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RATHINAM COLLEGE OF PHARMACY

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PRACTICAL MANUAL

THIRD YEAR B.PHARM (V-SEMESTER)

SUBJECTS: INDUSTRIAL PHARMACY-I{BP506P}

PHARAMACOLOGY-II{BP507P}

PHARMACOGNOSY AND PHYTOCHEMISTRY-II{BP508P}

Name :

Course : Bachelor of Pharmacy

Semester : 5 th semester

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INDUSTRIAL PHARMACY-I

B. PHARM 5th SEMESTER PREPARED

BY

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TABLE OF CONTENT

GENERAL LABORATORY INSTRUCTIONS

(A) Personal Hygiene:

1. Personal hygiene is enormously important during work in a pharmaceutical laboratory because people engaging in the preparation of medicinal products are working for patients who may already be ill.
2. Personnel should be well aware, trained, and should practice good health habits.
3. Smoking, eating, drinking, chewing, and the storage of food should be restricted in the laboratory areas.
4. Personnel suffering from an infectious disease or having open lesions on the exposed surface of the body should not engage in laboratory activities.

(B) Personal Protective Equipment (PPE):

1. A clean white coat (apron) should be worn to protect the person from the product, and conversely, the product should be protected from contamination by the person.
2. During the preparation and/or manufacturing process, safety equipment such as mouth masks, head caps, gloves, goggles, etc. must be used. Similarly, long hair should be tied back and properly covered with a head cap to ensure that any open cuts are covered.
3. It is the responsibility of the individual to ensure that the correct safety equipment is used and avoid direct contact with chemicals and APIs.

(C) Clean Work Area:

1. The cleanliness of the work area and equipment used during the compounding/preparation of medicaments is of paramount importance.
2. The risk of contaminating the final product with either dust, dirt, or microorganisms from the surroundings or from other ingredients from a previous preparation can be considerable if attention is not paid to the cleanliness of the work area and equipment.
3. Before starting to compound a product, the work area and equipment should be cleaned with a suitable solution, which must be allowed to dry fully.
4. Never use the apron outside the laboratory.

(D) Equipment Cleaning:

1. Equipment and utensils should be cleaned, stored, and where appropriate, sterilized to prevent contamination or carry-over of a material that would affect the quality of the intermediate or API beyond the official or other established specifications.
2. In cases where equipment is assigned to the continuous preparation of successive batches of the same intermediate or API, equipment should be cleaned at appropriate intervals to prevent the build-up and carry-over of contaminants (e.g., degradants or objectionable levels of microorganisms) that would alter the product quality

(E) Label Preparation:

The label for any pharmaceutical intermediate or finished product must be prepared before starting the compounding/preparation procedure. This will enable the product to be labelled as soon as it has been manufactured and packaged, eliminating the risk of the product being mislabeled and given to the wrong patient.

(F) Weighing and Measuring Procedure:

1. During weighing, using a clean balance pan as well as a spatula helps to prevent the mixing of different pharmaceutical ingredients, as many ingredients resemble each other.
2. After weighing or measuring each ingredient, label it using a paper piece or tag as soon as it has been weighed or measured.
3. Close the bottles/containers tightly after weighing and place them on the respective shelf/place after completing the weighing process.

EXPERIMENT NO: 01 **DATE:**

PREFORMULATION STUDIES ON PARACETAMOL/ASPIRIN/ OTHER DRUG

AIM:

To perform different preformulation studies of prepared granules.

REQUIREMENTS:

Measuring cylinder, Funnel, Sieves, Mortar & pestle, Spatula.

PRINCIPLE:

Preformulation is defined as the phase of research and development in which preformulation studies characterize physical and chemical properties of a drug molecule in order to develop safe, effective and stable dosage form. The objective of preformulation study is to develop the elegant, stable, effective and safe dosage form by establishing kinetic rate profile, compatibility with the other ingredients and establish Physico-chemical parameter of new drug substances. The major preformulation studies/parameters of granules are as follows:

1. **Bulk density:** It is defined as ratio of total mass of the powder to the bulk volume of powder. It gives an idea about tablet porosity and its relationship with disintegration time and hardness of a tablet. It is measured by pouring weighed powder into a measuring cylinder and the volume is noted down. It is expressed in gm/ml and is given by

$$D_b = M/V_o$$

Where, M= Mass of powder, V_o = Bulk volume of powder

2. **Tapped density:** It is defined as ratio of total mass of the powder to the tapped volume of powder. Tapped volume is measured by tapping the powder to constant volume. It is expressed in gm/ml and is given by:

$$D_t = M/V_t$$

Where, M= Mass of powder, V_t = Tapped volume of powder

3. **Angle of repose (Θ):** It is the maximum angle possible between surface of pile of powder and the horizontal plane, can be used to measure frictional forces in a powder.

$$\Theta = \tan^{-1}(h/r)$$

Where, Θ= angle of repose,

H height of the powder in cm,

R is the radius of heap of powder

Relationship between Angle of reposes and flow property

Angle of repose(θ)	Type of flow
<25	Excellent
25-30	Good
30-40	Passable
>40	Very poor

1. **Carr's Compressibility Index:** It indicates the ease with which a material can be induced to flow; it is expressed as a percentage and is given by

$$I = (Dt-Db)/Dt \times 100$$

Where, D is the tapped density of the powder. Db is the bulk density of the powder.

Relationship between Carr's index and flow property

Carr's index	Type of flow
5-15	Excellent
12-15	Good
15-22	Fair
23-30	Poor
33-38	Very poor
>40	Extremely poor

1. **Hausner's ratio:** It indicates the flow properties of the powder and is measured by the ratio of tapped density to the bulk density.

Hausner's ratio = (Tapped density)/ (Bulk density) ×100

Values of Hausner's ratio :< 1.25: good flow and > 1.25: poor flow

If Hausner's ratio is between 1.25-1.5, flow property can be improved by addition of glidants.

2. **Size and Size Distribution Analysis:** The particle-size distribution (PSD) of a powder, or granular material, is a list of values or a mathematical function that defines the relative amount, (typically by mass) of particles present according to size.

The size and shape distribution of the metal particles impacts powder behavior during die filling, compaction, and sintering, and therefore influences the physical properties of the parts created. In the pharmaceutical industry the size of active ingredients influences critical characteristics including content uniformity, dissolution and absorption rates.

Measurement Techniques:

1. Sieve Analysis
2. Air elutriation analysis
3. Photo analysis
4. Optical counting methods

5. Electro resistance counting methods
6. Sedimentation techniques
7. Laser diffraction methods

The way PSD is usually defined by the method by which it is determined. The most easily understood method of determination is sieve analysis, where powder is separated on sieves of different sizes. Thus, the PSD is defined in terms of discrete size ranges: e.g. "% of sample between 45 μm and 53 μm", when sieves of these sizes are used. The PSD is usually determined over a list of size ranges that covers nearly all the sizes present in the sample. However, the idea of the notional "sieve", that "retains" particles above a certain size, and "passes" particles below that size, is universally used in presenting PSD data of all kinds.

The PSD may be expressed as a "range" analysis, in which the amount in each size range is listed in order. It may also be presented in "cumulative" form, in which the total of all sizes "retained" or "passed" by a single notional "sieve" is given for a range of sizes. Range analysis is suitable when a particular ideal mid-range particle size is being sought, while cumulative analysis is used where the amount of "under-size" or "over-size" must be controlled.

PROCEDURE:

Bulk density and tapped density:

Pass a quantity of sample sufficient to complete the test through a sieve, if necessary, to break up agglomerates. Into a measuring cylinder of 100 ml, gently introduce, without compacting, approximately 15g of the test sample and weighed. Carefully level the powder without compacting, if necessary, and read the unsettled apparent volume to the nearest graduated unit. Calculate the bulk density by applying the above formula. The tapped volume is obtained by mechanically tapping the measuring cylinder containing the sample of 15 gm with a fixed drop of 14±2 mm at a nominal rate of 300 drops per mins until a constant volume is observed.

Then calculate the tapped density by using the above formula. After getting the value of bulk density and tapped density, **Carr's Compressibility Index and Hausner's ratio** is calculated by using the formula.

Angle of repose: The static angle of repose was measured according to the fixed funnel and free standing cone method. A funnel was clamped with its tip 2cm above a graph paper placed on a flat horizontal surface. The powders were carefully poured through the funnel. Block the orifice of the funnel by thumb. Fill the powder in the funnel and remove the thumb immediately. After emptying the powder from the funnel, measure the height of the pile and diameter.

Size and Size Distribution Analysis: Arrange all the sieves on the shaker one above the other

in increasing opening order i.e. decreasing sieve number, the one with powder sample occupying the upper most position. Weigh about 50g (W) of given sample and place it over the top sieve(Lowest sieve number).Shake the sieve either mechanically or electrically for a period of half an hour.

The powder retained on each sieve is collected and weighed separately. The percentage weight retained on each sieve is calculated by,

$$\text{Percentage powder retained} = \frac{\text{weight of powder that have retained over the sieve}}{\text{Weight of total powder taken for experiment}} \times 100$$

OBSERVATIONS

S.No	Sieve number passed or retained	Arthematic mean size of opening (µm)	Average size of the particle	Weight retained on a sieve (gm)	% weight retained	Cumulative percentage of oversized particles	Cumulative percentage of undersized
1	10/16	1350		W_1			
2	16/22	855		W_2			
3	22/40	517.5		W_3			
4	40/60	287.5		W_4			
5	60/85	142.5		W_5			
6	85/100	27.5		W6			

VIVA QUESTIONS:

- What is preformulation?
- What are true density and bulk density and tapped density? What is porosity?
- What is the role of bulkiness and compressibility of powder in the manufacturing of the dosage forms?
- What is void volume?
- What is the importance of angle of repose?
- What are the different methods used to determine size distribution analysis?

REPORT:

The preformulation parameters of the given sample were found to be:

Bulk density:

Tapped density:

Carr's Compressibility Index:

Hausner's ratio:

Angle of repose:

Size distribution analysis: The given sample is size separated by the sieves.

Their frequency distribution curve of the particle was plotted.

The average particle size----µm were found to be maximum of ---

% The average particle size----- µm were found to be minimum of -------%

The cumulative size distribution curve were also plotted and the total average particle size is found to be-- µm

INTRODUCTION TO TABLETS

Tablets may be defined as the solid unit dosage forms containing one or more medicaments and excipients, prepared either by molding or compression. It comprises a mixture of active substances and excipients in powder or granule form. The excipients include diluents, binders or granulating agents , glidants and lubricants to ensure efficient tablet compression, disintegrates to promote tablet break-up in the digestive tract , sweeteners or flavors to enhance taste and pigments to make tablets visually attractive.

ADVANTAGES:

1. Tablets offer the greatest compatibilities of all oral dosage forms for the greatest dose precision and the least content variability.
2. Their cost is lowest of all oral dosage form.
3. They are lightest and compact.
4. Easiest and cheapest to package and ship.
5. They have better physical and chemical stability and exert physiological activity of drug.
6. Special forms to facilitate patient compliance eg : - sustained release, extended release formulations.
7. Suitable for large scale economical production.

DISADVANTAGES:

1. Unsuitable for infants and children and patients who cannot swallow.
2. Delayed onset of action compared to liquid orals and parenterals.
3. Drugs with poor wetting, slow dissolution properties, optimum absorption high in GIT or combination of above features make tablet manufacturing difficult.
4. Bitter tasting drugs, drugs with objectionable odor or drugs that are sensitive to oxygen or atmospheric moisture may require encapsulation or entrapment prior to compression.

DIFFERENT TYPES OF TABLETS

They are generally divided as

A. Compressed tablets

B. Moulded tablets/ Tablets triturates.

CLASSIFICATION OF TABLETS ACCORDING TO USAGE:

(A) Tablets ingested orally:

1. Compressed tablet, e.g. Paracetamol tablet
2. Multiple compressed tablet

a. Layered tablets

b. Press coated/Dry coated Tablets

3. Repeat action tablet

4. Delayed release tablet, e.g. Enteric coated Bisacodyl tablet

5. Sugar coated tablet, e.g. Multivitamin tablet

6. Film coated tablet, e.g. Metronidazole tablet

7. Chewable tablet, e.g. Antacid tablet

(B) Tablets used in oral cavity:

1. Buccal tablet, e.g. Vitamin-C tablet

2. Sublingual tablet, e.g. Nitroglycerin tablet

3. Troches or lozenges

4. Dental cone

(C) Tablets used to prepare solution:

1. Effervescent tablet, e.g. Dispirin tablet (Aspirin)

2. Dispensing tablet, e.g. Enzyme tablet (Digiplex)

3. Hypodermic tablet

4. Tablet triturates e.g. Enzyme tablet

(D) Tablets administered by other Routes

1. Implantation tablets

2. Vaginal tablets

FORMULATION OF TABLETS

In addition to active ingredient, tablet contains a number of inert materials known as additives or excipients.

Different excipients are:

1. Diluents
2. Binders and adhesives
3. Disintegrants
4. Lubricants and glidants
5. Colouring agents
6. Flavoring agents
7. Sweetening agents

1. Diluents (Fillers)

Diluents are used to make required bulk of the tablet when the drug dosage is inadequate to produce the bulk. Secondary reason is to provide better tablet properties such as improve cohesion, to permit use of direct compression manufacturing or to promote flow.

a. Diluents for wet granulation

i. Lactose (hydrous): Most widely used. Lactose reacts with certain amine drugs / proteins in the presence of metal stearates (lubricants) resulting in the tablet discoloration with time. Such a reaction is known as **Millard reaction***(Browning reaction)*

ii. Anhydrous lactose

iii. Dicalcium phosphate and calcium sulfate: Excellent for water sensitive drugs because they contain appreciable water content and have low affinity to atmospheric moisture.

iv. Bentonite and kaolin

b. Diluents for dry granulation and direct compression

i. Spray dried lactose

ii. Directly compressible starches (corn, wheat or potato). They act as lubricant, binder and disintegrants

iii. Colloidal silica

iv. Sodium chloride used for dental cones

v. Mannitol, sorbitol, sucrose, dextrose (These agents can also be used as binder in solution form or for wet granulation)

2. Binders and Adhesives: These materials are added to hold powders together to form granules to promote cohesive compacts for directly compressed tablet.

Example: Acacia, tragacanth- Solution for 10-25% Conc. Cellulose derivatives- Methyl cellulose, Hydroxy propyl methyl cellulose, Polyvinylpyrrolidone (PVP)- 2% conc. Starch paste- 5-15% solution.

3. Disintegrants: Added to a tablet formulation to facilitate its breaking or disintegration when it comes in contact with water in GIT. Disintegrants acts by three mechanisms

a. Swelling e.g., alginates, starch, PVP ect Improving penetration of aqueous liquids (wetting agents) e.g., SLS, clays

b. Liberation of gas from effervescent base, e.g., $NaHCO_3$ and citric acid. **Superdisintegrants:** Swells up to ten fold within 30 seconds when contact water. Example: Crosscarmellose- cross-linked cellulose, Crosspovidone- cross-linked povidone (polymer), Sodium starch glycolate- cross-linked starch.

4. Lubricants: These are added for the following reasons

- Prevents adhesion of the tablet material to the surface of dies and punches.
- Reduce inter-particular friction; improve the rate of flow of tablet granulation.

- Facilitate ejection of the tablets from the die cavity.

Example: Lubricants- Stearic acid, Stearic acid salt – Stearic acid, Magnesium stearate, Talc, PEG (Polyethylene glycols).Glidants- Corn Starch – 5-10% conc, Talc-5% conc., Silica derivative – Colloidal silicas such as Cab-O-Sil, Syloid, Aerosil in 0.25-3% conc.

Glidants are intended to promote flow of the tablet granulation or powder materials by reducing the friction between the particles.

5. Coloring agent: The use of colors and dyes in a tablet has three purposes:

- It makes the tablet more esthetic in appearance.
- Colour helps the manufacturer to identify the product during its preparation.

All colorants used in pharmaceuticals must be approved and certified by the FDA *(food & Drug Administration*). Dyes are generally listed as FD&C (food, Drug & Cosmetic Dyes) dyes and D&C (Drug & Cosmetic Dyes).

Example: FD & C yellow 6-sunset yellow FD & C yellow 5- Tartrazine FD & C green 3- Fast Green FD & C blue 1- Brilliant Blue FD & C blue 2 – Indigo carmine D & C red 3- Erythrosine. D & C red 22 – Eosin Y

6. Flavoring agents: Flavors are usually limited to chewable tablets or other tablets intended to dissolve in the mouth. Flavor oils are added to tablet granulations in solvents, are dispersed on clays and other adsorbents or are emulsified in aqueous granulating agents (i.e. binder).Usually, the maximum amount of oil that can be incorporated to a granulation without influencing its tableting characteristics is 0.5 to 0.75% w/v.

6. Sweetening agents: The use of sweeteners is primarily limited to chewable tablets.

e.g. - Sugar.

Mannitol-72% as sweet as sugar, cooling & mouth filling effect

Saccharin- Artificial sweetener, 500 times sweeter than sucrose. Disadvantages: it has a bitter after taste and carcinogenic

Aspartame (Searle) - widely replacing saccharin. Disadvantage – lack of stability in presence of moisture

MANUFACTURING METHODS OF TABLETS

In the tablet-pressing process, it is important that all ingredients be dry, powdered, and of uniform grain size as much as possible. The main guideline in manufacture is to ensure that the appropriate amount of active ingredient is equal in each tablet so ingredients should be well-mixed. Compressed tablets are exerted to great pressure in order to compact the material. If a sufficiently homogenous mix of the components cannot be obtained with simple mixing, the ingredients must be granulated prior to compression to assure an even distribution of the active

compound in the final tablet. Two basic techniques are used to prepare powders for granulation into a tablet: wet granulation and dry granulation.

Powders that can be mixed well do not require granulation and can be compressed into tablets through Direct Compression.

The manufacturing of tablet dosage form is basically done by two methods, such as

1) Wet Granulation (most products)
2) Direct Compression

WET GRANULATION: Wet Granulation is a process of size enlargement whereby small particles are gathered into larger permanent aggregates in which the original particles can still be identified. Granulation usually refers to processes whereby agglomerates with sizes ranging from

0.1 to 2.0 mm are produced. The most important reasons for a granulation step prior to tableting are to:

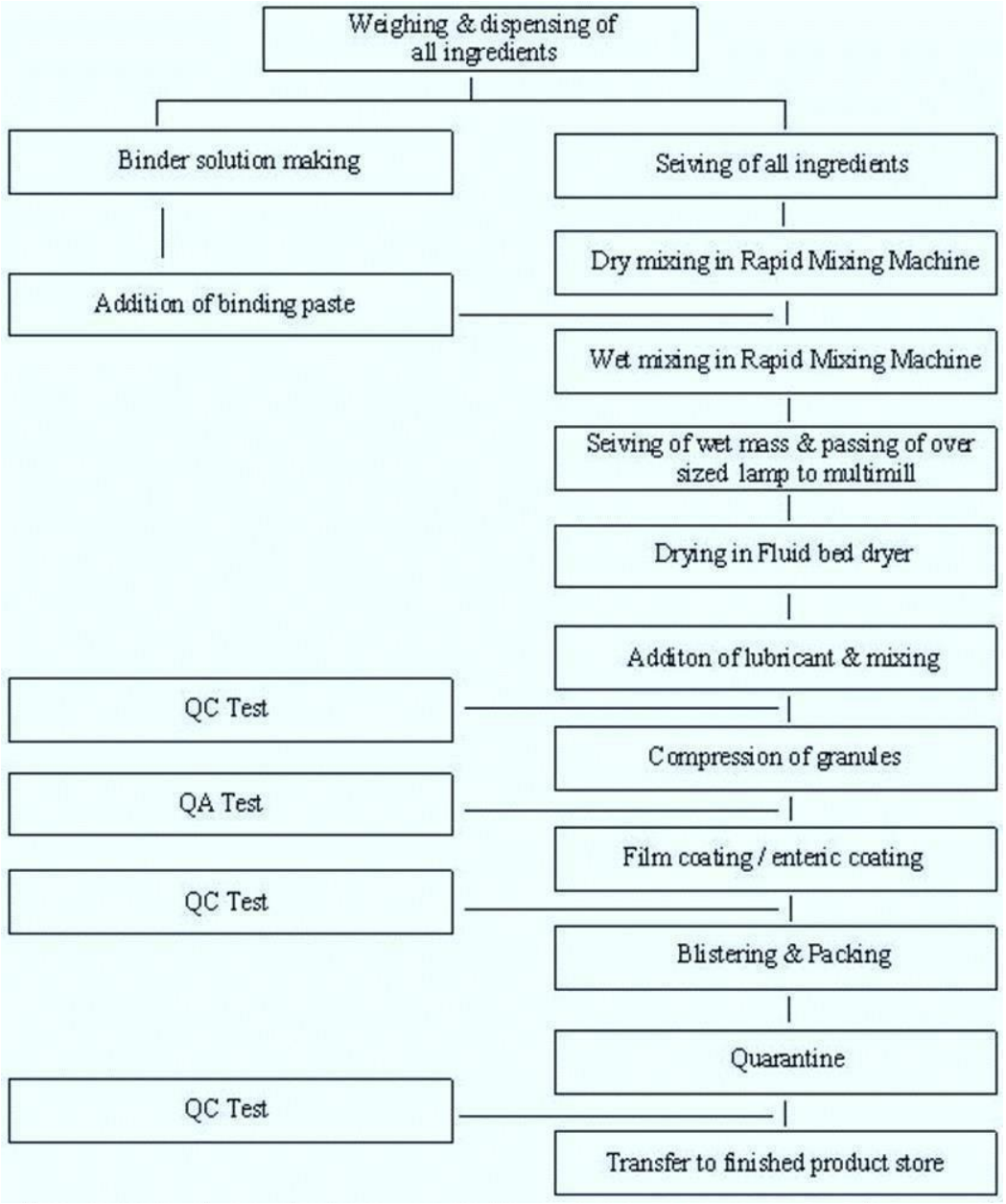
- Improve the flow properties of the mix and hence the uniformity of the dose.
- Prevent segregation of the ingredients.
- Improve the compression characteristics of the tablet mixture.
- Reduce dust during handling

The flow ability of the tablet mixture improves because the granules are larger and more spherical than the primary particles. Larger particles usually flow better than small particles (e.g. compare the flow ability of crystal sugar with powder sugar). In the hopper of tablet machines, small particles tend to segregate from the larger ones because of the vibration of the machine.

This causes higher concentrations of small particles at the bottom of the hopper. After granulation all particles are bound tight in the right amount in the granules, which prevents segregation of the small particles

PROCESS FLOW CHART

(Wet granulation method)



Equipment's used in wet granulation method:

1. Electronic Balance
2. Sieve
3. Rapid Mass Granulator (RMG)
4. Multimill

5. Fluid Bed Dryer
6. Double Cone Blender
7. Vat for the preparation of granulating fluid

DIRECT COMPRESSION: In the direct compression method, directly compressible filler (also called a filler-binder) is blended with the active(s), a lubricant and a disintegrating agent. Such free flowing directly compressible fillers make direct compression possible and practical. These include anhydrous lactose, unmilled dicalcium phosphate dihydrate, microcrystalline cellulose (e.g., Avicel PH 101), and modified (spray processed) lactose (e.g., Ludipress). Modified starch,

e.g. Starch 1500 flows better and compresses better than original starch, but are not as effective as other materials as the sole filler-binder. Generally, Starch 1500 is used as a component of a direct compression filler system, most likely for its disintegrating property, i.e., as a more compactible and better flowing substitute for starch. Certain materials like mannitol, sorbitol and modified sucrose are particularly useful in formulating direct compression chewable tablets.

Direct compression method can be classified as

a) Direct Compression with direct compressible materials and

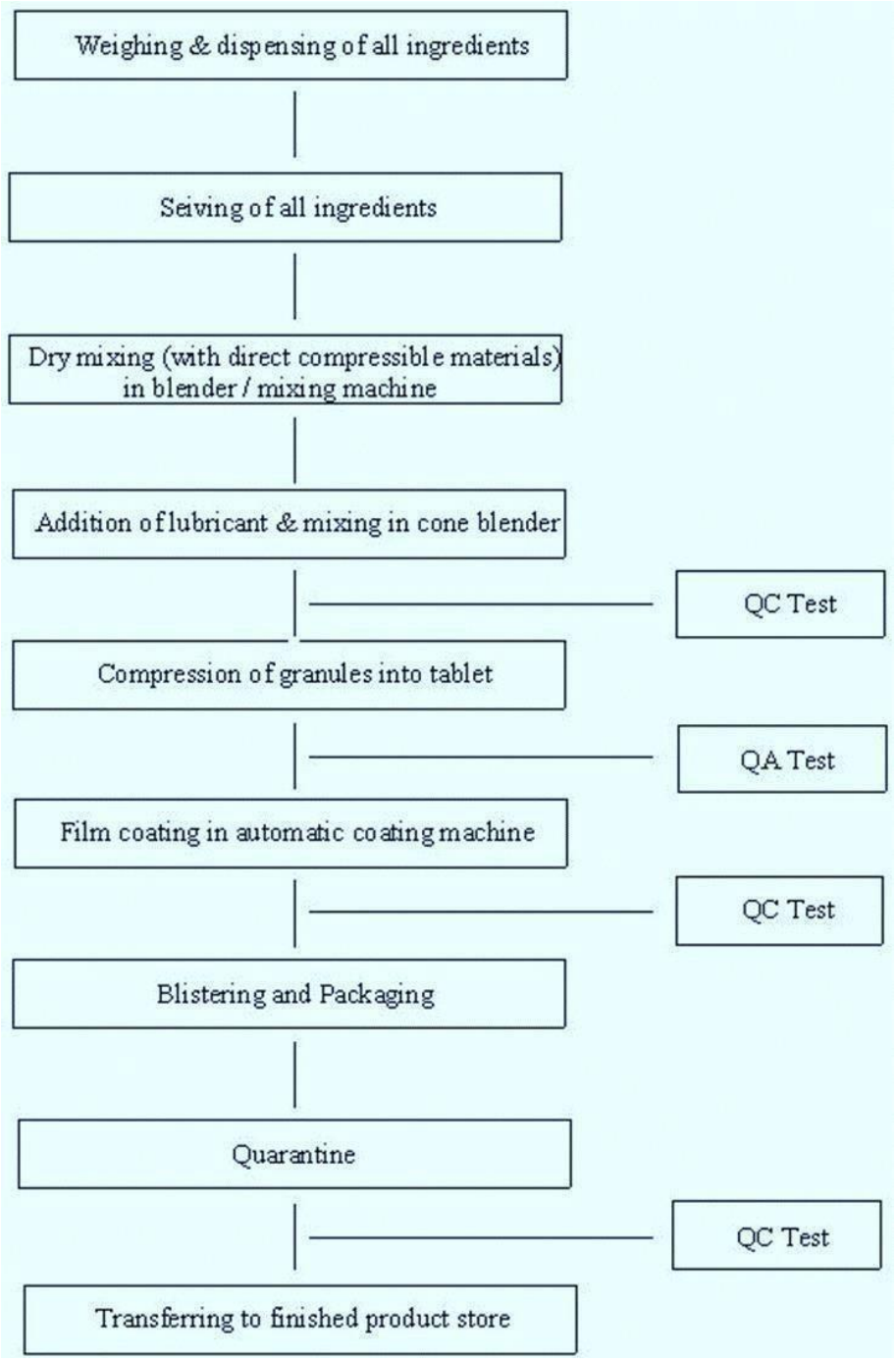
b) Direct Compression by Slugging method

Equipment's used in direct compression method:

1. Electronic Balance
2. Sieve
3. Double cone blender
4. Rotary Press

PROCESS FLOW CHART

(Direct Compression with direct compressible materials)



EXPERIMENT NO: 02 DATE:

PREPARATION OF PARACETOMOL TABLETS

AIM:

To prepare and submit 10 paracetamol (100 mg) tablets by wet granulation method.

REQUIREMENTS:

Mortar and pestle, spatula, beaker, Sieve

PRINCIPLE: Tablet is an important solid dosage form which is usually prepared with the aid of suitable pharmaceutical excipients. Tablets may vary with size, shape, cut, hardness, thickness. Their disintegration and dissolution characteristics and other aspects change depending on their intended use and method of manufacturing.

Compressed tablets are mainly prepared by 3 basic methods

- Wet granulation
- Dry granulation
- Direct compression

Wet granulation is the widely used method for the production of compressed tablets.

Steps involved in wet granulation method are

a) Weighing and blending of ingredients

b) Preparing a damp mass by adding wet binder

c) Converting the damp mass into wet granules

d) Drying of granules

e) Sizing the granules by dry screening

f) Addition of lubricants

g) Formation of tablets by compression

During the preparation process each step may influence the quality of tablet produced. In this preparation paracetamol used as API (antipyretic), lactose as adjuvant, starch (purified) as binding agent, starch monohydrate as disintegrant, magnesium stearate as lubricant and talc as Glidant.

INGREDIENTS TABLE (FORMULA):

Sl. No	INGREDIENTS	1-TABLET	10-TABLETS	PURPOSE
1	Paracetamol(Api)			Analgesic & Antipyretic
2	Starch (Purified)			Binding agent
3	Lactose Monohydrate			Diluent
4	Strarch Monohydrate			Disintegrant
5	Talc			Glidant
6	Mg.Stearate			Lubricant

PROCEDURE:

a) **Preparation of starch mucilage**: Dissolve 5mg of starch in 100ml of distilled water then resulting mixture is heated on a water bath until the starch is gelatinized by the formation of mucilage.

b) Divide disintegrating agent (starch monohydrate) into 2 portions to incorporate during wet granulation and after drying of granules to act as an intragranular and extra granular disintegrant.

c) **Wet Granulation**: Accurately weigh and mix the specified amount of paracetamol and other excipients (except half of the disintegrating agent and lubricant) until uniform powder is formed by geometric mixing.

d) A damp mass of the mixture is prepared by adding appropriate amount of the 5% starch mucilage and kneading by hand.

e) Wet mass is subsequently passes through a 6/10 mesh sieve/screen to form wet granules. Resulted granules are spread evenly on a large piece of paper in a tray and dried at 40ºC- 60ºC for 30min in an oven.

f) Dried granules are passed through a sieve 16 or 20 # and mixed with remaining half of the disintegrating agent and lubricant.

g) Resulting granules mixture is compressed in a tablet compression machine to obtain tablets.

h) Prepared tablets are stored properly for further evaluation.

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VIVA QUESTIONS:

- What is tablet?
- What are the advantages and disadvantages of tablet? What are the different types of tablet?
- What are the different methods of preparation of tablet?
- What are the basic ingredients used in wet granulation method? Give examples. What is the use of paracetamol?
- What are the steps involved in wet granulation method?

REPORT:

Paracetamol tablets were prepared by wet granulation method and submitted

EXPERIMENT NO: 03 DATE:

EVALUATION OF PARACETOMOL TABLETS

AIM:

To evaluate prepared paracetamol tablets.

REQUIREMENTS:

Beaker, Test tubes, Test apparatuses

PRINCIPLE:

EVALUATION PARAMETERS OF TABLETS

APPEARANCE:

Tablet from each formulation were randomly selected and organoleptic properties such as color, taste, and shape were evaluated.

HARDNESS TEST:

The tablet hardness is defined as the force required to break a tablet in a diametric direction. A tablet was placed between two anvils. Force was applied to anvils and crushing strength that causes the tablet to break was recorded. The hardness was measured using Monsanto hardness tester.

THICKNESS:

The thickness of tablets was determined using a Vernier calliper. Three tablets from each batch were used, and average values were calculated.

FRIABILITY TEST:

The friability of tablets was determined using Roche Friabilator. It is express in percentage (%). Ten or twenty tablets were initially weighed and revolved at 25 rpm for 4 min. The tablets were then reweighed after removal of fines and the percentage of weight loss was calculated. The % friability was then calculated by,

$$F = (W_{initial} - W_{fiinal}) \times 100 / W_{initial}$$

Acceptance criteria for % friability % weight loss should be less than 1%.

WEIGHT VARIATION TEST:

Twenty tablets were selected randomly from each batch and weighed individually on electronic balance. The individual weighed is then compared with average weight for the weight variations. The following percentage deviation in weight variation is allowed (U.S.P).

Average weight	% Difference
130 mg or less	10
130 – 324 mg	7.5
More than 324 mg	5

DISINTEGRATION TIME TESTING:

It was determine using USP tablet disintegration test apparatus, using 900 ml of distilled water without disk at room temperature. Test was performed on 6 tablets. One tablet each is kept in all six tubes. The tubes travel upward and downward in water at 37ºC±2ºC. The time taken for all the six tablets to break down and pass through the mesh at the bottom of the tube is noted. The tablets pass the test if all the six tablets disintegrate within the prescribed time (Less than 30 mins for uncoated tablets as per U.S.P).

IN VITRO DRUG RELEASE STUDY:

The release rate of paracetamol from tablets was determined using United States Pharmacopeia (USP) Dissolution Testing Apparatus Type-II. The dissolution test was performed using 900ml of

5.8pH phosphate buffer, at 37ºC±0.5ºC and 50 rpm. A sample (10ml) of the solution was withdrawn from the dissolution apparatus hourly and the samples were replaced with fresh dissolution medium. The samples were filtered through a 0.45µ membrane filter. Absorbance of these solutions was measured at 243 nm using a Thermospectronic-1 UV/V double-beam spectrophotometer. Cumulative percentage drug release was calculated using an equation obtained from a standard curve.

VIVA QUESTIONS:

- What are the different evaluation tests of tablet?
- What are the equipment's used to test hardness and friability of tablet?
- What is the disintegration time of uncoated tablet?
- What is the use of Vernier calliper?
- What is the range of acceptance of weight variation test of tablet as per U.S.P?

REPORT:

The evaluation tests are performed and all the tablets are found to be in the acceptable limits.

EXPERIMENT NO: 04 DATE:

PREPARATION OF ASPIRIN TABLETS

AIM:

To prepare and submit 10 Aspirin (100 mg) tablets by wet granulation method.

REQUIREMENTS:

Mortar and pestle, spatula, beaker, Sieve

PRINCIPLE:

Tablet is an important solid dosage form which is usually prepared with the aid of suitable pharmaceutical excipients. Tablets may vary with size, shape, cut, hardness, thickness. Their disintegration and dissolution characteristics and other aspects change depending on their intended use and method of manufacturing.

Compressed tablets are mainly prepared by 3 basic methods

- Wet granulation
- Dry granulation
- Direct compression

Wet granulation is the widely used method for the production of compressed tablets.

Steps involved in wet granulation method are

h) Weighing and blending of ingredients

i) Preparing a damp mass by adding wet binder

j) Converting the damp mass into wet granules

k) Drying of granules

l) Sizing the granules by dry screening

m) Addition of lubricants

n) Formation of tablets by compression

During the preparation process each step may influence the quality of tablet produced. In this preparation Aspirin used as API (Aspirin, also known as acetylsalicylic acid, is a medication used to treat pain, fever, or inflammation), lactose as adjuvant, acacia as binding agent, starch

monohydrate as disintegrant, magnesium stearate as lubricant and talc as Glidant.

INGREDIENTS TABLE (FORMULA):

Sl. No	INGREDIENTS	1 TABLET	10 TABLETS	PURPOSE
1	Aspirin (Api)			Treat Pain, Fever, Or Inflammation
2	Acacia			Binding agent
3	Lactose Monohydrate			Diluent
4	Strarch Monohydrate			Disintegrant
5	Talc			Glidant
6	Mg.Stearate			Lubricant

PROCEDURE:

a) Divide disintegrating agent (starch monohydrate) into 2 portions to incorporate during wet granulation and after drying of granules to act as an intragranular and extra granular disintegrant.

b) **Wet Granulation**: Accurately weigh and mix the specified amount of Aspirin and other excipients (except half of the disintegrating agent and lubricant) until uniform powder is formed by geometric mixing.

c) A damp mass of the mixture is prepared by adding appropriate amount of the acacia and drop wise addition of water.

d) Wet mass is subsequently passes through a 6/10 mesh sieve/screen to form wet granules. Resulted granules are spread evenly on a large piece of paper in a tray and dried at 40ºC- 60ºC for 30min in an oven.

e) Dried granules are passed through a sieve 16 or 20 # and mixed with remaining half of the disintegrating agent and lubricant.

f) Resulting granules mixture is compressed in a tablet compression machine to obtain tablets.

g) Prepared tablets are stored properly for further evaluation.

VIVA QUESTIONS:

- What is the use of aspirin tablet?
- What is the use of diluent and Glidant in tablet formulation? Give examples. Give some examples of binders used in tablet formulation.
- Whydisintegrating agents are used in 2 portions in tablet preparation?

REPORT:

Aspirin tablets were prepared by wet granulation method and submitted.

EXPERIMENT NO: 05 **DATE:**

EVALUATION OF ASPIRIN TABLETS

AIM:

To evaluate prepared Aspirin tablets.

REQUIREMENTS:

Beaker, Test tubes, Test apparatuses

EVALUATION PARAMETERS OF TABLETS:

APPEARANCE:

Tablet from each formulation were randomly selected and organoleptic properties such as color, taste, and shape were evaluated.

HARDNESS TEST:

The tablet hardness is defined as the force required to break a tablet in a diametric direction. A tablet was placed between two anvils. Force was applied to anvils and crushing strength that causes the tablet to break was recorded. The hardness was measured using Monsanto hardness tester.

THICKNESS:

The thickness of tablets was determined using a Vernier caliper. Three tablets from each batch were used, and average values were calculated.

FRIABILITY TEST:

The friability of tablets was determined using Roche Friabilator. It is express in percentage (%). Ten or twenty tablets were initially weighed and revolved at 25 rpm for 4 min. The tablets were then reweighed after removal of fines and the percentage of weight loss was calculated. The % friability was then calculated by,

$$F = (W_{initial} - W_{fiinal}) \times 100 / W_{initial}$$

Acceptance criteria for % friability % weight loss should be less than 1%.

WEIGHT VARIATION TEST:

Twenty tablets were selected randomly from each batch and weighed individually on electronic balance. The individual weighed is then compared with average weight for the weight variations. The following percentage deviation in weight variation is allowed (U.S.P).

Average weight	% Difference
130 mg or less	10
130 – 324 mg	7.5
More than 324 mg	5

DISINTEGRATION TIME TESTING:

It was determine using USP tablet disintegration test apparatus, using 900 ml of distilled water without disk at room temperature. Test was performed on 6 tablets. One tablet each is kept in all six tubes. The tubes travel upward and downward in water at 37ºC±2ºC. The time taken for all the six tablets to break down and pass through the mesh at the bottom of the tube is noted. The tablets pass the test if all the six tablets disintegrate within the prescribed time (Less than 30 mins for uncoated tablets as per U.S.P).

IN VITRO DRUG RELEASE STUDY:

The release rate of Aspirin from tablets was determined using United States Pharmacopeia (USP) Dissolution Testing Apparatus Type-II. The dissolution test was performed using 900ml of 5.8pH phosphate buffer, at 37ºC±0.5ºC and 50 rpm. A sample (10ml) of the solution was withdrawn from the dissolution apparatus hourly and the samples were replaced with fresh dissolution medium. The samples were filtered through a 0.45µ membrane filter. Absorbance of these solutions was measured at 265 nm using a Thermospectronic-1 UV/V double-beam spectrophotometer. Cumulative percentage drug release was calculated using an equation obtained from a standard curve.

VIVA QUESTIONS:

- How can we calculate friability of uncoated tablet?
- Which test apparatus is used for invitro drug release study?
- What are the different organoleptic properties are tested for tablet?

REPORT:

The evaluation tests are performed and all the tablets are found to be in the acceptable limits.

EXPERIMENT NO: 06 DATE:

FORMULATION OF FILM COATED TABLETS OF PARACETAMOL

AIM:

To prepare 10 tablets of paracetamol film coated tablets.

REQUIREMENTS:

Mortar and pestle, Sieve, Beaker, Glass rod

PRINCIPLE:

All drugs have their own characteristic, like some drugs are bitter in taste or have an unpleasant odor, some are sensitive to light or oxides, some are hygroscopic in nature. Because of this reasons, tablet coating is the choice of option to solve such problems in conventional dosage form. Tablet film coating is performed by two types, one is aqueous film coating (generally water is used as a solvent) and non-aqueous film coating (generally organic solvents are used). Some problems are associated with the non-aqueous film coating like safety of employees (as most of the solvents are dangerous, smell, and they are not good to breathe), atmospheric pollution etc. But key problem is with the approval of the regulatory authority. High quality aqueous film coating must be smooth, uniform and adhere satisfactorily to the tablet surface and ensure chemical stability of a drug. Coating may be applied to a wide range of oral solid dosage forms, including tablets, capsules, and multiparticulate and drug crystals. When coating composition is applied to a batch of tablets in a coating pan, the tablet surfaces become covered with a tacky polymeric film. Before the tablet surface dries, the applied coating changes from a sticky liquid to tacky semisolid and eventually to a non-stick dry surface. The entire coating process is conducted in a series of mechanically operated acorn-shaped coating pans of galvanized iron stainless steel or copper. The smaller pans are used for experimental, developmental, and pilot plant operations, while the larger pans for industrial production.

Necessity of Tablet Coating:

- A number of reasons can be suggested, like: The core contains a material which has a bitter taste in the mouth or has an unpleasant odour. Coating will protect the drug from the surroundings with a view to improve its stability.
- Coating will increase the ease by which a tablet can be ingested by the patient.
- Coating will develop the mechanical integrity; means coated products are more resistant

to mishandling (abrasion, attrition, etc.)

- The core contains a substance which is incompatible in the presence of light and subject to atmospheric oxidation, i.e. a coating is added to improve stability.
- The coated tablets are packed on high-speed packaging machine. Coating reduces friction and increases packaging rate.
- Coating can modify the drug release profile, e.g., enteric coating, osmotic pump, pulsatile delivery.

INGREDIENTS TABLE (FORMULA):

Name of the ingredient	Quantity (%w/w)
Cellulose acetate	6.3
PEG 400	0.7
Acetone	89
Deionized water	4

PROCEDURE:

Paracetamol uncoated tablets are prepared by wet granulation method. The prepared tablets are then coated with film coating solution prepared as below.

Film coating solution preparation: The coating solution was prepared by dissolving PEG in water followed by addition of this solution to acetone. Cellulose acetate was then added to the above mixture and stirred to achieve a clear solution.

The coating process was performed in a Vector Hi-Coater LDCS (batch size, 1.5 kg, with inclusion of placebo tablets) at a product temperature of 28ºC. Coated tablets were dried in a vacuum drying oven at 40ºC for 24 hours to remove residual solvent and moisture.

VIVA QUESTIONS:

- Why tablets are coated?
- What are the different types of tablet coating? What are the types of film coating?
- What are the different polymers used in tablet coating? Which equipment is used for tablet coating?

REPORT:

10 tablets of paracetamol film coated tablets are prepared and submitted.

EXPERIMENT NO: 07 DATE:

PREPARATION AND EVALUATION OF HARD GELATIN CAPSULES OF TETRACYCLINE HYDROCHLORIDE

AIM:

To prepare and evaluate hard gelatin capsules of tetracycline hydrochloride.

REQUIREMENTS:

Mortar and pestle, beaker, test tubes, spatula, glass rod, Test apparatuses

PRINCIPLE:

Hard gelatin capsule shells are used in most commercial medicated capsules. The community pharmacist also uses hard gelatin capsules in the extemporaneous compounding of prescriptions. The empty capsule shells are made of gelatin, sugar, and water. As such, they can be clear, colourless, and essentially tasteless; or they may be colored with various dyes and made opaque by adding agents such as titanium dioxide. Most commercially available medicated capsules contain combinations of colorants and opaquants to make them distinctive, many with caps and bodies of different colors. Gelatin is obtained by the partial hydrolysis of collagen obtained from the skin, white connective tissue, and bones of animals. In commerce, it is available in the form of a fine powder, a coarse powder, shreds, flakes, or sheets. Gelatin is soluble in hot water and in warm gastric fluid; a gelatin capsule rapidly dissolves and exposes its contents. Gelatin, being a protein, is digested by proteolytic enzymes and absorbed. Advantages of hard gelatin capsule are rapid drug release possible, flexibility of formulation and sealed HGCs are good barriers to atmospheric oxygen. Disadvantages of this dosage form are very bulky materials are a problem, filling equipment process is slower than tablets, generally more costly than tablets, but must judge on a case-by-case basis; concern over maintaining proper shell moisture content.

Tetracycline is used to treat a wide variety of infections, including acne. It is an antibiotic that works by stopping the growth of bacteria. This antibiotic treats only bacterial infections. It will not work for viral infections (e.g., common cold, flu). First Tetracycline hydrochloride granules are prepared by using wet granulation technique by using required ingredients. Then these granules are filled in the hard gelatin capsule shell

FORMULA:

Name of the ingredient	Quantity (mg)
Tetracycline hydrochloride	100
Microcrystalline cellulose	38
PVPK30	6

Magnesium stearate	4
Talc	2
Alcohol	q.s

PROCEDURE:

Formulation of Granules of Tetracycline hydrochloride:

Tetracycline hydrochloride granules were prepared by wet granulation method. Specified quantity of tetracycline hydrochloride, micro crystalline cellulose and PVP K30 will be weighed and mixed uniformly. Required quantity of alcohol drop wise incorporated to the blend. Wet granules will be passed through sieve #10 & air dried for 15 minutes. The dried granules will then be passed through sieve #22. Required quantity of magnesium stearate & talc were added to the granules. The prepared granules were then added to the Size #3 empty hard gelatin capsule.

Evaluation of prepared capsule of tetracycline hydrochloride:

Weight Variation Test: Twenty capsules were selected randomly from each batch and weighed individually on electronic balance. The individual weighed is then compared with average weight for the weight variations. The % difference should be 10%.

Disintegration time Testing: It was determine using disintegration test apparatus, using 900 ml of distilled water with disk (in case capsule floats) at room temperature. Test was performed on 6 capsules. One capsule each is kept in all six tubes. The tubes travel upward and downward in water at 37ºC±2ºC. The capsules pass the test if no drug or particles other than capsule fragments remained on the mesh or tube. The time taken for that is considered as disintegration time.

In vitro drug release study: The release rate of Tetracycline hydrochloride from capsule was determined using United States Pharmacopeia (USP) Dissolution Testing Apparatus Type-II. The dissolution test was performed using 900ml of 5.8pH phosphate buffer, at 37ºC±0.5ºC and 50 rpm. A sample (10ml) of the solution was withdrawn from the dissolution apparatus hourly and the samples were replaced with fresh dissolution medium.

The samples were filtered through a 0.45µ membrane filter. Absorbance of these solutions was measured at 344 nm using a Thermospectronic-1 UV/V double-beam spectrophotometer. Cumulative percentage drug release was calculated using an equation obtained from a standard curve.

VIVA QUESTIONS:

- Define capsule?
- What are the types of capsule?
- What are the advantages and disadvantages of hard gelatin capsules? What is the source and properties of gelatin?
- What is the use of tetracycline?
- What is the use of PVPK30 in the above formulation? What are the evaluation tests of capsules?

REPORT:

Tetracycline hydrochloride hard gelatin capsules were prepared and evaluated.

EXPERIMENT NO: 08 DATE:

PREPARATION OF CALCIUM GLUCONATE INJECTION

AIM:

To prepare and submit 10 ml Calcium gluconate injection.

REQUIREMENTS:

Beaker, Glass rod, Funnel, Filter paper, Ampoule

PRINCIPLE:

Injections are sterile solutions, emulsions or suspensions. They are prepared by dissolving, emulsifying or suspending an active ingredient and any other substances in water for injection. Injecting is the act of giving medication by use of syringe and needle to obtain the desired therapeutic effect taking into account the patient's safety and comfort. It is suitable for those drugs that are altered or not absorbed by other methods of administration.

Calcium gluconate is a mineral supplement and medication. As a medication it is used by injection into a vein to treat low blood calcium, high blood potassium, and magnesium toxicity. Supplementation is generally only required when there is not enough calcium in the diet. Calcium Gluconate is the calcium salt of gluconic acid, an oxidation product of glucose, and contains 9.3% calcium, which is about one-third of the calcium in strength of calcium chloride USP. Since it is soluble to the extent of only one part in 30 parts of cold water, the 10% solution is supersaturated and is stabilized by the addition of calcium saccharate tetrahydrate 0.46% w/v.

FORMULA:

Ingredients	1 ml injection	10 ml injection
calcium gluconate monohydrate	98 mg	
calcium saccharate tetrahydrate	4.6 mg	
Water for injection upto	1 ml	

PROCEDURE:

` Calcium gluconate monohydrate and calcium saccharate tetrahydrate are dissolved in

water for injection in a beaker and makes upto required volume. Filter it and take 1 ml of the filtrate. Then it is transferred into previously sterilized ampoules, sealed properly and sterilized by autoclaving.

USES:

It is used as mineral supplement and medication.

VIVA QUESTIONS:

- What is the use of calcium gluconate?
- What are the general methods to prepare injections?
- What is the use of calcium saccharate tetrahydrate?

REPORT:

Calcium gluconate injection were prepared and submitted

EXPERIMENT NO: 09 DATE:

PREPARATION OF ASCORBIC ACID INJECTION

AIM:

To prepare and submit 2 ml ascorbic acid injection.

REQUIREMENTS:

Beaker, Glass rod, Funnel, Filter paper, Ampoule

PRINCIPLE:

Injections are sterile solutions, emulsions or suspensions. They are prepared by dissolving, emulsifying or suspending an active ingredient and any other substances in water for injection. Injecting is the act of giving medication by use of syringe and needle to obtain the desired therapeutic effect taking into account the patient's safety and comfort. It is suitable for those drugs that are altered or not absorbed by other methods of administration. Ascorbic Acid (vitamin C) is a water-soluble vitamin. It occurs as a white or slightly yellow crystal or powder with a light acidic taste. It is an antiscorbutic product. Ascorbic Acid injection is a clear, colourless to slightly yellow sterile solution of Ascorbic Acid in Water for Injection, for intravenous, intramuscular or subcutaneous use.

FORMULA:

Ingredients	1 Ampoule	2 Ampoules
Ascorbic Acid	0.5 gm	1 gm
Water for injection upto	2 ml	4 ml

PROCEDURE:

Ascorbic acid is dissolved in water for injection in a beaker and makes upto required volume. Filter it and take 2 ml of the filtrate. Then it is transferred into previously sterilized ampoules, sealed properly and sterilized by autoclaving.

USE:

It is used as anti-scurvy.

VIVA QUESTIONS:

- What is injection?
- Whydrugs give in injection form? What is the use of ascorbic acid?
- What are the routes of administration of ascorbic acid injection

REPORT:

Ascorbic acid injection were prepared and submitted

EXPERIMENT NO: 10 **DATE:**

QUALITY CONTROL TEST OF (AS PER IP) MARKETED TABLETS AND CAPSULES

AIM:

Qualitycontrol test of marketed tablets and capsules as per I.P.

REQUIREMENTS:

Volumetric flask, Mortar and pestle, Pipette, Beaker, Stop watch, Measuring cylinder, Whatman filter paper, UV spectrophotometer, Dissolution apparatus.

PRINCIPLE:

Quality control is a procedure or set of procedures intended to ensure that a manufactured product or performed service adhere to a defined set of quality criteria or meets the requirement of the client or costumer. Quality is not an accident this is the result of intelligent effort. The quality in the pharmaceutical industry has become a very important and sensitive issue. In the pharmaceutical industry, it is essential for controlling the errors during the every stage in production process since total quality of the product must be ensured according to compendia of drugs. In order to determine the specifications of the finished product, the quality characteristics related to the manufacturing process should be taken into account. An appropriate specification for each aspect of quality studied during the phase of development and during the validation of the manufacturing process should be determined. At least those aspects considered to be critical should be the object of specifications routinely verified.

Content of active ingredients (Tablets/Capsules): For this test according to IP determine the amount of active ingredient(s) by the method described in the assay and calculate the amount of active ingredient(s) per tablet/ capsule. The result lies within the range for the content of active ingredient(s) stated in the monograph. This range is based on the requirement that 20 tablets/ capsules, or such other number as may be indicated in the monograph, are used in the assay. Where 20 tablets/ capsules cannot be obtained, a smaller number, which must not be less than 5, may be used, but to allow for sampling errors the tolerances are widened in accordance with Table 1. As specified by the IP requirements Table1 apply when the stated limits are between 90 and 110 percent. For limits other than 90 to 110 percent, proportionately smaller or larger allowances should be made.

Content of active ingredients test

weight of active ingredients in each tablet/capsule	Subtract from lower limit for samples of			Add to the upper limit for samples of		
	15	**10**	**5**	**15**	**10**	**5**
0.12 g or less	0.2	0.7	1.6	0.3	0.8	1.8
More than 0.12 g But less than 0.3 g	0.2	0.5	1.2	0.3	0.6	1.5
0.3 g or more	0.1	0.2	0.8	0.2	0.4	1.0

Uniformity of content for tablets: The content uniformity test is to ensure that every dosage form contains equal amount of drug substance i.e. active pharmaceutical ingredient within a batch. Mainly it is used for testing the consistency of bulk powders before or after compression, liquid orals before filling, also during filling of powders into capsules or liquids into vials or ampoules and amount of active pharmaceutical ingredient within individual unitsof tablets or capsules.

Normally testing is confirmed by performing specific assay to determine the content of drug material contained in particular dosage form. The content uniformity test is used to ensure that every tablet contains the amount of drug substance intended with little variation among tablets within a batch. Due to increase awareness of physiological availability, the content uniformity test has been included in the monographs of all coated and uncoated tablets and al capsules intended for oral administration. Randomly select 30 tablets. 10 of these assayed individually. The Tablet pass the test if 9 of the 10 tablets must contain not less than 85% andnot more than 115% of the labeled drug content and the 10th tablet may not contain less than 75% and more than 125% of the labeled content. If these conditions are not met, remaining 20 tablets assayed individually and none may fall outside of the 85 to 115% range.

Uniformity of content for capsules:

This test is applicable to capsules that contain less than 10 mg or less than 10 per cent w/w of active ingredient. For capsules containing more than one active ingredient carry out the test for each active ingredient that corresponds to the afore-mentioned conditions. The test should be carried out only after the content of active ingredient(s) in a pooled sample of the capsules has been shown to be within accepted limits of the stated content. Determine the content of active ingredient in each of 10 capsules taken at random using the method given in the monograph or by any other suitable analytical method of equivalent accuracy and precision.

The capsules comply with the test if not more than one of the individual values thus obtained is outside the limits 85 to 115per cent of the average value and none is outside the limits 75 to125per

cent. if maximum of three individual values are outside the limits 85 to 115 per cent of the average value repeat the determination using another 20 capsules. The capsules comply with the test if in the total sample of 30 capsules not more than three individual values are outside the limits 85 to 115 per cent and none is outside the limits 75 to 125per cent of the average value.

Dissolution Test:

Dissolution is the process by which a solid solute enters a solution. Dissolution is pharmaceutically defined as the rate of mass transfer from a drug substance into the dissolution medium or solvent under standardized conditions of liquid/solid interface, temperature and solvent composition. Dissolution is considered one of the most important quality control tests performed on pharmaceutical dosage forms and is now developing into a tool for predicting bioavailability, and in some cases, replacing clinical studies to determine bioequivalence. Two types of apparatus are generally used to carry out dissolution. Usually apparatus Type I (Paddle type) is employed in the evaluation of tablets (or capsule) containing poorly water soluble drugs while apparatus Type II (basket type) is used for partially water soluble drugs. This test is designed to determine compliance with the dissolution requirements for solid dosage administered orally. The test is intended for a apside or tablet. This test is provided to determine compliance with the dissolution equirements for solid dosage forms administered orally.

Dissolution Medium: Use the dissolution medium specified in the individual monograph. if the medium is a buffered solution, adjust the solution so that its pH is within 0.05 units of the pH specified in the monograph.

Method: Place the stated volume of the dissolution medium, free from dissolved air, into the vessel of the apparatus. Assemble the apparatus and warm the dissolution medium to 36.5° to 37.5°. Unless otherwise stated, place one dosage unit simultaneously and in a reproducible way in the apparatus, taking care to exclude air bubbles from the surface of the dosage unit. When Apparatus 1 is used, allow the tablet or capsule to sink to the bottom of the vessel priorto the rotation of the paddle.

A suitable device such as a sinker made up of stainless steel maybe used to keep the dosage unit horizontal at the bottom of the vessel for tablets or capsules that would otherwise float. When Apparatus II is used, place the tablet or capsule in a dry basket at the beginning of each test. Lower the basket into position before rotation. Operate the apparatus immediately at the speed of rotation specified in the individual monograph. Within the time interval specified, or at each of the times

stated, withdraw a specimen from a zone midway between the surface of the dissolution medium

and the top of the rotating blade or basket, not less than 10 mm from the waft of the vessel. Specimen withdrawal at each sampling time point should be from the same location either manually or automatically. Measure media temperature at each sampling time point, the inter-vessel temperature should agree within a range of 0.4°C. Except in the case of single sampling, add a volume of dissolution medium equal to the volume of the samples withdrawn. Filter the sample solution promptly through a membrane filter disc with an average pore diameter not greater than LORI. Discard the first few ml of the filtrate. Perform the analysis as directed in the individual monograph Repeat the whole operation five times. Where two or more tablets or capsules are directed to be placed together in the apparatus, carry out six replicate tests. The results are plotted as concentration versus time.

OBSERVATIONS:

Content of active ingredients of tablets

Tablet no	Drug content in each tablet (T1)	Average drugcontent (T2)	Difference in drug content (T1-T2)	% Difference	More than Less than official limit

Uniformity of drug content

Tablet no	Drug content in each tablet (T1)	Average drugcontent (T2)	Differencein drug content (T1-T2)	% Difference	More than Less than official limit

DISSOLUTION TEST

Test tube	Time(min)	Filtrate(ml)	Dilution fluid(ml)	Absorbance

RESULTS:

I. Tablet compliance on the specification of I.P. for content of active= Passes/Fails

II. Tablets compliance on the specification of I.P. for uniformity of content = Passes/Fails

III. The percentage of drug present in tablet dissolved in 30 min =

IV. Capsule compliance on the specification of I.P. for content of active =Passes/Fails

V. Capsules compliance on the specification of I.P. for uniformity of content = Passes/Fails

VI. The percentage of drug present in capsule dissolved in 30 min=----------%

EXPERIMENT NO: 11 DATE:

PREPARATION OF EYE OINTMENT

AIM:

To Prepare and submit 3 tubes each containing 4gm of eye ointment.

REQUIREMENTS:

Beaker, glass rod, measuring cylinder, water bath, spatula.

PRINCIPLE:

Antibiotics are popularly used in solution or in ointment for the ophthalmic route. Conventional ocular formulations such as emulsions, suspensions, and ointments are developed to improve solubility, precorneal residence time and ocular bioavailability of drugs. Along with drops, ointments are the most common way to treat many eye problems. Because the go right into eyes, they can start to work much faster than a medicine taken by mouth. Eye ointments are drugs in a greasy, semisolid form. Once ointment applied to eyes, it breaks into tiny drops. These hang out between eyeball and eyelid for a while. Ophthalmic ointments are another class of carrier systems developed for topical application. Ocular ointment comprises of mixture of semisolid and a solid hydrocarbon (paraffin). At has a melting point at physiological ocular temperature (34°C). The choice of hydrocarbon is dependent on biocompatibility. Ointments help to improve ocular bioavailability and sustain

FORMULA:

Ingredients	For 100 gm	For 10 ml
White soft paraffin	80gm	
Wool fat	10gm	
Liquid paraffin	10ml	

PROCEDURE:

1. Weigh and measure all the required ingredients of cold cream properly and keep them separately
2. Add white soft paraffin and wool fat in 100m1 beaker and melt them in order of melting point.

3. Add liquid paraffin to the main preparation with continuous stirring.
4. Transfer the prepared ointment to the suitable container
5. Add liquid paraffin to the main preparation with continuous stirring.
6. Transfer the prepared ointment to the suitable container

PRECAUTION:

Avoid contamination during use.

Uses:

1. Eye ointment use in acute or long – term problems.
2. Eye infections.
3. Inflammation condition.
4. Soreness, with dry-eye syndrome.

Examples of eye ointments: Chloramphenicol ointment, Tetracycline ointment, Hydrocortisone ointment

VIVA QUESTIONS:

- What are eye ointments?
- What are the different types of drugs it may contain?
- What are the formulation parameter

REPORT:

Eye ointment were prepared and submitted

EXPERIMENT NO: 12 DATE:

PREPARATION OF ATROPINE EYE DROPS

AIM:

To prepare and submit 10 ml of Atropine eye drop.

REQUIREMENTS:

Beaker, Glass rod, Measuring cylinder, Conical flask

PRINCIPLE:

Eye drops are saline-containing drops used as an ocular route to administer. Depending on the condition being treated, they may contain steroids, antihistamines, sympathomimetics, nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, antifungal, or topical anesthetics. Eye drops sometimes do not have medications in them and are only lubricating and tear-replacing solutions. Eye drops are also used for stopping itching and redness of the eyes. Atropine eye drop is used before eye examinations (e.g., refraction) and to treat certain eye conditions (e.g., uveitis). It belongs to a class of drugs known as anticholinergic. Atropine works by widening (dilating) the pupil of the eye.

FORMULA:

Ingredients	For 100 ml	For 10 ml
Atropine sulphate	1 gm	
Phenyl mercuric nitrate solution, 0.004% w/v	50 ml	
Purified water upto	100 ml	

PROCEDURE:

Weigh the medicament and dissolve it in the bactericidal solution in a small conical flask. Transfer it to a 10 ml measure, rinse the flask, and adjust the final volume with purified water. Sterilize it by autoclaving at 115ºC for 30 mins.

PRECAUTION:

Avoid contamination during use.

VIVA QUESTIONS:

- What is the use of Atropine sulphate eye drops?
- What is the use of Phenyl mercuric nitrate?
- Give some example of antibacterial agents used in eye drops
- How we can sterilize Atropine sulphate eye drops

REPORT:

Atropine eye drops were prepared and submitted

EXPERIMENT NO: 13 DATE:

PREPARATION OF COLD CREAM

AIM:

To prepare and submit 10gms of cold cream (w/o type of emulsion)

APPARATUS:

Beaker, glass rod, china dish, mortar and pestle, thermometer.

PRINCIPLE:

Cold cream is w/o type of emulsion, which when applied to the skin, a cooling effect is produced, due to the slow evaporation of water, present in emulsion. Cold cream is prepared by saponification reaction between and alkali-borax; i.e borax reacts with free fatty acids of bees wax and produce borax soap in-situ (ester of fatty acid). This soap acts as emulsifying agent. In cold cream, the internal phase is oil and external phase is water, hence it forms o/w type of emulsion. But after application on the skin, water evaporates and leads to phase inversion from o/w type to w/o type emulsion. Therefore oily phase, which is remaining (left) on the skin, gives emollient nature. Liquid paraffin is used as emollient and rose oil is used as perfume, to give a pleasant flavour to the cream.

INGREDIENTS TABLE (FORMULA):

Ingredients	Official formula	Working formula
White Bees Wax		
Liquid paraffin(emollient)		
Borax		
Water		
Perfume		

PROCEDURE:

Since there will be little wastage ((loss) during weighing and preparing, to manipulate these practical losses, calculate the ingredients for at least one or two grams extra, than prescribed.

1) Grate the white beeswax in to small pieces. Weigh the required quantity of white beeswax and liquid paraffin and melt in china dish, by heating on a water bath up to 70°C.
2) In a glass beaker, dissolve borax in water and heat up to 70°C
3) When both oily and aqueous phases reach the same temperature (70°C), gradually add borax solution to the melt of beeswax, with constant stirring.

4) Stir continuously until it becomes cool. When the temperature lowers to 40-45°C, incorporate rose oil and mix uniformly, until a homogenous semi solid mass is obtained.

Dispensing: weigh the prescribed quantity of cream on a butter paper and transfer to an ointment jar or metallic/plastic collapsible tube, close it thoroughly and label.

DIRECTION:

Apply to skin.

USES:

Cold cream is used as an emollient for the treatment of dry skin. Hence this becomes quite popular in winter season.

STORAGE:

Store in a cool place but do not allow to freeze.

Auxiliary label: FOR EXTERNAL USE ONLY

VIVA QUESTIONS:

- What are creams?
- How is cold cream prepared?
- What is the difference between ointments and creams? What is the use of liquid paraffin in cold cream?
- What is saponification?

REPORT:

10gms of cold cream were prepared and submitted.

EXPERIMENT NO: 14 DATE:

PREPARATION OF VANISHING CREAM

AIM:

To prepare and submit 10gms of vanishing cream (o/w type).

APPARATUS:

China dish, glass rod, beaker, Bunsen burner, thermometer

PRINCIPLE:

Vanishing cream is o/w type of emulsion, which when applied to the skin, it vanishes and leaves an almost invisible layer on it. Hence it is called as 'vanishing cream'. The layer left behind after application, acts as a base or foundation, for facial make up. Hence vanishing creams are also called as 'foundation creams'. Since water is an external phase, it will be quickly washed off with water.

The main ingredients of vanishing creams are stearic acid, alkali and water. Stearic acid gives a pearly white shining appearance to the cream, which on application gives a thin white film of free stearic acid. Soap is prepared in-situ by the chemical reaction between alkali and stearic acid, which is used as emulsifying agent.

Vanishing creams are o/w type emulsion; there is a possibility of evaporation of water from the external phase of emulsion. Therefore, glycerine, polyethylene glycol or alcohol are incorporated as humectants, to prevent the drying out of cream, since external phase of vanishing cream is aqueous, it should be protected from the contamination, from microorganisms by adding suitable preservatives, like methyl paraben or propyl paraben. These creams are also be scented pleasantly, using suitable perfumes in small quantities.

INGREDIENTS TABLE (FORMULA):

Ingredients	Official Formula(100 gm)	Working Formula
Stearic acid		
Potassium hydroxide		
Glycerine(humectants)		
Methyl paraben		
Water		
Perfume		

PROCEDURE:

- Melt stearic acid in china dish on water bath by heating up to 70^0C.
- In a beaker, Dissolve KOH, and methyl paraben (methyl parahydroxybenzoate) in water, add glycerin to it.
- Heat this aqueous solution up to 70^0C on water bath.

- When both aqueous and oil phases reaches the same temperature 70^0C, add aqueous phase to the melted stearic acid with continuous stirring.
- Remove the dish from heat and continue the stirring and when temperature reaches 40^0C, add perfume.
- Mix uniformly until it becomes cool and homogenous cream is obtained.

DISPENSING:

Weigh the prescribed quantity of cream on the butter paper and transfer to a wide mouthed, small, screw capped plastic or glass bottle or to collapsible tube, seal and label.

DIRECTION:

Used for external application. Apply to skin where ever necessary.

STORAGE:

Store in a cool place.

AUXILIARY LABEL: FOR EXTERNAL USE ONLY

USES:

Vanishing cream is used as foundation for holding the makeup preparation for longer period.

VIVA QUESTION:

1. What is vanishing cream?
2. What is the use of vanishing cream?
3. Why it is also called foundation cream?
4. What is the use of stearic acid and methyl paraben in vanishing cream?
5. What is humectant?

REPORT:

10gms of vanishing cream were prepared and submitted.

EXPERIMENT NO: 15 DATE:

EVALUATION OF GLASS CONTAINERS (AS PER IP)

AIM:

To carryout different evaluate tests of glass container as per I.P.

REQUIREMENTS:

Class container, Beaker, Conical flask, Burette, Mortar and pestle, Sieve

PRINCIPLE:

Glass containers may be colourless or coloured. Neutral glass is a borosilicate glass containing significant amounts of boric oxide, aluminum oxide, alkali and/or alkaline earth oxides. It has a high hydrolytic resistance and a high thermal shock resistance. Soda-lime- silica glass is a silica glass containing alkali metal oxides, mainly sodium oxide and alkaline earth oxides, mainly calcium oxide. It has only a moderate hydrolytic resistance.

According to their hydrolytic resistance, glass containers are classified as:

- Type I glass containers which are of neutral glass, with a high hydrolytic resistance, suitable for most preparations whether or not for parenteral use.
- Type II glass containers which are usually of soda-lime- silica glass with high hydrolytic resistance resulting from suitable treatment of the surface. They are suitable for most acidic and neutral, aqueous preparations whether or not for parenteral use.
- Type III glass containers which are usually of soda- lime-silica glass with only moderate hydrolytic resistance. They are generally suitable for non-aqueous preparations for parenteral use, for powders for parenteral use and for preparations not for parenteral use. Glass containers intended for parenteral preparations may be ampoules, vials or bottles. Glass is a common material to be used in either non sterile or sterile liquid dosage forms. It leaches alkali from its surface. Hence, a limit test for alkalinity is to be performed before using it for a particular product. USP and IP provide two tests to determine the chemical resistance of glass containers.

1. **<u>Powdered Glass Test</u>**

From the glass containers, alkaline constituents (oxides of sodium, potassium, calcium, aluminum, etc.) are leached into purified water under conditions of elevated temperatures. When the glass is powdered the leaching of alkali can be enhanced in the powdered is critical. The principle involved in the powdered glass test in estimate the amount of alkali leached form the

glass powder. The amount of acid that is necessary to neutralize the released alkali (a specified limit) is specified in the pharmacopoeia. The basic analysis is acid-base titration using methyl red indicator.

2. Water Attack Test

This is only for treated soda lime glass containers under the controlled humidity conditions which neutralize the surface alkali and glass will become chemically more resistant. The principle involved in the water attack test is to determine whether the alkali leached form the surface of a container is within the specified limits or not. Since the inner surface is under test entire container (ampoule) has to be used. The amount of acid that is necessary to neutralize the released alkali from the surface is estimated, the leaching of alkali is accelerated using elevated temperature for a specified time. Methyl red indicator is used to determine the end point. The basic is acid-base titration.

PROCEDURE:

Powdered glass test:

Step-1: **Preparation of glass specimen**: Few containers are rinsed thoroughly with purified water and dried with stream of clean air. Grind the containers in a mortar to a fine powder and pass through sieve no.20 and 50.

Step-2: **Washing the specimen**: 10gm of the above specimen is taken into 250 ml conical flask and wash it with 30 ml acetone. Repeat the washing, decant the acetone and dried ate specimen after which it is used within 48hr.

Step-3: 10gm sample is added with 50ml of high purity water in a 250ml flask. Place it in an autoclave at 121ºC±2ºC for 30min.Cool it under running water. Decant the solution into another flask, wash again with 15ml high purity water and again decant. Titrate immediately with 0.02N sulphuric acid using methyl red as an indicator and record the volume.

Water attack test:

Rinse thoroughly with high purity water. Fill each container to 90%of its overflow capacity with water and is autoclaved at 121ºC for 30min then it is cooled and the liquid is decanted which is titrated with 0.02N sulphuric acid using methyl red as an indicator. The volume of sulfuric acid consumed is the measure of the amount of alkaline oxides present in the glass containers.

Limits of alkalinity for glass containers

TESTS	CONTAINER	VOL.OF 0.02N H_2SO_4
Powdered glass test	Type I Type II Type III	1.0 8.5 15.0
Water attack test	Type II(100ml or below) Type II(above 100ml)	0.07 0.02

VIVA QUESTIONS:

- What are the different types of glass container? What are the major components of glass?
- What type of substances can be packed in type-III glass container? What is type – IV glass container? What is the use?
- Which indicator is used in powder glass test?
- What is the main principle involved in water attack

REPORT:

Evaluation test for glass containers were performed.

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PHARMACOLOGY-II

B. PHARM 5 th SEMESTER PREPARED

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EXPERIMENT NO: 1

DATE:

INTRODUCTION TO IN-VITRO PHARMACOLOGY AND PHYSIOLOGICAL SALT SOLUTIONS

AIM:

To study the Introduction about In-Vitro Pharmacology and Physiological salt solutions.

INTRODUCTION:

"In vitro" is a Latin word that means "within the glass" Therefore the studies which are done outside the living organism, inside glass (test tubes or Petri dishes) are known as In vitro studies. It is the experiment or observations done on the tissue outside of the living organism in a controlled environment, usually using Petri dishes and test tubes. In vitro processes, conditions are artificial and they are reconstructions of In vivo environments. Artificial conditions are formed by mixing the necessary components and reagents under controlled conditions inside a glassware in the laboratory. In vitro methods are widely used in pharmaceutical industry to produce large scale pharmaceuticals using microorganism due to ease of production and economic benefits.

INSTRUMENTS USED IN INVITRO STUDIES

1. DALE'S ORGAN BATH OR ISOLATED ORGAN BATH

- It is an apparatus used for studying the effect of drugs or chemical substances on isolated tissue *Invitro*.
- An inner glass tube or organ bath containing PSS and tissue and connected to reservoirthrough polythene or rubber tube.
- Aeration tissue holder to hold tissue and supply oxygen or air to perfusion fluid.
- An outer glass or bath made up of glass perplex filled with water, the temperature of which is checked with the help of thermometer and maintained at 37^0C for all mammalian experiments.
- A level of recording the response of the tissue on a kymograph drum.
- The isolated organ bath meant for research purpose also poses an inbuilt warming device. The thermostat, a fluid warming cell and a stirrer meant for automated temperature control of the water of the outer bath.
- The entire assembly mounted on a tripod stand.

2. SHERRINTON'S RESEARCH KYMOGRAPH

- It is the instrument in which physiogical response such as contraction and relaxation ofmuscles are recorded. It consists of heavy base and a vertical shaft.

Base hoof (legs): with adjustable levelling screw to keep drum horizontal on the

uneven surface.

- ❖ Slide hoof: to turn the drum on its side so that shaft becomes horizontal.
- ❖ Gear rods : arrangements with gear and the clutch to obtain desirable speed of the drum
- ❖ Drum cylinder: it bears ileum cylinder around which, paper is wrapped and smoked forrecording of trainings.

3. RECORDING LEVERS

They are used to record the contraction or relaxation of the isolated tissue preparation

PRINCIPLE

(i) Fulcrum: The pint around which the levers move on lever holder is the fulcrum.

(ii) Stylus: it is the writing point which records the tracing on the smoked paper of the drum. It is either made of celluloid parchments, aluminium foils or thin photographic or X-Rays.

MAGNIFICATION

The fulcrum (F) should be placed that there is some magnification of the actual concentration (response). In order to achieve this the distance between the writing point and the fulcrum and point of attachment of tissue (T). By adjustment of this relative distance from the fulcrum any of this relative distances from the fulcrum any degree of magnification can be obtained. Therefore lesser the inherent contractibility of the tissue higher the magnification needed for vice versa.

NAME OF TISSUE MAGNIFICATION

(i) Guinea pig ileum 5-10 times

(ii) Rat uterus 4-6 times

(iii) Frog rectus, abdominus rectus muscle 10 times

(iv) Rat fundus stripe 15 times

Application of LOAD/ TENSION

The muscle preparation has to be properly relaxed without affecting the normal tone and rhythmicand it also relaxes to its full length after warming. This is achieved by following ways:

Select the proper length of shorter and longer arm after fixing magnification to particular tissue andfix the fulcrum (plasticine) at the end of shorter arm, marked the point of tissues adjustment.

At equidistance ie, the distance between fulcrum and tissue from the (F). On the longer arm of leverdesired load required for particular tissue.

The tension (load) prescribed for various commonly used tissues as follows:

1) Guinea pig ileum 1g
2) Guinea pig trachea 0.2g
3) Guinea pig vas deference 0.5g
4) Rat colon 0.5g
5) Rat uterus 1g
6) Rat fundus 1g
7) Frog rectus, abdominal muscle 1g

The writing levers are light in weight and rigid and generally made of wood (straw) light aluminiumor stainless steel.

The levers are of two types:

1) Isotonic type: change in length due to contraction is recorded while the tension on the muscle remains the same. Eg; simple lever, frontal lever.

2) Isometric type: these levers are used in special circumstances foe instance when a twitch is produced by stimulating a muscle suspended between 2 rigid points all begging a strong spring. The muscle does not shorten but only creates a force or tension which is recorded the twitch is also much faster in action.eg: Parton's an isotonic lever will serve purpose well.

DIFFERENT TYPES OF LEVERS

1) **Simple lever**:

It is a simplest type of lever made up of wood (straw), stainless steel or aluminium. A celluloid writing tip (stylus) is attached to the end of the longer arm. The contraction is recorded as curved lines.

2) Frontal writing lever(writing frontally)

This lever is designed in such a way that the writing point rotates freely. This helps in reducing the tension between smoked paper and recording tip. The contraction is recorded as straight line.

3) Starlings' heart lever

This lever is used to record the contraction of the heart. The difference between this and other isotonic lever is that fulcrum lies at one end the point of attachment. It consists of a frame carrying a light lever arm with holes and notch supported by a fine adjustable hook.

4) Universal lever (brodies)

It is a lever of versatile utility. There is an adjustable spring supported which counter the pull on the writing arm. This spring help in bringing the pulled writing arm back to its original position. This lever is mainly used for recording sudden repetitive contraction of muscle or movement at a part of the body. Eg: contraction of a gastrocnemius muscle in response to sciatic nerve stimulation.

4. ARTERIAL CANNULA:

It is a small apparatus used in animal experiments to cannulate an artery (usually common carotid or femoral) for recording the blood pressure in anesthetised animal. Eg: dog cat rabbit and rat.

It consists of hollow bulb connected to these arms open into the cavity at bulb. The small globular arm is bevelled at its other end to help in its insertion in the artery. The size length and thickness of the arm depend on the size of artery cannulised to the

- Large thicker arm used for connecting the cannulised with the mercury manometer.
- The small thickened arm is used for flushing out and for removing the blood clot from the cavity at the cannula with the help of pump clip.
- During the experiment the entire cannula is filled up with a solution containing sodium citrate or heparin as anticoagulant.

5. VENOUS CANNULA

It is a small hollow glass tube about 4-5cm in length and 3-4mm in diameter and is used for cannulating. Weighing for intravenous administration of drugs or fluids during experiments on dog, cat and rabbit.

At one end there is a small globular projection with bevelled outer end help in its insertion into the vein. The base of this projection is thinner cetin the tied thread in place and prevents slippage at the cannula out of the vein.

A piece of rubber tube is slipped on the other thicker end of the cannula and is clamped with a punchclip to prevent back flow of the blood. The cannula is filled up with saline after cannulating the vein.

6. TRACHEAL CANNULA

It is a small metallic tubular device for cannulating trachea for:

a) Artificial expiration.
b) Recording of respiration in animals

They are of following types

1) Straight
2) Z-shaped
3) Y-shaped

- Straight and Z-shaped tracheal cannula are used whenever respiration is to be recorded. Oneend of this cannula is inserted into the lumen of trachea.
- The other end is connected to marry's tambour with the help of rubber tube.
- This cannula have adjustable slit. This is used to adjust the entry of air.
- Y-shaped tracheal cannula is used whenever artificial respiration is to be given. Stem of this Y cannula is inserted into the lumen of the trachea out of the remaining 2 arms;
- One is connected to the outlet of the respiration pump and other one to the outlet of the pumpwith the help of rubber tubing.
- Depending on the size of trachea, small or large tracheal cannula may be used in animalslike dog, cat, rabbit, guinea pig and rat.

7. Syringes

Simple glass syringes:

- It is also known as B and D syringes.
- B and D stands for Beckpon and Dikinson who manufactured it.
- It is a glass syringe with hollow floating distance.
- Available in 2cc, 5cc, 10cc, 50cc, and 100cc capacity.
- Sterilsed by autoclave.Tuberculin syringe:

It was used initially for the tuberculin test PD (pure protein derivative test).

Therefore the name tuberculin was given. Now days it is used for many other sensitivity test. Eg:Leronine tes.

I is 1ml capacity syringe and divided into 100 parts having further subdivision ie minimum 0.11mldrugs can be given 50 equal further division 0.02ml minimum drug can be given.

BIOASSAY

Definition:

Estimation of potency of an active ingredient in unit quantity of preparation is detection and measurement of concentration of substance preparation using biological method is known as bioassay.

Principle of Bioassay:

The basic principle of bioassay is to compare the test substance with internal standards of the same and find out how much test substance is required to produce same biological effect that are produced by the standard.

Methods of bioassay:

1. Quantal assay
2. Graded response assay

1. **Quantal assay**:

In this bioassay the dose of the standard thar of the unknown which provide the predominant or all or none, response is measured and their potency ratio is compared. Eg: digitalis induced cardiac arrest is in guinea pig or cat.

2. **Graded response assay**:

These are called graded response assay because the response to varying doses of drugs is graded and measured repeatedly of acetylcholine on frog rectus abdominal muscle.

2.1. **Matching bioassay**:

It is used when the test sample is too low. In this method a curve of which match is response with dose of standard is found by trial and error. From this the potency of unknown test solution can be calculated.

2.2. **Bracketing Bioassay**:

It is also used when the test sample is too small the observed test response with the drug is tracked between the one higher and one lower response of standard. The strength of the unknown can be found by sample interpolation of this tracked response on the dose axis.

2.3. **Interpolation method**:

It is based on the principle of log response. In the method LDR curve of the standard drug is obtained at 1st later 2nd and 3rd response of the unknown which fall in between linear portion of LDR curve are obtained by trial and error. Then interpolation of this response of log dose axis taking the antilog the concentration of this response can be found.

2.4. **Multiple point bioassay**:

a. Three point bioassay: The response of standard drug and one response is due to the test sample are taken into consideration. The test response should be intermediate between the 2 responses of the standard.

b. Four point bioassay: the Two response of standard drug and two response of test drug are made use should be linear portion of the concentration curve and also the ration between the dose should be perfectly 1:2 solution of test response are recorded in the random fashion.

Physiological salt solution

Physiological salt solution is prepared in deionised water free from metal ion. The salt and glucose is added to the water up to specified amount (1L). the solution is stirred and filtered. To prevent the precipitation Ca^{2+} and Mg salts are added at the end.

S No.	Constituent	Frog Ringer	Kerbs Ringer	Tyrode ringer	Ringer Lactate solution	Dejalon solution
1	NaCl	6.0	6.9	8	9	9
2	KCl	0.14	0.35	0.2	0.42	0.42
3	CaCl2	0.12	0.28	0.2	0.24	0.06
4	MgCl2	-	-	0.1-1.0	-	-
5	MgSO4.7H2O	-	1.28	-	-	-
6	NaH2PO4 (10% w/v)	-	-	0.04	-	-
7	K H2PO4 (10% w/v)	-	0.16	-	-	-
8	NaHCO3	0.2	2.1	1	0.5	0.5
9	Glucose	2	2	1	1	0.5

Purpose of each ingredient

1. Sodium Chloride (NaCl) To maintain iso osmolarity, isotonicity, excitability, contractility of the preparation
2. Potassium Chloride (KCl) To maintain ionic balance.
3. Calcium Chloride (CaCl2) Sodium To maintain the contractility of the preparation
4. bicarbonate (NAHCO3) To provide alkaline pH
5. Glucose To provide energy
6. Sodium or potassium Act as the buffer
7. Di hydrogen phosphate To stabilize the preparation
8. Sulphate To stabilize the preparation and hence to reduce the spontaneous activity.

REPORT:

EXPERIMENT NO: 2

DATE:

EFFECT OF DRUGS ON ISOLATED FROG HEART

Aim:

To study the effect of drugs (inotropic and chronotropic actions) on perfused frog heart.

Principle:

Drugs may influence the rate (chronotropy) and force (inotropy) of contraction of the heart. An increase in the heart rate is called a "positive chronotropic" response, while a "negative chronotropic" response is a decrease in the heart rate. Similarly, an increase in the force of contraction is called a 'positive inotropic' response and a decrease in the force of contraction is called a 'negative inotropic' response. Sympathomimetic amines such as adrenaline and noradrenaline produce positive inotropic and positive chronotropic response. Whereas parasympathomimetic such as acetylcholine produce negative inotropic and negative chronotropic response.

Requirements:

Animal: Frog

Apparatus: reservoir, tubing, screw clip, cannula, clamp, boss- head, thread, syringe and needle.

drugs: Adrenalin (stock solution 10 µg/ml) Noradrenaline (stock solution 10µg/ml) Acetylcholine (stock solution 10µg/ml) Calcium chloride (stock solution 10µg/ml) Potassium chloride (stock solution 10µg/ml)

Physiological solution: Frog ringer

Procedure:

1. Pith the frog and pin it to the frog board.

2. Give a mid-line incision on the abdomen. Remove the pectoral girdle and expose the heart.

3. Carefully remove the pericardium and put a few drops of frog ringer over the heart.

4. Trace the inferior vena cava, put a thread around it and give a small cut in order to insert the venous cannula which is in turn connected to a perfusion bottle containing frog ringer. Insert a cannula in the vein and tie the thread to assure the cannula in place.

5. Give a small cut in one of the aortae for the perfusate to come out. 6.

6. Adjust a proper venous pressure of 2-4 cm by altering the height of perfusion bottle. The effective venous pressure is the height in cm from level of the venous cannula and the ringer level in the perfusion bottle. Use of Marriott's bottle helps

in attaining the constant pressure. Start the perfusion by opening screw clamp attached to the tube.

7. Pass a thin pin hook through the tip of the ventricle, and with the help of a fine thread attached to the hook, tie it to the free limb of the universal lever, which is fixed to a stand. Adjust proper tension and magnification by altering the height of the lever. Record the normal contraction of the heart on the smoked drum.

8. Inject 0.1,0.2,0.5 and 1 ml of the stock solution of each drug in a sequential order and note the change in the rate and amplitude of contraction. Keep at least 5 min gap between the administration of each dose of the drug. The drug is administered by injecting the drug into the perfusion tube very close to the venous cannula. Take precautions to avoid any leakage of the drug from the tube, and the injection of air bubbles.

9. Label and fix the tracing with the fixing solution.

REPORT:

EXPERIMENT NO: 3

DATE:

EFFECT OF DRUGS ON BLOOD PRESSURE AND HEART RATE OF DOG

Aim:

To study the effect of drugs on blood pressure and heart rate of dog.

Principle:

The arterial blood pressure is defined as the pressure exerted by the blood on the walls of the blood vessels. Therefore, blood pressure = cardiac output × peripheral resistance. The heart and the blood vessels are under the control of autonomic nervous system. Both sympathetic and parasympathetic nerves supply the heart. Parasympathetic innervations are through the vagus supply the heart. Parasympathetic innervations are through the vagus whereas the sympathetic nerve supply to the heart comes from fibres arising from stellate or inferior cervical ganglion. The nervous supply to the blood vessels is principally from the sympathetic system. In general, sympathetic stimulation (administration of adrenaline and noradrenaline) increases cardiac output and resistance to flow leading to an increased blood pressure. On the other hand, parasympathetic stimulation (administration of acetylcholine) decreases cardiac output which lowers the blood pressure.

Requirements:

Animal – Dog (6-8 kg) Anesthetic pentobarbitone sodium (45 mg /kg, iv; prepare a stock solution containing 45mg/ml of the drug and per kg of body weight.

Drugs: adrenaline, noradrenaline, isoprenaline, acetylcholine (all 100 mg/ml stock solution), normal saline, and sodium citrate (10% w/v).

Equipment: Artery cannula, venous cannula, dog operating table, research kymograph (big), mercury manometer and surgical equipment.

Procedure:

1. Anaesthetize the dog with pentobarbitone (45 mg/kg) given intravenously.

2. Cannulate the femoral vein and connect it to a burette containing saline. Femoral vein cannulation is used for drug administration.

3. Cannulate carotid artery and mount the blood pressure using mercury manometer.

4. Record the base –line mean blood pressure response. Note the heart rate. Inject 3 or 5 µg/kg adrenaline through the femoral venous cannula. Note the sequence of the response, i.e., rise in blood pressure, heart rate, the vagal notch, blood pressure falling below the base line and recovery to Pre-drug base line. Wait for 5-10 min.

5. Similarly administer noradrenaline, isoprenaline and acetylcholine, one after the other. Give sufficient gap (5-10 min) between the effects of two drugs.

REPORT:

EXPERIMENT NO:4

DATE:

STUDY OF DIURETIC ACTIVITY OF DRUGS USING RATS/MICE (LIPSCHITZ TEST)

Aim:

To study the effects of various drugs (diuretics) on the output of urine.

Principle:

Diuretics are the compound which increases the flow of urine. Normal urine output in rats is very small (1-2 ml/rat/day). Hence to get the measurable quantity the animals are first hydrated. The urine output is increased after administration of diuretics like urea, hydro flumethiazide and furosemide. Increase in volume of urine is measured with the help of measuring cylinder and compared with the normal urine output.

Requirement:

Animal-Rats

Apparatus- metabolic cages, graduated measuring cylinder.

Drugs and solutions: Normal saline (0.9%), Urea (900 mg/kg; oral) Hydroflumethiazide (1mg/kg; oral) Furosemide (5mg/kg; oral)

Procedure:

1. Albino rats (150-200 g) are fasted (deprived of food and water) overnight and saline(25ml/kg) is administered orally with the help of oral feeding cannula.

2. Those animals are divided into four groups containing three rats in each as follows: (i) Ist group- only normal saline (Saline) (ii) IInd group- saline + Urea (900 mg/kg; oral) (iii) IIIrd group-saline +Hydroflumethiazide (1mg/kg; oral) (iv) IVth group-saline +furosemide (5mg/kg; oral)

3. After administration of drugs animals are placed in the four different metabolic cages.

4. Urine is collected in a measuring cylinder.

5. Time, when the first drop of urine is collected in a cylinder for each group is noted and the volume is recorded at intervals of 15 min for 3-4 hrs.

6. The difference in the volume collected at different time interval and total volume can be compared with various diuretics.

REPORT:

EXPERIMENT NO: 5

DATE:

DRC OF ACETYLCHOLINE USING FROG RECTUS ABDOMINIS MUSCLE

Aim:

To record a concentration response curve of acetylcholine using rectus abdominis muscle preparation of frog.

Principle:

Dose (concentration) - response curves demonstrate graded responses to drugs or agonists where an increase in response is recorded with a subsequent increase in the dose or the concentration of the drug. The dose –response curve is sigmoid or S-shaped. The first part (25% of graph) of the curve has poor discrimination between the doses. Whereas the middle portion of the curve shows greater sensitivity to different concentrations, and the responses to increasing concentrations are linearly differentiated. The last of the curve (plateau) shows the ceiling effect where no more increase in the response is seen with further increase in the dose. When the doses are increased in geometric progression (logarithmic intervals) and the response is plotted against logarithms of doses, the relationship is called log dose-response curve. Rectus abdominis muscle is a skeletal muscle, and the response of acetylcholine is described as nicotinic response.

Requirements:

Animals: Frog

Drugs: Acetyl choline stock solution (1mg/ml)

Physiological solution: Frog Ringer

Procedure

1. Pith or stun the frog and lay it on it back on the frog-dissecting board. Pin the four limbs.

2. Remove the skin on the abdomen and expose the rectus abdominis muscle.

3. Cut and prepare two rectus muscle preparations from each frog. Tie a thread to the top and bottom of each muscle preparation before detaching the muscle from the body of the frog.

4. Mount the preparation in up-right position in the organ bath containing frog Ringer solution under a tension of 1 g. There is no need of maintaining the bath temperature since it is an amphibian tissue preparation. Bubble the organ with air.

5. Relax the tissue for 45 min. during which period wash the tissue with fresh quantum of ringer for at least four times.

6. Record the concentrations due to acetylcholine using either simple sideway or frontal writing lever. Ninety second contact time and a total 5 min time cycle may be used for proper recording of the responses.

7. Record at least four responses to increasing doses of acetyl choline or till you get the maximum response. The maximum response is achieved if one gets same or slightly less response with a higher concentration. Properly label the graph, put the date, your name, and fix the tracing with the help of fixing solution.

8. Measure the height of the response (mm) and draw a dose (concentration) - response graph.

REPORT:

EXPERIMENT NO: 6

DATE:

EFFECT OF PHYSOSTIGMINE ON DRC OF ACETYLCHOLINE USING FROG RECTUS ABDOMINIS MUSCLE AND RAT ILEUM

Aim:

To record the effect of Physostigmine (eserine) on the concentration-response curve of acetylcholine using rat ileum preparation.

Principle:

Rat colon is an intestinal smooth muscle. Ach causes the contraction of the muscle by acting on muscarinic receptor. The spontaneous contraction of muscle preparation can be reduced by calcium level of physiological salt solution and maintain the water bath at room temperature. The muscle preparation obtained from a starved rat gives a stable preparation. Physostigmine increased the levels of Ach. Physostigmine is an anticholinesterase substance and it inhibits the metabolic break down of acetylcholine. As a result, the action of acetylcholine is potentiated. The concentration response curve of acetylcholine will be shifted to the left in the presence of physostigmine.

Requirements:

Animal – Frog

Drugs – Acetylcholine stock solution (1 mg/ml), Physostigmine stock solution (1mg/ml)

Physiological solution – modified ringer with less calcium.

Procedure:

1. Step 1 to 6 are same as previous experiment

2. Record a concentration response curve of acetylcholine using at least four doses.

3. Add physostigmine (2 µg/ml) to the reservoir containing frog ringer and irrigate the tissue with eserinised ringer 30 min.

4. Repeat the concentration-response curves of acetylcholine in the presence of physostigmine.

5. Label and fix both the concentration –response curves.

6. Plot both the concentration –response curves of acetylcholine, ie.one in the absence and the other in the presence of physostigmine. Note the potentiation in the response of acetylcholine.

REPORT :

EXPERIMENT NO: 7

DATE:

EFFECT OF ATROPINE ON DRC OF ACETYLCHOLINE USING FROG RECTUS ABDOMINIS MUSCLE AND RAT ILEUM

Aim:

To record the concentration-response curve of acetylcholine and its modification by atropine using rat ileum preparation.

Principle:

Rat colon is an intestinal smooth muscle. Acetylcholine causes the contractions of the smooth muscle by acting on muscarinic receptors. Atropine blocks muscarinic receptors in the smooth muscle. Therefore, atropine blocks acetylcholine induced contractions in rat colon. The concentration response curve of acetylcholine will be shifted to the right in the presence of atropine. The nature of antagonism is of competitive type. The spontaneous contractions of the preparations can be reduced by reducing the calcium content in physiological solution and maintaining the bath at room temperature (23+ 20 C). The muscle (colon) preparation obtained from an unstarved rat gives more stable contractions.

Requirements:

Animal: Rat (150-200g)

Drugs: Acetyl choline stock solution (1mg/ml), Atropine stock solution (1 mg/ml)

Physiological solution: Modified Ringer (contains less calcium)

Procedure:

1. Sacrifice the animal by cervical dislocation.

2. Cut open the abdomen and identify the colon. the right flexure, i.e., the subhepatic region of the colon where the ascending colon turns to become transverse colon, is cut out and placed in a shallow dish containing Modified Ringer's solution.

3. The lumen is gently cleaned and a 3 cm long tissue is mounted in the organ bath containing Modified Ringer's solution (pH 7.4) maintained at 25 0 C and bubbled with carbonated air. The preparation is allowed to equilibrate for 45 min under 500g tension.

4. Record the concentration dependent responses due to acetylcholine using frontal writing lever. Contact time 60 sec, and 5 min time cycle are kept for proper recording of the response.

5. Add atropine to the reservoir containing Modified Ringer's solution and irrigate the tissue with atropinised Modified Ringer for 20 min.

6. Repeat the concentration-response curve of acetylcholine in presence of atropine.

7. Label and fix the tracing, plot the graph as done in the earlier experiments.

8. Calculate EC 50 values and note the nature of antagonism.

Dose ratio = EC50 after atropine/ EC50 before atropine.

REPORT:

EXPERIMENT NO: 8

DATE:

BIO ASSAY OF HISTAMINE USING ISOLATED TISSUESBY MATCHING METHOD

Aim

To estimate the strength of unknown samples of Given drug using isolated tissues by matchingmethod

Principle

Simple type of graded assay is 1 for which no statistics are required and in which a constantdose of standard till an exact matching between the sample close to standard is achieved. The methodrather or in accurate and it is difficult to measure the margin of error. It does not give any paralismsof standard response of curve and unknown.

Requirements:

Animal	-	Rat
Drug	-	Histamine /acetylcholine
PSS	-	Modified ringer solution.

Procedure:

- Sacrifice the animal by cervical dislocation
- Cut open the abdomen and identify the isolated tissues
- The lumen gently washed mounted in organ bath contains modified ringer solutionmaintain at 25^0C and bubbled with carbonated air.
- The preparation is allowed for equilibrium for 45minutes under 0.45g tension.
- Record the concentration response curve due to ach after taking concentration of Ach.
- Initially the response of the unknown is recorded. Then takes the response of thestandard dose until matching with the test dose.
- In this method a constant dose of test is packed with varying dose of standard till theexact match is obtained.

REPORT:

EXPERIMENT NO: 9

DATE:

BIOASSAY OF OXYTOCIN USING RAT UTERINE HORN BY INTERPOLATION METHOD.

Aim:

To record the concentration –response curve of oxytocin using rat uterine horn by interpolation method.

Principle:

Oxytocin is a hormone secreted by the posterior pituitary gland. The rat uterine preparation is commonly used for the bioassay of oxytocin. The sensitivity of the uterus to oxytocin depends on the estrous cycle. The various stages of estrous cycle can be identified by preparing vaginal smears and observing under microscope. Rat uterus is highly sensitive. An adult female rat (2-3 months old) has an estrous cycle of five days.

The estrous cycle is divided into different stages.

1. Diestrus – characterized by presence of leukocytes in vaginal smear.

2. Proestrus /estrous – characterized by the presence of large number of nucleated epithelial cells.

3. Frank estrous –Presence of cornified epithelial cells.

4. Meta estrous or late estrous –presence of mixture of nucleated, cornified epithelial cells and leucocytes. If the rat is not in frank estrous stage, it can be induced by the administration of estrogen preparation, stilbestrol (0.1 mg/kg, sc:24 hrs. before) Frank estrus uterus is highly sensitive to oxytocin and hence preferred for bioassay than the diestrus uterus which is relatively less sensitive.

Requirements:

Animal: Female rat (120-150 g)

Drugs: Oxytocin, Stilbestrol

Physiological solution: De Jalon

Student organ bath

Procedure:

A. Preparation of animal:

Examine the vaginal smear under microscope to know about the proper stage of estrus cycle. If the rat is not in frank estrus, inject 0.1 mg/kg of stilbestrol and wait for 24 hr. (Vaginal smear is prepared by taking a drop of vaginal wash and putting

on the glass slide).

1. If the epithelial cells are present in the smear, it is said to be in frank estrous phase.

B. Isolation of tissue:

1. Animal is sacrificed by cervical dislocation.

2. Cut open the pelvic region and expose both the horns of uterus. Separate them gently from the surrounding fatty material and transfer them into a dish containing De Jalon's solution. When the rat is in estrus generally the uterus is fleshy and pink in color. 3. Then the uterus is cut longitudinally and a tissue portion of 2-3 cm long is taken and both ends are tied with the thread.

C. Mounting the tissue:

1. About 2-3 cm long tissue is mounted in organ bath containing De Jalon's solution at 320C along with proper aeration.

2. A tension of about 500 mg (0.5g) is applied and tissue is allowed to equilibrate for 45 min.

D. Recording of the response:

1. Record the DRC for the standard oxytocin solution is taken.

2. Record responses due to 0.1 ,0.2, or 0.4 ml of the test substance.

See that these responses would fall on the linear portion of the concentration – Response curve for the stand solution.

3. Label and fix the tracing.

4. Plot the concentration response curve due to standard acetyl choline solution.

Measure the heights of the contractions (response) due to different doses (A and B) of test solution. read the corresponding concentration from the standard curve.

REPORT:

EXPERIMENT NO: 10

DATE:

BIOASSAY OF SEROTONIN USING RAT FUNDUS STRIP BY THREE POINT BIOASSAY

Aim:

To find out the concentration of the given sample of 5 HT (5-hydroxy tryptamine) or serotonin by three-point bioassay using rat fundus strip preparation.

Principle:

Rat fundus is a very sensitive tissue for the study of the action of several naturally occurring substances like 5-hydroxy tryptamine, histamine, acetyl choline and bradykinin. Unlike the intestinal smooth muscle(ileum) this preparation is slow contracting and slow relaxing type. Rat fundus is generally employed for the bioassay of serotonin. The fundus (the upper part of the stomach) is grey in color and therefore, easily identified from pyloric part (pink in color). A zig-zag preparation of the fundal strip is prepared so as to expose maximum portion of the tissue to drug. The tissue is sensitive to 1 ng/ml of serotonin,0.05-1 ng/ml of histamine and 0.2-0.5 ng/ml of acetyl choline, respectively.

Principle of Three-point bioassay:

It is a method based on the assumption of dose-response relationship. Log dose response curve is plotted and the dose of the standard producing the same response as produced by the test sample is directly read from the graph so to estimate the potency of the test sample. In three-point bioassay, the DRC of standard, test samples are first obtained from the responses due to graded doses. From the DRC of standard, two standard doses are selected in such a way that they have produced 20% and80 % of the maximal response respectively and are designated as S1 and S2. The responses of these doses lie on the steepest and straightest part (linear) of the curve. From The DRC of test sample one test dose is selected such that it gives a response which lies in between the two standard responses i.e., it gives a greater response than S1 and a smaller response than S2 and is designated as T. After selecting the standard and test doses, the bioassay is performed by recording the standard and test responses in a randomized fashion. The pattern of addition of doses is S1 S2 T; S2, T, S1 and T, S1, S2 in 3 successive cycles. The mean values of height of the contraction for all the 3 doses are calculated and are used in plotting the graph so as to estimate the potency of the test sample.

Advantages: ➢ More precision ➢ Reliability. **Requirements:**

Animal: Rats (150-200 g, overnight fasted) Drug: Serotonin

(Stock solution 10 µg/ml)

Physiological solution: Krebs solution

Procedure:

1. Sacrifice the rat by a blow on the head and carotid bleeding. Cut open the abdomen and expose the stomach.
2. Identify the fundus of the stomach (upper part). Incise it from the junction of pyloric part and put it in the dish containing Krebs solution.
3. Incise the fundus from the lesser curvature and open it longitudinally. Give alternate zig zag cuts to make a fundal stip preparation. Tie both the ends with the thread and mount in the organ bath containing Krebs solution at 370 C. Aerate the tissue.
4. Apply 1 gm load and allow the preparation to equilibrate for 30 mins. Using frontal writing lever with 10-12 magnification record the contractions due to increase concentrations of serotonin. Since the muscle contracts slowly and relaxes slowly, a contact time of 90 sec, and 5 min time cycle.
5.. Select two doses form the DRC of standard drug, eliciting sub-maximal responses and bearing a dose ratio 1:2 preferentially and designate them as S1 and S2 and respectively.
6. Select one dose from the DRC of test solution in such a way that the response due to this dose lies preferentially between S1 and S2 and designate it as T.
7. Record 3 sets of responses due to S1 S2 and T adding them to organ bath in a randomized fashion as per Latin square design mentioned in the principle. The Latin square design of addition of doses is followed to ensure good randomization and to account for the fluctuating sensitivity of the tissue.
8. Measure various response to calculate the mean of each response (S1, S2, T)
9. Plot the graph with log dose on X-Axis and % of response on Y –axis and interpolate the T response on to the DRC of standard in between S1 and S2 so as to find the standard dose that gives an equivalent response of that of test.
10. Calculate the potency of the test drug by converting the log of the standard dose that has produced an equivalent response as that of test in to anti-log and report the potency as number of µ gms/ml.

11. Concentration of unknown = n1/t × anti-log {T-S 1 ×log n2/n1} Cs ----------
---------- S2-S1

Where, n1 = lower standard dose (n2) = Higher standard dose

(t)= test dose S1= response of n1 S2 = response of n2 T = response of t Cs= Concentration of standard

REPORT:

EXPERIMENT NO: 11

DATE:

BIO ASSAY OF GIVEN DRUG USING ISOLATED TISSUES BY FOURPOINT BIOASSAY

Aim:

To estimate the strength of unknown sample of given drug using isolated tissues by 4 point bioassay.

Requirement:

Animal	:	Rat
Drug	:	Acetylcholine
PSS	:	modified ringer solution

Principle:

4 point bioassay involves addition of 2 doses of standard, 2 doses of test solution and henceestimation of slope of line is obtained.

The same drug producing effect on both standard and test solution can be determined.

The begin with dose response curve of standard and test solution is plotted from this response which slows and finally linear relationship to the log close are selected from this range with both solution.A pair is chosen such that $S_1/S_2 = T_1/T_2$

=1:2 perfectly.

Response T_1 should be between the response S_1/S_2 for region T_1/T_2 same as S_1/S_2

From four point bioassay ABCD of the asset the mean of 4 response to each dose are calculated.

Procedure:

- Sacrifice the rat by cervical dislocation is placed on the dissection board.
- Isolate the colon, lie a thread to top and bottom of each muscle preparation on beforedetaching the muscle from body
- Mount the preparation in light solution
- Relax tissue for 45 minutes during which periodic wash the tissue with ringer solution atleast 4 times.

Record the concentration by using Ach by frontal writing lever at least for 10 seconds

- contact time 30 sec , base line 30 sec, response 45 sec, washing period 90 sec.Record the gradient response 45 sec , washing period which with standard solution ofach until peak obtained
- Select 2 concentration (A,B)standard drug sub maximal response (x_1 x_2) and bearing adose ratio 1:2
- Select two suitable volume of test solution by trial and error method in such a way thatthe response (T_1) due to course dose of test preferably between S1 and S2.
- The higher value of first solution selected should be such that dose ratio B/A – P/C
- All the responses (S_1, S_2, T_1 , T_2) due to linear part of standard dose.
- Standardization of tissue with concentration A1, record 4 set of responses due to A,B,C,D adding to organ bath in random fashion.
- Measure the various response to calculate the means of each response to calculate themean of each response S_1, S_2, T_1 , T_2 calculate the potency ratio.

Potency ratio = x/y1 (antilog(T2-S2)/(T2- T1)+(T1- S1)/(S2-S1)*log(x1/x2))

Where,

$X_1 X_2$	=	lower volume of standard drug (A)
	=	higher volume of standard drug (B)
Y_1	=	lower volume of test solution
S & T	=	mean weight of response

Determination of the strength of unknown ach using standard drug dilution factor test solution andpotency ration

Ctest = Cstd * potency ration * dilution factor of test

REPORT:

EXPERIMENT NO: 12

DATE:

DETERMINATION OF pD2 VALUE OF ATROPINE USING RAT ILEUM PREPARATION

Aim

To calculate pD2 value for atropine using acetylcholine as agonist employing rat ileumpreparation

Requirements

Animal : Rat (200-400g, overnight fasted)

Drugs : Acetylcholine stock solution (1mg/mL), Atropine stock solution (1mg/mL)Physiological solution : Tyrode solution.

Principle

pAx value is calculated to compare the potency of antagonists action acting on the same receptor. The pAx value is defined as the negative logarithm of the molar concentration of antagonist required to reduce the effect of a multiple dose (x) of the agonist to that of a single dose in the absence of antagonist. Higher the pAx value, more potent is the antagonist. The determination of pD2 (X=2) and pA10 (X=10) value have wider application. If the difference between the two values is found tobe 0.95 or very near, the antagonism is likely to be of competetive type. An antagonist acting on the same receptor will have same pD2 value in all the tissue or organ preparations.

Procedure

- The rat is anaesthetized and sacrificed by cervical dislocation.
- Cut open the abdomen and isolate the ileum to a watch glass containing Tyrode solution.
- Cut the ileum in to the small pieces 2-3 cm long.
- A 3cm long tissue is mounted in the organ bath containing Tyrode solution maintained at 32 –35°C and bubbled with O_2 or air.

- Record concentration-dependent response due to acetylcholine using a frontal writing lever.A tension of 0.5g is applied and to tissue is allowed to equilibrate for 30 min before adding drugsto the organ bath.
- Contact time of 60sec and a time cycle of 5mins is followed for the proper recording of theresponses
- Select two doses bearing 1:2 dose ratio and eliciting submaximal response (A, 2A) for pD2 valuedetermination.
- Standardise the tissue with the selected doses of acetylcholine. A tissue is said to be standardizedwhen it respond identical to the same dose of an agonist when repeated.
- Record the concentration due to the double dose of acetylcholine (2A) in presence of varyingconcentrations of atropine (B1, B2, B3,..)
- Determine the percentage response of this dose of acetylcholine (2A) in presence of variousconc. Of atropine.
- Plot a graph representing negative log of molar concentration of atropine employed along x-axisand percentage response along y-axis.
- pD2 value is defined as negative log of molar concentration of atropine require to reduce theeffect of dose 2A to A respectively.
- Readout the pD2 value for atropine from the graph directly. It corresponds to thepercentage response obtained with half the dose of Ach (A).

REPORT:

EXPERIMENT NO: 13

DATE:

DETERMINATION OF pD2 VALUE OF ATROPINE USING RAT ILEUM PREPARATION

Aim

To calculate pD2 value for atropine using acetylcholine as agonist employing rat ileumpreparation

Requirements

Animal: Rat (200-400g, overnight fasted)
Drugs: Acetylcholine stock solution (1mg/mL),Atropine stock solution (1mg/mL)
Physiological solution: Tyrode solution.

Principle

pA_X value is calculated to compare the potency of antagonists action acting on the same receptor. The pA_X value is defined as the negative logarithm of the molar concentration of antagonist required to reduce the effect of a multiple dose (x) of the agonist to that of a single dose in the absence of antagonist. Higher the pA_X value, more potent is the antagonist. The determination of pD2 (X=2) and pA_{10} (X=10) value have wider application. If the difference between the two values is found to be 0.95 or very near, the antagonism is likely to be of competetive type. An antagonist acting on thesame receptor will have same pD2 value in all the tissue or organ preparations.

Procedure

- The rat is anaesthetized and sacrificed by cervical dislocation.
- Cut open the abdomen and isolate the ileum to a watch glass containing Tyrode solution.
- Cut the ileum in to the small pieces 2-3 cm long.
- A 3cm long tissue is mounted in the organ bath containing Tyrode solution maintained at 32 –35°C and bubbled with O_2 or air.
- A tension of 0.5g is applied and to tissue is allowed to equilibrate for 30 min before adding drugsto the organ bath.
- Record concentration-dependent response due to acetylcholine using a frontal writing lever.

- Contact time of 60sec and a time cycle of 5mins is followed for the proper recording of theresponses
- Select two doses bearing 1:2 dose ratio and eliciting submaximal response (A, 2A) for pD2 valuedetermination.
- Standardise the tissue with the selected doses of acetylcholine. A tissue is said to be standardizedwhen it respond identical to the same dose of an agonist when repeated.
- Record the concentration due to the double dose of acetylcholine (2A) in presence of varyingconcentrations of atropine (B1, B2, B3,..)
- Determine the percentage response of this dose of acetylcholine (2A) in presence of variousconc. Of atropine.
- Plot a graph representing negative log of molar concentration of atropine employed along x-axisand percentage response along y-axis.
- pD2 value is defined as negative log of molar concentration of atropine require to reduce the effect of dose 2A to A respectivelyReadout the pD2 value for atropine from the graph directly. It corresponds to the percentage response obtained with half the dose of Ach (A).

REPORT:

EXPERIMENT NO: 14

DATE:

DETERMINATION OF PA2 VALUE OF PRAZOSIN USING RAT ANOCOCCYGEUS MUSCLE (BY SCHILDS PLOT METHOD)

Aim:

To determine the pA2 value of prazosin using rat anococcygeus muscle.

Principle:

The pA2 value is devised by Sir Heinz Otto Schild in Quantitative Pharmacology.In 1947 pA scale ,to express drug antagonism. pAx value is calculated to compare the potency of antagonists acting on the same receptor. The pAx value is defined as the negative logarithm of the molar concentration of the antagonist required to reduce the effect of a multiple dose(x) of the agonist to that of a single dose in the absence of antagonist. The meaning of pA2 is the affinity of the antagonist to the receptor. For antagonist, efficacy is 0 and affinity is one. Higher the pAx value, more potent is the antagonist. The determination of pA2 (X=2) and pA10 (X=10) values have wider applications. If the difference between these two values is found to be 0.95 or very near, the antagonism is likely to be of competitive type.an antagonist acting on the same receptor will have same pA2 value in all the tissue or organ preparations. Principle of schlids plot: ¬ calculation of pA2 value for an antagonist from the effects observed on isolated smooth muscles can be done in two-ways: using schild‟s plot procedure or schild‟ sequation. ¬ most commonly used method for estimating pA2 value. ¬ The Schild plot is a pharmacological method of receptor classification. ¬ Plot log (dose ratio-1) against negative log molar concentration of the antagonist(B) used (or directly against B) ¬ When the slope of the line so obtained is unity ,then the antagonism is competitive.

Requirements:

Animal: Rat (150-200 g,overnight fasted)

Drugs : Prazosin stock solution

Procedure:

Sacrifice the rat by a blow on the head and carotid bleeding

↓

Cut open the abdomen in the midline. Split the pelvis (Remove bladder and urethra)

↓

Cut through the colon at the pelvic brim. Pull forward the pelvic portion and clear the Delicate connective tissue behind until the anococcygeus muscle comes into view

↓

carefully clear the connective tissue to separate the two anococcygeus muscle. Mount the tissue in organ bath containing krebs solution maintaining 37·C equilibrate for 30minutes under 1g of tension

↓

After relaxation of tissue record the DRC standard of NE

↓

select one dose on nor-epinephrine which fall between 25-75% in DRC & labelled it as 2A

↓

Record 2A response of nor epinephrine in presence of increase in concentration of antagonist i.e. prazosin till 2A become A or less than A

NOTE: Administer prazosine and wail for 15 minutes than add specific dose of NE observe the

Response and washing till base line ….Now add 2A response dose without prazosine

REPORT:

EXPERIMENT NO: 15

DATE:

EFFECT OF SPASMOGENS AND SPASMOLYTICS USING RABBIT JEJUNUM.

Principle

Rabbit intestine is a smooth muscle which shows regular pendular movement(i.e continuous Contraction and relaxation). Therefore, to study the effect of drugs on the intestinal movement.Rabbit intestine is an ideal preparation, rabbit intestine is supplied by autonomic nervous system. It consist of muscarinic(m3) and adrenergic (α and β)receptors. Muscarinic receptor agonists like Ach produces contraction of rabbit intestine and physostigmine increases the spasm and pendular movements. These muscarinic actions and effects are blocked by muscarinic blockers like atropine.

Barium chloride acts by increasing tone of rabbit intestine and thereby increasing spasm and pendular movements of intestine.

Adrenaline acts on α and β adrenoceptors and exhibits an inhibitory influence of pendular movements of intestine. These action of adrenaline are blocked by α and β blockers.

Procedure

Fast a rabbit for 12 hour period.

Set up the assembly for rabbit intestine experiment.

Fill the outer jacket of student organ bath with water.

Set the thermostat of the student organ bath at 37®C and switch on the organ bath.

Fill the reservoir with tyrode solution and control the flow of tyrode solution to the inner organ bath

Through glass spiral using haemostatic forceps.

Once the temperature of tyrode solution reaches 37®C, kill a rabbit by giving a blow on its head and

Cutting carotid artery. Open the abdominal region and identify the intestine.

Cut and remove a few centimetre long of the intestine portion and immediately place it in the watch glass containing tyrode solution

Trim the mesentery and with gentle care clean the content of the intestine by pushing the tyrode Solution into the lumen. Utmost care should be taken to avoid any damage to the gut muscle

Take a piece of intestine of 3-4cm long and tie the thread to the top and the bottom ends without Closing the lumen, and amount the tissue in the inner organ tube containing tyrode solution maintained at 37®C and bubbled with o2 or air or carbogen. A tension of 0.5g is applied and the tissue Is allowed to equilibrate for 30 minutes before adding the drugs to the inner organ tube.

Record the normal pendular movement of rabbit intestine on the kymograph fixed on sherrington's drum for 30seconds. At the end of 30 seconds of pendular base line, inject 0.1ml of epinephrine(10µg/ml) and record the response for 30seconds. If sufficient response is not there increase dose to 0.2ml and so on.

Follow the washing period three times after recording the response by removing the tyrode solution present in inner organ tube and adding the fresh tyrode solution for 60 seconds. Due to washing, the writing point of frontal lever of normal pendular base line.

Add 0.1ml of propranolol(200µg/ml) into the inner organ tube allow it to act for 60 seconds and then record the response of epinephrine (10µg/ml) in presence of prpranolol. Follow the washing period for recovery.

Inject 0.1ml of acetyl choline(10µg/ml) and record the response for 30 seconds. If sufficient response is not there increase dose to 0.2ml and so on.

Add 0.1ml of physostigmine(10µg/ml) into the inner organ tube, allow it to act for 60seconds

And then record the response of acetylcholine(10µg/ml) in presence of physostigmine. Follow

The washing period for recovery.

Add 0.1ml of atropine sulphate(100µg/ml) into the inner organ tube, allow it to act for 60seconds

And then record the response of acetylcholine(10µg/ml) in presence of physostigmine. Follow

The washing period for recovery.

Record the response of different doses of barium chloride (10mg/ml) and follow the washing period for recovery.

REPORT:

EXPERIMENT NO: 16

DATE:

TO STUDY THE ANTI-INFLAMMATORY PROPERTY OF INDOMETHACIN AGAINST CARRAGEENAN-INDUCED ACUTE PAW OEDEMA IN RATS.

Aim:

To study the anti-inflammatory property of indomethacin against carrageenan induced acute paw oedema in rats.

Principle:

Inflammation is a tissue –reaction to infection, irritation or foreign substance. It is a part of the host defense mechanism but when it becomes great it is a hopeless condition. Aging is also considered to be an inflammatory response. There are several tissue factors or mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin and prostaglandins. This method is based upon the ability of anti-inflammatory agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent (carrageenan). The volume of the injected paw is measured before and after the application of irritants. The paw volume of treated animals is compared with control. **Plethysmograph is used to measure paw volume.**

Requirements:

Animal: Rats (150-200g)

Equipment: Plethysmograph (simple apparatus containing mercury. The mercury displacement due to dipping of the paw can be directly read from scale attached to the mercury column or adjusting the mercury level in the arm B to the original level by moving the arm B up/down and note the volume required in both the arms equal), syringe and needle

Drugs: carrageenan (1% w/v solution and inject 0.1 ml underneath the plantar region) Indomethacin (Dose 20 mg/kg, s.c, Prepare a stock solution containing 4mg/ml of the drug and inject 0.5 ml/100 g of body weight of the animal). Saline (0.9%)

Procedure:

1. Weigh the animals and number them.

2. Mark a mark on both the hind paws (right and left) just beyond tibio-tarsal junction, so that every time the paw is dipped in the mercury column upto the fixed mark to ensure constant paw volume.

3. Note the initial paw volume (both right and left) of each rat by mercury displacement method.

4. Divide the animals into two groups each comprising of at least four rats. To one group inject saline and to the second group inject indomethacin subcutaneously.

5. After 30 min inject 0.1 ml of 1% (w/v) carrageenan in the plantar region of the left paw of control as well as indomethacin –treated group. The right paw will serve as reference non- inflamed paw for comparison.

6. Note the paw volume of both legs of control and indomethacin-treated rats at 15,30,60, and 120 min after carrageenan challenge.

7. Calculate the percent difference in the right and left paw volumes of each animal of control and indomethacin –treated group. Compare the mean percent change in paw volume in control and drug–treated animals and express as per cent oedema inhibition by the drug.

REPORT:

EXPERIMENT NO: 17

DATE:

TO STUDY THE ANALGESIC EFFECT OF MORPHINE IN MICE USING HOT PLATE METHOD.

Aim:

To study the Analgesic effect of given drug (morphine) in mice using hot plate method (Eddy and Leimbach)

Principle:

Pain is an unpleasant feeling, which make us uncomfortable and reduce the physical as well as mental alertness. Although pain is also useful for us because it acts as a warning signal and it warns about something uncommon inside or outside our body. If the pain is minor, it may be tolerated but if the pain becomes severe it has to be managed at earliest. Analgesia is defined as a state of reduced awareness to pain, and analgesics are substances which decrease pain sensation (pain –killers) by increasing threshold to painful stimuli. The commonly used analgesics are aspirin, paracetamol (non-narcotic type) and morphine (narcotic type). Painful reaction in experimental animals can be applying noxious (unpleasant) stimuli such as (i) (ii) (iii) Thermal (radiant heat as a source of pain) Chemical (irritants such as acetic acid and bradykinin) Physical pressure (tail compression) In the laboratory commonly used procedures are tail-flick (tail-withdrawal from the radiant heat) method using analgesiometer, hot plate (jumping from the hot plate at 55ºC) method and acetic acid – induced writhing.

Requirements:

Animal: Mice (20-25 g)

Equipment: Eddy's hot plate

Drugs: Morphine sulphate (dose 5 mg/kg, sc, prepare a stock solution containing 0.5 mg/ml and inject 1 ml/100 g of body weight of mouse)

Procedure:

1. Weigh and number the mice.
2. Take the basal reaction –time by observing hind paw licking or jump response (whichever appears first) in animals when placed on the hot plate maintained at constant temperature (55º C). normally animals show such response in 6-8 sec. A cut off period of 15 sec is observed to avoid damage to the paws.
3. Inject morphine to animals and note the reaction time of animals on the hot plate at 15, 30, 60 and 120 min after the drug administration. As the reaction time increases with morphine,15 sec is taken as maximum analgesia and the animals are removed from the hot plate to avoid injury to the paws.
4. Calculate percent increase in reaction time (as index of analgesia) at each time interval.

REPORT:

EXPERIMENT NO: 18

DATE:

STUDY OF ANALGESIC EFFECT IN MICE BY TAIL IMMERSION METHOD

Aim:

To evaluate the analgesic activity of opioid by tail immersion method

Principle:

In this method heat is produced as a source of pain. The tail of animal are individually immersed in hot water contained in beaker maintained at constant temperature at 55^0C and the reaction time of animal such as tail flicking response is taken as an end point.

Requirements:

Animal - mice (20-25g)/ Rat (150-220g)

Drug - Diclofenac

Beaker, Thermometer.

P**rocedure**:

- Weigh and mark the animal
- Take the basal reaction time by observing the tail flicking response in animal whenimmersed in beaker containing hot water maintained at constant temperature at 55^0C.
- Normally animal shows such response in 6-8seconds
- A cut off time of 15 seconds is observed to avoid tail injury.
- Inject pentazocine through IP route and note the basal, reaction time and afteradministrated 15, 30, 45, 60, 90 and 120 minutes.
- The reaction time increased with pentazocine in 15 sec is taken as maximumanalgesic.
- Calculate the percentage of inhibition by following formula.

PIP = (T1-T0)/ T0* 100

T1 - Post latency

T0 - Pre latency

REPORT:

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PHARMACOGNOSY AND PHYTOCHEMISTRY-II

B. PHARM 5th SEMESTER PREPARED

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INTRODUCTION

Theory:

The microscope is one of the most commonly used instruments in medical, paramedical and clinical laboratories. It is used to study Cell Morphology, Histology, Histopathology and Microbiology. A microscope helps us to see microscopic objects that are too small and invisible to the naked eye. Description of Compound Microscope: The Compound microscope has the following main parts

1. The supporting system.

2. The focusing system.

3. The optical or magnifying system.

4. The illumination system including:

a) Source of light

b) Mirror

c) Condenser

1. The support system:

It is a framework to which various functional units are attached. It consists of the following:

a) Base: it is a heavy metallic, 'U' shaped or 'horseshoe' shaped base with supports the microscope on work table and provides maximum stability.

b) Pillars: There are two upright pillar that project up from the base and are attached to the 'C' shaped handle. This allows the microscope to be tilted at a suitable angle for comfortable observation.

c) Body tube: It is 16-17 cm long cylindrical tube fitted at the upper end of handle which is vertical or at an angle through which light passes via the eye piece to the observer's eye visualizing the image

d) The stage: The stage is a square platform with an aperture in its centre and fitted to the limb below the objective lenses. When the slide is placed on it, converging rays of light emerging from the condenser passes through slide and then objective lens into the body tube. It can be either the fixed stage or the mechanical stage. The fixed stage has two clips that hold the slide in position. The mechanical stage has a calibrated metal frame fitted on right side of stage. It has a spring mounted clip to hold the slide and two screw heads to move the slide from side to side, forward and backward. The Vernier scale is also attached to indicate degree of movement

2. The focusing system:

The focusing system consists of coarse and fine adjustment and screw heads are used for raising and lowering body tube for proper focusing the slide. The coarse adjustment moves the focusing system up or down through a large distance via a rack. The fine adjustment works in same way which requires several rotations to move the tube through a small distance. It is employed for accurate focusing. The optical or magnifying system: It consists of body tube, eyepiece and the nosepiece. The body tube is the present between upper end of objective and eyepiece. The eyepiece fits into top of body tube. They can be 5X, 6X, 8X, 10X or 15X. Each eyepiece has two lenses; the eye lens at the top and field lens at the bottom. The field lens collects divergent rays, passes through eye lens to further magnify the image. The Nosepiece has two parts; the fixed nosepiece and the revolving nosepiece. The fixed nosepiece holds the revolving nosepiece that carries interchangeable objective lenses. The objective lenses are spring loaded objectives of different magnifying powers. Different types of the objective lenses are low

power objective or 10X, high power objective or 45X, oil İmmersion objectives Of 100X and scanning objective 3X.

Magnification: Magnification is the ability to make small objects seen larger, such as making a microscopic organism visible. The objective lenses magnify the images as stated below: Low power objective 10X = 10 x 10 = 100 times. High power objective 45X = 45 x 10 = 450 times. oil immersion objective (100X) = 100 x 10 = 1000 times. oil immersion (100X) objective has very small aperture and deep focusing position i.e. 1 mm from the slide. The light rays coming from the slide (denser medium) are refracted by thin layer of air (rarer medium) away from the small aperture of the objective and results in faint image. If some other medium like cedar wood oil, paraffin or glycerin having same refractive index as that of glass is added on the slide, it removes the thin layer of air and forms a continuous medium. This avoids the refraction of light rays and results in sharp image.

2. The illumination system: The microscope will function only when proper illumination or lightning is provided. The illumination system is to provide uniform, soft and bright illumination. The illumination system consists of:

(a) Source of light: It may be external (natural day light, electric lamp or tube light) orinternal (electric inbuilt light source).

(b) A condenser: It is a system of lenses filled as short cylinder mounted below the stage.

(c) Mirror: A double sided mirror with one flat side and other concave is located below condenser and can be rotated in all directions. It focuses light rays into a solid cone of light onto the material under study and helps in resolving image.

(d) Iris diaphragm: Iris diaphragm is a thin opaque membranous structure fitted within condenser with a small lever on the side. The lever can adjust size of aperture of diaphragm and allows less or more light falling on slide, Procedure:

1. Examine the permanent slide/blood film/specimen first with naked eye.

2. Place the microscope on working table in an upright position, and raise the body tube approximately 7- 8 cm above the stage. Put the slide on the stage and using the mechanical stage, bring the specimen over the central aperture.

3. Select the low magnification objective (10X). ‘

4. Select and adjust the mirror (plane or concave) so that the light shines on the specimen

5. Adjust the condenser well down, and partly close the diaphragm to cut down excesslight.

6. Looking from the side, and using the coarse adjustment, brings the body tube down so that the low power lens is about 1 cm above the slide. Look into eyepiece and gently raise the tube till the slide tomes into focus.

7. Then choose the area of interest for viewing it under higher magnifications.

8. For focusing under high magnification, simply rotate the nosepiece so that the high magnification objective (45X) ’clicks’ into position. Raise the condenser to mid position and open the diaphragm to admit enough light. Use fine adjustment asrequired.

9. For focusing under oil immersion objective (1O0X}, raise the body tube 8-10 cm above De slide. Place a drop of cedar wood oil, paraffin or glycerin on &e slide. Looking from the side bring down the objective till it just enters the oil drop. Use other adjustment asrequired.

EXP NO: 1

DATE:

CINCHONA

Aim: To identify the Morphology, histology and powder characteristics, extraction detection of Cinchona

Synonym: Jesuit's bark, Peruvian bark, cinchona bark

Biological source:

Dried bark of cinchona species cinchona calisaya wedd, C.Ledgeriana Mocns, C.Officinalis L., C.Succirubra Pav or hybrids of either of the last two species with either of the first two. It contains not less than 6% of total alkaloids of cinchona.

Family: Rubiaceae

Morphology characterstics

Organoleptic characters:

Colour:

Odour:

Taste:

Shape:

Fracture:

Chemical constituents: Quinine, quinidine

Histology and powder characterstics

Transverse section

The dried bark are collected and washed with water for remove the foreign matters.the bark should be free from any microbial contamination.

The dried bark are soaked in water for overnight

The bark should be cutted thinny not thick with the help of blade.

The thin section are placed in the microslide at horizontal position with the help of brush.

Add 2 drops of phloroglucinol reagent as a staining agent and dried it.

Add few drops of dil.Hcl and dried it.

Glycerin is placed on thin section for moisturizing and clear the vision of section.

Chemical required:

Hydrochloric acid, Phloroglucinol, Glycerin, iodine, glycial acetic acid, sulphuric acid, bromine water, ammonia

Apparatus required

Test tube, test tube stand, holder, Watch Glass, microscope slide. Cover slip, Blade, Brush

Histology of cinchona

S.no	Chemical test	Observation	Inference
1.	Phloroglucinol+conc.HCI(1:1)		
2.	Dil.Iodine solution		
3.	Dil.Acetic acid		
4.	Dil.HCI		
5.	Sulphuric acid(6%)		

Powder microscopy:

Dried bark are grinded with using mixer

The powder through the sieve 60

The powder are examined by using phloroglucinol and con.Hcl.

Observe the microscopical character of powder cinchona.

Extraction:

Extraction of cinchona by hot maceration method.

2gm of powder sample is treated with boiling water till the chemical constistuents elute from the powder.

Chemical test

S.no	Chemical test	Observation	Inference
1.	Heat the powder with glacial acetic acid		
2	Treat the bark with conc.sulphuric acid and observe under UV light		
3.	Thalloquin test: Heat powder with few drops of bromine water,shake and strong ammonia		

Uses: Anti malarial, Bitter tonic and Anti pyretic

Observation

Report

REPORT

EXP NO: 2

DATE:

CINNAMON

Aim: To identify the Morphology, histology and powder characteristics, extraction detection of Cinnamon

Biological source:- Obtained from dried inner bark of the tree Cinnamomum zeylanicum.

Family:-Lauraceae.

Morphology characterstics

Colour:

Odour:

Taste:

Shape:

Fracture:

Chemical constituents:-

Eugenol, Cinnamic acid &Cinnamic aldehyde.

Uses:-Flavouring agent, Germicide, Stomachic & Diaphoretic.

Histology and powder characterstics

Transverse section

The dried bark are collected and washed with water for remove the foreign matters.the bark should be free from any microbial contamination.

The dried bark are soaked in water for overnight

The bark should be cutted thinny not thick with the help of blade.

The thin section are placed in the microslide at horizontal position with the help of brush.

Add 2 drops of phloroglucinol reagent as a staining agent and dried it.

Add few drops of dil.Hcl and dried it.

Glycerin is placed on thin section for moisturizing and clear the vision of section.

Chemical required

Hydrochloric acid, Phloroglucinol, Glycerin,iodine, acetic acid, sulphuric acid, osmic acid, Dil tincture alkane, ruthenium red, chloroform, ethanol, ferric chloride, phenylhydrazine hydrochloride , lead acetate, potassium permanganate,

Apparatus required

Watch Glass,microscope slide. Cover slip, Blade, Brush and Compound Microscope.

Histology

S.no	Chemical test	Observation	Inference
1.	Phloroglucinol+conc.HCI(1:1)		
2	Dil.Iodine solution		
3	Dil.Acetic acid		
4	Dil.HCI		
5	Sulphuric acid(6%)		
6	Ruthenium red		
7	1% osmic acid solution		
8	Dil tincture alkana		

Powder microscopy:

Dried bark are grinded with using mixer

The powder through the sieve 60

The powder are examined by using phloroglucinol and con.Hcl.

Observe the microscopical character of powder cinnamon.

Extraction

Extraction of cinnamon by hot maceration method and cold maceration method.

2gm of powder sample is treated with boiling water till the chemical constistuents elute from the powder.

2gm of powder sample is treated with 95 % alcohol within 24 hours.

2 gm of powder sample is treated with chloroform within 24 hours.

Chemical test

S.no	Chemical test	Observation	Inference
1.	Alcoholic extract of drug+one drop of ferric chloride solution		
2.	Chloroform extract of the drug + 10% aqueous solution of phenylhydrazine hydrochloride		
3.	Aqueous extract + 5% FeCI3		

4.	Aqueous extract + lead acetate reagent		
5.	Aqueous extract + potassium permanganate solution		

Uses: Flavouring agent, Germicide, Stomachic & Diaphoretic.

Observation

Report

REPORT

EXP NO: 3

DATE:

SENNA

Aim: To identify the Morphology, histology and powder characteristics , extraction detection of senna.

Synonym: senna ki patti, sonamukhi, Indian senna, Tinnevelley senna

Biological source:

It consist of dried leaflets of Cassia angustifolia Valh.

Family: Leguminosae

It contains Not less than 2.0% of glycosides calculated as sennoside B.

Morphology characterstics

Organoleptic characters:

Colour:

Odour:

Taste:

Shape:

Size:

Extra features:

Chemical constituents:

Anthroquinone glycosides mainly A,B,C and D.

Histology and powder characterstics

Transverse section :

The fresh leaves are collected and washed with water for remove the foreign matters.the fresh leaves should be free from any microbial contamination.

The midrib of leaves are cutting with using the help of blade.

The piece of leaves are mounted the v shaped cuuted potato. The placed leaves are cutted a thinny not thick.

The thin section are placed in the microslide at horizontal position with the help of brush.

Add 2 drops of phloroglucinol reagent as a staining agent and dried it.

Add few drops of dil.Hcl and dried it.

Glycerin is placed on thin section for moisturizing and clear the vision of section.

Chemical required

Hydrochloric acid, Phloroglucinol, Glycerin,Ruthenium red, dil.acetic acid, dil.Hcl, sulphuric acid, sudan-III, Benzene, ammonia

Apparatus required

Watch Glass, microscope slide. Cover slip, Blade, Brush and Compound Microscope, Test tube, test tube stand, holder.

Histology

S.no	Chemical test	Observation	Inference
1.	Phloroglucinol+conc.HCI(1:1)		
2	Ruthenium red		
3	Dil.Acetic acid		
4	Dil.HCI		
5	Sulphuric acid(6%)		
6	Sudan red III		

Powder microscopy:

Dried leaves are grinded with using mixer.

The powder through the sieve 60

The powder are examined by using phloroglucinol and con.Hcl.

Observe the microscopical character of powder senna.

Extraction :

Extraction of senna by hot maceration method.

2gm of powder sample is treated with boiling water till the chemical constistuents elute from the powder.

Chemical test

S.no	Chemical test	Observation	Inference
1.	Borntrager test for anthraquinone: Boil drug with dil sulphuric acid(hydrolysis) filter and cool.Add benzene or CCI4(immiscible organic solvents) shake and separate organic solvent layer in another test tube. Add strong ammonia solution,shake slightly and keep the test tube aside		

Uses: Irritant purgatives

Observation

REPORT

EXP NO: 4

DATE:

CLOVE

Aim: To identify the Morphology, histology and powder characteristics , extraction detection of clove.

Synonym: Lavang

Biological source:

It consists of dried flower buds of ***Eugenia caryophyllus***

Family: Myrtaceae

The oil contain Not less than 15% V/W of clove oil.

Morphology characterstics

Organoleptic character

Colour:

Odour:

Taste:

Shape:

Size:

Extra features:

Chemical constituents: Eugenol, iso eugenol, methyl and dimethyl furfuryl , α and β caryophylline, hydrolysable tannins.

Histology and powder characterstics

Transverse section

The dried clove are collected and washed with water for remove the foreign matters.the clove should be free from any microbial contamination.

The dried clove are soaked in water for overnight

The clove should be cutted thinny not thick with the help of blade.

The thin section are placed in the microslide at horizontal position with the help of brush.

Add 2 drops of phloroglucinol reagent as a staining agent and dried it.

Add few drops of dil.Hcl and dried it.

Glycerin is placed on thin section for moisturizing and clear the vision of section.

Chemical required

Hydrochloric acid, Phloroglucinol, Glycerin, KoH , Dil.HCl, sulphuric acid, sudan red-III

Apparatus required

Watch Glass,microscope slide. Cover slip, Blade, Brush and Compound Microscope.

Histology

S.no	Chemical test	Observation	Inference
1.	Phloroglucinol+conc.HCI(1:1)		
2.	Strong KOH solution		
3.	Dil.HCI		
4.	Sulphuric acid(60%)		
5.	Sudan red III		

Powder microscopy:

Dried clove are grinded with using mixer.

The powder through the sieve 60

The powder are examined by using phloroglucinol and con.Hcl.

Observe the microscopical character of powder clove

Extraction

Extraction of Clove by hot maceration method.

2gm of powder sample is treated with boiling water till the chemical constistuents elute from the powder.

S.no	Chemical test	Observation	Inference
1.	Aq.extract + lead aceate solution		
2.	Clove oil+alcohol+5% ferric chloride		
3.	Aq.extract + 5%ferric chloride		

Uses: carminative, aromatic, stimulant, anti septic, flavouring agent, dental analgesic oil

Observation

Report

EXP NO: 5

DATE:

EPHEDRA

Aim: To identify the Morphology, histology and powder characteristics , extraction detection of Ephedra.

Biological source:-Obtained from dried young stems of Ephedra gerardiana Wall stapf, and also of E. nebrodensis (Tineo) stapf collected in autumn.

It contains not less than 1.0% of total alkaloids calculated as ephedrine.

Family:-Ephedraceae.

Morphology characterstics

Colour:

Odour:

Taste:

Shape:

Size

Chemical constituents:: Ephedrine & Pseudoephedrine.

Histology and powder characterstics

Transverse section

The dried cephedra are collected and washed with water for remove the foreign matters.the ephedra should be free from any microbial contamination.

The dried ephedra are soaked in water for overnight

The ephedra should be cutted thinny not thick with the help of blade.

The thin section are placed in the microslide at horizontal position with the help of brush.

Add 2 drops of phloroglucinol reagent as a staining agent and dried it.

Add few drops of dil.Hcl and dried it.

Glycerin is placed on thin section for moisturizing and clear the vision of section.

Chemical requried

Hydrochloric acid, Phloroglucinol, Glycerin, Dragendorff's reagents, Mayer's reagents, Hager's reagents, Wagner's reagents, sodium hydroxide, copper sulphate, dil.Hcl.

Apparatus required

Watch Glass, microscope slide. Cover slip, Blade, Brush and Compound Microscope.

Histology

S.no	Chemical test	Observation	Inference
1.	Phloroglucinol+conc.HCI(1:1)		

Powder microscopy:

Dried powder are grinded with using mixer.

The powder through the sieve 60

The powder are examined by using phloroglucinol and con.Hcl.

Observe the microscopical character of powder ephedra.

Extraction

Extraction of ephedra by hot maceration method.

2gm of powder sample is treated with boiling water till the chemical constistuents elute from the powder.

Chemical test

S.no	Chemical test	Observation	Inference
1.	10mg drug+1ml water+0.2 ml dil HCI+0.1 ml copper sulphate+1ml sodium hydroxide;solution becomes violet add 1ml solvent ether,shake		
2.	Drug+Dragendorff's reagents		
3.	Drug+Mayer's reagents		
4.	Drug+Hager's reagents		
5.	Drug+Wagner's reagents		

Uses: Treatment of asthma, hay fever, and the common cold.

Observation

Report

EXP NO: 6

DATE:

FENNEL

Aim: To identify the morphological characters of given organised drug.

Synonym: Bari sauf, fructus foeniculi

Biological source:

Dried ripe fruits of cultivated species, Foeniculum vulgare Miller

Family: umbeliferae

It contains not less than 1.4% of volatile oil.

Morphology characterstics

Organoleptic characters:

Shape:

Colour:

Odour:

Taste:

Size:

Extra features:

Chemical constituents:

Volatile oil (4-6%), Anethole (50-60% of volatile oil), d-fenchone (10% of volatile oil), fixed oil (12-18%), proteins (14-22%). Minor constituents of the fennel include limonene, anisaldehyde and methyl chavicol.

Histology and powder characterstics

Transverse section

The dried fennal are collected and washed with water for remove the foreign matters.the fennal should be free from any microbial contamination.

The dried fennal are soaked in water for overnight

The fennal should be cutted thinny not thick with the help of blade.

The thin section are placed in the microslide at horizontal position with the help of brush.

Add 2 drops of phloroglucinol reagent as a staining agent and dried it.

Add few drops of dil.Hcl and dried it.

Glycerin is placed on thin section for moisturizing and clear the vision of section.

Chemical required

Hydrochloric acid, Phloroglucinol, Glycerin, alcoholic picric acid, sudan red-III

Apparatus required

Watch Glass,microscope slide. Cover slip, Blade, Brush and Compound Microscope.

Histology

S.no	Chemical test	Observation	Inference
1.	Phloroglucinol+conc.HCI(1:1)		
2	Alcoholic picric acid		
3	Sudan red III		

Powder microscopy:

Dried powder are grinded with using mixer.

The powder through the sieve 60

The powder are examined by using phloroglucinol and con.Hcl.

Observe the microscopical character of powder fennal .

Extraction

Extraction of fennal by hot maceration method.

2gm of powder sample is treated with boiling water till the chemical constistuents elute from the powder.

Uses: Stomachic, Aromatic, Diuretic, Carminative, Diaphoretic, Digestive, Pectoral, Antipyretic, Antimicrobial & Antiinflammtory.

Observation

Report

EXP NO: 7

DATE:

CORIANDER

Aim: To identify the morphological characters of given organised drug.

Synonym: Dhania, coriander fruit

Biological source:

Dried ripe fruits of Coriandrum sativum Linn.

Family: Umbelliferae

It contains not less than 0.3% of volatile oil.

Morphology characterstics

Organoleptic characters:

Shape:

Colour:

Odour:

Taste:

Size:

Extra Features:

Chemical constituents:

Volatile oil (0.2 -1%), coriandrol (D-Linalol 60-70%), Terpenes (20%), Fixed oil (13-20%), proteins (70%), small amount of borneol, geraniol, p-cymene and α-pinene are also present. Vitamin A also found in the coriander leaves.

Histology and powder characterstics

Transverse section

The dried coriander are collected and washed with water for remove the foreign matters.the coriander should be free from any microbial contamination.

The dried coriander are soaked in water for overnight

The coriander should be cutted thinny not thick with the help of blade.

The thin section are placed in the microslide at horizontal position with the help of brush.

Add 2 drops of phloroglucinol reagent as a staining agent and dried it.

Add few drops of dil.Hcl and dried it.

Glycerin is placed on thin section for moisturizing and clear the vision of section.

Chemical required

Hydrochloric acid, Phloroglucinol, Glycerin, alcoholic picric acid, sudan red-III

Apparatus required

Watch Glass,microscope slide. Cover slip, Blade, Brush and Compound Microscope.

Histology

S.no	Chemical test	Observation	Inference
1.	Phloroglucinol+conc.HCI(1:1)		
2.	Alcoholic picric acid		
3.	Sudan red III		

Powder microscopy:

Dried coriander are grinded with using mixer.

The powder through the sieve 60

The powder are examined by using phloroglucinol and con.Hcl.

Observe the microscopical character of powder coriander

Extraction

Extraction of coriander by hot maceration method.

2gm of powder sample is treated with boiling water till the chemical constistuents elute from the powder.

.**uses:** - Carminative, Stimulant.

Observation

Report

EXP NO: 8

DATE:

ISOLATION OF CAFFEINE FROM TEA DUST

Aim

To perform the isolation of caffeine from tea dust.

Caffeine

Caffeine, as 1,3,7-trimethyl-1H-purine-2,6-dione, represents a naturally occurring alkaloid within the methyl xanthine family. Exhibiting bitterness and an odorless state in its pure form, caffeine manifests as needle-like crystals. Found abundantly in tea, coffee beans, kola nuts, and cocoa beans, this compound stimulates the central nervous system, respiration, and cardiac activity. Ubiquitously present in everyday products such as soft drinks, tea, coffee, chocolates, pharmaceutical drugs, and skincare items, caffeine plays a pivotal role. This study focuses on the extraction of caffeine from various tea types, including used tea, utilizing dichloromethane as an organic solvent. Investigating the impact of temperature and residence time on the extraction efficiency, we observed a direct correlation between these factors and efficiency. Organic solvent-based caffeine extraction offers high efficiency, enabling the rapid and selective removal of caffeine from tea.

Chemical required

Tea dust, Sodium carbonate,Dichloromethane,Anhydrous sodium sulfate, Iodine, Methanol,Ethyl acetate,Acetic acid, potassium chlorate and dil.Hcl.

Apparatus required

Beaker, separating funnel, muslin cloth, heating mandle, funnel, filterpaper, watch glass, butter sheet, watch glass.

Procedure

8 g of tea powdered are weighed and put in each beaker Then, 100 mL of distilled water and 4.87 g of Na2CO3 were added. The mixed solution was heated until boiling at 100 ◦C for 30 min After boiling, the solution was filtered by using filter paper and a funnel. The obtained filtrate was cooled and shifted to another separating funnel. Approximately 15 mL of dichloromethane was added to this filtrate and then the funnel was tightly sealed by a stopper. The mixture was split into two layers; the lower layer was di-chloromethane and collected from the bottom of the funnel into the conical flask. The same process was repeated by adding another 15 mL of dichloromethane into the funnel. An anhydrous sodium sulfate mixture was added to a conical flask containing the mixture of dichloromethane and held for ten minutes. After the required time, the mixer was separated with the help of filter paper and a funnel. After the filtration process, the filtrate was weighed as the initial weight. The mixture was placed in a water bath for the evaporation of dichloromethane. A powder of caffeine in a light green color was visible when the dichloromethane was evaporated. The weight of this green-colored caffeine powder was the final weight. To calculate the total quantity of caffeine extracted, the initial weight was subtracted from the final weight.

Chemical test

s.no	Chemical test	Observation	Inference
1.	Sample+potassium chlorate and dil.Hcl evaporated and dryness and exposed to ammonia		

TLC

Dissolve 1 mg caffeine in chloroform or methanol. Spot the sample on TLC plate and elute it in ethyl acetate- methanol- acetic acid (8:1:1). Visualize the dried TLC plate by exposure to iodine vapour.

Calculation:

1. **Percentage yield of isolated caffeine**

Weight of the processed tea dust =

Weight of the isolated caffeine =

$$\text{Percentage yield of isolated caffeine} = \frac{\text{Weight of the isolated caffeine} \times 100}{\text{weight of the tea powder taken}}$$

2. **Rf Value of the isolated caffeine**

$$\text{Rf Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

1. The Percentage yield of isolated caffeine was found to be =

2. The Rf Value of the isolated caffeine was found to be=

EXP NO: 9

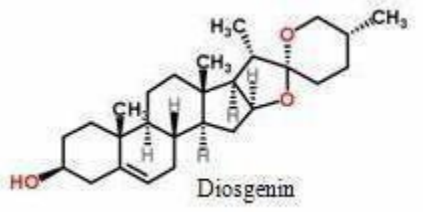
DATE:

ISOLATION OF DIOSGENIN FROM DIOSCOREA

Aim:

To perform isolation Diosgenin from Dioscorea

Diosgenin



Diosgenin is a steroidal saponin obtained by initial hydrolysis of dioscin which is present in the tubers of various dioscorea species such as D. deltoidea, D. floribunda, D composite Family: Dioscoreaceae. It is present to the extent of 2-5% in dioscorea tubers. Diosgenin is an important starting material for the synthesis of steroidal hormones. It is converted to 16-dehydropregnenolone acetate which is used as a substrate for various types of steroidal drugs such as corticosteroids, sex hormones, oral contraceptives, spiranolactones etc. Diosgenin is used as a pharmaceutical aid for the synthesis of various steroidal drug.

Chemical required:

Rhizomes powder, HCL, sodium bi carbonate, ethylacetate, ethanol, sulphuric acid, anisaldehyde

Apparatus required:

Soxhlet apparatus funnel , Round bottom flask, filter paper

Procedure:

Alcoholic extraction method:

1. The diosgenin tubers are cut into small pieces and dried under sun. The dried tubers are powdered, extracted with ethanol or methanol twice for 6-8hr.

2. Filter the extract and filtrate is concentrate to syrupy liquid which is then hydrolysed using HCL or H2SO4 for 2 to 12 hr. About 85% of the crude diosgenin is precipitate.

3. The precipitates are filtered, washed with water and purified with alcohol

TLC:

Dissolved 1 mg diosgenin in 1 ml methanol. silica gel-G plates spotted with the sample are eluted in solvent system Toluene : ethyl actate (7:3). The dried plates are sprayed with anisaldehyde- sulphuric acid reagent and heated at 110º C for 10 min. Dark spot is observed in day light at Rf Value 0.37

Calculation:

1. Percentage yield of isolated diosgenin

Weight of the dried powder =

Weight of the isolated diosgenin =

$$\text{Percentage yield of isolated diosgenin} = \frac{\text{Weight of the isolated diosgenin} \times 100}{\text{weight of the dried powder taken}}$$

2. Rf Value of the isolated diosgenin

$$\text{Rf Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

1. The Percentage yield of isolated diosgenin was found to be =

2 The Rf Value of the isolated diosgenin was found to be=

EXP NO: 10

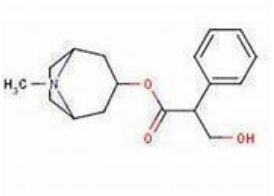
DATE:

ISOLATION AND DETECTION OF ATROPINE FROM DATURA STROMONIUM

Aim

To perform the isolation of Atropine from datura stromonium .

Atropine



Atropine is a tropane alkaloid from the members of the solanaceae family. It is present in Atropa belladonna, Datura stramonium, and hyoscyamus niger. Tropane alkaloids also known as solanaceous alkaloids, belladonna alkaloids which includes atropine, scopolamine (hyoscine), belladonine, hyoscyamine, apoatropine and norhyoscymine. Atropine isolated from the juice or the powdered drug. Hyoscyamus muticus having high alkaloidal content hence preferred for manufacturing of atropine and then D. staramonium is next in order. Atropine is used as an antispasmodic, mydriatic and anti cholinergic. Atropine first stimulates and then depresses the CNS. Atropine dilates the pupils of the eyes hence used in ophthalmology.

Chemical required

Datura leaves,sodium carbonate, ether, benzene, acetic acid

Apparatus required

Beaker, round bottom flask, vacuum filtration assembly.

Procedure:

1. The powdered drug material is thoroughly moistened with an aqueous solution of sodium carbonate and then extracted with ether or benzene.

2. The alkaloidal free bases are extracted from solvent with water acidified with acetic acid.

3. The acid solution is then shaken with solvent ether or acetone and dehydrated with anhydrous sodium sulphate before filtration.

4. Concentrate the filtrate which yields crude crystals of hyoscyamine and atropine from the solution.

5. The crude crystalline mass is dissolved in alcohol and sodium hydroxide solution is added and the mixture is allowed to stand until hyoscyamine is completely racemized to atropine which is indicated by the absence of optical activity.

6. Crude atropine is purified by crystallization from acetone. Atropine sulphate is the most important salt of atropine.

Chemical Test:

S.no	Chemical test	Observation	Inference
1.	**Vitali-morin test:** Sample solution +con.HNO3 boil and evaporated then add some potassium hydroxide		

TLC

1% atropine solution dissolved in 2N acetic acid is spotted over silica gel G plate and eluted in the solvent system of strong ammonia solution-methanol (1.5:100). TLC plates is sprayed with an acidified iodoplatinate solution.

Calculation:

1. Percentage yield of isolated Atropine

Weight of the dried powder =

Weight of the isolated Atropine =

$$\textbf{Percentage yield of isolated Atropine} = \frac{\textbf{Weight of the isolated Atropine} \times 100}{\textbf{weight of the dried powder taken}}$$

2. Rf Value of the isolated Atropine

$$\textbf{Rf Value calculation} = \frac{\textbf{Distance travelled by the solute}}{\textbf{Distance travelled by the solvent}}$$

Report:

1. The Percentage yield of isolated Atropine was found to be=

2. The Rf Value of the isolated Atropine was found to be=

EXP NO: 11

DATE:

ISOLATION AND DETECTION OF SENNOSIDES FROM SENNA

Aim:

To perform extract sennoside from senna.

Sennasides

Sennosides are obtained from cassia angustifolia (Tinnevelly senna), cassia acutifolia (Alexandrian senna). Sennosides are the dimeric anthroquinone glycosides. Sennoside A and B is a pair of stereoisomer containing rhein dianthrone (sennedine A and B) as the aglycon. Sennoside D and E are the dianthrone of aloe-emodin and rhein. Purgative activity of senna is mainly due to sennoside A and sennoside B while sennoside C and D exerts a powerful synergistic effect upon the purgative activity.

Chemical required

senna leaves or pods, benzene, ethanol, methanol.

Apparatus required

vacuum filter unit, electric shaker, desiccators

Procedure:

1. Extract 100 g powdered leaves with 300 ml benzene for 2 hr on electric shaker, filter in vacuum and distill off the solvent.
2. The dried marc extract with 300 ml 70% ethanol on shaker for 4-6 hrs, filter under vacuum and the marc re-extract with 200 ml of 70% methanol for 2 hr; filter and combine methanolic extract.
3. Concentrate ethanolic extract and acidify to PH 3.2 by addition of HCL with constant stirring. Set aside the mixture for 2 hr at 5ºC. Filter under vacuum and add 1g anhydrous calcium chloride in 13 ml denatured spirit with vigorous shaking.
4. Adjust PH of the solution to 8 by addition of ammonia solution and set aside for 2 hr; filter the solution by vacuum and dry precipitate over phosphorous pentoxide in a dessiccator.

Chemical test:

s.no	Chemical test	Observation	inference
1.	**Borntrager test:** Boil drug with dil sulphuric acid (hydrolysis). Filter and cool. Add benzene or CCL4 (Immisible organic solvents). Shake and separate organic solvent layer in another test tube. Add strong ammonia solution		

TLC

Dissolve 1 mg sennoside in 1 ml solvent containing equal volumes of ethyl acetate, n- propanol and water (upper layer). The silica gel –G plates spotted with the sample and eluted in solvent system ethyl acetate: n-propanol: water (4:4:3). The dried plate is exposed to vapours of ammonia for 5 min till the colour develops. Cover the plate with glass and heat at 110ºC for 5-10 min sennosides A and B develop two prominent spots.

Calculation:

1. Percentage yield of isolated sennoside

Weight of the dried powder =
Weight of the isolated sennoside =

$$\text{Percentage yield of isolated sennosides} = \frac{\text{Weight of the isolated sennoside} \times 100}{\text{weight of the dried powder taken}}$$

2. Rf Value of the isolated sennosides

$$\text{Rf Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

1. The Percentage yield of isolated sennoside was found to be =

2. The Rf Value of the isolated sennoside was found to be=

EXP NO:12

DATE:

SEPERATION OF SUGARS BY PAPER CHROMATOGRAPHY

Aim

To perform the separation of sugars by paper chromatography.

Principle

The term chromatography comes from the earlier times when the technique was used for the separation of colored plants pigments. chromatography is a technique for separation of closely related groups of compounds. The separation is brought about by differential migration along a porous medium and the migration is caused by the flow of solvent. Within limits chromatography can be divided into two types: partition and adsorption chromatography.

Paper chromatography is an example of liquid-liquid chromatography.

In this type of chromatography separation is due to differential partition of solutes between two liquid phases.

One liquid phase is bound to the porous medium for example; the water bound in the cellulose paper, this phase is referred to as, the stationary phase. The other liquid phase, the mobile phase flows along the porous medium.

As the mobile phase flows over the solute mixture, the individual solutes partition themselves between the aqueous stationary phase and the organic mobile phase relative to their solubility in the two phases.

The more soluble a solute in the mobile phase, the faster it will travel along the papper, and conversely, the mobile phase must be a mixture in which the compounds to be separated are soluble or partially soluble.

In paper chromatography solute or solute mixture is spotted in solution along a base line on a sheet of filter (whatman NO.1).The mobile phase(solvent) is allowed to flow over the spots either ascending the paper by capillary action or descending te paper by gravity.

The separation is measured in terms of a unit called Rf(relative rates of flow)with respect to the solvent front.

$$\text{Rf Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

The Rf value of a compound in a particulare solvent system is constant under identical conditions of the experiment, e.g.temperature,pH,etc.

Because most compounds are colorless the pots are visualized after separation by specific reagent. The location reagent is applied by spraying the paper or rapidly dipping it in a solution of the reagent in a volatile solvent. viewing under ultraviolet light is also useful since some compound which absorbs it strongly show up as dark spots against the florescent background of the paper.

Materials:

Paper: Whatman filter paper

Solvents:

n-butanol-acetic acid-water(4:1:5 v/v)

Spray Reagent:

Resorcinol reagent:

Mix 1% ethanolic solution of resorcinol and 0.2N HCI(1:1 v/v).spray the dried chromatograms and visualize spots by heating at 900c.

Procedure:

1. Place sufficient solvent into the bottom of the tank. cover the led and allow the tank to be saturated with the solvent.

2. Take a sheet of whattman 1 chromatography paper(about 9*10cm) and place it on a piece of clean paper on a bench.

3. Draw a fine line with a pencil along the width of the paper and about 1.5cm from the lower edge.

4. Along this line place four equality spaced (about 2cm apart)small circles with a pencil.

5. Label the paper at the top with the name of each of the sugars and label the last unknown.

6. Use a fine capillary or tooth pick to place the drops of the solutions of the sugars, glucose, fructose, maltose, lactose and the mixture.

7. After spotting dry the paper with hot air dryer for one minute, repeat this step again.

8. Place the spotted paper in the chromatographic tank and make the development by using the ascending technique.

9. Close the tank with lid allow the solvent to flow for about 30-45 minutes.

10. Remove the paper and immediately mark the position of the solvent front with a pencil.

11. After the chromatogram has dried spray the paper with the locating reagent.

12. You need to put the paper on the hot plate at low temperature or expose it to the hot air dryer, until the colored spots appear. The colors are stable for some weeks if kept in the dark and away from acid vapors.

13. Circle the position of each spot with pencil.

14. Calculate the Rf value for each spot and also for the spot the mixture contained.

Report

EXP NO: 13

DATE:

SEPERATION OF ACTIVE CONSTITUENTS OF CLOVE BY THIN LAYER CHROMATOGRAPHY

Aim

To perform the seperation of active constituents of clove by thin layer chromatography

Biological source: It consist of the dried flower buds of Eugenia caryophyllus belonging to the family: Myrtaceae.

Active constituents: It contains volatile oil, 14 to 20%, gallotannic acid, 10-13% caryophyllin, a white, odourless, tasteless, crystallizing silky needles, vanillin, eugenin. It contains more than 85% of eugenol.

Uses: Clove is an aromatic, flavouring agent, used as local anaesthetic agent in dentisty.

Procedure:

Preparation of plate:

Thin layer glass plates are used as supporting medium. Mix the adsorbent (30g) in a mortar to a smooth consistency with the required amount of water and spread the slurry on the plate so as to get a thickness of 1-2 mm. Allow it to dry and keep it for activation for 1 hr at 120º C. Cool and use the plates for separation.

Extraction of oil.

Take clove flower buds and powder them. Add toluene (5ml) to the powder (1g) and shake it for some times. Filter and concentrate the filtrate. Use this as sample.

Chemical required

Toluene, ethyl acetate, silicagel-G standard eugenol, vanillin sulphuric acid or ferric chloride solution

Mobile phase composition : Toluene: Ethyl Acetate (93:7)

Apparatus required

Tlc plate, Tlc chamber

Calculation:

$$\text{Rf Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

EXP NO:14

DATE:

SEPERATION AND IDENTIFICATION OF CURCUMINOIDS FROM CURCUMIN

Aim

To separate and identify the curcuminoid present in turmeric by thin layer chromatography.

Preparation of plate:

Thin layer glass plates are used as supporting medium. Mix the adsorbent (30g) in a mortar to a smooth consistency with the required amount of water and spread the slurry on the plate so as to get a thickness of 1-2 mm. Allow it to dry and keep it for activation for 1 hr at 120º C. Cool and use the plates for separation.

Sample preparation: The sample is extracted with methanol on a water bath. Cool and filter. The filtrate is used for TLC studies.

Chemical required

Chloroform, ethanol, glacial acetic acid, standard curcumin.

Apparatus required

Tlc plate, Tlc chamber

Mobile phase: chloroform: ethanol: glacial acetic acid (95:5:1)

Procedure:

The solvent system is prepared in the given ratio and then taken in a chamber and kept for saturation. The extract is placed as a spot on TLC plate and kept in the chamber at an angle of 45º. After 3/4th movement of solvent the plate is taken out and mark the solvent front. Then the plate is dried in air and detected in UV light.

Calculation:

$$\text{Rf Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

EXP NO: 15

DATE:

SEPERATION AND IDENTIFICATION OF PIPERINE FROM PEPPER

Aim

To identify piperine by performing thin layer chromatography.

Preparation of plate:

Thin layer glass plates are used as supporting medium. Mix the adsorbent (30g) in a mortar to a smooth consistency with the required amount of water and spread the slurry on the plate so as to get a thickness of 1-2 mm. Allow it to dry and keep it for activation for 1 hr at 120º C. Cool and use the plates for separation.

Chemical required

Toluene, ethyl acetate, vanillin sulphuric acid, standard piperine, silica gel-G

Apparatus required

Tlc plate, Tlc chamber,

Extract: Aqueous and alcoholic extract of piper nigrum

Stationary phase: Silica gel – G

Mobile phase : Toluene : Ethyl acetate (7:3)

Spraying agent : vanillin in sulphuric acid

Procedure:

Solvent system is prepared and taken in the chamber and kept it for saturation about 30 min. sample is placed on the plate above 1 cm of the plate by using capillary tube. Place the plate into the chamber at an angle of 45º. Develop the chromatogram by ascending technique till the solvent front moved by 3/4th of the TLC plates. The plates are withdrawn from the chamber and air dried. The plate is sprayed with vanillin sulphuric acid and dried in hot air oven. Appearance of yellow colour spot indicates the presence of piperine.

Calculation:

$$\text{Rf Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

DISTILLATION OF VOLATILE OILS AND DETECTION OF PHYTOCONSTITUENTS BY TLC

Volatile oil or essential oils or ethereal oils are the odorous principles of the plants. Being volatile at the temperature of boiling water or steam, mostly essential oils are hydro distilled or steam distilled for their separation from the crude drugs. colour, odour, taste, density, specific rotation, refractive index, boiling range and solubility are some important parameters used in determining the purity of volatile oils.

Methods of extraction of volatile oil

1. Distillation
2. Enfleurage
3. Expression
4. Maceration
5. solvent Extraction

Extraction by distillation

Most common method for the production of volatile oil is distillation>In general ,the term distillation applies to vaporization process in which the vapours evolved are recovered, usually by condensation.

Distillation apparatus is basically consists of three parts

a. Distillation flask: tlc plate, tlc chamber, silicagel.

b. condenser: provide cooling to avoid reflux of the distillate

c. Receiver: Allow separation of the oily layer from water in the distillate

EXP NO: 16

DATE

ISOLATION OF CARAWAY OIL FROM CARAWAY FRUIT BY HYDRO-DISTIILLATION METHOD. (VOLATILE OIL LIGHTER THAN WATER)

Aim

To isolation of caraway oil from caraway fruit by hydro-distiillation method. (volatile oil lighter than water)

Principle:

Hydro-distillation is based on distilling the drug with water and/or glycerine and collecting distillate in a graduated tube from which the aqueous portion of distillate is automatically returned to the distillation flask.

Biological source

The caraway oil obtained from the dried fruits of carum carvi.

Family: umbelliferae.

Dried ripe fruits of caraway should contain not less 2.5%v/w of volatile oil.

The characteristics of the caraway oil are as given below:

Colour: pale Yellow

Odour and taste: Aromatic and characteristic

Weight per ml: 0.90 to 0.91

Solubility: soluble in 8 parts of 80% alcohol

Optical rotation: At 250+700 + 800

Content of carvone: 53 to 63% w/w

Chemical required

Caraway powdered, tolune, ethyl acetate, vanillin sulphuric acid, carvone, tlc plate, tlc chamber, silicagel-G

Apparatus required

Heating mantle,Volatile distillation unit of 1 litre capacity.

Procedure:

1. Take 50g of powdered drug in 1 litre of distillation flask together with 250 ml of water.Add few pieces of porcelain to it in order to avoid bumping.

2. place the distillation flask on the heating mantleand set the distillation assembly.fill the graduated receiver with water avoiding any air bubbles.do not tighten the outlet near the upper end of the receiver.instead loosely pack it with cotton.

3. Distillation should be done for 4 hours.Allow the distillation to be collected in the graduated receiver in which the aqueous portion of the distillate is automatically separated and returned to the distillation flask.

4. Measure the volume of volatile oil which seperates out as the upper layer in the graduated tube and calculate the % v/w on a dry weight basis.

TLC of carvone:

Dissolve 1 mg of carvone in 1ml of methanol and apply the spot over silica gel- G plate. Elute the plate with toluene-ethyl acetate (93:7) as a solvent system. spray the dried plate with vanillin sulphuric acid

Storage: volatile oil should be stored in well closed well filled containers away from light and in cool place

Precautions:

1. Add a few pieces of porcelain to it in order to avoid bumping during distillation.to

2. Do not tighten the outlet near the upper end of the receiver. Instead loosely pack it with cotton.

Calculation:

1. **Percentage yield of isolated volatile oil**

Weight of the powder taken =

volume of the isolated volatile oil =

$$\text{Percentage yield of isolated volatile oil} = \frac{\text{volume of the isolated volatile oil} \times 100}{\text{weight of the powder taken}}$$

Rf Value of the carvone

$$\text{Rf Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

1. The Percentage yield of isolated volatile oil was found to be
2. The Rf Value of the carvone was found to be

EXP NO: 17

DATE

ISOLATION OF CLOVE OIL FROM CLOVE BUDS (VOLATILE OIL HEAVIER THAN WATER)

Aim

To isolation of clove oil from clove buds (volatile oil heavier than water)

Principle

Eugenol is 4-allyl-2-methoxy phenol obtained from the essential oil of clove buds Eugenia caryophyllus Family myrtaceae. clove oil contain 80 to 90% of eugenol. Dried clove buds are hydro distilled to yield the clove oil. Being heavier than water it makes a layer beneath water

The characteristics of the Eugenol are as given below:

Colour:colourless or pale yellow liquid

Odour:odour of clove and spicy

Taste:Pungent

Solubility:Alcohol,chloroform,and ether

Insolubility:water

Specific gravity:1.038 to 1.060

Refractive index:1.527 to 1.535

Content of Eugenol:15% w/w

Chemical required

Clove powdered, anhydrous sodium sulphate, eugenol, benzene, anisaldehyde, tlc plate, tlc chamber, silicagel-G

Apparatus required

Heating mantle,volatile distillation unit of litre capacity

Procedure:

1. Take 20gm powdered drug in a 1 litre of distillation flask together with 250ml of water. Add few pieces of porcelain to it in order to avoid bumping.

2. Place the distillation flask on the heating mantle and set the distillation assembly. Boil the mixture for 2 hours.

3. Seperate the oil and add few grams of anhydrous sodium sulphate to remove water residue

4. Calculate the oil content in ml per 100g of plant material

TLC of Eugenol:

Dissolve 1 mg of eugenol in 1 ml of methanol and apply the spot over silica gel-G plate. Elute the plate with pure benzene as a solvent system. Spray the dried plate with 1% anisaldehyde-sulphuric acid reagent and heat the plate at 110ºC for 10min. Eugenol shows the spot with dirty green colour at

Storage: volatile oil should be stored in well closed well filled containers away from light and in cool place

Precautions: Add a few pieces of porcelain to it in order to avoid bumping during distillation.

Calculation:

1. **Percentage yield of isolated volatile oil**

Weight of the powder taken =

volume of the isolated volatile oil =

$$\textbf{Percentage yield of isolated volatile oil} = \frac{\textbf{volume of the isolated volatile oil} \times 100}{\textbf{weight of the powder taken}}$$

2. **Rf Value of the eugenol**

$$\textbf{Rf Value calculation} = \frac{\textbf{Distance travelled by the solute}}{\textbf{Distance travelled by the solvent}}$$

Report:

1. The Percentage yield of isolated volatile oil was found to be
2. The Rf Value of the eugenol was found to be

EXP NO: 18
DATE

ASAFOETIDA

Synonyms:

Devil's drug, Hing, Gum asafoetida

Biological source:

It is an oleo-gum resin of living roos and rhizomes of ***Ferula foetida,***

F.rubicals, F asafoetida etc and other spices of Ferula belongs to family umbelliferae.

Physical Characteristics:

Colour: yellowish brown to reddish brown tears

Odour: Intense, penetrating, persistent alliaceous

Taste: Bitter,acrid,alliaceous

Shape: It occurs in two forms viz tears and masses

Tears are rounded or flattened more or less agglutinated together.

Mass consists of agglutinated tears with foreign mass like stone, earth, pieces of roots,

calcium sulphate and it is of inferior quality as compared to tears

Size:0.5×4.0cm in diameter

Solubility: Partly soluble in alcohol

Extra Feature:

Fresh tears are tough,dried are hard and brittle.Tears are internally milky white

yellowish,translucent or opaque mass consists of agglutinated tears with foreign materials and impurities

Chemical constituents:Resin(40 to 60%) mainly Asaresinotannol in free or combined form with ferulic acid,pinene,vanillin and asaresene;gum(20 to 25%) and volatile oil(4 to 20%) contains isobutyl propanyl disulphide which gives alliaceous odour to drug.

Uses: carminatives, laxative, antispasmodic, Nervine tonic, anthelmintic and digestive

It is used to treat flatulence colic, constipation, asthma, bronchitis, whooping cough and epilepsy.

It is also used as flavouring agent in sauces, pickles and curries.

Chemical required

Ethanol , alkali, H_2SO_4, Sand, HCL, ammonia, phloroglucinol

Apparatus required

Test tube, test tube stand, holder, water bath, morter and pestle

Chemical test

S.no	Experiment	Observation	Inference
1.	1 gm of asafoetida is mixed with 3 ml of water		
2.	0.5 gm of drug is shaken with 2ml of alcohol		
3.	1 gm of drug is triturated with 3 ml of water		
4.	The above emulsion mixed with alkali		
5.	Add 2 drops of H_2SO_4 to freshly fractured surface of asafoetida		
6.	Add 2 drops of con. HNO_3 to fracture surface		
7.	Combined umbelliferone test Triturate 1 gm of drug with sand , boil with 3 ml of Hcl and water for 5-10 min , filter; to the filtrate add equal volume of alcohol, ammonia . examine under UV light		
8.	Alcoholic extract is mixed with phloroglucinol and con.Hcl		

Report

EXP NO:19

DATE

BENZOIN

Synonyms: Sumatra benzoin, loban, siam benzoin

Biological source: Balsamic resin obtained from the incised stem of syntax benzoin or styrax paralleloneurus, s.tonkinensis belongs to family styraceae

Physical characteristics:

Colour: greyish brown or grey masses

Odour: agreeable and balsamic

Taste: sweetish and slightly acrid

Size: varying in size

Shape: tears, masses, lumps

Uses: Expectorant

Chemical constituents:

Sumatra benzoin: free balsamic acids(benzoic and cinnamic acid), Triterpenoid acids like summaresionlic acid

Siam benzoin: An ester coniferyl benzoate(75%) styol,vanillin and phenyl propyl cinnamate

Chemical required:

Ethanol, ether H2SO4, ferric chloride, potassium permanganate

Apparatus required

Test tube, test tube stand, holder, microslide, waterbath, microscope

Chemical test

S.no	Experiment	Observation	Inference
1.	Solubility a) 0.5 gm of drug is mixed with 5 ml of water b) 0.5 gm of drug is mixed with 2 ml of alcohol		
2.	Heat small quantity of drug in a test tube with a glass slide , cool the content of test tube. Examine glass slide under microscope		

3.	Heat 0.5 g slowly in a dry test tube. It melts and evoles irritating whitish fumes.		
4	To 2.5 gms of benzoin add 10 ml of ether , shake it well.pour 2to 3 ml of this extract in a porcelain dish. Add 2-3 drops of H2So4		
5	Add 1 gm of drug and shake with 3 ml of alcohol. Filter add to an alcoholic solution of ferric chloride		
6	Addd 4 ml of potassium permanganate solution to 1 gm of benzoin and warm		

Report

EXP NO:20
DATE

COLOPHONY

Synonym: Rosin, Rosina, colophonium, Amber resin, resin

Biological source: colophony is the solid residue obtained after distilling the oleo-resin from various species of pinus (p.palustris, p.longifolia, p.radiate etc)

Family: pinaceae

Physical characteristics:

Colour: amber or pale yellow

Odour: turpentine like

Taste: slightly bitter

Solubility: alcohol, ether, chloroform, and light petroleum

Insolubility: water

Extra features: brittle and readily fusible glassy masses

Standards:

Melting point: 75 to 85ºC

Acid value: Not less than 150

Saponification value: 188 to 192

Ash value: NMT 0.125%

Chemical constituents: colophony contains 90% of abietic aid (resin acid), 5 to 6 % of resene, and 0.5% of volatile oil. Other acid present are sapinic acid, pimaric acid.

Uses:

colophony posses stimulant and diuretic properties.

It is commonly used as ingredients of plasters and ointment.

Industrially it is used in manufacturing of varnishes, pint driers, printing ink, soaps, wood polishes, cements, paper, plastics and fire works.

Storage: colophony should be stored in large pieces in well closed containers away from Light.

Chemical required.

Acetic acid, con.Hcl, petroleum ether, copper acetate, litmus paper, ethanol

Apparatus required

Test tube, test tube stand, water bath, holder

s.no	Experiment	Observation	inference
1.	To a solution of powdered resin (0.1 g) in acetic acid (10 ml) one drop of conc. Sulphuric acid is added in a dry test tube.		
2.	To a petroleum ether solution of powdered Colophony twice its volume of dilute solution of copper acetate is shaken.		
3.	To alcoholic solution of Colophony sufficient water is added.		
4.	Alcoholic solution of Colophony turns blue litmus to red due to the presence of diterpenic acids.		
5.	Colophony mixed with alcohol		

Report

EXP NO: 21
DATE

ALOES

Synonym: musabbar, Ghritkumari, Aloe

Biological source: Aloe is the dried juice of the leaves of Aloe barbadensis mille (curaccao aloes), Aloe perrbaker, (socotrine aloes) Aloe ferox miller and its hybrid with Aloe africana miller and Aloe spiculata Baker (cape aloes). Family: Lilliaceae. It is obtained by incision of leaves at the base.

Physical characteristics:

Texture: solid waxy masses

Colour: Dark brown.

Odour: characteristic unpleasant odour

Taste: Bitter

Solubility: ethanol, alkali and glacial acetic acid; partly soluble in water, chloroform and ether.

Extra features: Hard and uneven porous fracture

Chemical constituents:

Aloes are the major sources of anthraquinone glycosides. Aloin is the mixture of three isomers namely barbaloin, β Barbaloin and isobarbaloin.

Aoe emodin, Aloe-resin, aloesone, aloetic acid, chrysophanic acid, chrysamminic acid, glactouronic acid, choline, saponins, coniferyl alcohol.

Uses:

Purgative and improve digestion, cosmetics.

Ointment of aloe is used in sun burns, thermal burns, radiation burns, abrasions and skin irritation.

At higher doses of aloes are abortifacient.

Used to prepare compound benzoin tincture in which it is pharmaceutical adjunct.

Stimulates immune system specially T4 cells.

It has greatest nutritional and therapeutic properties

Chemical required

Borax, ferric chloride, dil.Hcl, bromine, sosium nitrate, dil.acetic acid, nitric acid, copper sulphate,

sodium chloride, ethanol

Apparatus required

Test tube, test tube stand, water bath, holder

Chemical test

S.no	Chemical test	Observation	Inference
1.	Borax test Take 10 ml of aloe solution and to it add 0.5 gm of borax and heat		
2.	Modified borntrager's test To 0.1 gm of drug , 5 ml of 5% solution of ferric chloride is added followed by the addition of 5 ml dilute hydrochloric acid.the mixture is heated on water bath for 5-6 min and cooled. And organic solvent (Benzene or chloroform) is added		
3.	Bromine Test: To 5 ml of aloe solution, add equal volume of bromine solution		
4.	Nitrous Acid Test: To 5 ml of aloe solution, add little of sodium nitrite and few drops of dilute acetic acid;		
5.	Nitric Acid Test: 2 ml of concentrated nitric acid is added to 5 ml of aloe solution		
6.	Cupraloin Test: 1 ml of the aloe solution is diluted to 5 ml with water and to it 1 drop of copper sulphate solution is added. Bright yellow colour is produced which on addition of 10 drops of saturated solution of sodium chloride changes to purple and the colour persist if 15–20 drops of 90% alcohol is added.		

Report

EXP NO:22

DATE

MYRRH

Synonym: Myrrha, Gum-myrrh, Bol

Biological source: myrrh is the oleo-gum-resin obtained from incision from the stem of commiphora molmol

Family: burseraceae.

Physical characteristics:

Colour: externally reddish internally brown

Odour: Agreeably aromatic

Taste: Aromatic, bitter, acrid

Size: 1.5-3.0 cm in diameter

Shape: irregular tears or lumps

Solubility: partly soluble in alcohol and ether

Insolubility: water

Extra features: fractured surface in granular, brittle, and translucent

Chemical constituents: volatile oil (10%) which are terpenes, cuminic aldehyde, eugenol, gum (60%), resin (25 to 40%) which contains ether soluble resin acids, α,β, and γ commiphoric acid.

Uses: carminatives, antiseptic, uterine stimulant, protective, used in gargles and mouth wases

Chemical required

Ether, bromine, nitric acid

Apparatus required

Test tube, test tube stand, water bath, holder

Chemical test

S.no	Chemical test	Observation	Inference
1.	Triturate with water		
2.	Ether extract of drug with bromine vapours		
3.	Moistened with nitric acid solution		

Report