic acid.

In the tests and assays of the Pharmacopoeia, this solution is specified as “0.1 N perchloric acid:. Thus the solution in anhydrous glacial acetic acid is to be used unless the words “in dioxan” are stated.

Non-Aqueous Titration

Protect the solution and titrant from atmospheric carbon dioxide and moisture throughout the determination. Use the titrant, solvent and indicator specified in the individual monograph. The titrant is standardised using the same method, solvent and indicator as specified for the substance.

Method A

Dissolve the prescribed quantity of the substance being examined in a suitable volume of anhydrous glacial acetic acid, warming and cooling if necessary, or prepare a solution as directed in the monograph and determine the equivalence point potentiometrically using 0.1 M perchloric acid as titrant, unless otherwise specified in the monograph. Potentiometric titration may be carried out using a glass electrode and a standard reference electrode, e.g. calomel reference electrode containing saturated solution of potassium chloride in water. Potentiometric titrations may also be carried out by using a glass electrode and a saturated solution of potassium chloride in water has been replaced by a saturated solution of potassium chloride in methanol. It must be ensured that no leakage of salt- bridge solution occurs. Alternatively, a combined electrode may be used. The junction between the calomel electrode

and the titration liquid should have a reasonably low electrical resistance and there should be minimum of transfer of liquid from one side to the other. The connections between the potentiometer and the electrode system must be made according to the manufacturer's instructions to avoid problems of instability.

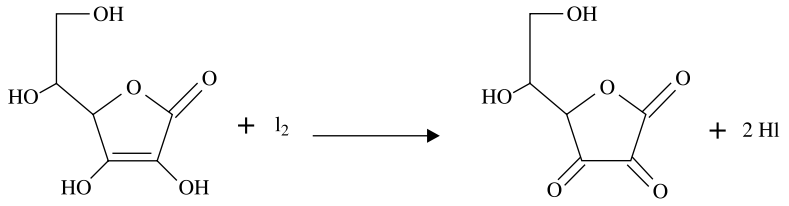
When the temperature (t_2) of the titrant at the time of the assay is different from the temperature (t1) of the tirant when it was standardised, multiply the volume of the titrant required by [1 + 0.0011 ($t_1 - t_2$) and calculate the result of the assay from the corrected volume.

Report : The given sample contains _____ mg of chloroquine.

Experiment No. 21

Assay of Ascorbic Acid

Aim: To estimate the amount of Ascorbic acid present in the given sample.
Apparatus: Burette, Conical flask, Pipette and Electronic weighing machine.
Chemicals required: Ascorbic acid, Iodine, Sodium thiosulfate, starch solution and sulfuric acid.
Principle: Ascorbic acid is also known as vitamin C, which is assayed, iodometrically by titrating against standard iodine. It is a redox titration where iodine acts as self-indicator. Vitamin C undergoes oxidation and forms dehydroascorbicacid and iodine undergoes reduction to form iodide. Standardization of iodine is done by titrating with Sodiumthiosulfate. In this reaction Sodiumthiosulfate is oxidized to sodiumtetrathionate.



Ascorbic acid — Dehydro ascorbic acid

$I_2 + Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2\,NaI$

Procedure: Weigh accurately 0.2 ml of Ascorbic acid into a 250 ml conical flask. Dissolve it in a mixture of 80 ml of freshly boiled and cooled water and 20 ml of 0.1 M sulfuric acid. Titrate with 0.05 M iodine using starch solution as indicator until solution turns blue.
Standardization of Iodine: Transfer 6.025 gm of Sodiumthiosulfate into a 250 ml volumetric flask. Dissolve in water adjust to volume and shake. Pipette 25 ml of 0.05 M Iodine solution into a conical flask and titrate with standard Sodiumthiosulfate solution until just colorless.
Equivalent Factor: 1 ml of 0.05 M Iodine is equivalent to 0.0881 gm of Ascorbic acid

Report: The amount of ascorbic acid present in the given sample was found to be _____ mg.

Experiment No.22

Assay of Benzylpenicillin Sodium

Aim: To estimate the amount of benzyl penicillin present in the given sample.
Apparatus: Burette, Conical flask, Pipette and Electronic weighing machine.
Chemicals required: 1 M nitric acid, 1 M sodium hydroxide, 0.02 M mercuric nitrate.
Assay: Weigh accurately about 50 mg, dissolve in 5 ml of water, add 5 ml of 1M sodium hydroxide and allow to stand for 15 minutes. Add 5 ml of 1M nitric acid, 20 ml of acetate buffer pH 4.6 and 20 ml of water and titrate at 35–40º with 0.02 M mercuric nitrate. Titrate slowly so that the titration takes about 15 minutes. Determine the end-point potentiometrically using a platinum or mercury indicator

electrode and a mercury-mercurous sulphate reference electrode. (Cleaning the reference electrode with dilute nitric acid and the indicator electrode with a 10% w/v solution of sodium thiosulphate followed by rinsing with distilled water is recommended after each assay). Ignore any preliminary inflection on the titration curve. Each ml of 0.02 M mercuric nitrate is equivalent to 0.007450 g of total penicillins, calculated as $C_{16}H_{17}KN_2O_4S$.

To 0.25 g, accurately weighed, add 25 ml of water and 25 ml of acetate buffer pH 4.6 and shake until solution is complete. Titrate immediately at room temperature with 0.02 M mercuric nitrate determining the end-point as above. Each ml of 0.02 M mercuric nitrate is equivalent to 0.007450 g of degradation products, calculated as $C_{16}H_{17}KN_2O_4S$.

Calculate the percentage content of total penicillins and the percentage content of degradation products. The difference between the two percentages is the content of penicillins.

Benzylpenicillin Potassium intended for use in the manufacture of injectable preparations complies with the following additional requirements.

Sodium Hydroxide, xM: Solutions of any molarity xM may be prepared by dissolving 40x g of Sodium Hydroxide in sufficient water to produce 1000 ml.

Nitric Acid, xM: Solutions of any molarity xM may be prepared by diluting 63x ml of nitric acid to 1000 ml with water.

Acetate Buffer pH 4.6: Dissolve 5.4 g of sodium acetate in 50 ml of water, add 2.4 ml of glacial acetic acid and dilute with water to 100 ml. Adjust the pH, if necessary.

Mercuric Nitrate 0.02 M: Dissolve 6.85 g of Mercuric Nitrate in 20 ml. of 1M nitric acid and add sufficient water to produce 1000 ml.

Standardize the solution in the following manner.

Dissolve 15mg. of sodium chloride in 0 ml of water and tirtrate it with the mercuric nitrate solution determining the end point potentiometrically , using a platinum or mercury indicator electrode and a mercury-mercurous sulphate reference electured. Each ml of 0.02 M mercuric nitrate is equivalent to 0.002338 g of NaCl.

Nitric Acid, Dilute: Contains approximately 10 % w/w of HNO_3. Dilute 106 ml of nitric acid to 1000 ml with water.

Report : The given sample contains _____ mg of benzylpenicillin.

Experiment No.23

Assay of Dapsone Tablets

Aim: To estimate the amount of dapsone present in the given sample.

Requirements: $NaNO_2$, Hydrochloric acid, Beaker, Volumetric flask, Distilled water, Analytical balance, Weight box, Burette, and Conical flask.

Principle: Assay of dapsone involes diazotizatin titration. Under controlled experimental conditions the reaction is quantitative and can be used for the determination of most of the drugs containing a free primary amino group. Primary aromatic amines react with sodium nitrite in acid solution to form diazonium salts. The end point in this titration method is located virtually by using starch iodide paste as indicator. A small amount of iodide included in the titration mixture is converted to iodine by excess of nitrous acid, this is detected using starch indicator. Titration with nitrous acid is used in pharmacopoeial assays of the following: Benzocaine, procaine, dapsone, primaquine, suphacetamide, sulphadoxine, sulphamethizole.

Procedure: Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 0.25 g of dapsone and dissolve in a mixture of 15 ml of water and 15 ml of 2 M hydrochloric acid. Cool the solution to about 15º and carry out the nitrite titration, Perform a blank determination and make any necessary correction.

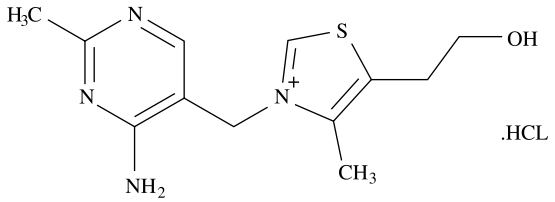
Equivalent factor: Each ml of 0.1 M sodium nitrite is equivalent to 0.01242 g of $C_{12}H_{12}N_2O_2S$.

Report : The given sample contains _____ mg of dapsone.

Experiment No.24

Assay of Thiamine Hydrochloride (Vitamin B1)

Aim: To estimate the amount of thiamine present in the sample.
Requirements: formic acid, glacial acetic acid, Measuring cylinder, Volumetric flask, Distilled water, Analytical balance, Weight box, Burette, and Conical flask.



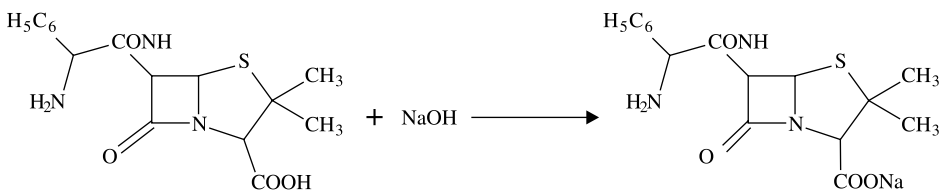
Thiamine hydrochloride

Procedure: Weigh accurately about 0.15 g, dissolve in 5 ml of anhydrous formic acid, add 65 ml of anhydrous glacial acetic acid and 10 ml of mercuric acetate solution, with stirring.Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically. Carry out a blank titration.
Equivalent Factor: 1 ml of 0.1 M perchloric acid is equivalent to 0.01686 g of $C_{12}H_{17}ClN_4OS$, HCl.
Report: The given sample of contains _____ mg of thiamine hydro chloride.

Experiment No.25

Assay of Ampicillin

Aim: To find out % purity of Ampicillin present in the given sample.
Apparatus: Burette, conical flask, pipette and electronic weighing machine.
Chemicals required: Ampicillin capsules, 0.1 N oxalic acid, 0.1 N Sodium hydroxide and phenolphthalein indicator.
Principle: Ampicillin is official in I.P .It is semi synthetic penicillin, which is used as a broad-spectrum antibiotic. The principle involved in assay is acidimetric titration. Ampicillin is stable to acids & being acidic in nature it can be assayed directly by titrating with base. It is a monobasic acid, which consists of one carboxylic acid, and therefore it consumes 1mole of NaOH solution. Thus it can be estimated by no.of moles of NaOH consumed in the titration. The titration is done with NaOH after dissolving the sample in the free water dissolving the sample in free water (freshly boiled and cooled).



Procedure

- Weigh the powder content present in 20 capsules.
- Weigh accurately quantity of powder equivalent to 0.5 g of Ampicillin.

- Transfer the powder into a conical flask containing 25 ml of freshly boiled and cooled distilled water.
- Keep this solution aside for 10 min and then add phenolphthalein indicator.
- Titrate the solution with 0.1 N NaOH until permanent pink color is obtained.

Equivalent Factor: 1 ml of 0.1 N NaOH ≡ 0.03495 gm of Ampicillin

Report: The amount of ampicillin present in the given sample was found to be — and % purity was found to be_____

Experiment No.26

Estimation of Alkaloid (by Gravimetry)

Aim: To estimate the amount of caffeine citrate(M.Wt = 386.3) present in the sample by gravimetry.
Requirements: NaoH, Chloroform, Beaker, Measuring cylinder, Volumetric flask, Distilled water, Analytical balance, Weight box, Burette, and Conical flask.
Principle: It is a gravimetric estimation. Whereby caffeine is extracted out from the solution of caffeine citrate. Sodium hydroxide solution is added to separate citric acid from caffeine citrate. Then caffeine is extracted out from aq solution using chloroform since caffeine is more soluble in chloroform.

1 mole of caffeine citrate equivalent to 1 mole of caffeine

386.3 g equivalent to 194.2 g caffeine

1.989 g equivalent to 1 g of caffeine

Procedure: Weigh accurately about 1.0 g, and dissolve in 50 ml of water. Add 10 ml of 1 M sodium hydroxide and extract with successive portions, each of 25 ml of chloroform until extraction is complete. Wash the combined extracts with 2 ml of water. Evaporate the chloroform and dry the residue of anhydrous caffeine at 80°C for four hrs, cool and weigh.

Gravimetric factor =caffeine (194.2)/caffeine citrate(386.2) =0.5027

Wt of ppt = X

$$\textbf{Percentage of desired constituent} = \frac{\textbf{Wt of ppt(X)} \times \textbf{Gravimetric factor} \times 100}{\textbf{Wt of sample}}$$

Report: The percentage purity of caffeine citrate was found to be_____

Experiment No.27

Estimation of Phosphoric Acid

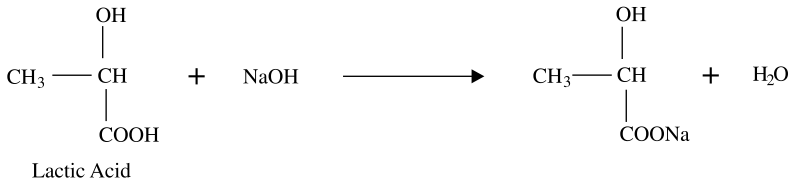
Aim: to estimate the amount of phosphoric acid present in the sample.
Requirements: NaCl, NaOH, oxalic acid, measuring cylinder, volumetric flask, distilled water, analytical balance, weight box, burette, and conical flask.
Principle: It is a simple neutralization titration. When a weak acid is titrated with a strong base the salt produced in the reaction is completely hydrolysed and the pH of the resultant solution at the end point is alkaline. phenolphthalein is used as indicator. In this titration NaOH is standardized by using oxalic acid as the primary standard.
Procedure: Weigh accurately about 1.0 g, add a solution of 10 g of sodium chloride in 30 ml of water and titrate with 1 M sodium hydroxide using dilute phenolphthalein solution as indicator.
Equivalent factor: 1 ml of 1 M sodium hydroxide is equivalent to 0.04900 g of H_3PO_4.

Standardization Of NaOH: Take 10 ml of 0.1 N oxalic acid solution into a conical flask and add 2–3 drops of phenolphthalein indicator. Titrate the contents of the flask against NaOH solution until a permanent pink color is obtained. Repeat the titration to get similar values.
Report: The given sample contains _____mg of phosphoric acid. Phosphoric acid contains not less than 84.0 per cent w/w and not more than 90.0 per cent w/w of H_3PO_4.

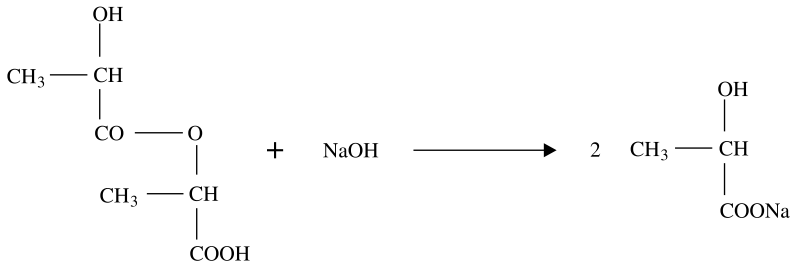
Experiment No.28

Estimation of lactic acid

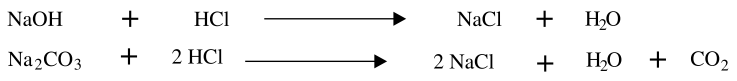
Aim: To estimate the amount of lactic acid present in the given sample.
Apparatus required: Burette, Conical flask, Pipette
Chemicals required: HCl, Lactic acid, Na_2CO_3, NaOH, Phenolphthalein, Methyl orange indicator.
Principle: Lactic acid consists of a mixture of lactic acid, lactoyl lactic acid and various other condensed polymers derived from lactic acid and its condensation products. Hydrolysis of lactoyl lactic acid and the condensation polymers with excess NaOH produces lactic acid, which together with the free lactic acid originally present in the sample is neutralized by NaOH solution. The excess of NaOH is back titrated with standard HCl solution.



Lactic Acid



Lactanoyl Lactate

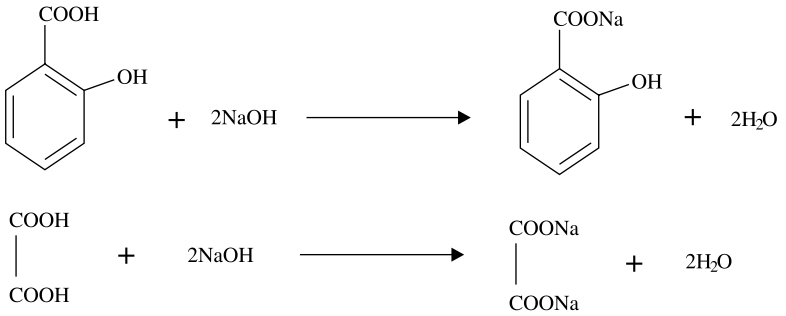


Procedure: Weigh accurately about 1 gm of lactic acid into an iodine flask. Add 10 ml of H_2O to the mixture then to it add 20 ml of 1 M NaOH solution, stoppered the flask. Shake well and allow it to stand for 30 min. Titrate the excess of NaOH with 1 M HCl using phenolphthalein as an indicator until pink color is discharged.
Equivalent factor: Each ml of 1 M NaOH is equivalent to 0.09008 gms of lactic acid.
Standardisation of HCl: Pipette out 10 ml of standard solution of Na_2CO_3 into a conical flask. Add 50 ml of water and 2–3 drops of methyl orange indicator. Titrate the solution with 1 M HCl. Repeat the titration till concurrent values are observed.
Report: The amount of lactic acid in the given sample is found to be_____ gms

Experiment No.29

Estimation of Salicylic Acid

Aim: To estimate the amount of salicylic acid present in the sample.
Requirements: NaOH, Oxalic acid, Measuring cylinder, Volumetric flask, Distilled water, Analytical balance, Weight box, Burette, and Conical flask.
Reference: I.P 1996
Principle: It is a simple neutralization titration. When a weak acid is titrated with a strong base the salt produced in the reaction is completely hydrolysed and the pH of the resultant solution at the end point is alkaline. Phenol red is used as indicator. In this titration NaOH is standardized using oxalic acid as the primary standard.

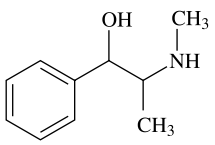


Procedure: Weigh accurately about 0.3 gms of salicylic acid and dissolving 50 ml of ethanol. Add 20 ml of water and titrate with 0.1 M NaOH using phenol red solution as an indicator until reddish violet color is obtained.
Equivalent factor: 0.1 M NaOH is equivalent to 0.01381 gms of Salicylic acid
Standardization Of NaOH: Take 10 ml of 0.1 N oxalic acid solution into a conical flask and add 2–3 drops of phenolphthalein indicator. Titrate the contents of the flask against NaOH solution until a permanent pink color is obtained. Repeat the titration to get similar values.
Report: The given sample of salicylic acid contains _____mg

Experiment No.30

Estimation of Ephedrine by Degradation Method

Aim: To estimate the amount of ephedrine present in the sample.
Requirements: Ephedrine, Ammonia, sulphuric acid, Measuring cylinder, Volumetric flask, Distilled water, Analytical balance, Weight box, Burette, and Conical flask.
Reference: I.P 1996
Principle: Ephedrine is an alkaloid which is a strong base and soluble in aq solution. Ephedrine has two asymmetric carbon atoms thus there are four optically active isomers.The erythro racemate is called ephedrine.It is degraded by acids like sulphuric acid, perchloric acid,hydrochloric acid to different acid products. It is degraded to benzoic acid by sulphuric acid.



Ephedrine

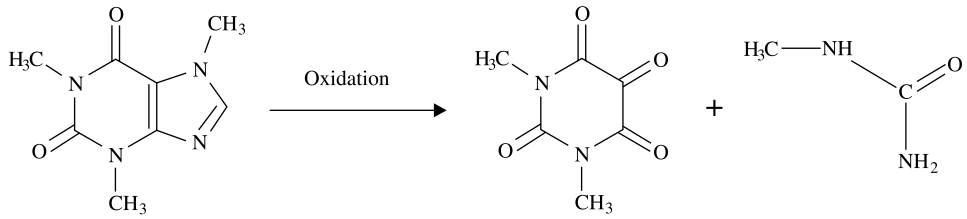
Procedure: Weigh accurately a quantity of powdered material equivalent to 100 mg of ephedrine. Suspend in water and basify with ammonia solution. Extract with four 25 ml portions of ether. Evaporate ether at low temperature and take the residue in 10 ml of 0.1 N sulphuric acid. Excess acid is titrated with 0.1 N sodium hydroxide using methyl red solution as indicator. Alternatively wash ether layer

with water till neutral. Add exactly 50 ml of 0.02 N sulphuric acid and evaporate ether layer completely. Cool, add 2–3 drops of methyl red solution as indicator and titrate with 0.02 N sodium hydroxide.
Equivalent Factor: Each ml of 0.02 N sulphuric acid is equivalent to 0.004033 g of ephedrine.
Report: The given sample of ephedrine contains_____mg

Experiment No.31

Estimation of Caffeine

Aim: To estimate the amount of caffeine present in the sample.
Requirements: NaOH, HCl,$HClO_4$, Measuring cylinder, Volumetric flask, Distilled water, Analytical balance, Weight box, Burette, and Conical flask.
Reference: I.P 1996
Principle: Caffeine is 3, 7-dihydro-1, 3, 7-trimethyl-1*H*-purine-2, 6-dione or its monohydrate. Caffeine is a weak base with bitter taste and forms salts with strong acids. Sodium hydroxide solution is added to separate citric acid from caffeine citrate. Then caffeine is extracted out from aq solution using chloroform since caffeine is more soluble in chloroform. On oxidation with perchloric acid or potassium chlorate in hydrochloric acid, it undergoes degradation to dimethyl alloxan and methyl urea.



Caffeine | Dimethyl alloxan | Methylurea

Procedure: Transfer accurately weighed quantity of powdered material equivalent to 100 mg of caffeine to a separator having 15 ml of Water. Add about 10 ml of 1 N HCl, shake well and extract with five 30 ml portions of chloroform. Combine the chloroform layers, add about 20 ml of 0.1 N NaOH and 15 ml of Water to the combined chloroform layers. Shake well and reject aq layer. Then chloroform layer is washed twise with 20 ml of water. Reject aq layer and filter chloroform layer through cotton plug into a dry 500 ml conical flask. Evaporayte chloroform to dryness. Dissolve the residue in 2 ml chloroform, add 10 ml of benzene and 10 ml of acetic anhydride. Then titrate with 0.1 N perchloric acid using 0.2 ml of solution of neutral red (0.2% w/v solution o in acetic acid). Carry out the blank determination and make necessary correction.
Equivalent Factor: Each ml of 0.1 N perchloric acid is equivalent to 0.01942 g of caffeine
Report: The given sample of contains _____ mg of caffeine.

Experiment No.32

Determination of Eugenol in Clove Oil

Aim: To determine the phenolic content in clove oil.
Requirements: Graduated volumetric flask, Clove oil, Potassium Hydroxide.
Principle: Clove oil is the oil distilled from the dried flower buds of Syzygium *aromaticum*. It contains NLT 85% w/w and not more than 95% w/w of phenolic substances chiefly Eugenol.

Eugenol content is determined by treating the oil with a solution of KOH and measuring the material that is not dissolved. The phenolic eugenol dissolves in KOH and measuring the material that is not dissolved. The phenolic material (Terpenes) forms a separate layer on the surface of the liquid. The

volume of undissolved oil should be between 1–1.5 ml. Before use the flask should be cleaned with H_2SO_4 and well rinsed with water to free the flask from any greasy material.

Procedure: Place 70 ml of 5% aqueous potassium hydroxide solution into 100 ml volumetric flask. Pipette 10 ml of the sample into 100 ml volumetric flask. Pipette 10 ml of the sample into the KOH solution. Shake thoroughly at 5 min intervals during ½ hr. Gradually add more aqueous KOH so that the undissolved oil is slowly raised into the graduated neck of the flask.

Allow to stand for not les than 24 hrs. Record the volume of undissolved oil.

Limit: The volume of undissolved oil should be between 1 and 1.5 ml.

Category: Eugenol is used as local analgesic used in dentistry and as pharmaceutical aid (flavouring agent).

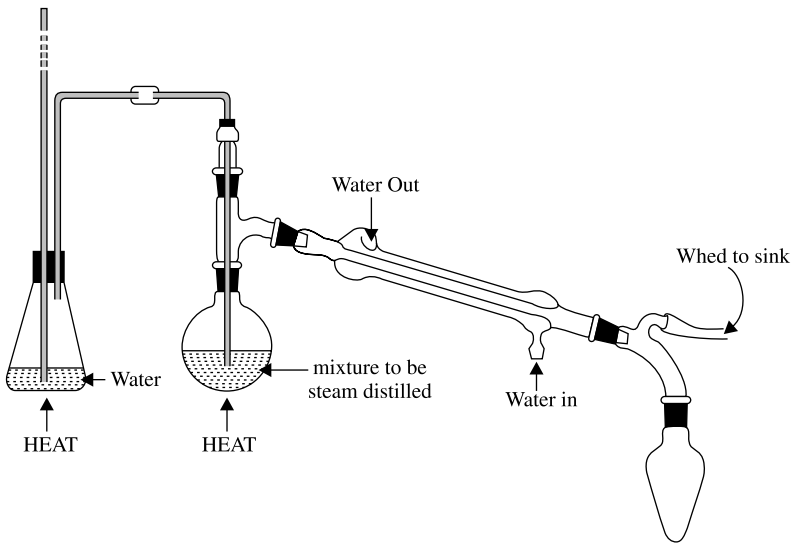
Report: The undissolved non-phenolic matter was observed to be 1.2 ml. therefore the eugenol content was found to be within the limits.

Experiment No.33

Volatile Oil Production by Steam Distillation

Aim: Volatile production by using the technique of steam distillation.

Apparatus:



The steam is generated from bolt headed flask ‘a’, which is provided with a long safety tube dipping well below the surface of water & passes into the bolt head flask ‘b’, by the wide tube. Bend as shown in the fig.

The short glass tube in ‘a’ terminating in a screw clip is optional; it prevents the contents of ‘b’ sucking back into the generator when the steam distillation is stopped. Other wise the rubber tube connecting ‘a’ & ‘b’ must be detached immediately & flame beneath ‘a’ is removed.

Caution: steam rushes out.

The steam distillation is maintained at an angle so as to prevent the solution in ‘b’ from being splashed into the entrance of outlet tube & this being blown over mechanically into the condenser; distillate is collected in flask ‘a’. E is a glass adapter, which facilitates the collection of the distillate, but may be omitted if desired. The flask ‘a’ is heated on asbestos centered wire gauge supported on a tripod

as is also the flask 'b'. If the lab posses an external steam supply, the trap replaces the steam generator 'a'. It will remove the foreign matter & also the water present in steam.

The screw clip is opened from time to time to allow the accumulated water.

Theory: Steam distillation is a means of separating & purifying organic compounds. Essentially, the operation consists in volatizing a substance, which is insoluble or sparingly soluble in water. Provided the organic compound as an appreciable vapour pressure. It will distill with the steam. It cam be readily separated from the distillate since it is immiscible with water. Steam distillation takes place at a temp below boiling point of organic substances. This renders possible purification of many substances of high boiling point of low temp distillation & is particularly valuable, when the substances undergo decomposition when distillation allowed at atmospheric pressure. It is also of importance in the separation of desired organic compound.

Report : Volatile oil was obtained by using steam distillation and submitted.

4

MONOGRAPH ANALYSIS OF THE FOLLOWING COMPOUNDS

Introduction to Monograph

The requirements stated in the monographs of the Pharmacopoeia apply to articles that are intended for medicinal use but not necessarily to articles that may be sold under the same name for other purposes. A monograph is to be construed in accordance with any general monograph or notice or any appendix, note or other explanatory material that is contained in this edition and that is applicable to that monograph. All statements in the monographs given under the heading 'STANDARDS' constitute standards except where a specific general notice indicates otherwise. An article is not of pharmacopoeial quality unless it complies with all of the requirements stated. The requirements given in the monographs are not framed to provide against all possible impurities, contaminants or adulterants; they provide appropriate limitation of potential impurities only. Material found to contain an impurity, contaminant or adulterant not detectable by means of the prescribed tests is not of pharmacopoeial quality if the nature or amount of such substances found is objectionable under conditions in which the article is customarily employed or is incompatible with good pharmaceutical practice.

Added Substances

An official substance, as distinguished from an official preparation, contains no added substances except when specifically permitted in the individual monograph. Unless otherwise specified in the individual monograph, or elsewhere in the General Notices, suitable substances may be added to an official preparation or finished device to enhance its stability, usefulness or elegance, or to facilitate its preparation. Such auxiliary substances shall be harmless in the amounts used, shall not exceed the minimum quantity required to provide their intended effect, shall not impair the therapeutic efficacy or the bioavailability or safety of the preparation and shall not interfere with the tests and assays prescribed for determining compliance with the official standards. Particular care should be taken to ensure that such substances are free from harmful organisms. Thus, freedom and responsibility for an official preparation have been conferred on the manufacturer who will need to satisfy the appropriate authority on the purpose of the added substance(s) and innocuousness of such added substance(s). The addition of colouring and flavouring agents, unless permitted in the individual monograph or in the general mono-

graphs, is not official. Where the addition of colouring is permitted, any colouring agent used shall be one that is included in the list of colours prescribed under the Drugs and Cosmetics Rules, 1945.

Ingredients and Processes

Official preparations are prepared from ingredients that comply with the requirements of the compendial monographs for those individual ingredients for which monographs are provided. Where water is used as an ingredient it meets the requirements for Purified Water, for Water for Injection, or for one of the sterile forms of water covered by a monograph in this Pharmacopoeia. A dosage form shall be formulated with the intent to provide 100% of the quantity of each ingredient stated on the label. Where the content of an ingredient is known to decrease with time, an amount in excess of that stated on the label may be added at the time of manufacture to assure compliance with the content requirements of the monograph throughout the life period of the product. The limits stated in the monographs allow for such overages, for analytical errors, for unavoidable variations in manufacturing and for deterioration to an extent considered insignificant under practical conditions. The specified tolerances are based on the assumption that good manufacturing practices have been followed in the production of an article from suitable raw materials.

Monographs for certain official preparations include a full formula together with, in some cases, directions for their preparation. Such full formulae and directions are intended for preparation of small quantities for short-term supply and use. When so prepared, no deviation from the stated formula and directions is permitted. If, however, such a preparation is manufactured on a larger scale with the intention that it may be stored and distributed, deviations from the formula and directions stated in the monograph are permitted provided that the ingredients used comply with the compendial requirements and with the general notice on **Added Substances** and that the final product meets the following criteria:

a. compliance with all of the requirements stated in the monograph, and
b. retention of the essential characteristics of the preparation made strictly according to the forula and directions of the Pharmacopoeia.

If a preparation is intended to be stored over a period of time, deterioration due to microbial contamination may be inhibited by the addition to the formula of a suitable antimicrobial preservative. In such circumstances the label states the concentration of the antimicrobial preservative and the appropriate storage conditions. It is implied that such a preparation will be effectively preserved according to the appropriate criteria applied and interpreted as described in the test for effectiveness of antimicrobial preservatives in a pharmaceutical preparation.

The direction that a preparation must be freshly prepared indicates that it must be made not more than 24 hours before it is used.

Monographs

The format within a given monograph is such that after the official title, the primarily informational portions of the text appear first, followed by the text comprising requirements, which are given under the heading ‘STANDARDS.’

Titles

The main titles of monographs in the Pharmacopoeia are given in bold capitals. The International Nonproprietary Names (INN) approved by the World Health Organization have been preferred for the main title of a new drug substance over other names that may be in use for it. For example, Ormeloxifene Hydrochloride is the main title of the monograph of the drug substance popularly known as Centchroman Hydrochloride or Centchroman. Subsidiary or abbreviated titles or synonyms, where included, have the same significance as the main titles.

The main titles of formulated preparations are drawn from the full non-proprietary name of the active ingredient or ingredients while the synonym represents any other name that may also be commonly recognised in practice. For example, tablets containing ranitidine hydrochloride are official under the main title of 'Ranitidine Hydrochloride Tablets' whereas 'Ranitidine Tablets' is recognised as a synonym.

Capital Letters in the Text

The names of the Pharmacopoeial substances, preparations and other materials in the text are printed with capital initial letters and these infer that materials of Pharmacopoeial quality must be used.

Italics

Italic types have been used for the systematic names of plants and micro-organisms, and for some sub-headings and some parts of the chemical names. Italic types have also been used for words which refer to reagents, substances, processes or a physical characteristic that is defined or described in an appendix. For example, methanol, absorbance, mercuric sulphate solution, thin-layer chromatography, which imply compliance with the requirements specified in the appropriate appendix.

Chemical Formulae

When the chemical structure of an official substance is known or generally accepted, the graphic and molecular formulae and the molecular weight are given at the beginning of the monograph. This refers to the chemically pure substances and is not to be regarded as an indication of the purity of the drug. From the statements of standards of purity and strength and in descriptions of processes of assay, it will be evident from the context that the formulae denote the chemically pure substances.

Where the absolute stereochemical configuration is specified, the International Union of Pure and Applied Chemistry (IUPAC) R/S and E/Z systems of designation have been used. If the substance is an enantiomer of unknown absolute stereochemistry, the sign of the optical rotation, as determined in the solvent and under the conditions specified in the monograph, has been attached to the systematic name. An indication of sign of rotation and/or configuration has also been given where this is incorporated in a trivial name that appears on an IUPAC preferred list.

Chemical Names

Chemical names have been provided in the monographs which have titles which specify substances that are distinctly definable chemical entities. These are names sanctioned and employed by the IUPAC.

Atomic Weights

The atomic weights adopted are the values given in the Table of Relative Atomic Weights 1989 published by the IUPAC. The values are based on the carbon-12 scale (Appendix 15.1).

Category

The statement of category is provided for information and is indicative of the medical or pharmaceutical basis for recognition in the Pharmacopoeia. It generally represents an application of the best known pharmacological action of the article or of its active ingredient. In the case of pharmaceutical aids it may indicate the more common usage of the article. The statement is not intended to limit in any way the choice or use of the article nor to indicate that it has no other activity or use.

Dose

Doses mentioned in the Pharmacopoeia are intended merely for general guidance and represent, unless otherwise stated, the average range of quantities which are generally regarded as suitable for adults when administered by mouth. They are not to be regarded as binding upon the prescribers. The medical practitioner will exercise his own judgment and act on his own responsibility in respect of the amount

of any therapeutic agent he may prescribe or administer or the frequency of its administration. If it is usual to administer a drug by a method other than by mouth, the single dose suitable for that method of administration is mentioned. In the case of some preparations notes have been given below the statement of doses to show the approximate quantities of active ingredients contained in the maximal doses as information for the prescriber.

Usual Strength

The statement on the usual strength(s) of a preparation given in the individual monograph indicates the strength(s) usually marketed for information of the pharmacist and the medical practitioner. It does not imply that a strength other than the one(s) mentioned in the individual monograph meeting all the prescribed requirements cannot be manufactured and marketed with the approval of the appropriate authority.

Description

Statements given under this heading are not to be interpreted in a strict sense and are not to be regarded as analytical requirements, although they may help in the preliminary evaluation of the integrity of an article. Where, however, description is included under the heading 'STANDARDS', the article shall comply with this requirement.

Where substance is described as 'odourless', the following method of examination applies: Examine a sample of not more than 25 g immediately after opening the package. If any odour is noticeable, transfer the sample rapidly to an open container and re-examine after 15 minutes. If the odour is still discernible, the sample does not comply with the description 'odourless'.

Statements on taste are provided only in cases where this property is a guide to the acceptability of the material (for example, a substance used primarily for flavouring).

Solubility

Statements on solubility given under the heading 'Solubility' are not standards or tests for purity but are provided primarily as information. Where, however, a quantitative solubility test is given under 'STANDARDS', the substance shall comply with this requirement.

Statements of solubilities are indicated by a descriptive phrase and are intended to apply at 20–30º. The following table indicates the meanings of the terms used in statements of approximate solubilities.

Descriptive term	Approximate volume of solvent in millilitres per gram of solute
very soluble	*less than 1*
freely soluble	*from 1 to 10*
soluble	*from 10 to 30*
sparingly soluble	*from 30 to 100*
slightly soluble	*from 100 to 1000*
very slightly soluble	*from 1000 to 10,000*
insoluble or practically insoluble	*more than 10,000*

The term 'partly soluble' is used to describe a mixture of which only some of the components dissolve.

Storage

Statements under the heading 'Storage' constitute non-mandatory advice. The substances and preparations of the Pharmacopoeia are to be stored under conditions that prevent contamination and, as far as

possible, deterioration. Precautions that should be taken in relation to the effects of the atmosphere, moisture, heat and light are indicated, where appropriate, in the individual monographs.

Specific directions are given in some monographs with respect to the temperatures at which Pharmacopoeial articles should be stored, where it is considered that storage at a lower or higher temperature may produce undesirable results. The conditions are defined by the following terms.

Cold-Any temperature not exceeding 8º and usually between 2º and 8º. A refrigerator is a cold place in which the temperature is maintained thermostatically between 2º and 8º.

Cool-Any temperature between 8º and 25º. An article for which storage in a cool place is directed may, alternately, be stored in a refrigerator, unless otherwise specified in the individual monograph.

Room temperature-The temperature prevailing in a working area.

Warm-Any temperature between 30º and 40º.

Excessive heat-Any temperature above 40º.

Protection from freezing-Where, in addition to the risk of breaking of the container, freezing results in loss of strength or potency or in destructive alteration of the characteristics of an article the label on the container bears an appropriate instruction to protect from freezing.

Storage under non-specific conditions-Where no specific storage directions or limitations are given in the individual monograph, it is to be understood that the storage conditions include protection from moisture, freezing and excessive heat.

Containers

The container is the device that holds the article. The immediate container is that which is in direct contact with the article at all times. The closure is a part of the container.

The requirements, guidance and information on containers for pharmaceutical use are given in Appendices 11.1 and 11.3. Further requirements and guidance are provided in certain general and individual monographs for dosage forms in the Pharmacopoeia. These are for general application to containers of the stated category. In view of the wide variety of containers available and possible new developments, the publication of a specification does not exclude the use of other containers provided their use is justified and agreed to by the appropriate authority.

The container is designed so that the contents may be taken out for the intended purpose in a convenient manner. It provides the required degree of protection to the contents from the environmental hazards.

The container should not interact physically or chemically with the article placed in it so as to alter the strength, quality or purity of the article beyond the official requirements.

Prior to its being filled, the container should be clean. Special precautions and cleaning procedures may be necessary to ensure that each container is clean and that extraneous matter is not introduced into or onto the article.

Light-resistant Container-A light-resistant container protects the contents from the effects of actinic light by virtue of the specific properties of the material of which it is made. Alternatively, a clear and colourless or a translucent container may be made light-resistant by means of an opaque (light-resistant) covering and/or stored in a dark place; in such cases, the label on the container should bear a statement that the opaque covering or storage in dark place is needed until the contents have been used up.

Well-closed Container-A well-closed container protects the contents from extraneous solids and liquids and from loss of the article under normal conditions of handling, shipment, storage and distribution.

Tightly-closed Container-A tightly-closed container protects the contents from contamination by extraneous liquids, solids or vapours, from loss or deterioration of the article from effervescence, deliquescence or evaporation under normal conditions of handling, shipment, storage and distribution. A tightly-closed container must be capable of being tightly reclosed after use. Where a tightly-closed con-

tainer is specified, a hermetically sealed container may be used for a single dose of an article. A gas cylinder may be considered to be a metallic, tightly-closed container designed to hold gas under pressure.

Hermetically Sealed Container-A hermetically sealed container is impervious to air or any other gas under normal conditions of handling, shipment, storage and distribution. It may be closed by fusion of the material of the container as in ampoules or may be sealed by appropriate means other than by fusion of the material as in a 'sealed container' used for a powder for injection in an individual monograph of the Pharmacopoeia.

Single Unit Container-A single unit container is one that is designed to hold a quantity of the drug product intended for administration as a single dose or a single finished device intended for use promptly after the container is opened. The immediate container and/or outer container or protective packaging is so designed as to show evidence of any tampering with the contents.

Single Dose Container-A single dose container is intended for articles for parenteral administration and is designed to hold a quantity of the drug equivalent to a single dose.

Unit Dose Container-A unit dose container is a single unit container for articles intended for administration by other than parenteral route as a single dose, direct from the container.

Multiple Unit Container-A multiple unit container is a container that permits withdrawal of successive portions of the contents without changing the strength, quality or purity of the remaining portion.

Multiple Dose Container - A multiple dose container is a multiple unit container for articles intended for parenteral administration only.

Tamper-evident Container-A tamper-evident container is fitted with a device or mechanism that reveals irreversibly whether the container has been opened.

Labelling

In general, the labelling of drugs and pharmaceuticals is governed by the Rules made under the Drugs and Cosmetics Act, 1940. The statements that are given in the monographs under the heading 'Labelling' are included as recommendations. Practical considerations where the individual container is too small, as in certain vaccines, to accommodate the information stated under 'Labelling' may demand alternative means of making the required information available to the user. Such matters as the exact form of wording to be used and whether a particular information should appear in the label on the immediate container or the primary label as well as, or alternatively, on the package/leaflet/insert are, in general, outside the scope of the Pharmacopoeia.

Expression of Standards

Where the standard for the content of a substance in a monograph is expressed in terms of the chemical formula for that substance an upper limit exceeding 100% may be stated. Such an upper limit applies to the result of the assay calculated in terms of the equivalent content of the specified chemical formula. For example, the statement 'contains not less than 99.0 per cent and not more than 101.0 per cent of $C_7H_6O_2$' implies that the result of the assay is not less than 99.0% and not more than 101.0%, calculated in terms of the equivalent content of $C_7H_6O_2$. When the result of an assay or test is required to be calculated with reference to the dried, anhydrous or ignited substance, to the substance free from a specified solvent or to the peptide content, the determination of loss on drying, water content, loss on ignition, content of the specified solvent or peptide content is carried out by the method prescribed in the relevant test in the monograph.

Expression of Strengths

The term 'per cent' or more usually the symbol '%' is used with one of four different meanings in the expression of concentrations according to circumstances. In order that the meaning to be attached to the expression in each instance is clear, the following notation is used.

Per cent w/w (% w/w) (percentage weight in weight) expresses the number of grams of solute in 100 g of product.

Per cent w/v (% w/v) (percentage weight in volume) expresses the number of grams of solute in 100 ml of product.

Per cent v/v (% v/v) (percentage volume in volume) expresses the number of millilitres of solute in 100 ml of product.

Per cent v/w (% v/w) (percentage volume in weight) expresses the number of millilitres of solute in 100 g of product.

Usually, the strength of solutions of solids in liquids is expressed as percentage weight in volume, of liquids in liquids as percentage volume in volume and of gases in liquids as percentage weight in weight.

When the concentration of a solution is expressed as parts per million (ppm), it means weight in weight, unless otherwise specified.

When the concentration of a solution is expressed as parts of dissolved substance in parts of solution, it means parts by weight (g) of a solid in parts by volume (ml) of the final solution; or parts by volume (ml) of a liquid in parts by volume (ml) of the final solution; or parts by weight (g) of a gas in parts by weight (g) of the final solution.

When the concentration of a solution is expressed in molarity designated by the symbol M preceded by a number, it denotes the number of moles of the stated solute contained in sufficient Purified Water (unless otherwise stated) to produce 1 litre of solution.

Limits, Significant Figures and Tolerances

When limits of content are given in a monograph they are determined by the method prescribed therein.

When limits are expressed numerically, the upper and lower limits of a range are inclusive so that the range consists of the two values themselves and all intermediate values, but no values outside the limits. The limits expressed in monograph standards and tests, regardless of whether the values are expressed as percentages or as absolute numbers, are considered significant to the last digit shown.

The limits stated in monographs are based on data obtained in normal analytical practice. No further tolerances are to be applied to the limits prescribed to determine whether the article being examined complies with the requirements of the monograph.

Limit of Impurities

In certain tests, the concentration of impurity is given in parentheses either in parts per million by weight (ppm) or as a percentage where the limit exceeds 500 ppm. These figures are approximations only; conformity with the requirements is determined on the basis of compliance with the stated test.

Abbreviated Statements in Monographs

Incomplete sentences are employed in parts of the monographs for directness and brevity (for example, Iodine Value: Not more than.....; Wt. per ml: Between .....and.....). Where the tests are so abbreviated, it is to be understood that the appendix referred to provides the method to be followed and that the values specified are the required limits.

Other Requirements

In the monographs on dosage forms, under the sub-heading ‘Other requirements’, it is stated that the article complies with the requirements of tests stated under the general monograph on the relevant preparation (dosage form). Details of such tests are provided in the general monographs under the heading ‘STANDARDS’.

General Monographs

These are monographs which describe official preparations and include requirements of general application and requirements of tests which apply to all the monographs for the relevant dosage forms, unless otherwise specified in the individual monograph.

Percentage of Ethanol

All statements of percentages of ethanol, such as under the sub-heading 'Ethanol Content', refer to percentage, by volume, of C_2H_5OH at 15.56º. Where reference is made to "C_2H_5OH", the chemical entity (C_2H_5OH) possessing absolute (100%) strength is intended.

Assays and Tests

Apparatus: A specification for a definite size or type of container or apparatus in a test or assay is given merely as a recommendation. Where volumetric flasks or other exact measuring or weighing devices are specified, this or other equipment of at least equivalent accuracy may be employed.

In order to obtain solutions having concentrations that are adaptable to the working range of the instrument being used, solutions of proportionally higher or lower concentrations may be prepared, according to the solvents and proportions thereof that are specified in the procedure.

Water-bath: The term 'water-bath' means a bath of vigorously boiling water unless water at some other temperature is indicated.

Desiccator: The term 'desiccator' means a tightly-closed container of suitable size and design that maintains an atmosphere of low moisture content by means of silica gel or phosphorus pentoxide or other suitable desiccant.

Reagents and Solutions

The reagents required for the assays and tests of the Pharmacopoeia are defined in appendices showing their nature, degree of purity and the strengths of the solutions to be made from them. The requirements set out in these appendices are not intended to imply that the materials are suitable for use in medicine; reagents not covered by monographs in the Pharmacopoeia shall not be claimed to be of IP quality.

For convenience, reagents specially required for certain tests may be listed in the concerned appendix. For example, the support materials used in chromatographic analysis are described in the appendices relating to gas chromatography and high performance liquid chromatography.

The term 'analytical reagent grade of commerce' implies that the chemical is of a high degree of purity wherein the limits of various impurities are known. Where it is directed to use a 'general laboratory reagent grade of commerce' it is intended that a chemically pure grade material, not necessarily required to be tested for limiting or absence of certain impurities, is to be used.

Unless otherwise specified in the individual monograph, all solutions called for in assays and tests are prepared with Purified Water.

Reference Substances and Standard Preparations

Reference Substances and Standard Preparations of antibiotics and other substances are authentic specimens that have been verified for suitability for use as comparison standards in some assays and tests of the Pharmacopoeia. The words 'IP Reference Substance' are abbreviated to 'RS' wherever mentioned in the monographs or in the appendices.

All IP Reference Substances and Standard Preparations are issued under the direction of the Ministry of Health & Family Welfare, Government of India. They are official reference substances to be used in case of doubt or dispute. Laboratory working standards may be prepared for routine analysis provided they are standardised at regular intervals with reference to those issued by the Ministry of Health & Family Welfare.

Solvents

Where the name of the solvent is not stated, the term 'solution' implies a solution in water and the water used complies with the requirements of the monograph on Purified Water. The term 'distilled water' indicates Purified Water prepared by distillation.

Procedures

The assays and tests provided for determining compliance with Pharmacopoeial standards of identity, strength, quality and purity are the official methods on which the standards of the pharmacopoeia depend. These are sufficiently robust to permit replication by analysis in a wide range of laboratories. However, the analyst is not precluded from employing alternative methods including automated procedures and methods of micro-analysis if it is known that the method used will give a result of equivalent accuracy. Automated procedures utilising the same basic chemistry as the test procedures given in a monograph may also be used to determine compliance. Such alternative or automated procedures must be validated. In the event of doubt or dispute, the methods of analysis, the reference materials and the reference spectra of the Pharmacopoeia are alone authoritative and only the result obtained by the procedure given in this Pharmacopoeia is conclusive.

Unless otherwise prescribed, the assays and tests are carried out at a temperature between 20º and 30º.

Where it is directed that an analytical operation is to be carried out 'in subdued light', precautions should be taken to avoid exposure to direct sunlight or other strong light. Where it is directed that an analytical operation is to be carried out 'protected from light', precautions should be taken to exclude actinic light by using low-actinic glassware, working in a dark room or similar procedures.

In assays the approximate quantity to be taken for examination is indicated but the quantity actually used must not deviate by more than 10% from that stated. Reagents are measured and the procedures are carried out with an accuracy commensurate with the degree of precision implied by the standard stated for the assay. In tests the stated quantity to be taken for examination must be used unless any divergence can be taken into account in conducting the test and calculating the result. The quantity taken is accurately weighed or measured with the degree of precision implied by the standard or, where the standard is not stated numerically (for example, in tests for Clarity and Colour of Solution), with the degree of precision implied by the number of significant figures stated. Reagents are measured and the procedures are carried out with an accuracy commensurate with this degree of precision.

In the performance of assay or test procedures, not less than the specified number of dosage units should be taken for analysis. Proportionately larger or smaller quantities than the specified weights and volumes of assay or test substances and Reference Standards or Standard Preparations may be taken, provided the measurement is made with at least equivalent accuracy and provided that any subsequent steps, such as dilutions, are adjusted accordingly to yield concentrations equivalent to those specified and are made in such manner as to provide at least equivalent accuracy.

Where it is directed in the assay of Tablets to "weigh and powder not less than" a given number, usually 20, of the tablets, it is intended that a counted number of tablets shall be weighed and reduced to a fine powder. Likewise, where it is directed in the assay of Capsules to remove, as completely as possible, the contents of not less than a given number, usually 20, of the capsules, it is intended that a counted number of capsules should be carefully opened and the contents quantitatively removed, combined, mixed, and weighed accurately. The portion of the powdered tablets or the mixed contents of the capsules taken for assay is representative of the whole tablets or capsules respectively, and is, in turn, weighed accurately. The result of the assay is then related to the amount of active ingredient per tablet in the case of tablets and per capsule in the case of capsules from the weight of contents of each capsule.

Expressions such as 25.0 ml, 100.0 ml and 5.0 g, used with respect to volumetric or gravimetric measurements, indicate that the quantity is to be 'accurately measured' or 'accurately weighed' within the limits stated under 'Volumetric Glassware' or under 'Weights and Balances'.

The term 'transfer' is used generally to indicate a quantitative operation.

Blank determination

Where it is directed that 'any necessary correction' be made by a blank determination, the determination is to be done using the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but omitting the substance being examined.

Dilution

Where it is directed that a solution be diluted 'quantitatively and stepwise', an accurately measured quantity is to be diluted by adding water or other solvent, in the proportion indicated, in one or more steps. The relatively larger errors associated with the use of small-volume volumetric apparatus must be borne in mind when carrying out dilutions.

Drying to Constant Weight

The specification 'dried to constant weight' means that the drying shall be continued until two consecutive weighings do not differ by more than 0.50 mg per g of substance taken, the second weighing following an additional hour of drying.

Filtration

Where it is directed to filter, without further qualification, it is intended that the liquid be filtered through suitable filter paper or equivalent device until the filtrate is clear.

Identification Test

The tests described under this sub-heading, however specific, are not necessarily sufficient to establish absolute proof of identity. They provide a means of verifying that the article being examined is in accordance with the label on the container. Failure of an article taken from a labelled container to meet the requirements of a prescribed identification test indicates that the article may be mislabelled or substituted. Other tests and specifications in the monograph often contribute to establishing or confirming the identity of the article being examined.

The identification reactions of ions and groups of substances are brought together in an appendix instead of being frequently repeated in the monographs.

In certain monographs alternative series of identification tests are given; compliance with either one or the other set of tests is adequate to verify the identity of the article. When tests for infra-red absorption are applied to material extracted from formulated preparations, strict concordance with the specified spectrum may not always be possible, but nevertheless a close resemblance between the spectrum of the extracted material and the specified spectrum should be achieved.

Ignition to Constant Weight

The specification 'ignite to constant weight' means that the ignition shall be continued at $800^{\circ} \pm 25^{\circ}$, unless otherwise indicated, until two consecutive weighings do not differ by more than 0.50 mg per g of substance taken, the second weighing following an additional 15-minute ignition period.

Indicators

Where the use of an indicator solution is specified in an assay or test, approximately 0.1 ml of the solution shall be added, unless otherwise directed.

Loss on Drying and Water

Where absorbed water or water of hydration is determined by drying under specified conditions, the test is generally given under the heading 'Loss on drying'. It must, however, be understood that the loss in weight also represents residual volatile constituents including organic solvents as well as water.

Where the determination is done by the titrimetric method, the test is generally given under the heading 'Water'.

Negligible

This term indicates a quantity not exceeding 0.50 mg.

Cautionary Statements

A number of substances described in the monographs and some of the reagents specified for use in the tests and assays may be hazardous to health unless adequate precautions are taken. Attention is drawn to particular hazards in certain monographs and in statements pertaining to certain reagents by means of a italicised statements; the absence of such a statement should not be taken to mean that no hazard exists.

Pressure Measurements

Pressure has been indicated in terms of kiloPascals (kPa).

Temperatures

Unless otherwise specified, all temperatures in this Pharmacopoeia are expressed in degrees Celsius (centigrade) and all measurements are made at 25º.

Time Limit

In the conduct of assays and tests, 5 minutes shall be allowed for the reaction to take place unless otherwise directed.

Weights and Measures

The metric system of weights and measures is employed in the Pharmacopoeia. All measures are required to be graduated at 25º and all measurements involved in the analytical operations of the Pharmacopoeia are intended, unless otherwise stated, to be made at that temperature. Graduated glass apparatus used in analytical operations should comply with the requirements stated in Appendix 1.5.

Calculation of Results

In determining compliance with a numerical limit in an assay or test, the result should be calculated to one decimal place more than the significant figures stated and then rounded up or down as follows: if the last figure calculated is 5–9, the preceding figure is increased by 1; if it is 4 or less, the preceding figure is left unchanged.

Biological and Microbiological Assay and Tests

General considerations like design and statistical analysis of the usually applicable methods of biological assays and tests are dealt with in Appendix 2.1. Appendix 9.1 describes various considerations applying to microbiological assay of antibiotics.

Fiducial limits of error are stated in biological assays. In all cases fiducial limits of error are based on a probability of 95% ($p = 0.95$). For antibiotics for which the monograph specifies a microbiological assay the material is not of pharmacopoeial quality if the upper fiducial limit of error is less than the stated potency. For such antibiotics the required precision of the assay is stated in the monograph in terms of the fiducial limits of error about the estimated potency. For other substances and preparations for which the monograph specifies a biological assay, unless otherwise stated, the precision of the assay is such that the fiducial limits of error expressed as a percentage of the estimated potency are within a range not wider than that obtained by a factor of ten the square roots of the limits given in the monograph for the fiducial limits of error about the stated potency.

The methods of biological assay are provided for two purposes, namely, to ascertain the purity of the material and to determine the total activity of the drug in the container. Monographs may require the assay to be carried out for one or both of these purposes.

When the assay is being used to ascertain the purity of the material, the stated potency means the potency stated on the label in terms of Units or micrograms per gram, Units or micrograms per milligram or Units or micrograms per millilitre. When no such statement appears on the label, the stated potency means the fixed or minimum potency required in the monograph. This interpretation of potency applies in all cases where the monograph specifically directs otherwise.

When the assay is being used to determine the total activity of the drug in the container, the stated potency means the total number of Units stated on the label or if no such statement appears, the total activity calculated in accordance with the instructions in the monograph.

Where Units are referred to in an assay or test, the Unit for a particular substance or preparation is the specific biological activity contained in such an amount of the respective primary standard as the Ministry of Health & Family Welfare, Government of India indicates. The necessary information should normally be available when the primary standard is provided.

Wherever possible the primary standard is the respective International Standard or Reference Preparation and the Unit is that defined by the World Health Organisation for international use (International Unit).

Unless otherwise directed, animals used in an assay or test are healthy animals, drawn from a uniform stock, that have not previously been treated with any material that will interfere with the assay or test.

Vegetable Drugs

The macroscopical characteristics of a vegetable drug include those features that can be seen by the unaided eye or by the use of a hand lens. The diagnostic characteristics given under a powdered vegetable drug are to be read in conjunction with the microscopical characteristics given under the whole drug.

Vegetable drugs are required to be as free as practicable from micro-organisms, insects and other animal contaminants including animal excreta. They shall not show any abnormal discoloration, odour or other evidence of deterioration.

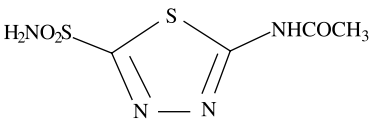
Vegetable drugs may be protected from insect infestation or microbiological contamination by the use of suitable additives or processes that do not leave harmful residue

Experiment No: 1

Acetazolamide

$C_4H_6N_4O_3S_2$, Mol. Wt. 222.24

Acetazolamide is N-(5-sulphamoyl-1, 3, 4-thiadiazol-2-yl)-acetamide.



Category: Carbonic anhydrase inhibitor; used in the treatment of glaucoma.

Dose: Initial dose, 500 mg; subsequent doses, 250 mg every six hours.

Description: White to faintly yellowish-white, crystalline powder; odourless.

Solubility: Slightly soluble in ethanol (95%); very slightly soluble in water; practically insoluble in chloroform and in ether. It is soluble in dilute solutions of alkali hydroxides.

Storage: Store in well-closed containers.

Standards

Acetazolamide contains not less than 98.5 per cent and not more than 101.0 per cent of $C_4H_6N_4O_3S_2$, calculated with reference to the dried substance.

Identification: Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A: The infra-red absorption spectrum, is concordant with the reference spectrum of acetazolamide or with the spectrum obtained from acetazolamide RS. If the spectra are not concordant, dissolve the substance being examined as well as the reference substance, if used, in methanol, evaporate the solutions to dryness and prepare new spectra

B: The light absorption in the range 230 to 260 nm of a 0.003% w/v solution in 0.01M sodium hydroxide exhibits a maximum at about 240 nm; absorbance at about 240 nm, 0.49 to 0.53 (amendment 3). The light absorption in the range 260 to 360 nm of a 0.00075% w/v solution in 0.01 M sodium hydroxide exhibits a maximum at about 292 nm; absorbance at about 292 nm, 0.43 to 0.4.

C: To about 20 mg in a test-tube add 4 ml of 2 M hydrochloric acid and 0.2 g of zinc powder and immediately place a piece of lead acetate paper over the mouth of the tube; the paper exhibits a brownish-black colour.

D: To about 25 mg add 5 ml of water, 4 drops of 1 M sodium hydroxide and 2 drops of cupric sulphate solution; a bluish-green colour or precipitate is produced.

Silver-reducing substances: Mix 5 g with 25 ml of ethanol (95%), add 125 ml of water, 10 ml of nitric acid and 5 ml of 0.1 M silver nitrate, stir for 30 minutes and filter. Wash the residue with water, mix the filtrate and washings and titrate the excess of silver nitrate in the mixture with 0.05 M ammonium thiocyanate using ferric ammonium sulphate solution as indicator; not less than 9.5 ml of 0.05 M ammonium thiocyanate is required.

Heavy metals: Not more than 20 ppm, determined by Method C on 1.0 g dissolved in a mixture of 10 ml of 1M sodium hydroxide and 15 ml of water.

Related substances: Carry out the method for thin-layer chromatography, using silica gel GF254 as the coating substance and a freshly prepared mixture of 50 volumes of 2-propanol, 30 volumes of ethyl acetate and 20 volumes of strong ammonia solution as the mobile phase. Do not line the walls of the tank and allow to saturate for 1 hour before development. Apply separately to the plate 20 ml of each of two solutions of the substance being examined in a mixture of equal volumes of ethanol (95%) and ethyl acetate containing (1) 0.50% w/v and (2) 0.0050% w/v. After removal of the plate, allow it to dry in air and examine under ultra-violet light (254 nm). Any secondary spot in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2)

Sulphated ash: Not more than 0.1%,

Loss on drying: Not more than 0.5%, determined on 2.5 g,

Assay: Weigh accurately about 0.4 g, dissolve in 90 ml of dimethylformamide and carry out Method A for non-aqueous titration, using 0.1 M tetrabutylammonium hydroxide as titrant and determining the end-point potentiometrically (Take precautions to prevent absorption of atmospheric carbon dioxide). Perform a blank determination and make any necessary correction. Each ml of 0.1M tetrabutylammonium hydroxide is equivalent to 0.02222 g of $C_4H_6N_4O_3S_2$.

Experiment No: 2

Aminophylline (Theophylline and Ethylenediamine**)**

$(C_7H_8N_4O_2)_2$, $C_2H_8N_2$ Mol. Wt. 420.43 (anhydrous) Aminophylline is a stable mixture or combination of theophylline and ethylenediamine. It may be anhydrous or may contain not more than two molecules of water of hydration.

Category: Bronchodilator.

Dose: Orally, 100–300 mg; by slow intravenous injection, 250–500 mg.
Description: White or slightly yellowish granules or powder; odour, slightly ammoniacal. On exposure to air it gradually loses ethylenediamine and absorbs carbon dioxide with liberation of free theophylline. Even in the absence of light, it is gradually decomposed on exposure to a humid environment, the degradation being faster at higher temperatures.
Solubility: Freely soluble in water (the solution usually becomes turbid on standing); practically insoluble in ethanol and in ether.
Storage: Store in tightly-closed, light-resistant containers and protect from atmospheric carbon dioxide.

Standards

Aminophylline contains the equivalent of not less than 84.0 per cent and not more than 87.4 per cent of theophylline, $C_7H_8N_4O_2$, and the equivalent of not less than 13.5 per cent and not more than 15.0 per cent of ethylenediamine, $C_2H_8N_2$, both calculated with reference to the anhydrous substance.
Identification Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A: Dissolve 1 g in 10 ml of water and add 2 ml of dilute hydrochloric acid drop wise, with shaking. Separate the precipitate by filtration and reserve the filtrate for test E. Wash the precipitate with successive small quantities of cold water, recrystallise from hot water and dry at 100–105º. The infra-red absorption spectrum, of the crystalline powder is concordant with the reference spectrum of theophylline or with the spectrum obtained from theophylline RS.
B: The melting range of the dried crystalline powder obtained in test A is between 270º and 274º.
C: To 10 mg of the dried crystalline powder obtained in test A add 1 ml of hydrochloric acid in a porcelain dish and 0.1 g of potassium chlorate and evaporate to dryness on a water-bath; invert the dish over a vessel containing a few drops of dilute ammonia solution; the residue acquires a purple colour. Add a few drops of dilute sodium hydroxide solution; the colour is discharged.
D: Saturate in water a portion of the dried crystalline powder obtained in test A and add tannic acid solution; a precipitate soluble in excess of the reagent is produced.
E: To the filtrate obtained in test A add 0.2 ml of benzoyl chloride, make alkaline with 2M sodium hydroxide and shake vigorously. Filter, wash the precipitate with 10 ml of water, dissolve in 5 ml of hot ethanol (95%) and add 5 ml of water. The melting range of the precipitate, after washing and drying at 100–105º, is between 248–252ºC.

Heavy metals: Not more than 20 ppm, determined by Method A on a 8% w/v solution.
Related substances: Carry out the method for thin-layer chromatography, using silica gel GF254 as the coating substance and a mixture of 40 volumes of 1-butanol, 30 volumes of acetone, 30 volumes of chloroform and 10 volumes of strong ammonia solution as the mobile phase. Apply separately to the plate 10 ml of each of the following solutions. For solution (1) dissolve 0.2 g of the substance being examined in 2 ml of water with the aid of heat and dilute to 10 ml with methanol. For solution (2) dilute 1 volume of solution (1) to 200 volumes with methanol. After removal of the plate, allow it to dry in air and examine under ultra-violet light (254 nm). Any secondary spot in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).
Sulphated ash: Not more than 0.1%.
Water: Not more than 1.5% w/w (for anhydrous), determined on 2 g dissolved in 20 ml of pyridine. Between 3.0 and 8.0% w/w (for hydrate), determined on 0.5 g.
Assay: For theophylline — Weigh accurately about 0.25 g, add 50 ml of water and 8 ml of dilute ammonia solution and warm gently on a water-bath until complete solution is effected. Add 20.0 ml of 0.1M silver nitrate, mix and boil for 15 minutes. Cool to between 5º and 10º for 20 minutes, filter at a pressure not exceeding 2.75 kPa and wash the precipitate with three quantities, each of 10 ml, of water. Acidify the combined filtrate and washings with nitric acid and add an excess of 3 ml of the acid. Cool,

add 2 ml of ferric ammonium sulphate solution, and titrate with 0.1 M ammonium thiocyanate. Each ml of 0.1 M silver nitrate is equivalent to 0.01802 g of $C_7H_8N_4O_2$.

Ammonium Thiocyanate, xM: Solutions of any molarity xM may be prepared by dissolving 76.12x g in sufficient water to produce 1000 ml.

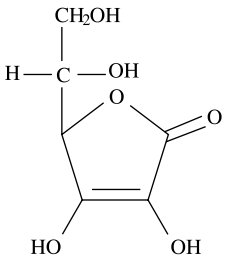
Ferric Ammonium Sulphate Solution: An 8.0 % w/v solution of ferric ammonium sulphate.

For ethylenediamine — Weigh accurately about 0.25 g and dissolve in 30 ml of water. Titrate with 0.1 M hydrochloric acid using methyl orange solution as indicator. Each ml of 0.1 M hydrochloric acid is equivalent to 0.003005 g of $C_2H_8N_2$.

Experiment No: 3

Ascorbic Acid

Vitamin C; L-Ascorbic Acid



Mol.formula: $C_6H_8O_{6,m}$, Mol. Wt. 176.13 Ascorbic Acid is (R)-5-[(S)-1, 2-dihydroxyethyl)-3, 4-dihydroxy-5(H)-furan-2-one.

Category: Vitamin (antiscorbutic) and pharmaceutical aid (antioxidant).

Dose: Prophylactic, 25–75 mg daily; therapeutic, not less than 250 mg daily, in divided doses.

Description: Colourless crystals or white to very pale yellow crystalline powder; odourless. On exposure to light it gradually darkens.

Solubility: Freely soluble in water; sparingly soluble in ethanol (95%); insoluble in chloroform, in ether and in benzene.

Storage: Store in tightly-closed, light-resistant containers and avoid contact with metals. It undergoes rapid decomposition in solutions in contact with air.

Standards

Ascorbic Acid contains not less than 99.0 per cent and not more than 100.5 per cent of $C_6H_8O_6$.

Identification Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A: The infra-red absorption spectrum, is concordant with the reference spectrum of ascorbic acid or with the spectrum obtained from ascorbic acid RS.

B: Add 2 ml of a 2% w/v solution to a few ml of 2, 6-dichlorophenolindophenol solution; the solution is decolorised.

C: Dilute 1 ml of a 2% w/v solution with 5 ml of water and add 1 drop of a freshly prepared 5% w/v solution of sodium nitroprusside and 2 ml of dilute sodium hydroxide solution. Add 0.6 ml of hydrochloric acid dropwise and stir; the yellow colour turns blue.

D: To 2 ml of a 2% w/v solution add 2 ml of water, 0.1 g of sodium bicarbonate and about 20 mg of ferrous sulphate, shake and allow to stand; a deep violet colour is produced. Add 5 ml of 1M sulphuric acid; the colour disappears.

pH: Between 2.2 and 2.5, determined in a 5.0% w/v solution.

Clarity and colour of solution: A 5.0% w/v solution in carbon dioxide-free water is clear, and not more intensely coloured than reference solution BYS7.

Specific optical rotation: Between +20.5º and +21.5º, determined in a 10% w/v solution.

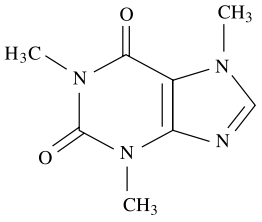
Light absorption: Absorbance of a 0.001% w/v solution in 0.01M hydrochloric acid at the maximum at about 244 nm, about 0.56.

Oxalic acid: Dissolve 0.25 g in 5 ml of water and neutralise to litmus paper with 2 M sodium hydroxide. Add 1 ml of 2 M acetic acid and 0.5 ml of 0.5 M calcium chloride. Any opalescence, after 60 minutes, is not more intense than that produced by treating 5 ml of a solution prepared by dissolving 70 mg of oxalic acid in 500 ml of water in a similar manner (0.3%).

Heavy metals: Not more than 20 ppm, determined by Method A on 1.0 g dissolved in 25 ml of water.
Sulphated ash: Not more than 0.1%.
Assay: Weigh accurately about 0.1 g and dissolve in a mixture of 100 ml of freshly boiled and cooled water and 25 ml of 1 M sulphuric acid. Immediately titrate with 0.05 M iodine, using starch solution as indicator until a persistent blue-violet colour is obtained. Each ml of 0.05 M iodine is equivalent to 0.008806 g of $C_6H_8O_6$.

Experiment No: 4

Caffeine



$C_8H_{10}N_4O_2$ Mol.Wt.194.19(anhydrous)
$C_8H_{10}N_4O_2, H_2O$ Mol.Wt.212.21(monohydrate)

Caffeine is 3, 7-dihydro-1, 3, 7-trimethyl-1H-purine-2, 6-dione or its monohydrate.
Category: Central nervous system stimulant.
Dose: 300 to 600 mg.
Description: Silky white crystals or white glistening needles or white crystalline powder, odourless; sublimes readily.
Solubility: Freely soluble in chloroform and in boiling water; sparingly soluble in water and in ethanol (95%); slightly soluble in ether.
Storage: Store in tightly-closed containers.
Labelling: The label states whether it is anhydrous or monohydrate.

Standards

Caffeine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_8H_{10}N_4O_2$, calculated with reference to the dried substance.
Identification: Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

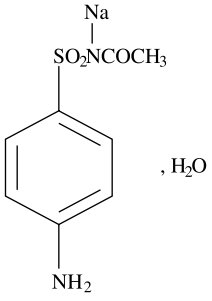
A: The infra-red absorption spectrum, of the substance being examined after drying at 100º for 1 hour is concordant with the reference spectrum of caffeine or with the spectrum obtained from caffeine RS.
B: To 10 mg in a porcelain dish, add 1 ml of hydrochloric acid and 0.1 g of potassium chlorate and evaporate to dryness on a water-bath. Expose the residue to the vapours of dilute ammonia solution; a purple colour is produced which disappears on addition of a solution of a fixed alkali.
C: To a saturated solution add a few drops of tannic acid solution; a white precipitate is produced which is soluble in excess of the reagent.
D: To 5 ml of saturated solution add 1.5 ml of 0.05 M iodine, the solution remains clear. Add a few drops of dilute hydrochloric acid; a brown precipitate is formed which dissolves on neutralisation with sodium hydroxide solution.
E: Melts between 234º and 239ºC, determined after drying at 100º for 1 hour.

Acidity or alkalinity: Dissolve 0.2 g in 10 ml of boiling water and cool. Add 0.1 ml of bromothymol blue solution. The solution is coloured green or yellow. Titrate with 0.02M sodium hydroxide to a blue colour; not more than 0.1 ml is required.
Clarity and colour of solution: A 1.0% w/v solution is clear, and colourless,
Arsenic: Mix 3.3 g with 3 g of anhydrous sodium carbonate, add 10 ml of bromine solution and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in 16 ml of brominated hydrochloric acid and 45 ml of water. Remove the excess of bromine with 2 ml of stannous chloride solution AsT. The resulting solution complies with the limit test for arsenic, (3 ppm).

Heavy metals: Not more than 20 ppm, determined by Method A, on 25 ml of a solution prepared in the following manner. Mix 2.0 g with 5 ml of 0.1M hydrochloric acid and 45 ml of water, warm gently until solution is complete and cool to room temperature.
Related substances: Carry out the method for thin-layer chromatography, using silica gel GF254 as the coating substance and a mixture of 40 volumes of 1-butanol, 30 volumes of chloroform, 10 volumes of strong ammonia solution and 3 volumes of acetone as the mobile phase. Apply separately to the plate 10ml of each of the following solutions of the substance being examined in a mixture of 3 volumes of chloroform and 2 volumes of methanol containing (1) 2.0% w/v and (2) 0.010% w/v. After removal of the plate, allow it to dry in air and examine under ultra-violet light (254 nm). Any secondary spot in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).
Sulphated ash: Not more than 0.1%.
Loss on drying: Not more than 0.5% (for the anhydrous form) and 8.5% (for the monohydrate form), determined on 1 g by drying at 100º for 1 hour.
Assay: Weigh accurately about 0.18 g and dissolve with warming in 5 ml of anhydrous glacial acetic acid. For Caffeine Hydrate, use material previously dried at 100–105º. Cool, add 10 ml of acetic anhydride and 20 ml of toluene, Dissolve the prescribed quantity of the substance being examined in a suitable volume of anhydrous glacial acetic acid, warming and cooling if necessary, or prepare a solution as directed in the monograph and determine the equivalence point potentiometrically using 0.1 M perchloric acid as titrant, unless otherwise specified in the monograph. Potentiometric titration may be carried out using a glass electrode and a standard reference electrode, e.g. calomel reference electrode containing saturated solution of potassium chloride in water. Potentiometric titrations may also be carried out by using a glass electrode and a saturated solution of potassium chloride in water has been replaced by a saturated solution of potassium chloride in methanol. It must be ensured that no leakage of salt- bridge solution occurs. Alternatively, a combined electrode may be used. The junction between the calomel electrode and the titration liquid should have a reasonably low electrical resistance and there should be minimum of transfer of liquid from one side to the other. The connections between the potentiometer and the electrode system must be made according to the manufacturer's instructions to avoid problems of instability. When the temperature (t_2) of the titrant at the time of the assay is different from the temperature (t_1) of the tirant when it was standardised, multiply the volume of the titrant required by [1 + 0.0011 ($t_1 - t_2$) and calculate the result of the assay from the corrected volume. determining the end-point potentiometrically. Perform a blank determination and make any necessary correction.
Each ml 0.1 M perchloric acid is equivalent to 0.01942 g of $C_8H_{10}N_4O_2$.

Experiment No: 5

Sulphacetamide Sodium



, H_2O

$C_8H_9N_2NaO_3S,H_2O$ Mol. Wt. 254.24 Sulphacetamide Sodium is the monohydrate of the **sodium salt of N[1]-acetylsulphanilamide**.
Category: Antibacterial.
Description: White or yellowish white, crystalline powder; odourless.
Solubility: Freely soluble in water; slightly soluble in ethanol (95%); practically insoluble in chloroform and in ether.
Storage: Store in tightly-closed, light-resistant containers.

Standards

Sulphacetamide Sodium contains not less than 99.0 per cent and not more than 101.0 per cent of $C_8H_9N_2NaO_3S$, calculated with reference to the anhydrous substance.

Identification Test A may be omitted if tests B, C, D, E and F are carried out. Tests B, C, D and E may be omitted if tests A and F are carried out.

A: The infra-red absorption spectrum, is concordant with the reference spectrum of sulphacetamide sodium or with the spectrum obtained from sulphacetamide sodium RS.

B: The light absorption in the range 230–360 nm of a 0.001% w/v solution in citro-phosphate buffer pH 7.0 exhibits a maximum at about 255 nm; absorbance at about 255 nm, 0.66 to 0.72.

C: Dissolve 1 g in 10 ml of water, add 6 ml of 2M acetic acid and filter. Wash the precipitate with a small volume of water and dry at 105º for 4 hours. The melting range of the precipitate is between 181º and 185º.

D: Dissolve 0.1 g of the precipitate obtained in test C in 5 ml of ethanol (95%), add 0.2 ml of sulphuric acid and heat; ethyl acetate, recognizable by its odour, is produced.

E: Dissolve 1 mg of the precipitate obtained in test C in 5 ml of water with the aid of heat. The solution gives the reaction of primary aromatic amines, producing an orange-red precipitate.

F: A 5% w/v solution gives the reactions of sodium salts.

pH: Between 8.0 and 9.5, determined in a 5% w/v solution.

Clarity and colour of solution: A 5.0% w/v solution in carbon dioxide-free water is clear, and not more intensely coloured than reference solution GYS_4.

Heavy metals: Not more than 20 ppm, determined by Method B on 12 ml of a solution prepared by dissolving 2.5 g in sufficient distilled water to produce 25 ml, adding 25 ml of 2 M acetic acid, shaking for 30 minutes and filtering. Use lead standard solution (1 ppm Pb) to prepare the standard,

Sulphate: Dissolve 1.5 g in sufficient distilled water to produce 25 ml, add 25 ml of 2 M acetic acid, shake for 30 minutes and filter. 25 ml of the filtrate complies with the limit test for sulphates,

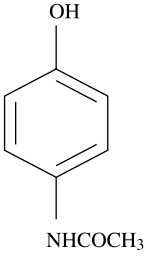
Related substances: Carry out the method for thin-layer chromatography, using silica gel HF254 as the coating substance and a mixture of 50 volumes of 1-butanol, 25 volumes of ethanol, 25 volumes of water and 10 volumes of strong ammonia solution as the mobile phase. Apply separately to the plate 5 ml of each of four solutions in water containing (1) 10.0% w/v of the substance being examined, (2) 0.050% w/v of sulphanilamide, (3) 0.025% w/v of sulphanilamide and (4) 0.050% w/v sulphanilamide in solution (1). After removal of the plate, allow it to dry in air and spray with 4-dimethyl aminobenzaldehyde solution a freshly prepared 2% w/v solution of 4-dimethylaminobenzaldehyde in a mixture of 55 volumes of hydrochloric acid and 45 volumes of water. Any secondary spot in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3). The chromatogram obtained with solution (4) shows two clearly separated spots.

Water: Between 6.0 and 8.0% w/w, determined on 0.2 g.

Assay: Weigh accurately about 0.25 g, dissolve in a mixture of 50 ml of water and 20 ml of 2 M hydrochloric acid, add 3 g of potassium bromide, cool in ice and carry out the nitrite titration, Each ml of 0.1M sodium nitrite is equivalent to 0.02362 g of $C_8H_9N_2NaO_3S$.

Dimethylaminobenzaldehyde Solution: Dissolve 0.2 g of 4-dimthylaminobenzaldehyde in 20 ml of ethanol (95%) and add 0.5 ml of hydrochloric acid. Shake the solution with activated charcoal and filter. The colour of the solution is not more intense than that of freshly prepared 0.0001 M iodine. Prepare immediately before use.

Experiment No: 6



Paracetamol (Acetaminophen)

$C_8H_9NO_2$, Mol. Wt. 151.16 and Paracetamol is 4-hydroxyacetanilide.

Category: Analgesic; antipyretic.

Dose: 500 mg to 1 g every 4 to 6 hours, upto 4 g daily, in divided doses.

Description: White crystals or white, crystalline powder.

Solubility: Freely soluble in ethanol (95%) and in acetone; sparingly soluble in water; very slightly soluble in dichloromethane and in ether.
Storage: Store in well-closed, light-resistant containers.

Standards

Paracetamol contains not less than 99.0 per cent and not more than 101.0 per cent of $C_8H_9NO_2$, calculated with reference to the dried substance.
Identification Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

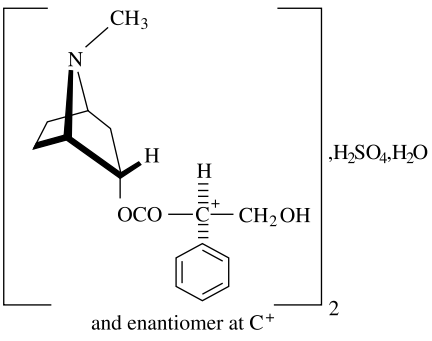
A: The infra-red absorption spectrum, is concordant with the reference spectrum of paracetamol or with the spectrum obtained from paracetamol RS.
B: Dissolve 50 mg in sufficient methanol to produce 100 ml. To 1 ml of this solution add 0.5 ml of 0.1 M hydrochloric acid and dilute to 100 ml with methanol. Protect the resulting solution from bright light and immediately measure the absorbance at the maximum at about 249 nm; absorbance at about 249 nm, about 0.44.
C: Boil 0.1 g in 1 ml of hydrochloric acid for 3 minutes, add 10 ml of water and cool; no precipitate is produced. Add 0.05 ml of 0.0167 M potassium dichromate; a violet colour develops which does not turn red.
D: Gives the reaction of acetyl groups.
E: Melts between 168º and 172º.

Heavy metals: Not more than 10 ppm, determined on 2.0 g Method B.
4-Aminophenol: Dissolve 0.50 g in sufficient methanol (50%) to produce 10 ml. Add 0.2 ml of freshly prepared alkaline sodium nitroprusside solution, mix and allow to stand for 30 minutes. Any blue colour in the solution is not more intense than that in 10 ml of a solution prepared at the same time and in the same manner containing 0.5 g of 4-aminophenol-free paracetamol and 0.5 ml of a 0.005% w/v solution of 4-aminophenol in methanol (50%) (50 ppm).
Related substances: Carry out the method for thin-layer chromatography, using silica gel GF254 as the coating substance and a mixture of 65 volumes of chloroform, 25 volumes of acetone and 10 volumes of toluene as the mobile phase but allowing the solvent front to ascend 14 cm above the line of application. Apply separately to the plate 200 m l of solution (1) and 40 m l of each of solutions (2), (3) and (4). For solution (1) transfer 1.0 g of the substance being examined, finely powdered, to a ground-glass stoppered 15-ml centrifuge tube, add 5 ml of peroxide-free ether, shake mechanically for 30 minutes and centrifuge at 1000 rpm for 15 minutes or until a clear supernatant liquid is obtained. For solution (2) dilute 1 ml of solution (1) to 10 ml with ethanol (95%). Solution (3) contains 0.005% w/v of 4-chloroacetanilide in ethanol (95%). For solution (4) dissolve 0.25 g of 4-chloroacetanilide and 0.1 g of the substance being examined in sufficient ethanol (95%) to produce 100 ml. After removal of the plate, dry it in a current of warm air and examine under ultra-violet light (254 nm). Any spot corresponding to 4-chloroacetanilide in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). Any other secondary spot in the chromatogram obtained with solution (2) is not more intense than the spot in the chromatogram obtained with solution (3). The chromatogram obtained with solution (4) shows two clearly separated spots, the spot corresponding to paracetamol having the lower Rf value.
Sulphated ash: Not more than 0.1%.
Loss on drying: Not more than 0.5%, determined on 1 g by drying in an oven at 105º.
Assay: Weigh accurately about 0.5 g, dissolve in a mixture of 10 ml of water and 50 ml of 1 M sulphuric acid. Boil under reflux condenser for 1 hour, cool and dilute to 100.0 ml with water. To 20.0 ml of the solution add 40 ml of water, 40 g of water in the form of ice, 15 ml of 2 M hydrochloric acid and 0.1 ml of ferroin solution and titrate with 0.1 M ceric ammonium sulphate until a yellow colour is

produced. Perform a blank determination and make any necessary correction. Each ml of 0.1 M ceric ammonium sulphate is equivalent to 0.00756 g of $C_8H_9No_2$.

Experiment No: 7

Atropine Sulphate



$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$, Mol. Wt. 694.84. Atropine Sulphate is (1R, 3r, 5S)-3-tropoyloxytropanium sulphate monohydrate.

Category: Anticholinergic; antidote to cholinesterase inhibitors.

Dose: As anticholinergic, orally, 250 mg to 2 mg daily in single or divided doses; by subcutaneous, intramuscular, or by intravenous injection, 400 mg to 600 mg four to six times a day; as antidote to cholinesterase inhibitors, by intravenous injection, 2 to 4 mg initially, followed by intramuscular injection, 2 mg repeated every 5 to 10 minutes.

Description: Colourless crystals or white, crystalline powder; odourless.

Solubility: Very soluble in water; freely soluble in ethanol (95%) and in glycerin; practically insoluble in chloroform and in ether.

Storage: Store in well-closed, light-resistant containers.

Standards

Atropine Sulphate contains not less than 99.0 per cent and not more than 101.0 per cent of $(C_{17}H_{23}NO_3)_2$, H_2SO_4, calculated with reference to the anhydrous substance.

Identification: Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A: The infra-red absorption spectrum, is concordant with the reference spectrum of atropine sulphate or with the spectrum obtained from atropine sulphate RS.

B: To a 2% w/v solution add sodium hydroxide solution, filter and transfer the precipitate with water. Dry the precipitate at 60º. To 5 mg of the residue add 5 drops of fuming nitric acid and evaporate to dryness on a water-bath. Cool the faintly yellow coloured residue and add 2 ml of acetone and 4 drops of a 3% w/v solution of potassium hydroxide in methanol; a violet colour is produced.

C: Gives the reaction of alkaloids.

D: A 5% w/v solution gives the reactions of sulphates.

pH: Between 4.5 and 6.2, determined in a 2.0% w/v solution.

Specific optical rotation: Between –0.50º to +0.05º, determined in a 10% w/v solution, using a 2-dm tube, (distinction from hyoscyamine).

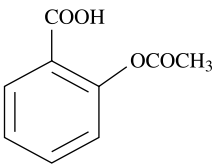
Apoatropine: Absorbance of a 0.1% w/v solution in 0.01 M hydrochloric acid at about 245 nm, not more than 0.4 (about 0.5%).

Foreign alkaloids and decomposition products: Carry out the method for thin-layer chromatography, using silica gel G as the coating substance and a mixture of 90 volumes of acetone, 7 volumes of water and 3 volumes of strong ammonia solution as the mobile phase. Apply separately to the plate 10 ml of each of three solutions of the substance being examined in methanol containing (1) 2.0% w/v, (2) 0.020% w/v and (3) 0.010% w/v. After removal of the plate, dry it at 105º for 15 minutes. Allow it to cool to room temperature and spray with dilute potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram

obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).
Sulphated ash: Not more than 0.2%.
Water: Not more than 4.0% w/w, determined on 0.5 g.
Assay: Weigh accurately about 0.5 g, dissolve in 30 ml of anhydrous glacial acetic acid and carry out for non-aqueous titration, determining the end-point potentiometrically. Dissolve the prescribed quantity of the substance being examined in a suitable volume of anhydrous glacial acetic acid, warming and cooling if necessary, or prepare a solution as directed in the monograph and determine the equivalence point potentiometrically using 0.1 M perchloric acid as titrant, unless otherwise specified in the monograph. Potentiometric titration may be carried out using a glass electrode and a standard reference electrode, e.g. calomel reference electrode containing saturated solution of potassium chloride in water. Potentiometric titrations may also be carried out by using a glass electrode and a saturated solution of potassium chloride in water has been replaced by a saturated solution of potassium chloride in methanol. It must be ensured that no leakage of salt- bridge solution occurs. Alternatively, a combined electrode may be used. The junction between the calomel electrode and the titration liquid should have a reasonably low electrical resistance and there should be minimum of transfer of liquid from one side to the other. The connections between the potentiometer and the electrode system must be made according to the manufacturer's instructions to avoid problems of instability. When the temperature (t_2) of the titrant at the time of the assay is different from the temperature (t_1) of the tirant when it was standardised, multiply the volume of the titrant required by [1 + 0.0011 ($t_1 - t_2$) and calculate the result of the assay from the corrected volume. Perform a blank determination and make any necessary correction. Each ml of 0.1 M perchloric acid is equivalent to 0.06768 g of $(C_{17}H_{23}NO_3)_2, H_2SO_4$.

Experiment No: 8

Aspirin (Acetylsalicylic Acid)



$C_6H_8O_4$, Mol. Wt. 180.16 Aspirin is 2-acetoxybenzoic acid.
Category: Analgesic; antipyretic; antirheumatic; antithrombotic.
Dose: As analgesic and antipyretic, 300–600 mg four to six times a day; as antirheumatic, 1–2 g four to six times a day, upto 10 g daily; as antithrombotic, 75 mg daily.
Description: Colourless crystals or white, crystalline powder; odourless or almost odourless.
Solubility: Freely soluble in ethanol (95%); soluble in chloroform and in ether; slightly soluble in water.
Storage: Store in tightly-closed containers in a cool, dry place.

Standards

Aspirin contains not less than 99.5 per cent and not more than 100.5 per cent of $C_9H_8O_4$, calculated with reference to the dried substance.
Identification Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

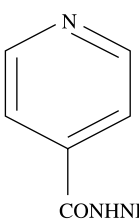
A: The infra-red absorption spectrum is concordant with the reference spectrum of aspirin or with the spectrum obtained from aspirin RS.
B: Boil about 0.5 g with 10 ml of sodium hydroxide solution for 3 minutes, cool and add 10 ml of dilute sulphuric acid; a white, crystalline precipitate is produced and the odour of acetic acid is perceptible. Filter, dissolve the precipitate in about 2 ml of water and add ferric chloride test solution; a deep violet colour is produced.

C: To the filtrate obtained in test B add 3 ml of ethanol (95%) and 3 ml of sulphuric acid and warm; the odour of ethyl acetate is perceptible.
D: Melts at about 142º.

Clarity and colour of solution in ethanol: A 1% w/v solution in ethanol (95%) is clear, and not more intensely coloured than reference solution BS8.
Clarity of solution in alkali: A 5% w/v solution in warm 5% w/v solution of sodium carbonate is clear.
Chloride: Boil 1.75 g with 75 ml of water for 5 minutes, cool, add sufficient water to restore the original volume and filter. 25 ml of the filtrate complies with the limit test for chlorides (430 ppm).
Sulphate: 10 ml of the filtrate obtained in the test for **Chloride** complies with the limit test for sulphates, (600 ppm).
Arsenic: Mix 5 g with 3 g of anhydrous sodium carbonate, add 10 ml of bromine solution and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite, and dissolve the cooled residue in 16 ml of brominated hydrochloric acid and 45 ml of water. Remove the excess of bromine with 2 ml of stannous chloride AsT. The resulting solution complies with the limit test for arsenic (2 ppm).
Heavy metals: Not more than 10 ppm, determined by the following method. Dissolve 2 g in 25 ml of acetone, add 1 ml of water and 10 ml of hydrogen sulphide solution; any colour produced is not more intense than that produced by mixing 25 ml of acetone, 1.0 ml of lead standard solution (20 ppm Pb) and 10 ml of hydrogen sulphide solution.
Readily carbonisable substances: Dissolve 0.50 g in 5 ml of sulphuric acid (containing 94.5% to 95.5% w/w of H_2SO_4); any colour produced is not more intense than that of reference solution BYS4.
Salicylic acid: Dissolve 2.5 g in sufficient ethanol (95%) to produce 25.0 ml (test solution). To each of two matched Nessler cylinders add 48 ml of water and 1 ml of a freshly prepared acid ferric ammonium sulphate solution. Into one cylinder pipette 1.0 ml of a freshly prepared 0.010% w/v solution of salicylic acid and into the other pipette 1.0 ml of the test solution. Mix the contents of the cylinders; after 30 seconds, the colour in the cylinder containing the test solution is not more intense than that in the cylinder containing the standard solution (0.1%).
Sulphated ash: Not more than 0.1%.
Loss on drying: Not more than 0.5%, determined on 1 g by drying over phosphorus pentoxide at a pressure of 1.5 to 2.5 kPa.
Assay: Weigh accurately about 1.5 g, dissolve in 15 ml of ethanol (95%), add 50.0 ml of 0.5 M sodium hydroxide, boil gently for 10 minutes, cool and titrate the excess of alkali with 0.5M hydrochloric acid using phenol red solution as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of sodium hydroxide required. Each ml of 0.5M sodium hydroxide is equivalent to 0.04504 g of $C_9H_8O_4$.

Experiment No: 9

Isoniazid(Isonicotinylhydrazid; INH)



$C_6H_7N_3O$, Mol. Wt. 137.14 Isoniazid is isonicotinohydrazide.
Category: Antitubercular.
Dose: 300 mg daily or upto 1 g twice weekly.
Description: Colourless crystals or white, crystalline powder; odourless.
Solubility: Freely soluble in water; sparingly soluble in ethanol (95%); slightly soluble in chloroform; very slightly soluble in ether.
Storage: Store in well-closed, light-resistant containers.

Standards

Isoniazid contains not less than 98.0 per cent and not more than 101.0 per cent of $C_6H_7N_3O$, calculated with reference to the dried substance.

Identification Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A: The infra-red absorption spectrum is concordant with the reference spectrum of isoniazid or with the spectrum obtained from isoniazid RS.

B: Dissolve 0.1 g in 2 ml of water, add a warm solution of 0.1 g of vanillin in 10 ml of water, allow to stand and scratch the inside of the container with a glass rod; a yellow precipitate is produced. The melting range of the precipitate, after recrystallisation from 5 ml of ethanol (70%) and drying at 105º is between 226º and 231º.

C: Melts between 170º and 174º.

pH: Between 6.0 and 8.0, determined in a 5.0% w/v solution.

Clarity and colour of solution: A 5.0% w/v solution in carbon dioxide-free water is clear, and not more intensely coloured than reference solution BYS7.

Heavy metals: Not more than 20 ppm, determined by Method B on 1.0 g.

Hydrazine and related substances: Carry out the method for thin-layer chromatography, using silica gel GF254 as the coating substance and a mixture of 50 volumes of ethyl acetate, 20 volumes of acetone, 20 volumes of methanol and 10 volumes of water as the mobile phase. Apply separately to the plate 5 m l of each of the following solutions. For solution (1) dissolve 1.0 g of the substance being examined in sufficient of a mixture of equal volumes of acetone and water to produce 10 ml. For solution (2) dissolve 50 mg of hydrazine sulphate in 50 ml of water and dilute to 100 ml with acetone; to 10 ml of this solution add 0.2 ml of solution (1) and dilute to 100 ml with a mixture of equal volumes of acetone and water. After removal of the plate, allow it to dry in air and examine under ultra-violet light (254 nm). Any secondary spot in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Spray with dimethylaminobenzaldehyde reagent (amendment 4) and examine in daylight. The additional spot (due to hydrazine) in the chromatogram obtained with solution (2) is more intense than any corresponding spot in the chromatogram obtained with solution (1).

Sulphated ash: Not more than 0.1%.

Loss on drying: Not more than 0.5%, determined on 1 g by drying in an oven at 105º.

Assay: Weigh accurately about 0.25 g, dissolve in sufficient water to produce 100.0 ml. To 20.0 ml of the resulting solution add 100 ml of water, 20 ml of hydrochloric acid and 0.2 g of potassium bromide and titrate slowly with continuous shaking with 0.0167 M potassium bromate using 0.05 ml of methyl red solution as indicator, until the red colour disappears. Each ml of 0.0167 M potassium bromate is equivalent to 0.003429 g of $C_6H_7N_3O$.

Experiment No: 10

Phenobarbitone

Phenobarbital $C_{12}H_{12}N_2O_3$ Mol. Wt. 232.2 Phenobarbitone is 5-ethyl-5-phenylbarbituric acid. Phenobarbitone contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{12}N_2O_3$, calculated on the dried basis.

Description. Colourless crystals or a white, crystalline powder; odourless.

Identification Test A may be omitted if tests B, C, D and E are carried out. Tests C, D and E may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry. Compare the spectrum with that obtained with phenobarbitone RS or with the reference spectrum of phenobarbitone. B. Determine the melting point of the substance under examination and of a mixture of equal quantities of the substance under examination and phenobarbitone RS. The difference between the melting points, which are about 175º, is not greater than 2º. C. Complies with the test for identification of barbiturates. D. Dissolve about 20 mg in

5 ml of ethanol, add a drop of cobalt chloride solution and a drop of dilute ammonia solution; a violet colour is produced. E. Gives the reaction of non-nitrogen substituted barbiturates.

Tests

Appearance of solution. A 10.0 per cent w/v solution in a mixture of 20 volumes of 2 M sodium hydroxide and 30 volumes of water is clear, and not more intensely coloured than reference solution YS6.
Acidity. Mix 1.0 g with 50 ml of water, boil for 2 minutes, allow to cool, filter and adjust the volume to 50 ml. To 10 ml of the filtrate add 0.15 ml of methyl red solution; not more than 0.1 ml of 0.1 M sodium hydroxide is required to change the colour of the solution from orange-yellow to pure yellow.
Related substances. Complies with the test for related substances in barbiturates.
Sulphated ash. Not more than 0.1 per cent.
Loss on drying. Not more than 1.0 per cent w/w, determined on 1.0 g by drying in an oven at 105º for 2 hours.
Assay. Weigh accurately about 0.1 g, dissolve in 5 ml of pyridine, add 0.25 ml of thymolphthalein solution and 10 ml of silver nitrate-pyridine reagent and titrate with 0.1 M ethanolic sodium hydroxide until a pure blue colour is obtained. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required. 1 ml of 0.1 M ethanolic sodium hydroxide is equivalent to

0.01161 g of $C_{12}H_{12}N_2O_3$.

Storage. Store protected from moisture.

Experiment No: 11

Phenytoin Sodium

Diphenylhydantoin Sodium $C_{15}H_{11}N_2NaO_2$ Mol. Wt. 274.3

Phenytoin Sodium is 4-oxo-5,5-diphenyl-2-imidazolidin-2-olate Phenytoin Sodium contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{15}H_{11}N_2NaO_2$, calculated on the anhydrous basis.

Description. A white powder; odourless; somewhat hygroscopic.
Identification Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out. A. Determine by infrared absorption spectrophotometry . Compare the spectrum with that obtained with phenytoin sodium RS or with the reference spectrum of phenytoin sodium. B. Dissolve 0.25 g in 5 ml of water and acidify with dilute hydrochloric acid; a white precipitate is produced. C. Dissolve 0.1 g in 10 ml of a 10 per cent w/v solution of pyridine, add 1 ml of cupric sulphate with pyridine solution and allow to stand for 10 minutes; a blue precipitate is produced. D. Incinerate 0.1 g; the residue after neutralisation with hydrochloric acid and addition of 2 ml of water gives the reactions of sodium salts.

Tests

Appearance of solution: Suspend 1.0 g in 5 ml of water and dilute to 20 ml with 0.1 M sodium hydroxide; the solution is clear, and not more intensely coloured than reference solution BYS6.
Free phenytoin: Dissolve 0.3 g in 10 ml of a mixture of equal volumes of pyridine and water and add 0.5 ml of dilute phenolphthalein solution and 3 ml of silver nitrate-pyridine reagent. Not more than 1.0 ml of 0.1 M sodium hydroxide is required to change the colour of the solution to pink.
Related substances: Determine by thin-layer chromatography , coating the plate with silica gel GF254. Mobile phase. A mixture of 45 volumes of chloroform, 45 volumes of 2-propanol and 10 volumes of strong ammonia solution. Test solution. Dissolve 0.4 g of the substance under examination in 10 ml of

methanol. Reference solution (a). A 0.04 per cent w/v solution of the substance under examination in methanol. Reference solution (b). A 0.02 per cent w/v solution of benzophenone in methanol. Apply to the plate 10 µl of each solution. After development, dry the plate at 80º for 5 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and any spot corresponding to benzophenone is not more intense than the spot in the chromatogram obtained with reference solution (b).
Heavy metals: 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).
Water: Not more than 3.0 per cent, determined on 1.0 g.
Assay: Weigh accurately about 0.18 g, suspend in 2 ml of water, add 8 ml of 0.05 M sulphuric acid and heat gently for 1 minute. Add 30 ml of methanol, cool. Titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically. After the first inflection, stop the addition of sodium hydroxide, add 5 ml of silver nitrate solution in pyridine, mix and continue the titration until a second inflection is reached. Record the volume of 0.1 M sodium hydroxide added between the two inflections. 1 ml of 0.1 M sodium hydroxide is equivalent to 0.02743 g of $C_{15}H_{11}N_2NaO_2$.
Storage: Store protected from moisture.

Experiment No: 12

Phensuximide

$C_{11}H_{11}NO_2$ M. Wt: 189.21 1-Methyl-3-phenyl-2, 5-pyrrolidinedione; N-Methyl-2-phenylsuccinimide; N-Methyl-α-phenyl-succinimide.
Limits: Phensuximide contains not less than 97% and not more than 103% of $C_{11}H_{11}NO_2$ calculated on the anhydrous basis.
Packing and storage: Preserve in tight containers.
Melting range: Between 68°C – 74°C
Residue on ignition: Not more than 0.5%
Limit of cyanide: Dissolve 1 g in 10 ml of warm alcohol and add 3 drops of ferrous sulfate,1 ml of 1 N NaOH, and a few drops of ferric chloride. Warm gently and finally acidify with 2 N sulphuric acid; no blue precipitate or blue color is formed within 15 min.
Assay: Transfer about 200 mg of phensuximide accurately weighed to a 50 ml volumetric flask. Dissolve in 40 ml of alcohol, dilute with alcohol to volume and mix.Transfer 5 ml of this solution to a 50 ml volumetric flask, dilute with alcohol and mix. Concomitantly determine the absorbances of this solution and of a standard solution of phensuximide in the same medium having a known conc of about 400 µg per ml in 1cm cells at the wavelength of maximum absorbance at about 258 nm, with a suitable spectrometer, using alcohol as the blank. Calculate the quantity in mg of $C_{11}H_{11}NO_2$ in the phensuximide taken by the formula.

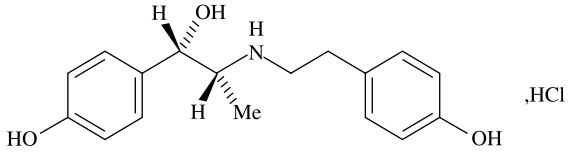
0.5C (Atest/A standard)

In Which C is the conc in micro gram per ml in the standard solution and Atest, A standard Are absorbance of solution from phensuximide test and standard solution.

Experiment No: 13

Ritodrine Hydrochloride

$C_{17}H_{21}NO_3$, HCl M.Wt: 323.8
Action and use: Beta2-adrenoceptor agonist; bronchodilator.



and/or enantiomer

Preparations: Ritodrine Injection Ritodrine Tablets.
Definition: Ritodrine Hydrochloride is erythro-1-(p-hydroxyphenyl)-2-(4-hydroxyphenethylamino) propan- 1-ol hydrochloride. It contains not less than 97.0% and not more than 103.0% of $C_{17}H_{21}NO_3$, HCl, calculated with reference to the dried substance.
Characteristics: A white or almost white, crystalline powder. Freely soluble in water; soluble in absolute ethanol ; practically insoluble in acetone and in ether .
Identification: A. The infrared absorption spectrum, is concordant with the reference spectrum of ritodrine hydrochloride (RS 313).

B. A 1% w/v solution yields reaction A characteristic of chlorides.

Tests

Acidity: pH of a 2% w/v solution, 4.5 to 6.0.
Heavy metals: 12 ml of a 10.0% w/v solution complies with limit test A for heavy metals
Use: lead standard solution (2 ppm Pb) to prepare the standard (20 ppm).
Related substances: Carry out the method for liquid chromatography, using the following solutions. Solution (1) contains 0.10% w/v of the substance being examined in the mobile phase. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) dissolve about 20 mg of the substance being examined in 50 ml of the mobile phase, add 5.6 ml of sulphuric acid and sufficient of the mobile phase to produce 100 ml, mix and heat at a temperature of 85° for 2 hours. Cool to room temperature, carefully mix 10 ml of the cooled solution with 8 ml of a 10% w/v solution of sodium hydroxide and allow to cool (generation of threo-diastereoisomer). The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with end-capped octylsilyl silica gel for chromatography (7 μm) (Zorbax C8 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of a solution containing 6.6 g of diammonium hydrogen orthophosphate and 1.1 g of sodium heptanesulphonate in 700 ml of water and 300 ml of methanol ; the mixture adjusted to pH 3.0 with an 85% v/v solution of orthophosphoric acid and (c) a detection wavelength of 214 nm. The test is not valid unless, in the chromatogram obtained with solution (3), the resolution factor between the two principal peaks (ritodrine and the threo-diastereoisomer) is at least 1.5. In the chromatogram obtained with solution (1), identify any peaks corresponding to tyramine (relative retention time to ritodrine, 0.3), 'hexahydroketone II' (0.65), 'hexahydroketone I' (0.85), threo-diastereoisomer (1.15) and 'aminoketone' (2.3). In the chromatogram obtained with solution (1), (a) the area of any peak corresponding to hexahydroketone II is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3% [response relative to ritodrine 0.35]), (b) the areas of any peaks corresponding to tyramine and aminoketone are not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2% each), (c) the area of any peak corresponding to hexahydroketone I is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4% [response relative to ritodrine 0.5]), (d) the area of any peak corresponding to the threo-diastereoisomer is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%) and (e) the area of any other secondary peak is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).
Loss on drying: When dried at 105° for 2 hours, loses not more than 1.0% of its weight. Use 1 g.
Sulphated ash: Not more than 0.2%.

Assay

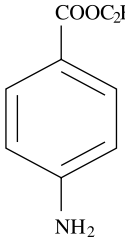
Carry out the method for liquid chromatography, Appendix III D, using the following solutions. Solution (1) contains 0.02% w/v of the substance being examined in the mobile phase. Solution (2) contains 0.02% w/v of ritodrine hydrochloride BPCRS in the mobile phase. For solution (3) dissolve about 20 mg of the substance being examined in 50 ml of the mobile phase, add 5.6 ml of sulphuric acid and

sufficient of the mobile phase to produce 100 ml, mix and heat at a temperature of 85° for 2 hours. Cool to room temperature, carefully mix 10 ml of the cooled solution with 8 ml of a 10% w/v solution of sodium hydroxide and allow to cool (generation of threo-diastereoisomer). The chromatographic procedure described under Related substances may be used. The test is not valid unless, in the chromatogram obtained with solution (3), the resolution factor between the two principal peaks (ritodrine and the threo-diastereoisomer) is at least 1.5. Calculate the content of $C_{17}H_{21}NO_3$, HCl in the substance being examined from the chromatograms obtained and from the declared content of $C_{17}H_{21}NO_3$, HCl in ritodrine hydrochloride BPCRS.

Storage: Ritodrine Hydrochloride should be kept in an airtight container.

Experiment No: 14

Benzocaine



$C_9H_{11}NO_2$ Mol. Wt. 165.19 Benzocaine is ethyl 4-aminobenzoate.

Category: Local anaesthetic.

Description: Colourless crystals or white, crystalline powder; odourless.

Solubility: Freely soluble in ethanol (95%), in chloroform and in ether; very slightly soluble in water. It is soluble in dilute acids.

Storage: Store in well-closed, light-resistant containers.

Standards

Benzocaine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_9H_{11}NO_2$, calculated with reference to the dried substance.

Identification Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A: The infra-red absorption spectrum, is concordant with the reference spectrum of benzocaine or with the spectrum obtained from benzocaine RS.

B: Dissolve 10 mg in 1 ml of water with the aid of one drop of dilute hydrochloric acid and add 2 drops of a 10% w/v solution of sodium nitrite and 2 drops of a solution of 10 mg of 2-naphthol in 5 ml of sodium hydroxide solution; a deep red colour is produced. On setting aside the solution for some time, a scarlet precipitate is produced.

C: Dissolve 0.2 g in 10 ml of water with the aid of dilute hydrochloric acid (solution A) and divide into 2 parts. To one part of solution A add iodine solution; a precipitate is obtained (distinction from orthocaine).

D: To the other part of solution A add potassium mercuri-iodide solution; no precipitate is obtained (distinction from procaine).

Melting range: Between 88º and 92º, **Acidity or alkalinity:** Dissolve 0.5 g in 5 ml of ethanol (95%), add 10 ml of water and one drop of phenolphthalein solution; no pink colour is produced. Add 0.5 ml of 0.01 M sodium hydroxide; the solution develops a pink colour.

Clarity and colour of solution: A 5.0% w/v solution in ethanol (95%) is clear, and colourless,

Heavy metals: Not more than 10 ppm, determined on 2.0 g by Method B,

Chloride: Dissolve 0.2 g in 5 ml of ethanol (95%) previously acidified with a few drops of dilute nitric acid and add few drops of silver nitrate solution; no turbidity is produced immediately.

Sulphated ash: Not more than 0.1%.

Loss on drying: Not more than 0.5%, determined on 1 g by drying over phosphorus pentoxide at a pressure of 1.5 to 2.5 kPa,

Assay: Weigh accurately about 0.4 g and dissolve in a mixture of 25 ml of hydrochloric acid and 50 ml of water. Cool to 10º and carry out the nitrite titration. Each ml of 0.1M sodium nitrite is equivalent to 0.01652 g of $C_9H_{11}NO_2$.

Experiment No: 15

Indomethacin

$C_{19}H_{16}ClNO_4$ Mol. Wt. 357.8 Indomethacin is 1-(4-chlorobenzoyl)-5-methoxy-2- methylindol-3-yl acetic acid. Indomethacin contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{16}ClNO_4$, calculated on the dried basis.

Description. A white to pale yellow, crystalline powder; odourless or almost odourless.

Identification: Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out. A. Determine by infrared absorption spectrophotometry.

Compare the spectrum with that obtained with indomethacin RS or with the reference spectrum of indomethacin. Examine the substances in the solid state without recrystallisation. B. When examined in the range 230 nm to 360 nm, a 0.0025 per cent w/v solution in a mixture of 90 volumes of methanol and 10 volumes of 1 M hydrochloric acid shows an absorption maximum only at about 320 nm; absorbance at about 320 nm, about 0.45. C. Dissolve 0.1 g in 10 ml of ethanol (95 per cent), heating gently if necessary. To 0.1 ml add 2 ml of a freshly prepared mixture of 1 volume of a 25 per cent w/v solution of hydroxylamine hydrochloride and 3 volumes of 2 M sodium hydroxide. Add 2 ml of 2 M hydrochloric acid and 1 ml of ferric chloride solution and mix; a violet-pink colour develops.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with a suspension of silica gel HF254 in a 4.68 per cent w/v solution of sodium dihydrogen phosphate.

Mobile phase. A mixture of 70 volumes of ether and 30 volumes of light petroleum (50º to 70º).

Prepare the following solutions immediately before use. Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of methanol. Reference solution. A 0.01 per cent w/v solution of the substance under examination in methanol. Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals: 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash: Not more than 0.1 per cent.

Loss on drying: Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.45 g, dissolve in 75 ml of acetone and titrate under nitrogen with carbonate-free 0.1 M sodium hydroxide using 0.2 ml of phenolphthalein solution as indicator. Carry out a blank titration. 1 ml of 0.1 M sodium hydroxide is equivalent to 0.03578 g of $C_{19}H_{16}ClNO_4$.

Storage. Store protected from light.

Experiment No: 16

Diclofenac Sodium

$C_{14}H_{10}C_{l2}NNaO_2$ Mol. Wt. 318.1 Diclofenac Sodium is sodium 2-[(2,6-dichlorophenyl)- amino] phenylacetate. Diclofenac Sodium contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{14}H_{10}C_{l2}NNaO_{2,}$ calculated on the dried basis.

Description. A white to slightly yellowish crystalline powder; slightly hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diclofenac sodium RS or with the reference spectrum of diclofenac sodium. B. To 1 ml of a 0.4 per cent w/v solution in methanol add 1 ml of nitric acid; a dark red colour develops. C. In the

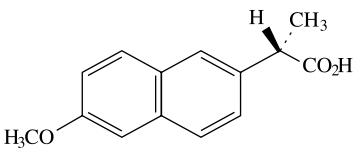
test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution. C. A 1 per cent w/v solution gives the reaction of sodium salts.

Tests

Appearance of solution. A 5.0 per cent w/v solution in methanol is clear, and not more intensely coloured than reference solution BYS6.
pH. 6.5–8.5, determined on a 1.0 per cent w/v solution.
Light absorption. Absorbance of a 5.0 per cent w/v solution in methanol at about 440 nm, not more than 0.050.
Related substances. Determine by liquid chromatography. Test solution. Dissolve 50 mg of the substance under examination in methanol and dilute to 50 ml with the same solvent. Reference solution. Dilute 2 ml of the test solution to 100 ml with methanol. Dilute 1 ml of this solution to 10 ml with methanol. Chromatographic system – a stainless steel column 25 cm × 4.6 mm, packed with end-capped octylsilyl silica gel (5 µm), – mobile phase: a mixture of 34 volumes of a solution containing 0.5 g per litre of phosphoric acid and 0.8 g per litre of sodium dihydrogen phosphate adjusted to pH 2.5 with phosphoric acid, and 66 volumes of methanol, – flow rate. 1 ml per minute, – spectrophotometer set at 254 nm, – a 20 µl loop injector. Inject the test solution and the reference solution. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent); the sum of the areas of all peaks other than the principal peak is not greater than 2.5 times that of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution.
Heavy metals. 1.0 g complies with the limit test for heavy metals, Method B (10 ppm).
Loss on drying. Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105º for 3 hours.
Assay. Weigh accurately about 0.2 g and dissolve in 50 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically. Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.03181 g of $C_{14}H_{10}C_{l2}NNaO_2$.
Storage. Store protected from light.

Experiment No: 17

Naproxen



$C_{14}H_{14}O_3$ M.Wt: 230.3
Action and use: Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.
Preparations:

- Naproxen Oral Suspension
- Naproxen Suppositories
- Naproxen Tablets
- Gastro-resistant Naproxen Tablets

Definition: (2S)-2-(6-Methoxynaphthalen-2-yl) propanoic acid.
Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: White or almost white, crystalline powder.
Solubility: Practically insoluble in water, soluble in ethanol (96 per cent) and in methanol.

Identification

First identification A, D.

Second identification A, B, C.

A. Specific optical rotation: + 59 to + 62 (dried substance). Dissolve 0.50 g in ethanol (96 per cent) R and dilute to 25.0 ml with the same solvent.
B. Melting point: 154–158 ºC.
C. Dissolve 40.0 mg in methanol R and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with methanol R. Examined between 230 nm and 350 nm (2.2.25), the solution shows 4 absorption maxima, at 262 nm, 271 nm, 316 nm and 331 nm. The specific absorbances at the absorption maxima are 216–238, 219–241, 61–69 and 79–87, respectively.
D. Infrared absorption spectrophotometry. Comparisonınaproxen CRS.

Tests

Appearance of solution

The solution is clear and not more intensely coloured than reference solution BY7.

Dissolve 1.25 g in methanol R and dilute to 25 ml with the same solvent.

Enantiomeric purity

Liquid chromatography. Protect the solutions from light. Test solutionıDissolve 25.0 mg of the substance to be examined in tetrahydrofuran R and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 2.5 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of racemic naproxen CRS in 10 ml of tetrahydrofuran R and dilute to 100 ml with the mobile phase.

Column

- size: l = 0.25 m, Ø = 4.6 mm;
- stationary phase: silica gel G- temperature: 25 ºC.

Mobile phaseıglacial acetic acid R, acetonitrile R, 2-propanol R, hexane R (5:50:100:845 V/ V/V/V).

Flow rate 2 ml/min.

Detection Spectrophotometer at 263 nm.

Injection 20 µl.

Run time 1.5 times the retention time of naproxen (retention time = about 5 min).

System suitabilityıReference solution (b):

- resolution: minimum 3 between the peaks due to impurity G and naproxen.

Limit

- impurity G: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent).

Related substances

Liquid chromatography. Protect the solutions from light.

Test solution Dissolve 12 mg of the substance to be examined in the mobile phase and dilute to 20 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 6 mg of bromomethoxynaphthalene R (impurity N), 6 mg of 1-(6-methoxynaphthalen-2-yl) ethanone R (impurity L) and 6 mg of (1RS)-1-(6- methoxynaphthalen-2-yl) ethanol R (impurity K) in acetonitrile R and dilute to 10 ml with the same solvent. To 1 ml of this solution add 1 ml of the test solution and dilute to 50 ml with the mobile phase. Dilute 1 ml of this solution to 20 ml with the mobile phase.

Column

- *size:* l = 0.10 m, Ø = 4.0 mm;
- *stationary phase:* octadecylsilyl silica gel for chromatography R (3 µm);
- *temperature:* 50ºC.

Mobile phase Mix 42 volumes of acetonitrile R and 58 volumes of a 1.36 g/l solution of potassium dihydrogen phosphate R previously adjusted to pH 2.0 with phosphoric acid R. Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 1.5 times the retention time of impurity N.

Relative retention With reference to naproxen (retention time = about 2.5 min): impurity K = about 0.9; impurity L = about 1.4; impurity N = about 5.3.

System suitability Reference solution (b):

- resolution: minimum 2.2 between the peaks due to impurity K and naproxen.

Limits

- impurity L: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- unspecified impurities: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- total: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent); disregard limit: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the reference solution using 2 ml of lead standard solution (10 ppm Pb) R.

Loss on drying Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105ºC for 3 h.

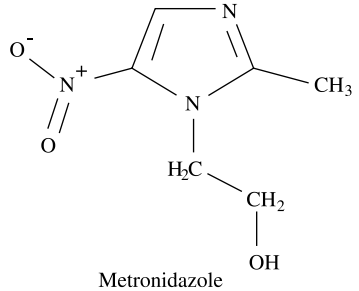
Sulphated ash Maximum 0.1 per cent, determined on 1.0 g.

Assay

Dissolve 0.200 g in a mixture of 25 ml of water and 75 ml of methanol . Titrate with 0.1 M sodium hydroxide, using 1 ml of phenolphthalein solution as indicator. 1 ml of 0.1 M sodium hydroxide is equivalent to 23.03 mg of $C_{14}H_{14}O_3$.

Storage Protected from light.

Experiment No: 18



Metronidazole

Metronidazole

$C_6H_9N_3O_3$ Mol. Wt. 171.2

Metronidazole is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol. Metronidazole contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_9N_3O_3$, calculated on the dried basis.

Description. A white or yellowish, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out. A. Determine by infrared absorption spectrophotometry. Compare the spectrum with that obtained with metronidazole RS or with the reference spectrum of metronidazole. B. When examined in the range 230 nm to 360 nm, a 0.001 per cent w/v solution in 0.1 M hydrochloric acid shows an absorption maximum at about 277 nm and a minimum at about 240 nm; absorbance at about 277 nm, between 0.365 and 0.395. C. Heat about 10 mg in a water-bath with 10 mg of zinc powder, 1 ml of water and 0.25 ml of 2 M hydrochloric acid for 5 minutes and cool. The solution gives the reaction of primary aromatic amines.

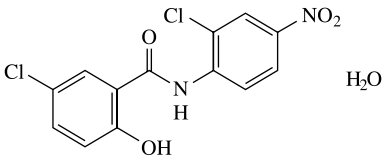
Tests

Appearance of solution. A 5.0 per cent w/v solution in 1 M hydrochloric acid is not more opalescent than opalescence standard OS_2, and not more intensely coloured than reference solution GYS_4.
Related substances. Determine by thin-layer chromatography, coating the plate with silica gel GF254. Mobile phase. A mixture of 80 volumes of chloroform, 10 volumes of diethylamine, 10 volumes of ethanol (95 per cent) and 1 volume of water. Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of acetone. Reference solution. A 0.003 per cent w/v solution of the substance under examination in acetone. Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.
Heavy metals. 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).
Sulphated ash. Not more than 0.1 per cent.
Loss on drying. Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.
Assay. Weigh accurately about 0.15 g, dissolve in 50 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically. Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.01712 g of $C_6H_9N_3O_3$.
Storage. Store protected from light and moisture.

Experiment No: 19

Niclosamide

Anhydrous Niclosamide
$C_{13}H_8C_{l2}N_2O_4$ Mol. Wt. 327.1



Niclosamide is 2′, 5-dichloro-4′-nitrosalicylanilide. Niclosamide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{13}H_8C_{l2}N_2O_4$, calculated on the dried basis.
Description. A yellowish white to yellowish, fine crystals or powder; odourless.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out. A. Determine by infrared absorption spectrophotometry. Compare the spectrum with that obtained with niclosamide RS or with the reference spectrum of niclosamide. B. Heat 50 mg with 5 ml of 1 M hydrochloric acid and 0.1 g of zinc powder in a water-bath for 10 minutes, cool and filter. To the filtrate add 1 ml of a 0.5 per cent w/v solution of sodium nitrite and allow to stand for 3 minutes. Add 2 ml of a 2 per cent w/v solution of ammonium sulphamate, shake, allow to stand for 3 minutes and add 2 ml of a 0.5 per cent w/v solutionof N- (1-naphthyl) ethylenediamine dihydrochloride; a violet colour is produced. C. Heat the substance under examination on a copper wire in a non-luminous flame; a green colour is imparted to the flame.

Tests

Chlorides. To 2.0 g add a mixture of 40 ml of water and 1.2 ml of 5 M acetic acid, boil for 2 minutes, cool and filter; 10 ml of the filtrate diluted to 15 ml with water complies with the limit test for chlorides (500 ppm). **2-Chloro-4-nitroaniline**. Not more than 100 ppm, determined by the following method. Boil 0.25 g with 5 ml of methanol, cool, add 45 ml of 1 M hydrochloric acid, heat to boiling, cool, filter and dilute the filtrate to 50.0 ml with 1 M hydrochloric acid. To 10.0 ml of this solution add 0.5 ml of a 0.5 per cent w/v solution of sodium nitrite and allow to stand for 3 minutes. Add 1.0 ml of a 2 per cent w/v solution of ammonium sulphamate, shake, allow to stand for 3 minutes and add 1.0 ml of a 0.5

per cent w/v solution of N- (1-naphthyl) ethylenediamine dihydrochloride. Any pinkish violet colour produced is not more intense than that obtained in a solution prepared at the same time and in the same manner using 10.0 ml of a solution prepared by diluting 2.0 ml of a0.00050 per cent w/v solution of 2-chloro-4-nitroaniline in methanol to 20 ml with 1 M hydrochloric acid and beginning at the words "add 0.5 ml of a 0.5 per cent w/v solution of sodium nitrite.....". **5-Chlorosalicylic acid.** Not more than 60 ppm, determined by the following method. Boil 1.0 g with 15 ml of water for 2 minutes, cool, filter through a membrane filter (pore size 0.45 μm), wash the filter and dilute the combined filtrate and washings to 20 ml with water (solution A). Dissolve 30 mg of 5-chlorosalicylic acid in 20 ml of methanol and add sufficient water to produce 100.0 ml. Dilute 1.0 ml of this solution to 100.0 ml with water (solution B). To 10.0 ml of each of solutions A and B add separately 0.1 ml of ferric chloride solution; any violet colour produced in solution A is not more intense than that obtained in solution B.
Sulphated ash. Not more than 0.1 per cent.
Loss on drying. Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105º for 4 hours.
Assay. Weigh accurately about 0.3 g, dissolve in 80 ml of a mixture of equal volumes of acetone and methanol. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically. Carry out a blank titration. 1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.03271 g of $C_{13}H_8C_{12}N_2O_4$.
Storage. Store protected from light and moisture.

Experiment No: 20

Aciclovir

$C_8H_{11}N_5O_3$ Mol. Wt. 225.2 Aciclovir is 2-amino-9-[2-hydroxyethoxy)methyl]-1,9-dihydro-6H-purin-6-one.Aciclovir contains not less than 98.5 per cent and not more than 101.0 per cent of $C_8H_{11}N_5O_3$, calculated on the anhydrous basis.
Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry, Compare the spectrum with that obtained with aciclovir RS.

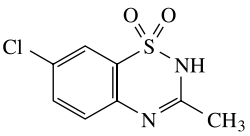
Tests

Appearance of solution. A 1.0 per cent w/v solution in 0.1 M sodium hydroxide is clear (2.4.1), and not more intensely coloured than reference solution YS7.
Related substances. Determine by thin-layer chromatography, coating the plate with silica gel GF254. Mobile phase. A mixture of 80 volumes of dichloromethane, 20 volumes of methanol and 2 volumes of strong ammonia solution. Prepare the following solutions immediately before use. Test solution. Dissolve 1.0 g of the substance under examination in 100 ml of dimethyl sulphoxide. Reference solution. A 0.005 per cent w/v solution of acyclovir impurity RS in dimethyl sulphoxide. Apply to the plate 10 μl of each solution. Keep the spots compact by drying in a current of warm air and allow the plate to cool. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot with Rf value greater than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).
Sulphated ash : Not more than 0.1 per cent.
Water. Not more than 6.0 per cent, determined on 0.5 g.

Assay. Weigh accurately about 0.15 g and dissolve in 60 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02252 g of $C_8H_{11}N_5O_3$.
Storage. Store protected from light and moisture.

Experiment No: 21

Diazoxide



$C_8H_7ClN_2O_2S$ M.Wt: 230.7
Action and use: Vasodilator; Treatment of hypertension.
Preparations: Diazoxide Injection, Diazoxide Tablets
Definition: Diazoxide contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 7-chloro-3-methyl-2H-, 2, 4-benzothiadiazine 1, 1-dioxide, calculated with reference to the dried substance.
Characters: A white or almost white, fine or crystalline powder, practically insoluble in water, freely soluble in dimethylformamide, slightly soluble in alcohol. It is very soluble in dilute solutions of the alkali hydroxides.

Identification

First identification B.
Second identification A, C, D.

A. Dissolve 50.0 mg in 5 ml of 1 M sodium hydroxide and dilute to 50.0 ml with water R. Dilute 1.0 ml of this solution to 100.0 ml with 0.1 M sodium hydroxide. Examined between 230 nm and 350 nm, the solution shows an absorption maximum at 280 nm and a shoulder at 304 nm. The specific absorbance at the maximum is 570–610.
B. Examine by infrared absorption spectrophotometry, comparing with the spectrumobtained with diazoxide CRS. Examine the substances prepared as discs using potassium bromide R.
C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (b).
D. Dissolve about 20 mg in a mixture of 5 ml of hydrochloric acid R and 10 ml of water R. Add 0.1 g of zinc powder R. Boil for 5 min, cool and filter. To the filtrate add 2 ml of a 1 g/l solution of sodium nitrite R and mix. Allow to stand for 1 min and add 1 ml of a 5 g/l solution of naphthylethylenediamine dihydrochloride R. A red or violet-red colour develops.

Tests

Appearance of solution

Dissolve 0.4 g in 2 ml of 1 M sodium hydroxide and dilute to 20 ml with water R. The solution is clear and not more intensely coloured than reference solution Y7.

Acidity or alkalinity

To 0.5 g of the powdered substance to be examined add 30 ml of carbon dioxide-free water R, shake for 2 min and filter. To 10 ml of the filtrate add 0.2 ml of 0.01 M sodium hydroxide and 0.15 ml of methyl red solution R. The solution is yellow. Not more than 0.4 ml of 0.01 M hydrochloric acid is required to change the colour of the indicator to red.

Related substances

Examine by thin-layer chromatography, using silica gel GF254 R as the coating substance.

Test solution (a) Dissolve 0.1 g of the substance to be examined in a mixture of 0.5 ml of 1 M sodium hydroxide and 1 ml of methanol R and dilute to 5 ml with methanol R. Test solution (b) Dilute

1 ml of test solution (a) to 5 ml with a mixture of 1 volume of 1 M sodium hydroxide and 9 volumes of methanol R. Reference solution (a) Dilute 0.5 ml of test solution (a) to 100 ml with a mixture of 1 volume of 1 M sodium hydroxide and 9 volumes of methanol R. Reference solution (b) Dissolve 20 mg of diazoxide CRS in a mixture of 0.5 ml of 1 M sodium hydroxide and 1 ml of methanol R and dilute to 5 ml with methanol R. Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 7 volumes of concentrated ammonia R, 25 volumes of methanol R and 68 volumes of chloroform R. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Loss on drying: Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash: Not more than 0.1 per cent, determined on 1.0 g.

Assay: Dissolve 0.200 g with gentle heating in 50 ml of a mixture of 1 volume of water R and 2 volumes of dimethylformamide R. Titrate with 0.1 M sodium hydroxide, determining the endpoint potentiometrically. Carry out a blank titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 23.07 mg of $C_8H_7ClN_2O_2S$.

Experiment No: 22

Busulphan

$C_6H_{14}O_6S_2$ Mol. Wt. 246.29 Busulphan is 1, 4–butanediol dimethanesulphonate.

Category: Cytotoxic.

Dose: 2–4 mg daily; maintenance dose, 0.5–2 mg daily.

Description: White or almost white, crystalline powder.

Solubility: Freely soluble in acetone, in chloroform, and in acetonitrile; very slightly soluble in water, in ethanol (95%) and in ether.

Storage: Store in tightly-closed, light-resistant containers.

Standards

Busulphan contains not less than 99.0 per cent and not more than 100.5 per cent of $C_6H_{14}O_6S_2$, calculated with reference to the dried substance.

Identification: Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A: The infra-red absorption spectrum, is concordant with the reference spectrum of busulphan or with the spectrum obtained from busulphan RS.

B: Carry out the method for thin-layer chromatography, using silica gel G as the coating substance and a mixture of equal volumes of acetone and toluene as the mobile phase. Apply separately to the plate 5 l of each of two solutions in acetone containing (1) 1.0% w/v of the substance being examined and (2) 1.0% w/v of busulphan RS. After removal of the plate, dry it in a current of hot air, spray with anisaldehyde solution and heat at 120˚. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C: Heat 0.1 g with 5 ml of 1M sodium hydroxide until a clear solution is obtained and allow to cool. To 2 ml of the solution add 0.1 ml of a 3% w/v solution of potassium permanganate; the purple colour changes to violet, then to blue and finally to green. Filter and add 1 ml of ammoniacal silver nitrate solution; a precipitate is produced.

D: Fuse 0.1 g with 0.1 g of potassium nitrate and 0.25 g of potassium hydroxide, cool and dissolve the residue in 5 ml of water. Acidify with dilute hydrochloric acid and add a few drops of barium chloride solution; a white precipitate is produced.

E: Melts at about 116º.

Acidity: Dissolve 0.2 g in 50 ml of warm ethanol previously neutralised to methyl red solution and titrate with 0.1M sodium hydroxide using methyl red solution as indicator; not more than 0.05 ml of 0.1 M sodium hydroxide is required.

Clarity and colour of solution: Dissolve 0.25 g in 20 ml of acetonitrile, dilute to 25 ml with water and examine immediately. The solution is clear, and not more intensely coloured than reference solution BS_6,

Sulphated ash: Not more than 0.1%,

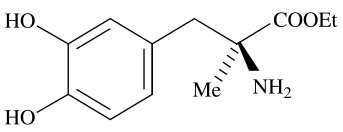
Loss on drying: Not more than 2.0%, determined on 1 g by drying over phosphorus pentoxide at 60º at a pressure of 1.5 to 2.5 kPa.

Assay: Weigh accurately about 0.25 g and shake with 50 ml of water. Boil under a reflux condenser for 30 minutes and, if necessary, restore the initial volume with water. Allow to cool and titrate with 0.1 M sodium hydroxide, using 0.3 ml of dilute phenolphthalein solution as indicator, until a pink colour is produced. Each ml of 0.1M sodium hydroxide is equivalent to 0.01232 g of $C_6H_{14}O_6S_2$

Experiment No: 23

Methyldopa

$C_{10}H_{13}NO_4$, 1½ H_2O Mol. Wt. 238.2



,HCl

Methyldopa is 3-(3,4-dihydroxyphenyl)-2-methyl-L-alanine sesquihydrate. Methyldopa contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{10}H_{13}NO_4$, calculated on the anhydrous basis.

Description. A white to yellowish white, fine powder which may contain friable lumps.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out. A. Determine by infrared absorption spectrophotometry. Compare the spectrum with that obtained with methyldopa RS or with the reference spectrum of methyldopa. B. When examined in the range 230 nm to 360 nm, a0.004 per cent w/v solution in 0.1 M hydrochloric acid shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.46. C. Determine by thin-layer chromatography, coating the plate with microcrystalline cellulose. Mobile phase. A mixture of 50 volumes of 1-butanol, 25 volumes of glacial acetic acid and 25 volumes of water. Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of 1 M hydrochloric acid. Reference solution. A 1 per cent w/v solution of methyldopa RS in 1 M hydrochloric acid. Apply to the plate 5 µl of each solution. After development, dry the plate in a current of warm air, and spray with a solution freshly prepared by mixing equal volumes of a 10 per cent w/ v solution of ferric chloride and a 5 per cent w/v solution of potassium ferricyanide. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. D. To 10 mg add 3 drops of a 0.4 per cent w/v solution of ninhydrin in sulphuric acid; a dark purple colour is produced within 5 to 10 minutes. Add 0.15 ml of water; the colour changes to pale brownish yellow.

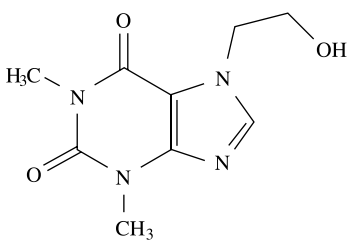
Tests

Appearance of solution. A 4.0 per cent w/v solution in 1 M hydrochloric acid is not more intensely coloured than reference solution BYS6 or BS6.

Acidity. Dissolve 1.0 g in 100 ml of carbon dioxide-free water with the aid of heat, add 0.15 ml of methyl red solution and titrate with 0.1 M sodium hydroxide; not more than 0.5 ml is required to produce a pure yellow colour.
Optical rotation. –1.10º to –1.23º, determined in a solution prepared by dissolving a quantity containing 2.2 g of the anhydrous substance in 50.0 ml of aluminium chloride solution.
3-Methoxy compound and related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose. Mobile phase. A mixture of 65 volumes of 1-butanol, 25 volumes of water and 15 volumes of glacial acetic acid. Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of a mixture of 96 volumes of methanol and 4 volumes of 7 M hydrochloric acid. Reference solution (a). A 0.005 per cent w/v solution of 3-methoxymethyldopa RS in methanol. Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a). Apply to the plate 10 µl of each of the test solution and reference solution (a) and 20 µl of reference solution (b). After development, dry the plate immediately in a current of warm air and spray with a mixture of 5 volumes of a 5 per cent w/v solution of sodium nitrite and 45 volumes of a 0.3 per cent w/v solution of 4-nitroaniline in a mixture of 80 volumes of hydrochloric acid and 20 volumes of water. Dry it in a current of warm air and spray with a 20 per cent w/v solution of sodium carbonate and examine immediately. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.
Heavy metals. Dissolve 2.0 g in 10 ml of water, add 2 ml of dilute acetic acid and dilute to 25 ml with water. The solution complies with the limit test for heavy metals, Method B (10 ppm).
Sulphated ash. Not more than 0.1 per cent.
Water. 10.0–13.0 per cent, determined on 0.4 g.
Assay. Weigh accurately about 0.4 g and dissolve in 15 ml of anhydrous formic acid, 30 ml of anhydrous glacial acetic acid and 30 ml of dioxan. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02112 g of $C_{10}H_{13}NO_4$.
Storage. Store protected from light and moisture.

Experiment No: 24

Etofylline



$C_9H_{12}N_4O_3$ M.Wt: 224.2
Action and use
Non-selective phosphodiestarase inhibitor (xanthine); treatment of reversible airways obstruction.
Definition: Etofylline contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 7-(2-hydroxyethyl)-1,3-dimethyl-3, 7-dihydro-1H-purine-2, 6-dione, calculated with reference to the dried substance.

Characters: A white or almost white, crystalline powder, soluble in water, slightly soluble in alcohol.

Identification First identificationıB, C. Second identification A, C, D.

A. Melting point: 161–166°C.
B. Examine by infrared absorption spectrophotometry , comparing with the spectrum obtained with etofylline CRS. Examine the substances as discs prepared using 0.5 mg to 1 mg of the substance to be examined in 0.3 g of potassium bromide R.
C. Dissolve 1 g in 5 ml of acetic anhydride R and boil under a reflux condenser for 15 min. Allow to cool and add 100 ml of a mixture of 20 volumes of ether R and 80 volumes of light petroleum

R. Cool in iced water for at least 20 min, shaking from time to time. Filter, wash the precipitate with a mixture of 20 volumes of ether R and 80 volumes of light petroleum R, recrystallise from alcohol R and dry in vacuo. The crystals melt at 101º–105ºC.

D. It gives the reaction of xanthines.

Tests

Solution S: Dissolve 2.5 g in carbon dioxide-free water R and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear and colourless.

Acidity or alkalinity

To 10 ml of solution S add 0.25 ml of bromothymol blue solution R1. The solution is yellow or green. Not more than 0.4 ml of 0.01 M sodium hydroxide is required to change the colour of the indicator to blue.

Related substances

Examine by thin-layer chromatography , using silica gel HF254 R as the coating substance. Test solutionıDissolve 0.3 g of the substance to be examined in a mixture of 20 volumes of water R and 30 volumes of methanol R and dilute to 10 ml with the same mixture of solvents. Prepare immediately before use. Reference solution (a) Dilute 1 ml of the test solution to 100 ml with methanol R. Reference solution (b) Dilute 0.2 ml of the test solution to 100 ml with methanol R. Reference solution (c) Dissolve 10 mg of theophylline R in methanol R, add 0.3 ml of the test solution and dilute to 10 ml with methanol R. Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of concentrated ammonia R, 10 volumes of ethanol R and 90 volumes of chloroform R. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides: Dilute 2.5 ml of solution S to 15 ml with water R. The solution complies with the limit test for chlorides (400 ppm).

Heavy metals: 12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard using lead standard solution (1 ppm Pb) R.

Loss on drying: Not more than 0.5 per cent,determined on 1.0g by drying in an oven at 105ºC.

Sulphated ash: Not more than 0.1 per cent, determined on 1.0 g.

Assay

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached. Dissolve 0.200 g in 3.0 ml of anhydrous formic acid R and add 50.0 ml of acetic anhydride R. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically. 1 ml of 0.1 M perchloric acid is equivalent to 22.42 mg of $C_9H_{12}N_4O_3$.

Storage: Store protected from light.

5

IDENTIFICATION AND ESTIMATION OF DRUG METABOLITES FROM BIOLOGICAL FLUIDS

Experiment No: 1

Estimation of Diphenyl Hydantoin in Blood or Urine

Aim: To estimate the amount of diphenyl hydantoin in blood or urine samples
Apparatus: graduated measuring cylinder, separator
Chemicals required: NaOH, $CHCl_3$, $KMnO_4$
Procedure: Place 10 ml of the sample in a separator and adjust the pH to about 6–7 by drop wise addition of 0 .5 M HCl. Add 100 ml of $CHCl_3$ and shake vigorously for 3 minutes. Filter through a phase separating paper into a glass stoppered graduated measuring cylinder and record the volume. Add 1molar 5 ml NaOH and shake thoroughly for 3minutes. Allow to separate and transfer the alkaline layer (4 ml) to a 250 ml RBF and evaporate to approx. 1 ml by means of a rotary evaporator at 50–60ºC under reduced pressure. Cool, add a solution of $KMnO_4$ in 1 M NaOH (1%, 20 ml), heptane (5 ml) and reflux the contents with stirring for 30 min. Cool and record the absorption spectra of the heptane layer over the region of 220–340 nm using a blank of heptane. Calculate the amount of diphenylhydantoin by reference to a calibration curve (0–20 micro grams/ml) prepared by treating aq. Solutions of the drug to identical procedures.

Report : The amount of diphenylhydantoin was found to be _____ mg.

Experiment No: 2

Estimation of Diphenhydramine by Acid dye Technique

Aim: To estimate the amount of diphenhydramine Ascorbic acid present in the given sample by acid dye technique.
Apparatus: graduated measuring cylinder, separator.
Chemicals required: NaOH, HCl

Procedure: To blood plasma (5 ml) add 0.1 M NaOH (5 ml) and extract with hexane (25 ml). Reject the lower aq. layer and extract the hexane layer with 2 M HCl (6 ml). Transfer the acid extract to the small separator and add 2 M NaOH (10 ml). Extract with the special chloroform solvent (10 ml) and reject the aq. Phase. Shake the organic layer with methyl orange solution (0.5 ml) centrifuge the organic layer to remove excess reagent. To the clear chloroform extract (5 ml) add acid alcohol (0.5 ml) and measure the absorbance at 535 nm. Prepare a calibration curve using standard solutions of diphenhydramine (0–10 μg/ml) treated as described for plasma.

Report : The amount of diphenhydramine was found to be _____ mg.

Experiment No: 3

Estimation of Barbiturate in Plsma or Urine

Aim: To estimate the amount of barbiturate present in the given sample.
Apparatus: graduated measuring cylinder, separator.
Chemicals required: NaOH, $CHCl_3$, H_2SO_4
Procedure: Transfer plasma or urine (5 ml) to a separator and add $CHCl_3$ (75 ml). Shake thoroughly and allow to separate. Filter the chloroform extract and transfer an aliquot of the filtrate (50–60 ml accurately measured) to a clean separator. Add 0.45 M NaOH (8 ml) and shake thoroughly. Run off the lower chloroform layer, transfer the alkaline solution to a centrifuge tube and centrifuge until clear. Dilute the clear supernatant solution (2 ml) with (a) 0.45 M NaOH (1 ml) (b) boric acid solution (1 ml) and (c) 1 msulphuric acid (1 ml) in 1 cm cells. Record the absorption spectra of each solution (225–300 nm) against an appropriate blank solution obtained by treating 5 ml water as described for plasma. Record also the difference absorption spectra of (1) the solution in 0.45 M NaOH (pH approx. 13.5) against the equimolar solution in borate buffer (pH 10.5) and (2) the solution in 0.45 M NaOH (pH approx. 13.5) against the equimolar solution in sulphuric acid (pH 0.5).

A positive ΔA around 260 nm and a negative ΔA around 240 nm in the difference spectrum of the pH 13.5 solution against the pH 10.5 solution is reasonable evidence of the presence of a barbiturate, because few other substances exhibit these spectral transformations between pH 13.5 and pH 10.5 for quantitative purposes the larger ΔA at the maximum around 255 nm in the difference spectrum of NaOH solution against the H_2SO_4 solution should be measured. The calculation of the concentration of the barbiturates in plasma is based upon the proportional relationship that exists between ΔA of the sample solution and that of std.solutions of the appropriate reference barbiturate (5 ml of a 20 μg/ml solution) carried through the procedure.

Report : The amount of barbiturate was found to be _____ mg.

6

DETERMINATION OF PARTITION COEFFICIENT OF COMPOUNDS FOR QSAR ANALYSIS

Experiment No: 1

Partition Coefficient for the Distribution of Iodine between Carbon Tetrachloride and Water

Aim: To determine the partition coefficient for distribution of iodine between CCl_4 and water.

Principle: When a solute is distributed between two solvents which are immiscible so as to form two separate layers in contact with each other, the ratio of the equilibrium concentrations of the solute in the two solvents (C_1/C_2) will be a constant at a given temperature. This constant is called the partition coefficient for that system.

Procedure: Take two stoppered 250 cm_3 iodine flasks A and B. Fill burette with a saturated solution of iodine in CCl_4 and transfer 30 cm^3 iodine solution into flask A and 25 cm^3 iodine solution into flask B. Add 5 cm_3 of pure CCl_4 into flask B only to make the volumes same. Then pour 150 cm^3 of water each into the two flasks. Put the stoppers and shake the flasks on a mechanized shaker for 5 minutes. Then let the bottles rest for 15 minutes so that the layers separate. While waiting, fill one burette with 0.1N thiosulphate solution. From this, transfer 10 cm^3 into the volumetric flask and make up to 100 cm^3 to get 0.01 N thiosulphate solution. Fill the second burette with this solution. From iodine flask A, pipette out 5 cm^3 of organic layer into a conical flask (organic layer should not be contaminated by the aqueous layer; close the upper tip of the pipette with your finger while inserting the bottom tip to the very bottom of the organic layer and only then remove your finger). Add 5 cm^3 of 10% KI solution and titrate against 0.1 N thiosulphate solution, without indicator till the colour becomes yellow, and continue using starch indicator till the blue colour just disappears. Pipette out 40 cm^3 of aqueous layer from the same bottle into the conical flask, (adding starch at the beginning itself since the colour is light) and titrate against 0.01 N thiosulphate solution. Repeat the two titrations for iodine flask B also as a duplicate. Calculate partition coefficient for each flask.

Calculation

Flask No.	Volume of 0.1N thio (V1)	Volume of 0.01N thio (V2)	Partition Coefficient K
A			
B			

Concentrations are proportional to volumes of thio used.
Since this is a ratio, volumes may be used directly.
Volume of 0.01N thio = iodine in 40 ml octanol = $V_1 \times 10 \times 8$ ml
Volume of 0.01N thio = iodine in 40 ml water = V_2 ml

$$K = \frac{V_1 \times 10 \times 8}{V_2}$$

Result: Partition coefficient of iodine between CCl_4 and water = (1) _____ (2) _____

Experiment No: 2

Partition Coefficient for the Distribution of Phenyl Butazone between Octanol and Water

Aim: To determine the partition coefficient for distribution of phenyl butazone between octanol and water.

Principle: When a solute is distributed between two solvents which are immiscible so as to form two separate layers in contact with each other, the ratio of the equilibrium concentrations of the solute in the two solvents (C1/C2) will be a constant at a given temperature. This constant is called the partition coefficient for that system.

Procedure: Take two stoppered 250 ml **iodine flasks A and B.** Fill **burette** with a saturated solution of phenyl butazone in octanol and transfer 30 ml phenyl butazone solution into flask A and 25 ml phenyl butazone solution into flask B. Add 5 ml of pure octanol into flask B only to make the volumes same. Then pour 150 ml of water each into the two flasks. Put the stoppers and shake the flasks on a mechanized shaker for 5 minutes. Then let the bottles rest for 15 minutes so that the layers separate. While waiting, fill one burette with 0.1 N NaOH solution. From this, transfer 10 ml into the volumetric flask and make up to 100 ml to get 0.01 N NaOH solution. Fill the second burette with this solution. From iodine flask A, pipette out 5 ml of organic layer into a conical flask (organic layer should not be contaminated by the aqueous layer; close the upper tip of the pipette with your finger while inserting the bottom tip to the very bottom of the organic layer and only then remove your finger). and titrate against 0.1 N NaOH solution by using bromothymol blue indicator. Pipette out 40 ml **of** aqueous layer from the same bottle into the conical flask, and titrate against 0.01 N NaOH solution. Repeat the two titrations for iodine flask B also as a duplicate. Calculate partition coefficient for each flask.

Calculation

Flask No.	Volume of 0.1N NaOH (V1)	Volume of 0.01N NaOH (V2)	Partition Coefficient K
A			
B			

Concentrations are proportional to volumes of NaOH used.
Since this is a ratio, volumes may be used directly.
Volume of 0.01 N NaOH = Phenyl butazone in 40 ml octanol = $V_1 \times 10 \times 8$ ml

$$K = \frac{V_1 \times 10 \times 8}{V_2}$$

Result: Partition coefficient of phenyl butazone between octanol and water = (1) _____ (2) _____

Experiment No: 3

Partition Coefficient for the Distribution of Methyldopa between Octanol and Water

Aim: To determine the partition coefficient for distribution of methyldopa **between octanol** and water.
Principle: When a solute is distributed between two solvents which are immiscible so as to form two separate layers in contact with each other, the ratio of the equilibrium concentrations of the solute in the two solvents (C_1/C_2) will be a constant at a given temperature. This constant is called the partition coefficient for that system.
Procedure: Take two stoppered 250 ml **iodine flasks A and B.** Fill burette with a saturated solution of methyldopa in octanol and transfer 30 ml methyldopa solution into flask A and 25 ml methyldopa solution into flask B. Add 5 ml of pure octanol into flask B only to make the volumes same. Then pour 150 ml of water each into the two flasks. Put the stoppers and shake the flasks on a mechanized shaker for 5 minutes. Then let the bottles rest for 15 minutes so that the layers separate. While waiting, fill one burette with 0.1 M perchloric acid solution. From this, transfer 10 ml into the volumetric flask and make up to 100 ml to get 0.01 M perchloric acid solution. Fill the second burette with this solution. From iodine flask A, pipette out 5 ml of organic layer into a conical flask (organic layer should not be contaminated by the aqueous layer; close the upper tip of the pipette with your finger while inserting the bottom tip to the very bottom of the organic layer and only then remove your finger), and titrate against 0.1 M perchloric acid solution by using crystal violet solution as indicator. Pipette out 40 ml **of** aqueous layer from the same bottle into the conical flask, and titrate against 0.01 M perchloric acid solution. Repeat the two titrations for iodine flask B also as a duplicate. Calculate partition coefficient for each flask.

Calculation

Flask No.	Volume of 0.1 M perchloric acid (V1)	Volume of 0.01 M perchloric acid (V2)	Partition Coefficient K
A			
B			

Concentrations are proportional to volumes of NaOH used.
Since this is a ratio, volumes may be used directly.
Volume of 0.01 M perchloric acid = methyldopa in 40 ml octanol = $V_1 \times 10 \times 8$ ml
Volume of 0.01 M perchloric acid = methyldopa in 40 ml water = V_2 ml

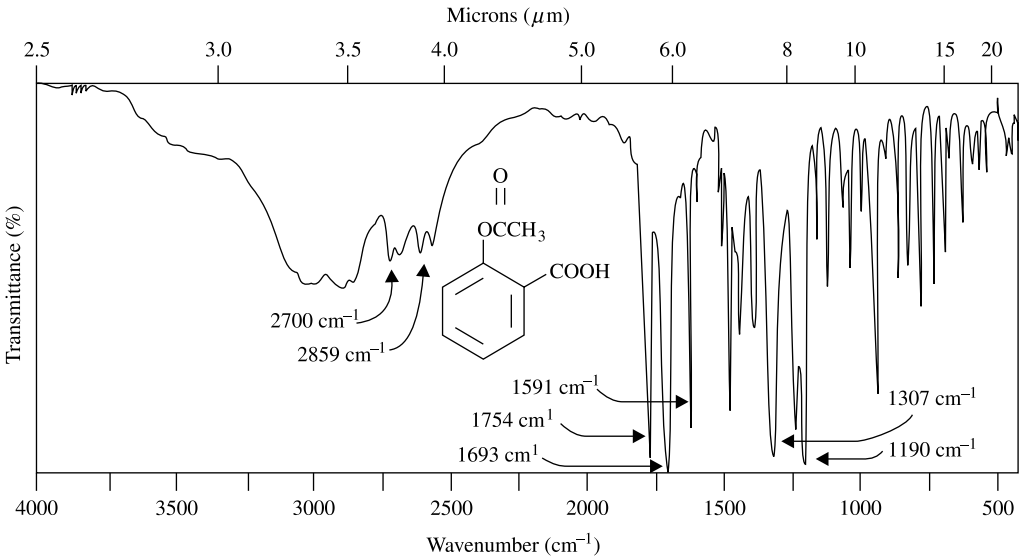
$$K = \frac{V_1 \times 10 \times 8}{V_2}$$

Result: Partition coefficient of methyldopa between octanol and water = (1) _____ (2) _____

7

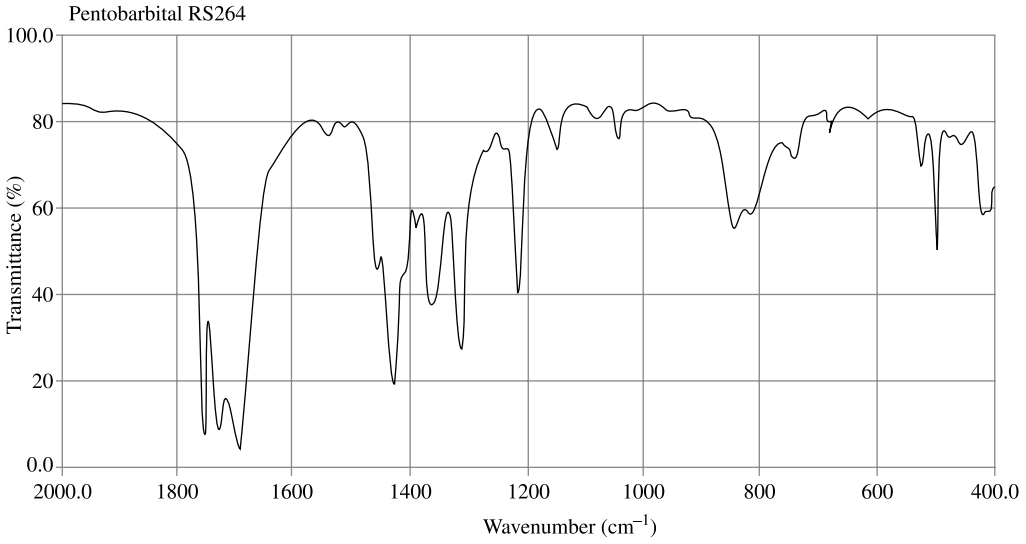
I.R SPECTRA OF SOME OFFICIAL MEDICINAL COMPOUNDS

1. Aspirin

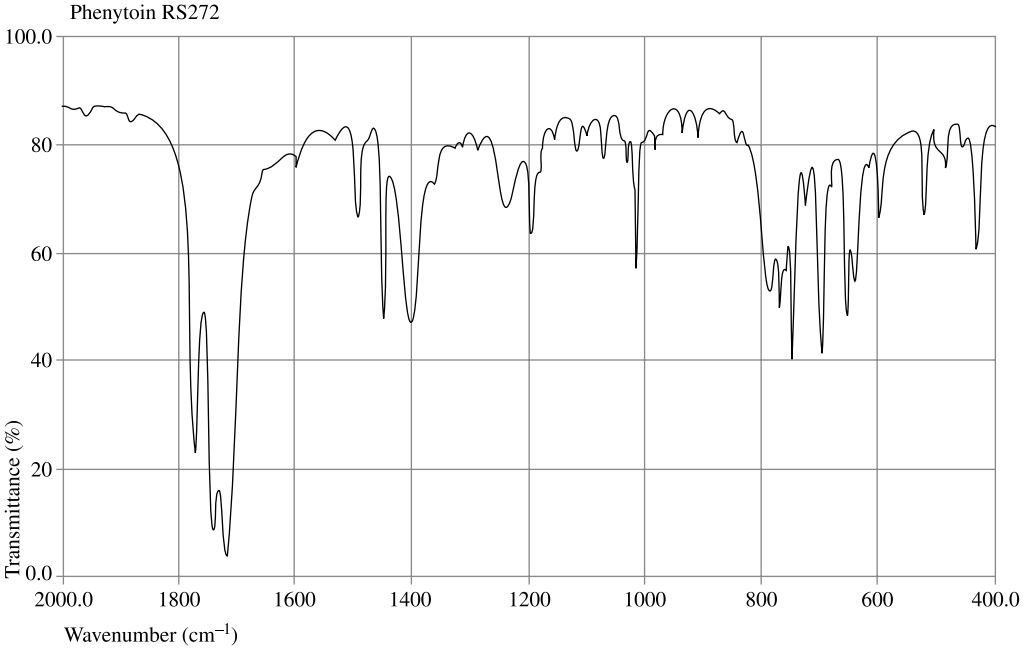


Infrared spectrum of acetylsalicylic acid (aspirin) ir $CHCl_3$.

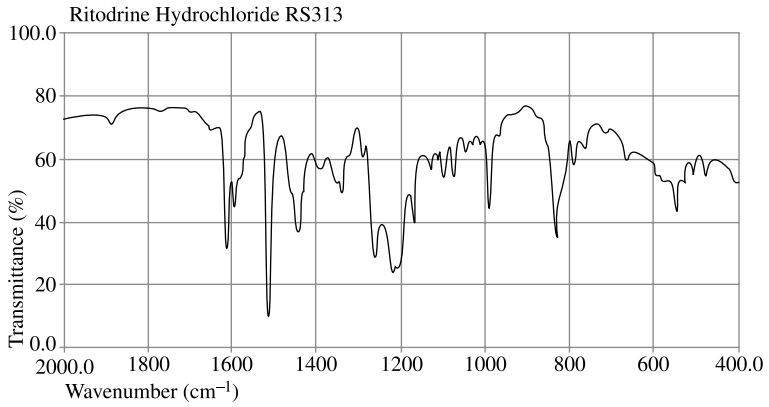
2. Pentobarbital



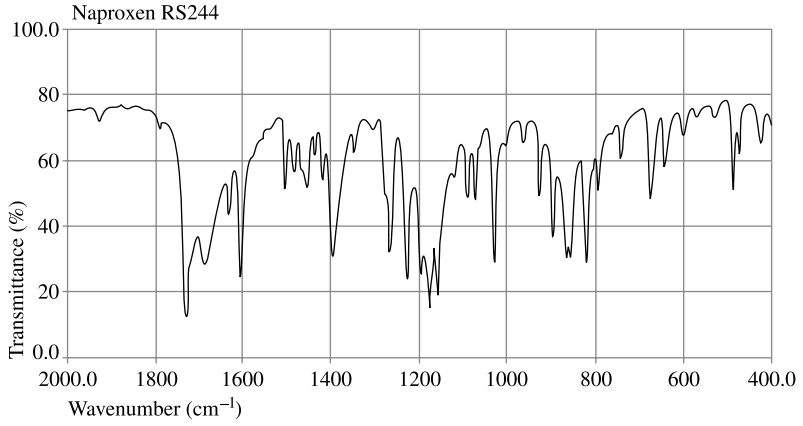
3.Phenytoin



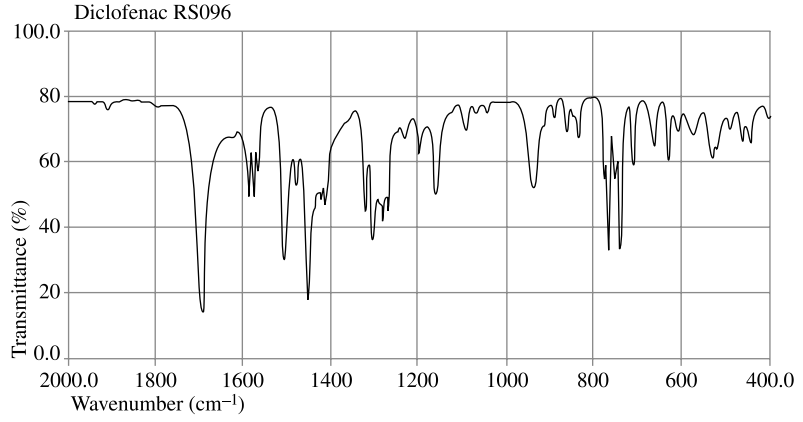
4.Ritodrine Hydrochloride



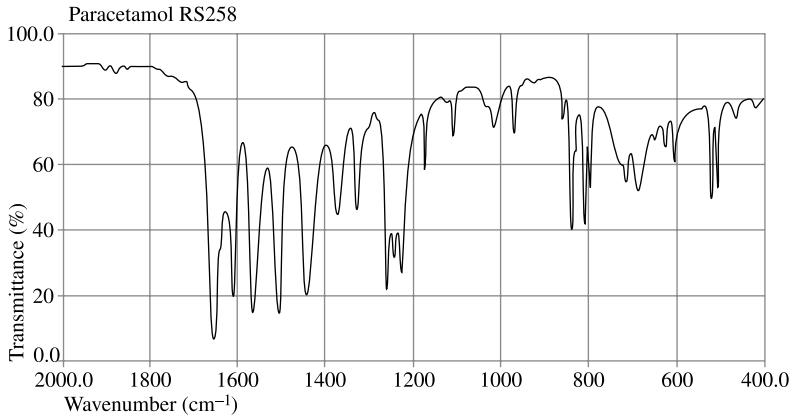
5. Naproxen



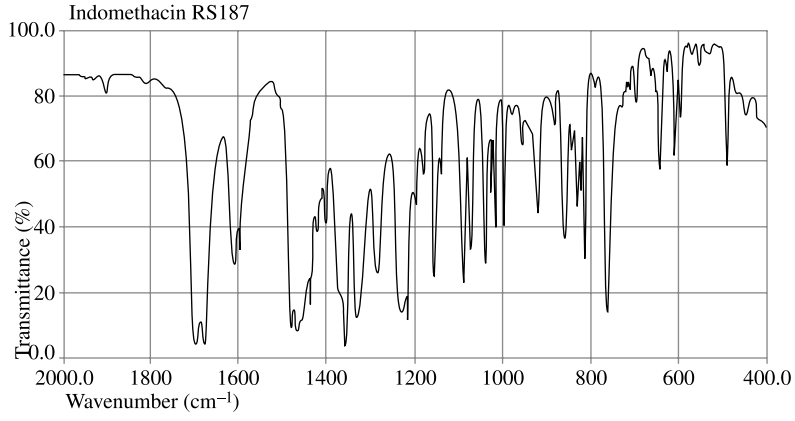
6. Diclofenac



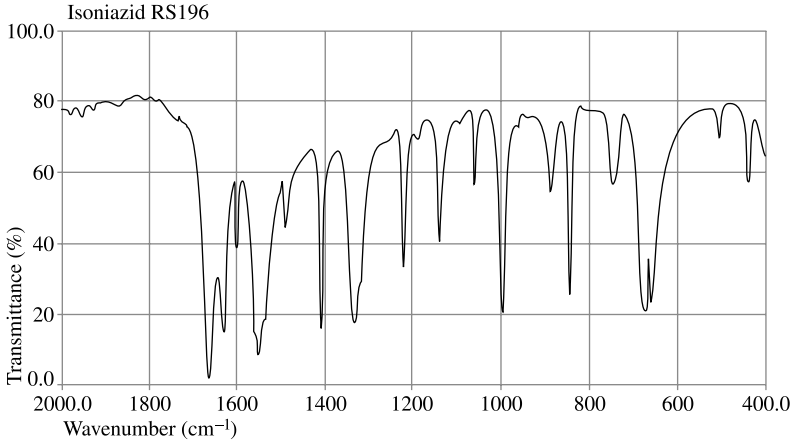
7. Paracetamol



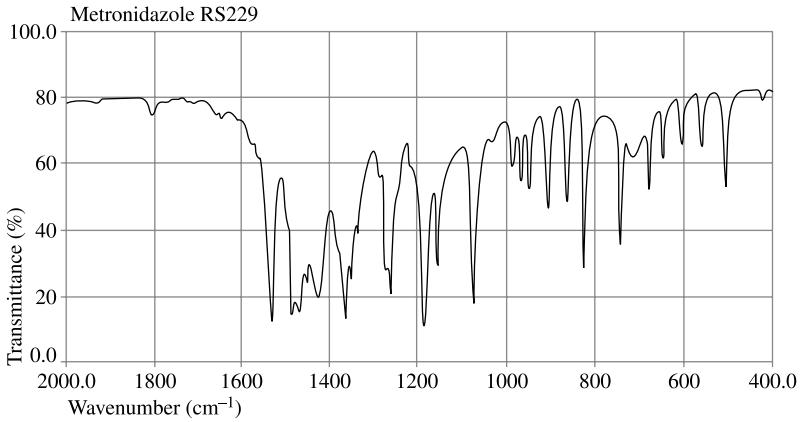
8. Indomethacin



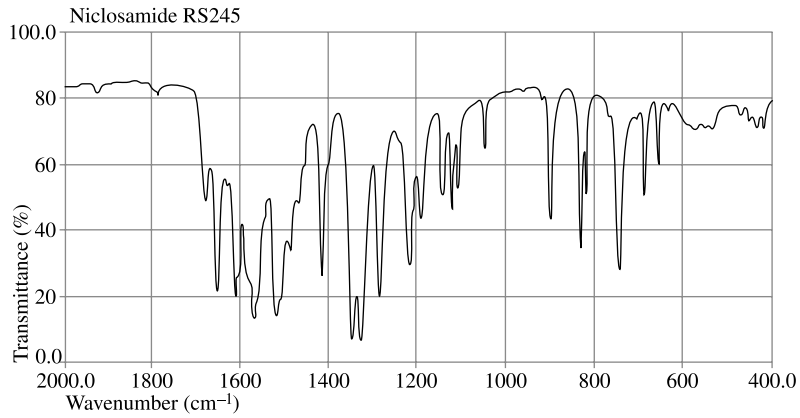
9. Isoniazid



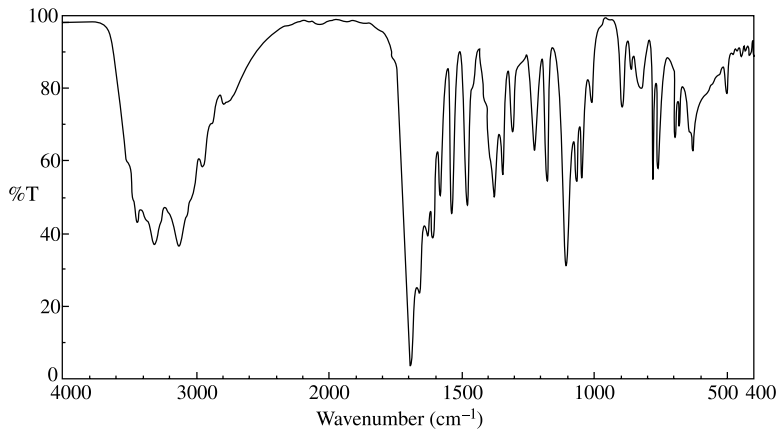
10.Metronidazole



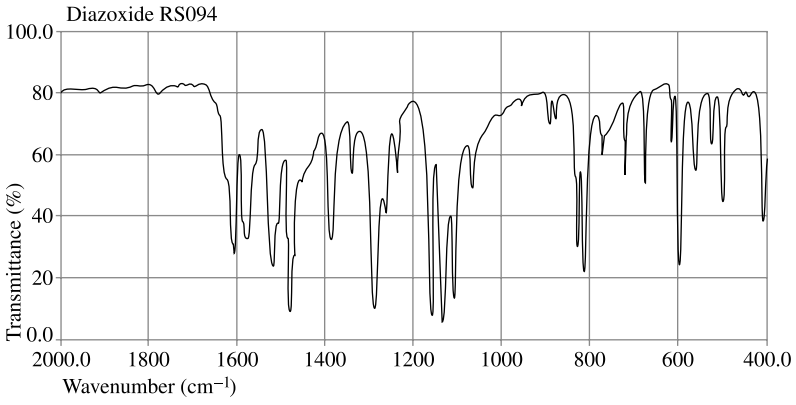
11.Niclosamide



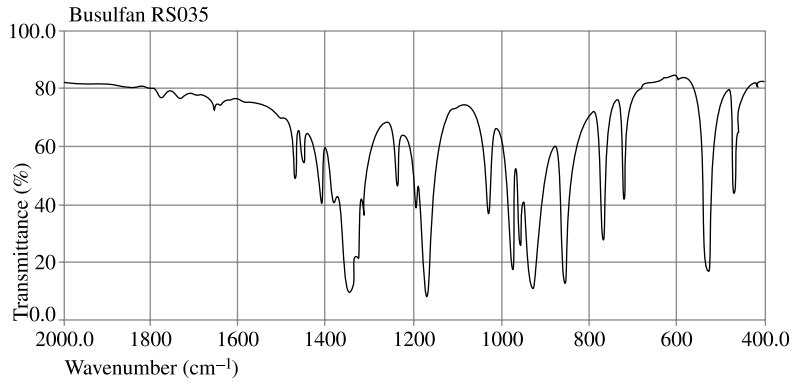
12.Acyclovir



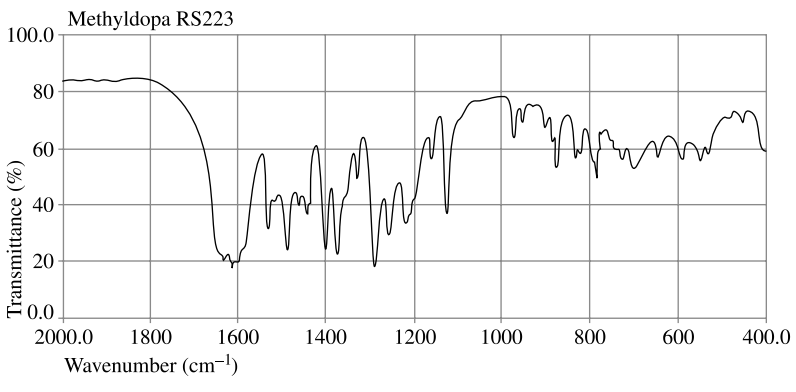
13.Diazoxide



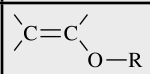
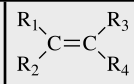
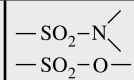
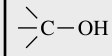
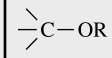
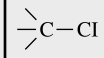
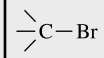
14. Busulfan



15 Methyldopa



Interpretation of ir Spectra

Functional group	Structure	V (cm^{-1})	Intensity
A mine		*3300 - 3500*	
Terminal acetylenes		*3300*	*strong*
Imines		*1480 -1690*	*weak to medium*
Enol ethers		*1600 -1660*	*strong*
Alkenes		*1640 - 1680*	*strong medium*
Nitro groups		*1500 - 1650* *1250 - 1400*	*strong*
Sulfoxides		*1010 - 1070*	*strong*
Sulfones		*1300 - 1350* *1100 - 1150*	*strong*
Sulfonamides and Sulfonate esters		*1140 - 1180* *1300 -1370*	*strong*
Alcohols		*1000 - 1260*	*strong*
Ethers		*1085 - 1150*	*strong*
Alky] fluorides		*1000 - 1400*	*strong*
Alkyl chlorides		*580 - 780*	*strong*
Alkyl bromides		*560 -800*	*strong*
Alkyl iodides		*500 - 600*	*strong*

Characteristic Infrared Bands of Aliphatic Hydrocarbons

Wavenumber (cm^{-1})	Assignment
	Alkanes
2960	*Methyl symmetric C–H stretching*
2930	*Methylene asymmetric C–H stretching*
2870	*Methyl asymmetric C–H stretching*
2850	*Methylene symmetric C–H stretching*

1470	*Methyl asymmetrical C–H bending*
1465	*Methylene scissoring*
1380	*Methyl symmetrical C–H bending*
1305	*Methylene wagging*
1300	*Methylene twisting*
720	*Methylene rocking*
	Alkenes
3100-3000	*=C–H stretching*
1680-1600	*C=C stretching*
1400	*=C-H in–plane bending*
1000-600	*=C–H out–of–plane bending*
	Alkynes
3300-3250	*=C–H stretching*
2260-2100	*C=C stretching*
700-600	*=C–H bending*

Characteristic infrared bands of Oxygen containing compounds

Wave number (cm^{-1})	**Assignment**
	Alcohol and phenols
3600	*Alcohol O–H stretching*
3600	*Phenol O–H stretching*
3550–3500	*C–O stretching*
1300-1000	*C–O stretching*
	Ethers
1100	*C-O-C stretching*
	Aldehydes and ketones
2900-2700	*Aldehyde C—H stretching*
1740-1720	*Aliphatic aldehyde C=O stretching*
1730-1700	*Aliphatic ketone C=O stretching*
1720-1680	*Aromatic aldehyde C=O stretching*
1700–1680	*Aromatic ketone C=O stretching*
	Esters
1750–1730	*Aliphatic C=O stretching*
1730–1705	*Aromatic C=O stretching*
1310–1250	*Aromatic C-O stretching*
1300–1100	*Aliphatic C-O stretching*

	Carboxylic acids
3300-2500	*O-H stretching*
1700	*C=O stretching*
1430	*C—O—H in-plane bending*
1240	*C—O stretching*
930	*C—O—H out-of-plane bending*
	Anhydrides
I840-1800	*C=O stretching*
I780-1740	*C=O stretching*
1300-1100	*stretching*

Characteristic infrared bands of Amines

Wavenumber (cm^{-1})	Assignment
3335	*N-H stretching (doublet for primary amines; single for secondary amines)*
2780	*N-CH_2 stretching*
1615	*NH_2 scissoring, N-H bending*
1360-1250	*Aromatic C-N stretching*
1220-1020	*Aliphatic C-N stretching*
850-750	*NH_2: wagging and twisting*
715	*N-H wagging*

Characteristic infrared bands of Amides

Wavenumber (cm^{-1})	Assignment
3360-3340	*Primary amide NH_2 asymmetric stretching*
3300-3250	*Secondary amide N–H stretching*
3190-3170	*Primary amide NH_2 symmetric stretching*
3100-3060	*Secondary amide amide II overtone*
1680-1660	*Primary amide C=O stretching*
1680-1640	*Secondary amide C=O stretching*
1650-1620	*Primary amide NH_2 bending*
1560-1530	*Secondary amide N-H bending, C-N stretching*
750-650	*Secondary amide N-H wagging*

Characteristic infrared bands of Halogens

Wavenumber (cm^{-1})	Assignment
1300-10 00	*C-F stretching*
800-400	*C-X stretching (X = F, CL Br or I}*

Characteristic infrared bands of Sulphur compounds

Wavenumber (cm^{-1})	Assignment
700-600	*C-S stretching*
550-450	*S-S stretching*
2500	*S-H stretching*
1390-1290	*SO_2 asymmetric stretching*
I 190-1120	*SO_2 symmetric stretching*
1060 -1020	*S=O stretc hing*