RATHINAM COLLEGE OF PHARMACY

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PRACTICAL MANUAL

FIRST YEAR B. PHARM (I-SEMESTER)

SUBJECTS: HUMAN ANATOMY AND PHYSIOLOGY I
PHARMACEUTICAL ANALYSIS I
PHARMACEUTICS I
PHARMACEUTICAL INORGANIC CHEMISTRY

Name **:**

Course : Bachelor of Pharmacy

Semester : I semester

HUMAN ANATOMY AND PHYSIOLOGY I

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GENERAL LABORATORY INSTRUCTIONS

The Anatomy laboratory is a hands-on learning environment.

Safety in the laboratory is the first priority for students and instructors.
To ensure a safe Anatomy laboratory, a list of rules has been developed and provide to you. These rules must be followed at all times.

General Guidelines

1. Conduct yourself in a responsible manner at all times in the laboratory.
2. Follow all written and verbal instructions carefully. If you do not understand a direction or part of a procedure, ask the instructor before proceeding.
3. Do not eat food, drink beverages, or chew gum in the laboratory. Do not use laboratory glassware as containers for food or beverages.
4. Perform only those experiments authorized by the instructor. Never do anything in the laboratory that is not called for in the laboratory procedures or by your instructor. Carefully follow all instructions, both written and oral. Unauthorized experiments are prohibited.
5. Be prepared for your work in the laboratory. Never fool around in the laboratory. Horseplay, practical jokes, and pranks are dangerous and prohibited.
6. Observe good housekeeping practices. Work areas should be kept clean and tidy at alltimes. Table tops and lab chairs should be cleaned at the end of lab class.
7. Lab material, lab chairs, microscopes, interfaces and other equipment should be returned totheir proper storage location.
8. Bring only your laboratory instructions, worksheets, and/or reports to the work area. Othermaterials (books, purses, backpacks, etc.) should be stored in a safe place.
9. Be alert and proceed with caution at all times in the laboratory. Notify the instructor immediately of any unsafe conditions you observe.
10. Labels and equipment instructions must be read carefully before use. Set up and use the prescribed apparatus as directed in the laboratory instructions or by your instructor.

1. Experiments must be personally monitored at all times. You will be assigned a laboratory station at which to work. Do not wander around the room, distract other students, or interfere with the laboratory experiments of others.
2. Know what to do if there is a fire drill during a laboratory period; turnoff electrical equipment.
3. When using knives and other sharp instruments, always carry with tips and points pointing down and away. Always cut away from your body. Never try to catch falling sharp instruments. Grasp sharp instruments only by the handles.
4. Examine glassware before each use. Never use chipped or cracked glassware. Never use dirty glassware. If you do not understand how to use a piece of equipment, ask the instructor for help.

General instruction to histology

Histological slides should be handled gently and special care should be taken not to damage the slides. Practical record book should be handled gently and neatly.

Students are strictly advised to return the histological slides and the microscope in a good condition before leaving histology practical hall. Drawing the diagrams in histology is an art.

A proper knowledge about different cells, their staining characters and a bit of neatness makes the histological diagrams more attractive and meaningful.

Use HB pencils, good quality eraser, sharpener, and a transparent scale.

Always develop a habit of drawing a circle and writing the diagrams within it, see to it the picture should not come out of the circle's margin.

Always try to label the diagrams on one side of the picture (usually on right side). Make sure that the picture and its beauty is not destroyed while labeling.

Ex. No: Date:

STUDY OF COMPOUND MICROSCOPE

A compound microscope is an indispensable instrument in any biological laboratory.

It is used for passive observation of structural details of a cell, tissue or organ in sections

A modern compound microscope has following structural components

1. Non-Optical Components
2. Optical Components

1. NON-OPTICAL COMPONENTS

- Base (Foot)
- Pillar
- Arm (Limp)
- Inclination Joint
- Stage
- Body tube
- Draw tube
- Rack and pinion
- Adjustment screws
- Automatic Stop

Base (foot): It is U or horseshoe-shaped metallic structure that supports the whole microscope

Pillar: It is a short upright part that connects to base as well as arm

Arm (Limb): It is a curved metallic handle that connects with the arm by inclination joint. Itsupports stage and body tube

Inclination Joint: It is used for tilting the microscope if required for observation in sittingposition.

Stage: It is a metallic platform with a central hole fitted to the lower part of the arm. Microscopic slides held on the stage by either simple side clips or by a mechanical stage clip.

Body tube: It is meant for holding ocular and objective lenses at its two ends

The end holding ocular lens is called head while the end containing 3-4 objective lens is called nose piece

The body tube has an internal pathway for the passage of light rays which form the enlarged image or microscopic objects

Draw tube: It is a small tube that remains fixed at the upper end of the body tube. It holds eyepiece or ocular lens.

Rack and pinion: The microscope has a rack and pinion attached either to body tube or the stage for bringing the object under focus.

Adjustment screws: There are two pairs of screws for moving the body tube in relation tostage, larger for coarse adjustment and smaller for fine adjustment.

In fine adjustment the body tube or stages moves for extremely short distances.

In coarse adjustment the body tube or stage can move up and distance.

In coarse adjustment is meant for briefly objective lens at a proper distance from the object so as to form image of the same at the ocular end. Fine adjustment is required to obtain sharp image.

Automatic Stop: It is a small screw fitted at lower end or rack and pinion.

It is meant for stopping the downward sliding of the body tube so as to prevent the damage of objective lens and the slide.

2. OPTICAL COMPONENTS

Optical components are simple optical elements that are used in the construction of optical systems.

- **Diaphragm**
- **Condenser**
- **Reflector (Mirror)**
- **Objective Lenses**
- **Ocular Lens or Eyepiece**

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- **Ocular Lens or Eyepiece**

Diaphragm:

It is flitted just below the stage for regulating the amount of light failing on the object.

Diaphragm is of two types, disc and iris

Condenser:

It is attached below the diaphragm. Condenser can be moved up and down to focuslight on the object.

Reflector (Mirror):

It is attached just above the base. Both its surface bear mirrors, plane on one side and concave on other side.

Plane side is used in strong light and concave side in weak light.

Reflector directs the light on the object through the condenser and diaphragm system.

Objective Lenses:

They are fitted over the nose piece. Objective lenses are of two 10 three types – low power (commonly 10X or 5X), high power (commonly 45X) and oil immersion (commonly 100X, can be more)

An objective lens is not a simple lens but compound lens. It forms real inverted image of the object inside the body tube

Ocular Lens or Eyepiece:

It is lens through which image of the microscopic object is observed. It also takes part in magnification.

Depending upon magnification, the eye piece is of four types-5X, 10X, 15X, and 20 X.

Advanced microscope has two eye pieces so that both the eyes can be used.

Microscope head having device for using two eye pieces is called binocular head. It contains a number of internal mirrors and prisms for the passage of light

WORKING PRINCIPLE OF COMPOUND MICROSCOPE

The compound microscope is most commonly used in clinical and educational laboratories.

It has a combination of lenses that enhances both magnifying power as well as the resolving power. The specimen or object, to be examined is usually mounted on a transparent glass slide and positioned on the specimen stage between the condenser lens and objective lens.

A beam of visible light from the base is focused by a condenser lens onto the specimen.

The objective lens picks up the light transmitted by the specimen and create a magnified image of the specimen called primary image inside the body tube.

This image is again magnified by the ocular lens or eye piece.

When higher magnification is required, the nose piece is rotated after low power focusing to bring the objective of higher power (generally 45X) in line with the illuminated part of the slide. The objective lens comes very near the cover slip but it does not touch the same.

Only fine adjustment it moved for proper focusing.

More light may be required. After observation under high power, the nose piece is rotated to bring back the slide under low power.

Occasionally very high magnification it required (e.g. for observing bacterial cell).

In that case, oil immersion objective lens (usually 100X) is employed.

After focusing under low power a drop of immersion oil (e.g. cedar oil, olive oil) placed over the illuminated part of the cover-slip.

The nose piece is rotated to bring the oil immersion lens in line with die specimen. It comes in contact with the oil.

By using fine adjustment only, the specimen is brought under focus. Immersion oil increases the sharpness of the image.Soon after observation, both the lens and the slide are cleared of the oil by fine cotton cloth or lens paper.

The common light microscope is also called bright field microscope because the image is produced a brightly illuminated field.

The image appears darker because the specimen or object is denser and somewhat opaque than the surroundings. Part of the light passing through or object is absorbed.

PRECAUTIONS WHILE USING THE COMPOUND MICROSCOPE:

1. While transporting the microscope one hand is placed under the base and the other hand on the handle. The microscope must not be used by placing it on the edge of the working table. The microscope must not be tilted when counting cells in a counting chamber or when using the oil immersion objectives

2. While transporting the microscope one hand is placed under the base and the other hand on the handle. The microscope must not be used by placing it on the edge of the working table. The microscope must not be tilted when counting cells in a counting chamber or when using the oil immersion objectives
3. The objective must not be moved down while looking into the eye piece. Such a maneuver might risk breaking the slide
4. All traces of cedar wood oil must be removed from the objective with a clean flannel dipped in Xylol. Foreign bodies such as dust must be kept away from the eye piece and objective
5. While changing the objective it should be noted that the objective clicks into its proper position
6. Examination of the specimen under low and high power should always precede the examination under oil immersion objective

OTHER TYPES OF MICROSCOPE

1. The simple microscope which refers to a magnifying glass
2. The binocular microscope which has two eye pieces. The dissection microscope whichis a type of binocular used for micro dissection
3. The ultra-microscope which uses ultraviolet light instead of ordinary light and has a magnification of 4000 times. The phase contrast microscope that uses principles of light refraction to produce an image with good contrast
4. The fluorescence microscope that uses a fluorescent dye to stain tissues which are studied under the microscope
5. The Transmission electron microscope that uses a beam of electrons instead of light and electron magnetic fields instead of glass lenses. This microscope obtains a magnification of up to 1 lakh times
6. The scanning electron microscope is similar to the transmission electron microscope. It is used for three-dimensional study of surface topography of cells and objects.

REPORT:

HISTOLOGY

Ex.No: Date:

MICROSCOPIC STUDY OF EPITHELIAL AND CONNECTIVE TISSUES

Objective: 1

Aim: To study the epithelial tissues.

Requirements:

Permanent histological slides

Microscope

Principle:

Epithelium is derived from all the three germs layers Endoderm, Ectoderm and Mesoderm.

Epithelial tissue is composed of single or multiple layers of cells arranged closely in the form of continuous sheets which are held together by numerous cell junction on basal surface called the basement membrane

Epithelial tissues lines the outer surfaces of organs and blood vessels throughout the body, as well as the inner surfaces of cavities in many internal organs.

Example: Epidermis, the outermost layer of the skin.

Epithelial tissue consists of apical surface,lateral and basal surface.

Apical surface is the free end of the epithelium and face towards surface of the body or other cavity or lumen of an internal organ. Apical surface also contain cilia.

Lateral surface is joined with adjacent cells on both sides with the help of desmosome or gap junctions.

Basal surface is the lower end of the epithelial tissue and is attached to the basement membrane, which is site of attachment and provides support to the epithelial cells.

It is consists of two layers: 1. **Basal lamina, 2. Reticular lamina**

1. Basal lamina is secreted by the epithelial cells and consists of protein like lamina and collagen, glycoproteins and proteoglycans.

2. Reticular lamina lies below the basal lamina and consists of collagen proteins secreted by underlying the connective tissue.

Epithelial tissue does not have any blood vessels which supply blood and oxygen.

Classification of epithelium

Epithelial tissue is classified into three major types.

1. **Simple epithelium**
2. **Compound epithelium or Stratified epithelium**
3. **Specialized epithelium**

1. <u>Simple Epithelium</u>

Simple epithelium is a single layer of cells with every cell in direct contact with the basement membrane that separates it from the underlying connective tissue.

In general, it is found where absorption and filtration occur.

The thinness of the epithelial barrier facilitates these processes.

In general, simple epithelial tissues are classified by the shape of their cells.

The four major classes of simple epithelium are

1. **Simple squamous epithelium**
2. **Simple cuboidal epithelium**
3. **Simple columnar epithelium**
4. **Pseudo stratified epithelium**

Types	Description	Location	Functions
Simple Squamous Epithelium	It is consist of single layer of flat cells with nuclei at the center and arranged closely on the basement membrane	It lines the heart, blood and lymph vessels, kidneys, peritoneum, pericardium, pleural membrane, ear drum and small intestine	Filtration Secretion Diffusion

Simple Cuboidal Epithelium	It is consist of simple layer of cube shaped or hexagonal cells with their nuclei at the center, arranged close together on the basement membrane	It forms the covering of ovary, tubules of kidneys and duct of gland, posterior surface of retina. It is also forms the secretory portion of the glands: thyroid gland and pancreas	Secretion Absorption
Simple columnar epithelium ***(i) Ciliated***	It is consist of single layer of long rectangular column shaped cells with their nuclei arranged linearly near the base and structure called cilia at their apex.	It perform the lining of bronchioles, uterus, fallopian tubes, testes, paranasal sinuses, ventricular of the brain and spinal central canal of spinal cord	Cilia help in expulsion of mucus and other foreign substances and in moving oocytes from ovaries into uterus.
(ii) Non- Ciliated	It is consist of single layer of non-ciliated, long rectangular column shaped cells. These cells bear microvilli and goblet cells.	It is lines the entire length of GIT, gall bladder and duct of the glands	Absorption Secretion

Pseudo Stratified epithelium	It is consist of single layer of the cells attached to the basement with their nuclei at different heights. It is ciliated form contain cilia and goblet cells which secrete mucus while non- ciliated forms lacks both cilia and goblet cells	Lining of air passages of upper respiratory tract. Lining of the duct of glands, epididymis and portion of the male urethra	• Secretion of mucus goblet cells • Movement of mucus away from the free surfaces of the organs (lungs) • Absorptive and protective

II. <u>Compound or Stratified epithelium</u>

Stratified epithelial tissue is formed of several layers of epithelial cells of different shapes representing newly formed and mature cells.

It is divided into Stratified squamous epithelium, Stratified Cuboidal epithelium, Stratified Columnar epithelium and Transitional Epithelium.

Types	**Description**	**Location**	**Functions**
Stratified Squamous Epithelium	It is most common type of all the stratified epithelia in the body. It is contains cuboid or columnar cells at the base and squamous cells in the apical layers. **Types** • i. Keratinized stratified epithelium • ii. Non-keratinized stratified epithelium	Lines of the super ficial layers of skin, mouth, oesophagus, pharynx, vagina and tongue.	• It is protect the surface of the underlying organs from injury, water loss, UV rays and foreign invasion

	i. Keratinized stratified epithelium The Epidermis(most superficial layer) of the skin is composed of stratified squamous epithelialcells that contain large quantities of the protein. Keratin is a tough fibrous protein that offers protection from abrasion and water loss. The dermis, which lies deep to the epidermis, is composed of connective tissue. **iii. Non-keratinized stratified epithelium** These are Layers of living squamous epithelium formed by cuboidal cells. Found in moist surface like, buccal cavity, oesophagus, vagina		• Impervious to water, resistant to mechanical damage • Protection from mechanical damage
Stratified Cuboidal epithelium	It is consist of two or more layers of the cell with apical cuboidal cells and basal irregularly shaped cells	It forms the lining of the ducts of sweat glands, excretory glands and part of the male urethra.	• Protection from wear and tear or injury • Absorption and secretion to a limited extend.
Stratified Columnar epithelium	It is made up of apical columnar cells and basal irregularly shaped cells.	It is forms the lining of urethra, excretory duct of glands like esophageal glands, part of the conjunctiva of the eye and part of the anal mucus membrane	• Protection from injury or wear and tear. • Secretion

Transitional Epithelium	It is consist of more than two layer of the cell with basal cuboidal cells or columnar cells and apical squamous (when stretched) to dome like or rounded cells (when relaxed)	It line the urinary system	• It allows stretching and relaxation of urethra, urinary bladder and ureters.

III. <u>Specialized Epithelium</u>

Specialized Epithelial tissue is a class of epithelial tissues that have very specific and specialized functions and structures. This tissue is classified into four type Glandular epithelium, Germinal epithelium, Sensory epithelium and Ciliated epithelium

Types	Description	Location	Functions
Glandular Epithelium	Glands are composed of cuboidal and columnar epithelium and are specialized for secretion. • Based on the no. of cells, glands are of two types: • **Unicellular gland-** • It isindividual cell is modified into glandular cell. • **Multi cellular gland-** It is Number of glandular cells are aggregated to form multicellular gland	Goblet cell Sweat glands	Secrete enzymes, hormones and mucus etc Secrete oils
Germinal epithelium	These are cuboidal or columnar epithelium layer found in the gonads	Found in the gonads ovary and testicle.	Supportive cells to the developing sperm.

Sensory epithelium	These are modified columnar epithelium having sensory hair (cilia) at the free surface lies between columnar Epithelium.	Nasal cavity and tongue	Perceive the stimulus
Ciliated epithelium	These are elongated columnar epithelium having numerous cilia at the free end.	Found in Ureter, respiratory passage, spinal cord and oviduct	Moisten and protect the airway
Pseudo Stratified epithelium	It is consist of single layer of the cells attached to the basement with their nuclei at different heights. It is ciliated form contain cilia and goblet cells which secrete mucus while non- ciliated forms lacks both cilia and goblet cells	Lining of air passages of upper respiratory tract. Lining of the duct of glands, epididymis and portion of the male urethra	• Secretion of mucus goblet cells • Movement of mucus away from the free surfaces of the organs (lungs) • Absorptive and protective

Objective: 2

Aim: To study the Connective tissue.

Requirements:

Permanent histological slides

Microscope

Principle:

Connective tissue (CT) is one of the four basic types of tissue, along with epithelial tissue, muscle tissue, and nervous tissue. It develops from the mesoderm.

Connective tissue is found in between other tissues everywhere in the body, including the nervous system.

In the central nervous system, the three outer membranes (the meninges) that envelop the brain and spinal cord are composed of connective tissue.

All types of connective tissues are derived from embryonic connective tissue called mesenchyme.

Altogether connective tissue is composed of extracellular matrix and connective tissue cells. Connective tissue fibers provide support and strength to the tissues and mainly composed of structural proteins.

Three main types of fibers are present in the extra cellular matrix.Collagen fibers, elastic fibers and reticular fibers

CLASSIFICATION OF CONNECTIVE TISSUE

Connective tissue divided into four types.

a) Loose connective tissue

b) Dense connective tissue

c) Supportive connective tissue

d) Fluid connective tissue

a. Loose connective tissue

Loose connective tissue consists of loosely arranged fibers embedded in the ground substances between the connective tissue cells.

Three types of loose connective tissues are

i. Adipose tissue, ii. Areolar connective tissue, iii. Reticular connective tissue

Location	Description		Functions
i. Adipose tissue	It is consists mostly of fat storage cells (Adipocytes), with little extracellular matrix White adipose tissue is most abundant. It can appear yellow and owes its color to carotene and related pigments from plant food. White fat contributes mostly to lipid storage and can serve as insulation from cold temperatures and mechanical injuries.	Subcutaneous layer of the skin. Around the kidneys, Heart,joints, in bone marrow, Eye, socket neck and clavicle region.	• Stores fat • Reduce the loss of the heat through skin • Give shape to the body organs • Support and protect the organs from injury or damage
ii. Areolar connective tissue	Areolar tissue shows little specialization. It contains all the cell types and fibers and it is distributed in a random, web-like fashion. Areolar tissue underlies most epithelia and represents the connective tissue component of epithelial membranes.	It fills the spaces between muscle fibers, surrounds blood and lymph vessels.	Supports organs in the abdominal cavity.

iii.Reticular connective tissue	It derives its name from the Latin *reticulus*, which means "little net." It is a mesh-like, supportive framework for soft organs. Reticular cells produce the reticular fibers that form the network onto which other cells attach.	Lymphatic tissue, spleen, liver, red bone marrow and around the blood vessels	• Supporting framework for soft organs such as lymphatic tissue, the spleen, and the liver. • Filter and remove damaged blood vessels and bacteria and lymph nodes

b. Dense connective tissue

Dense connective tissue contains more collagen fibers than does loose connective tissue.

As a consequence, it displays greater resistance to stretching. There are three major categories of dense connective tissue:

i. Dense regular connective tissue
ii. Dense irregular connective tissue
iii. Elastic connective tissue

Types	Description	Location	Functions
i. Dense regular connective tissue	Dense regular connective tissue fibers are parallel to each other, enhancing tensile strength and resistance to stretching in the direction of the fiber orientations.	Ligaments and tendons are made of dense regular connective tissue.	• Provides tensile strength and resistance to stretching

ii. Dense irregular connective tissue	It is consist of fibroblasts and irregularly arranged thick. White collagen fibers throughout the ground substance	Pericardium of heart, heart vessels, dermis layer of the skin, around the kidneys and liver	• It provides strength and toughness to various body organs and tissues
iii. Elastic connective tissue	It consist of a network of thin yellow, branching elastic fibroblasts scattered in the spaces between the fibers	Lungs walls of the arteries, bronchiole tubes, trachea, vocal cords and some ligaments	• Expiration • Prevent the excessive dilation • Maintain blood flow

c. Supportive connective tissue

Supporting connective tissue comprises bone and cartilage, Supporting connective tissue comprises bone and cartilage.

Two major forms of supportive connective tissue: i. Cartilage ii. Bone

i. Cartilage

- Cartilage is non-vascular connective tissue and it is present end of the bones and joint**s.**
- It is tough, hard but a flexible connective tissue. It can resist strain and can absorb mechanical shock.
- It consists of solid or semisolid matrix in which cartilage cell (chondrocytes) and fibres are embedded. Cartilage is enclosed in a sheath of white fibrous connective tissue called below the perichondrium, there is layer chondroblast cell, which form chondrocytes.
- Chondrocytes are dispersed in the matrix and occur in the fluid filled space called lacunae. Each lacunae contain 2-4 chondrocytes. Each chondrocytes is a large, angular cell with distinct nucleus.

Cartilage divide into three types: **Hyaline cartilage , Fibrous cartilage & Elastic cartilage**

j. Cartilage

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Types	Description	Location	Functions
A. Hyaline cartilage	The matrix is glass like semitransparent, homogenous and has fine collagen fibers. It is slightly elastic and compressible.	Found in nose, larynx, trachea, breast bone and end of the long bones	Provides flexibility, support and smooth surface for movements of the joints
B. Fibrous cartilage	The matrix has bundles of densely packed white collagen fibers It is considered as intermediate between hyaline and elastic cartilage	Found in intervertebral discs, pubis symphysis and ligaments and tendons of the bones	Providing strength, rigidity and support to the structures
C. Elastic cartilage	The matrix is semi opaque and has network of yellow elastic fibers It is highly elastic and flexible. The tissue recover the shape quickly	Found in-external ear, epiglottis, and pharynx (Eustachian tube)	Provides strength and elasticity

d. Fluid connect tissue

Fluid or liquid connective tissue is consist of blood and lymph

Blood

Blood is a fluid connective tissue that travels through tubular vessels.

• Blood consists of cells and cell fragments collectively called formed elements. These formed elements are erythrocytes, leukocytes(white blood cells), and platelets.

Contains dissolved protein fibers in a watery ground substance.

Together, the dissolved protein fibers and the watery ground substance form an extracellular matrix called plasma.

• The dissolved protein fibers are modified to become insoluble and form a clotting meshwork if a blood vessel or tissue becomes damaged.

- Blood cells: RBC, WBC& Platelets
- Fibers: Fibrinogens
- Ground substance:Protein

Functions

• Phagocytosis of bacteria &Release of antimicrobial chemicals.
- Secrete histamine (a vasodilator), which increases blood flow to a tissue
- Secrete heparin (an anticoagulant), which promotes mobility of other WBCs by preventing clotting. Secrete antibodies, Serve in immune, memory &Clotting of blood
- Destroy cancer cells, cells infected with viruses, and foreign cells
- Secrete antibodies, Serve in immune, memory &Clotting of blood

Lymph

Lymph is usually a clear, colorless fluid, similar to blood plasma but low in protein.

Its composition varies substantially from place to place. After a meal, for example, lymph draining from the small intestine has a milky appearance because of its high lipid content.

Lymph leaving the lymph nodes contains a large number of lymphocytes—indeed, this is the main supply of lymphocytes to the bloodstream.

REPORT:

EX NO **DATE**

MICROSCOPIC STUDY OF MUSCULAR AND NERVOUS TISSUES

Objective: 1

Aim: To study the muscular tissue.

Requirements:

Permanent histological slides

Microscope

Principle:

Muscle tissue is a richly vascularized, cellular tissue containing elongated cells called as muscle fibres or myocytes.

These myocyte consist of actin and myosin flament which provide sufficient enrgy for contraction and relaxation of the muscles tissue by utilizing ATP.

Apart from provide movements within the body, muscle tissue stores and moves substances within the body.

Contractility and conductivity are the two primary functions of any muscle tissue.

Muscle tissue is divided into three types based on location, functional and structural characters.

Classification of Muscular Tissue

1. **Smooth muscle**
2. **Cardiac muscle**
3. **Skeletal muscle**

1. Smooth muscle

Smooth muscle is a type of muscle tissue which is used by various systems to apply pressure to vessels and organs. Smooth muscle is an involuntary non-striated muscle.

Location

Smooth muscle cells are found in the walls of hollow organs, including the stomach, intestines, urinary bladder and uterus, and in the walls of passageways, such as

the arteries and veins of the circulatory system, and the tracts of the respiratory, urinary,and reproductive systems.

These cells are also present in the eyes and are able to change the size of the iris and alter the shape of the lens.

In the skin, smooth muscle cells cause hair to stand erect in response to cold temperature or fear.

Properties

Smooth muscle is composed of sheets or strands of smooth muscle cells

These cells have fibers of actin and myosin which run through the cell and are supported by a framework of other proteins.

A smooth muscle contract under certain stimuli as ATP is freed for use by the myosin.

The amount of ATP released depends on the intensity of the stimuli.

The actin and myosin filaments in smooth muscle are arranged in a stacked pattern across the cell.

This “staircase” arrangement of actin and myosin is much different than the structure in skeletal and cardiac muscle.

In skeletal and cardiac muscle, the actin filaments are attached to Z plates, which hold many actin filaments and show up as dark bands under the microscope.

In smooth muscle, the actin and myosin fibers are arranged an angle to each other as they run through the cell.

Functions:

- The smooth muscle in the uterus helps a woman to push out her baby.
- In the bladder, smooth muscle helps to push out urine.
- Smooth muscle determines the flow of blood in the arteries.
- Smooth muscles move food through the digestive tract.
- In one's eye the pupillary sphincter muscles is responsible for shrinking the size of the pupil.
- In arteries, smooth muscle movements maintain the arteries' diameter.

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The actin and myosin filaments in smooth muscle are arranged in a stacked pattern across the cell.

This “staircase” arrangement of actin and myosin is much different than the structure in skeletal and cardiac muscle.

In skeletal and cardiac muscle, the actin filaments are attached to Z plates, which hold many actin filaments and show up as dark bands under the microscope.

In smooth muscle, the actin and myosin fibers are arranged an angle to each other as they run through the cell.

Functions:

- The smooth muscle in the uterus helps a woman to push out her baby.
- In the bladder, smooth muscle helps to push out urine.
- Smooth muscle determines the flow of blood in the arteries.
- Smooth muscles move food through the digestive tract.
- In one's eye the pupillary sphincter muscles is responsible for shrinking the size of the pupil.
- In arteries, smooth muscle movements maintain the arteries' diameter.

- Smooth muscle regulates air flow in lungs.
- Smooth muscle helps sperm to move along the reproductive tract.
- Smooth muscle in the lungs helps the airways to expand and contract as necessary
- Skin stands on end when piloerector muscles contract.
- To prevent urination during ejaculation by men, the sphincter muscle constricts.
- The tunica dartos is a smooth muscle that contracts or expands to regulate temperature of the testicles.
- Smooth muscles in arteries and veins are largely responsible for regulation of blood pressure.

2. Cardiac muscles

Cardiac muscle (also called **heart muscle** or **myocardium**) is one of three types of vertebrate muscles

It is an involuntary, striated muscle that constitutes the main tissue of the walls of the heart.

The myocardium forms a thick middle layer between the outer layer of the heart wall (the epicardium) and the inner layer (the endocardium), with blood supplied via the coronary circulation.

It is composed of individual heart muscle cells (cardiomyocytes) joined together by intercalated discs, encased by collagen fibres and other substances that form the extracellular matrix.

Cardiac muscle can be likened to the wall of a house. Most of the wall is taken up by bricks, which in cardiac muscle are individual cardiac muscle cells or cardiomyocytes.

The mortar which surrounds the bricks is known as the extracellular matrix, produced by supporting cells known as fibroblasts.

In the same way that the walls of a house contain electrical wires and plumbing, cardiac muscle also contains specialized cells for conducting electrical signals rapidly (the cardiac conduction system), and blood vessels to bring nutrients to the muscle cells and take away waste products (the coronary arteries, veins and capillary network)

Intercalated discs

The cardiac syncytium is a network of cardiomyocytes connected to each other by intercalated discs that enable the rapid transmission of electrical impulses through the network, enabling the syncytium to act in a coordinated contraction of the myocardium.

Gap junctions: Gap junctions are part of the intercalated discs. When one cardiac muscle cell is stimulated to contract, a gap junction transfers the stimulation to the next cardiac cell. This allows the muscle to contract in a coordinated way.

***Desmosomes*:** Like gap junctions, desmosomes are also found within intercalated discs. They help hold the cardiac muscle fibers together during a contraction.

***Nucleus*:** The nucleus is the "control center" of a cell. It contains all of the cell's genetic material. While skeletal muscle cells can have multiple nuclei, cardiac muscle cells typically only have one nucleus.

Functions

Cardiac muscle tissue works to keeps heart pumping through involuntary movements.It does this through specialized cells called pacemaker cells.

These control the contractions of your heart. Nervous system sends signals to pacemaker cells that prompt them to either speed up or slow down your heart rate.

Pacemaker cells are connected to other cardiac muscle cells, allowing them to pass along signals. This results in a wave of contractions of your cardiac muscle, which creates your heartbeat.

3. Skeletal muscles

Skeletal muscle is one of three major muscle types, the others being cardiac muscle and smooth muscle.

It is a form of striated muscle tissue, which is under the voluntary control of the somatic nervous system.

Most skeletal muscles are attached to bones by bundles of collagen fibers known as tendons.

Skeletal muscle is comprised of a series of muscle fibers made of muscle cells. These muscle cells are long and multinucleated

At the ends of each skeletal muscle a tendon connects the muscle to bone. This tendon connects directly to the *epimysium*, or collagenous outer covering of skeletal muscle.
Underneath the epimysium, muscle fibers are grouped into bundles called *fascicles.*
These fascicles are surrounded by another protective covering formed from collagen. The *perimysium*, as it is called, allows nerve and blood vessels to make their way through the muscle.

Each fascicle is formed from tens to hundreds of bundled muscle fibers. Each muscle fiber is formed from a chain of multinucleated muscle cells.

These fibers are then protected by another layer called the *endomysium* as they are bundled into fascicles. Each muscle cell has distinct regions when viewed under a microscope.

These are known as *sarcomeres*, and give skeletal muscle a banded or *striated* appearance. Each sarcomere is a complex of proteins, which operates to contract the muscle.

Sarcomeres are formed from *actin* and *myosin*, as well as a number of associated helper proteins. The filaments seen between the dark bands are actin and myosin filaments

Actin is accompanied by a number of proteins which help stabilize it and provide a pathway for muscle contraction. The two most important are *troponin* and *tropomyosin*.

Tropomyosin surrounds the actin filament, and stops the heads of myosin from attaching. Troponin locks tropomyosin in place until receiving the signal to contract.

Myosin is a fiber composed of many interlaced tails of individual myosin units. The heads of the units stick above the fiber and are attracted to the actin filament.

Functions: Skeletal muscle cells are excitable and are subject to depolarization by the neurotransmitter acetylcholine, released at the neuromuscular junction by motor neurons

- Supports the body and maintain posture, Facilitates movement,
- Protects internal organs and Generate heat

Objective:2

Aim: To study the nervous tissue.

Requirements:

Permanent histological slides

Microscope

Principle:

Nervous tissue, also called neural tissue or nerve tissue, is the main tissue component of the nervous system.

The nervous system regulates and controls bodily functions and activity and consists of two parts. The central nervous system (CNS) comprising the brain and spinal cord, and the peripheral nervous system (PNS) comprising the branching peripheral nerves.

Nervous tissue is composed of neurons, also called nerve cells, and neuroglial cells. Four types of neuroglia found in the CNS are astrocytes, microglial cells, ependymal cells, and oligodendrocytes.

Two types of neuroglia found in the PNS are satellite cells and Schwann cells. In the central nervous system (CNS), the tissue types found are grey matter and white matter.

The tissue is categorized by its neuronal and neuroglial components Nervous tissue is made up of different types of nerve cells, all of which have an axon.

An axon is the long stem-like part of the cell that sends action potential signals to the next cell. Bundles of axons make up the nerves in the PNS and tracts in the CNS.

Classification

Neurons are classified both functionally and structurally.

Functional classification:

- Sensory neurons (afferent): Relay sensory information in the form of an action potential (nerve impulse) from the PNS to the CNS

- Motor neurons (efferent): Relay an action potential out of the CNS to the proper effector(muscles, glands)
- Interneurons: Cells that form connections between neurons and whose processes are limited to a single local area in the brain or spinal cord

Structural classification:

- **Multipolar neurons:** Have 3 or more processes coming off the soma (cell body). They are the major neuron type in the CNS and include interneurons and motor neurons.
- **Bipolar neurons:** Sensory neurons that have two processes coming off the soma, one dendrite and one axon
- **Pseudo unipolar neurons:** Sensory neurons that have one process that splits into two branches, forming the axon and dendrite
- **Unipolar brush cells:** Are excitatory glutamatergic interneurons that have a single short dendrite terminating in a brush-like tuft of dendrioles. These are found in the granular layer of the cerebellum.

Neuroglia encompasses the non-neural cells in nervous tissue that provide various crucial supportive functions for neurons. They are smaller than neurons, and vary in structure according to their function.

Classification of nervous tissue

In the central nervous system:

- Grey matter is composed of cell bodies, dendrites, unmyelinated axons, protoplasmic astrocytes (astrocyte subtype), satellite oligodendrocytes (non-myelinating oligodendrocyte subtype), microglia, and very few myelinated axons.
- White matter is composed of myelinated axons, fibrous astrocytes, myelinating oligodendrocytes, and microglia.

In the peripheral nervous system:

- Ganglion tissue is composed of cell bodies, dendrites, and satellite glial cells.
- Nerves are composed of myelinated and unmyelinated axons, Schwann cells surrounded by connective tissue.

The three layers of connective tissue surrounding each nerve are:

Endoneurium: Each nerve axon, or fiber is surrounded by the endoneurium, which is also called the endoneurial tube, channel or sheath. This is a thin, delicate, protective layer of connective tissue.

Perineurium: Each nerve fascicle containing one or more axons, is enclosed by the perineurium, a connective tissue having a lamellar arrangement in seven or eight concentric layers.

This plays a very important role in the protection and support of the nerve fibers and also serves to prevent the passage of large molecules from the epineurium into a fascicle.

Epineurium: The epineurium is the outermost layer of dense connective tissue enclosing the (peripheral) nerve.

Functions:

Sensory Function

• Sensory receptors gather information &• Information is carried to the CNS

Integrative Function

Sensory information used to create: • Sensations • Memory • Thoughts • Decisions

Motor Function

• Decisions are acted upon & Impulses are carried to effectors

REPORT:

OSTEOLOGY

EXNO DATE

IDENTIFICATION OF AXIAL BONES

Aim: To study and identify the Axial skeletal bones

Requirements: Skeleton and bone models

Principle:

The axial skeleton can be defined as those bones within the long axis of the body, which include the skull, vertebral column, ribs, sternum, hyoid bone and Auditory ossicle.

The skull and vertebral column work together to protect the central nervous system (Brain &Spinal cord)

The vertebral column also serves as the central support axis for the body as a whole.

The ribs and sternum (along with the vertebral column) form the thoracic cage, which supports and protects the heart and lungs.

All these bones are situated around the central longitudinal axis of the body.

The axial skeleton made up of 80 bones.

- 22 bones are of skull
- 26 bones are of vertebral column
- 25 bones are of thorax
- 6 Auditory ossicles
- 1 Hyoid bone

1. The skull

- The bony structure of head referred as skull
- Cranial bones & facial bones together constitute the skull
- Highly organized and complex structure, Made up of 22 bones
- Situated on the superior end of the backbone
- Bones are joined by sutures
- Only the mandible is attached by a freely movable joint

Functions of Skull

- Protects and supports sensitive organs of the body: Brian, Eyes and Ears
- Movement in the head are possible due to presence of cranial bone, which provide large area of attachment to various muscles
- Skull enclose various cavities which helps the organs to communicate with environment
- The serve as support system for entry into the digestive and respiratory system
- The mandible and maxilla contain alveolar ridges in which teeth are present
- Chewing of the food is possible because of the presence of the mandible
- Facial bones form the framework of the face

i. Cranial bones

The cranium considered as a protective cover for the brain and is made up of eight cranial bones. It is made up of two portions known as base and vault that surrounds and covers it.

Cranial vault otherwise known as calvaria is the area within which the brain is enclosed and cranial base of floor portion of the skull on which the brain rests.

All the bones of the cranium are interconnected via immovable joints (sutures)

The eight cranial bones you should be able to identify are:

- The singular frontal, occipital, ethmoid, and sphenoid
- The paired temporal and the paired parietal(1 Frontal bone, 2 Parietal bones, 2 Temporal bones, 1 Occipital bone. 1 Sphenoid bone)

Frontal bone

This is the bone of the forehead. It forms part of the orbital cavities (eye sockets) and the prominent ridges above the eyes, the supraorbital margins. Just above the supraorbital margins, within the bone

There are two air-filled cavities or sinuses lined with ciliated mucous membrane which haveopenings into the nasal cavity.

The coronal suture joins the frontal and parietal bones. The bone originates in two parts joined in the midline by the frontal suture.

Misalignment of this bone may affect: Change in voice tone, Sinus congestion, Loss of smell, Sinus headaches, Eye strain, or pain

Parietal bones

These bones form the sides and roof of the skull.

They articulate with each other at the sagittal suture, with the frontal bone at the coronal suture, with the occipital bone at the lambdoidal suture and with the temporal bones at the squamous sutures

The inner surface is concave and is grooved by the brain and blood vessels.

Misalignment of these bones may cause one to experience: Visual problems, Attentionproblems, Sleep issues and Headaches/migraines.

Temporal bones

These bones lay one on each side of the head and form immovable joints with the parietal, occipital, sphenoid and zygomatic bones

Each temporal bone has several important features

The squamous part is the thin fan-shaped part that articulates with the parietal bone.

The zygomatic process articulates with the zygomatic bone to form the zygomatic arch

The mastoid part contains the mastoid process, a thickened region behind the ear.

It contains a large number of very small air sinuses which communicate with the middle ear and are lined with squamous epithelium.

The petrous portion forms part of the base of the skull and contains the organs of hearing (the spiral organ) and balance.

The temporal bone articulates with the mandible at the temporomandibular joint, the only movable joint of the skull.

Immediately behind this articulating surface is the external auditory meatus (auditory canal), which passes inwards towards the petrous portion of the bone.

Occipital bone

This bone forms the back of the head and part of the base of the skull. It has immovable joints with the parietal, temporal and sphenoid bones.

Its inner surface is deeply concave and the concavity is occupied by the occipital lobes of the cerebrum and by the cerebellum.

The occiput has two articular condyles that form hinge joints with the first bone of the vertebral column, the atlas. Between the condyles there is the foramen magnum (meaning Targe hole') through which the spinal cord passes into the cranial cavity

Sphenoid bone

This bone occupies the middle portion of the base of the skull and it articulates with the occipital, temporal, parietal and frontal bones. On the superior surface in the middle of the bone there is a little saddle-shaped depression, the hypophyseal fossa (sellaturcica) in which the pituitary gland rests.

The body of the bone contains some fairly large air sinuses lined by ciliated mucous membrane with openings into the nasal cavity.

Ethmoid bone

The ethmoid bone occupies the anterior part of the base of the skull and helps to form the orbital cavity, the nasal septum and the lateral walls of the nasal cavity

On each side are two projections into the nasal cavity, the upper and middle conchae or turbinated processes. It is a very delicate bone containing many air sinuses lined with ciliated epithelium and with openings into the nasal cavity

a. Facial bones

The facial skeleton is sometimes called the *membranous viscerocranium*, the facial skeleton consists of fourteen bones in the face

Inferior nasal concha (2), Lacrimal bones (2), Mandible (1), Maxilla (2), Nasal bones (2), Palatine bones (2), Vomer (1), Zygomatic bones (2).

Maxilla (or) Upper jaw

This originates as two bones but fusion takes place before birth.

The maxilla forms the upper jaw, the anterior part of the roof of the mouth, the lateral walls of the nasal cavity and part of the floor of the orbital cavities

Nasal bones

These are two small flat bones, which form the greater part of the lateral and superior surfaces of the bridge of the nose

The outer surface is concavo-convex from above downward, convex from side to side; it is covered by the procerus and nasalis muscles, and perforated about its center by a foramen, for the transmission of a small vein.

The inner surface is concave from side to side, and is traversed from above downward, by a groove for the passage of a branch of the nasociliary nerve

Lacrimal bones

These two small bones are posterior and lateral to the nasal bones and form part of the medial walls of the orbital cavities. Each is pierced by a foramen for the passage of the nasolacrimal duct which carries the tears from the medial canthus of the eye to the nasal cavity

Vomer

The vomer is a thin flat bone which extends upwards from the middle of the hard palate to form the main part of the nasal septum. Superiorly it articulates with the perpendicular plate of the ethmoid bone

Palatine bones

These are two L-shaped bones

The horizontal parts unite to form the posterior part of the hard palate and the perpendicular parts project upwards to form part of the lateral walls of the nasal cavity

At their upper extremities they form part of the orbital cavities

Inferior conchae

Each concha is a scroll-shaped bone which forms part of the lateral wall of the nasal cavity and projects into it below the middle concha. The superior and middle conchae are parts of the ethmoid bone

Mandible

This is the only movable bone of the skull. It originates as two parts which unite at the midline.

Each half consists of two main parts: a curved body with the alveolar ridge containing the lower teeth and a ramus which projects upwards almost at right angles to the posterior end of the body

At the upper end the ramus divides into the condi/lar process which articulates with the temporal bone to form the temporomandibular joint and the coronoid process that gives attachment to muscles and ligaments. The point where the ramus joins the body is the angle of the jaw.

2. The vertebral column

In a human's vertebral column there are normally thirty-three vertebrae; the upper twenty-four are articulating and separated from each other by intervertebral discs, and the lower nine are fused in adults, five in the sacrum and four in the coccyx or *tailbone*.

7 Cervical, 12 Thoracic, 5 Lumbar,5 Sacrum (five fused bones) and 4 Coccyx (four fused bones)

- Vertebrae separated by intervertebral discs
- The spine has a normal curvature
- Each vertebrae is given a name according to its location

Characteristics of typical vertebrae:

The body & the vertebral (neural)

- The body of each vertebra is situated anteriorly
- They are smallest in the cervical region and become larger towards the lumbar region
- The vertebral (neural) arch encloses a large vertebral foramen.
- The ring of bone consists of two pedicles that project backwards from the body and two laminae

- The neural arch has four articular surfaces: two articulate with the vertebra above and two with the one below.
- The vertebral foramina form the vertebral (neural) canal that contains the spinal cord

Cervical vertebrae

The cervical vertebrae are the bones within the neck. The vertebrae are the bones that make up the backbone of vertebrate animals.

These cervical bones are the smallest in the body in mammals. The bones are identified as C1 through C7, and they increase in size as you move down the body.

These are important for the movement of the head and neck, including the action of nodding. The cervical vertebrae has varying structures depending on their location in the neck.

C1, or the atlas, is the only vertebra without a body; instead, it articulates with C2, the axis, at the odontoid process, a bony knob.

C2 has a large perpendicular process known as the dens, and its body extends down, covering part of C3.

The vertebrae C3 through C6 have a more regular shape. They have small bodies, with pedicles that extend backwards and laterally.

These vertebrae have large vertebral foramen in the transverse processes, which the vertebral artery, veins, and inferior cervical ganglion pass through.

Thoracic vertebrae

In vertebrates, thoracic vertebrae compose the middle segment of the vertebral column, between the cervical vertebrae and the lumbar vertebrae.

In humans, there are twelve thoracic vertebrae

The first and ninth through twelfth vertebrae contain certain peculiarities

First thoracic vertebra (T1): The first thoracic vertebra has, on either side of the body

The superior articular surfaces are directed upward and backward; the spinous process is thick, long, and almost horizontal.

The transverse processes are long, and the upper vertebral notches are deeper than those of the other thoracic vertebrae. The thoracic spinal nerve 1 (T1) passes out underneath it

Second thoracic vertebra (T2): The thoracic spinal nerve 2 (T2) passes out underneath it. The second thoracic vertebra is larger than the first thoracic vertebrae

Third thoracic vertebra (T3): The thoracic spinal nerve 3 (T3) passes out underneath it.

Fourth thoracic vertebra (T4): The fourth thoracic vertebra, together with the fifth, is at the same level as the sternal angle. The thoracic spinal nerve 4 (T4) passes out underneath it.

Fifth thoracic vertebra (T5): The fifth thoracic vertebra, together with the fourth, is at the samelevel as the sternal angle.

The human trachea divides into two main bronchi at the level of the 5th thoracic vertebra, but may also end higher or lower, depending on breathing. The thoracic spinal nerve 5 (T5) passes out underneath it.

Sixth thoracic vertebra (T6): The thoracic spinal nerve 6 (T6) passes out underneath it.

Seventh thoracic vertebra (T7): The thoracic spinal nerve 7 (T7) passes out underneath it.

Eighth thoracic vertebra (T8): The eighth thoracic vertebra is, together with the ninth thoracic vertebra, at the same level as the xiphi sternum.

The thoracic spinal nerve 8 (T8) passes out underneath it.

Nineth thoracic vertebra (T9): The thoracic spinal nerve 9 (T9) passes out underneath it. The xiphisternum (or xiphoid process of the sternum) is at the same level in the axial plane

Tenth thoracic vertebra (T10): It doesn't have any kind of facet below, because the following ribs only have one facet on their heads.

The thoracic spinal nerve 10 (T10) passes out underneath it.

Eleventh thoracic vertebra (T11): The spinous process is short, and nearly horizontal in direction. The transverse processes are very short, tuberculated at their extremities, and do not have articular facets.

Twelfth thoracic vertebra (T12): The twelfth thoracic vertebra has the same general characteristics as the eleventh, but may be distinguished from it by its inferior articular surfaces being convex and directed lateral ward, like those of the lumbar vertebrae

Lumbar vertebrae

The lumbar vertebrae are, in human anatomy, the five vertebrae between the rib cage and the pelvis.

They are the largest segments of the vertebral column and are characterized by the absence of the foramen transversarium within the transverse process (since it is only found in the cervical region) and by the absence of facets on the sides of the body (as found only in the thoracic region).

They are designated L1 to L5, starting at the top. The lumbar vertebrae help support the weight of the body, and permit movement.

Sacrum

This consists of five rudimentary vertebrae fused to form a triangular or wedge-shaped bone with a concave anterior surface.

The upper part, or base, articulates with the 5th lumbar vertebra.

On each side it articulates with the ilium to form a sacroiliac joint, and at its inferior tip it articulates with the coccyx.

The anterior edge of the base, the promontory, protrudes into the pelvic cavity.

Coccyx

Commonly referred to as the tailbone

This consists of the four terminal vertebrae fused to form a very small triangular bone, the broad base of which articulates with the tip of the sacrum.

It comprises three to five separate or fused coccygeal vertebrae below the sacrum, attached to the sacrum by a fibrocartilaginous joint, the sacrococcygealsymphysis, which permits limited movement between the sacrum and the coccyx.

The coccyx is formed of three, four or five rudimentary vertebrae. It articulates superiorly with the sacrum. In each of the first three segments may be traced a rudimentary body and articular and transverse processes; the last piece (sometimes the third) is a mere nodule of bone.

All the segments lack pedicles, laminae and spinous processes.

It has been claimed that the coccyx is not entirely useless in humans, based on the fact that the coccyx has attachments to various muscles, tendons and ligaments.

However, these muscles, tendons and ligaments are also attached at many other points, to stronger structures than the coccyx.

Functions of the vertebral column

These include the following.

• Collectively the vertebral foramina form the vertebral canal which provides a strong bony protection for the delicate spinal cord lying within it.

• The pedicles of adjacent vertebrae form intervertebral foramina, one on each side, providing access to the spinal cord for spinal nerves, blood vessels and lymph vessels.

The numerous individual bones enable a certain amount of movement.

• It supports the skull.

• The intervertebral discs act as shock absorbers, protecting the brain.

• It forms the axis of the trunk, giving attachment to the ribs, shoulder girdle and upper limbs, and the pelvic girdle and lower limbs.

3. The hyoid bone

Its name is derived from Greek*hyoeides*, meaning 'shaped like the letter upsilon (υ)
The hyoid bone (lingual bone or tongue-bone) is a horseshoe-shaped bone situated in the anterior midline of the neck between the chin and the thyroid cartilage.

- The only bone that does not articulate with another bone

The hyoid is anchored by muscles from the anterior, posterior and inferior directions, and aids in tongue movement and swallowing.

The hyoid bone provides attachment to the muscles of the floor of the mouth and the tongue above, the larynx below, and the epiglottis and pharynx behind.
The hyoid bone is classed as an irregular bone and consists of a central part called the body, and two pairs of horns, the greater and lesser horns.

Blood is supplied to the hyoid bone via the lingual artery, which runs down from the tongue to the greater horns of the bone.

The suprahyoid branch of the lingual artery runs along the upper border of the hyoid bone and supplies blood to the attached muscles.

4. Thoracic cage

The bones of the thorax or thoracic cage are:• 1 sternum

• 12 pairs of ribs

• 12 thoracic vertebrae.

Sternum or breast bone

This flat bone can be felt just under the skin in the middle of the front of the chest.

The manubrium is the uppermost section and articulates with the clavicles at the sternodavicular joints and with the first two pairs of ribs.

The body or middle portion gives attachment to the ribs.

The xiphoid process is the tip of the bone.

It gives attachment to the diaphragm, muscles of the anterior abdominal wall and the lineaalba.

Ribs

There are 12 pairs of ribs which form the bony lateral walls of the thoracic cage and articulate posteriorly with the thoracic vertebrae.

The first 10 pairs are attached anteriorly to the sternum by costal cartilages, some directly and some indirectly).

The last two pairs (floating ribs] have no anterior attachment.

Thoracic cage Sternum

The sternal end is attached to the sternum by a costal cartilage, i.e. a band of hyaline cartilage.

The superior border is rounded and smooth while the inferior border has a marked groove occupied by the intercostal blood vessels and nerves.

The first rib does not move during respiration. The spaces between the ribs are occupied by the intercostal muscles. During inspiration, when these muscles contract, the ribs and sternum are lifted upwards and outwards, increasing the capacity of the thoracic cavity

5. Auditory Ossicles

The auditory ossicles are three bones in either middle ear that are among the smallest bones in the human body.

They serve to transmit sounds from the air to the fluid-filled labyrinth (cochlea).

The absence of the auditory ossicles would constitute a moderate-to-severe hearing loss.

The term "ossicle" literally means "tiny bone".

Though the term may refer to any small bone throughout the body, it typically refers to the malleus, incus, and stapes (hammer, anvil, and stirrup) of the middle ear.

REPORT:

EX. NO: **DATE:**

IDENTIFICATION OF APPENDICULAR BONES

Aim: To study and identify the Appendicular skeletal bones

Requirements: Skeleton and bone models

Principle:

- The appendicular skeleton is the portion of the skeleton of vertebrates consisting of the bones that support the appendages.
- The appendicular skeleton includes the skeletal elements within the limbs, as well as supporting pectoral and pelvic girdle.
- Unlike the axial skeleton, the appendicular skeleton is unfused. This allows for a much greater range of motion
- The appendicular skeleton comprises 126. Functionally it is involved in locomotion (lower limbs) of the axial skeleton and manipulation of objects in the environment (upper limbs).
- The appendicular skeleton forms during development from cartilage, by the process of endochondral ossification.

The appendicular skeleton is divided into six major regions:

1. **The shoulder (Pectoral) girdle**
2. **The upper limbs**
3. **The pelvic (pelvis) girdle**
4. **The lower limbs**

1. Shoulder (Pectoral) girdle

- Composed of four bones
  - 2 Clavicle – collarbone (Left and right clavicle)

  - 2 Scapula – shoulder blade (Left and rightScapula)

- These bones allow the upper limb to have exceptionally free movement

The clavicle - collarbone

- The clavicle is a long bone which has a double curve. It articulates with the manubrium of the sternum at the sternoclavicular joint and forms the acromioclavicular joint with the acromion process of the scapula.
- The clavicle provides the only bony link between the upper limb and the axial skeleton
- It serves as a rigid support from which the scapula and free limb suspended; an arrangement that keeps the upper limb away from the thorax so that the arm has maximum range of movement. Acting as a flexible, crane-like strut, it allows the scapula to move freely on the thoracic wall.
- Covering the cervicoaxillary canal, it protects the neurovascular bundle that supplies the upper limb.
- Transmits physical impacts from the upper limb to the axial skeleton

Scapula or shoulder blade

- The scapula is a flat triangular-shaped bone, lying on the posterior chest wall superficial to the ribs and separated from them by muscles.
- At the lateral angle there is a shallow articular surface, the glenoid cavity which, with the head of the humerus, forms the shoulder joint.

On the posterior surface there is a spinous process that projects beyond the lateral angle of the bone that overhangs the shoulder joint, called the acromion process.

It articulates with the clavicle at the acromioclavicular joint.

The coracoid process, a projection from the upper border of the bone, gives attachment to muscles that move the shoulder joint.

2. The upper limb

The upper limb consists of the following bones:

i. ***Arms and forearms (6 bones)*** - Left and right humerus (2) (arm), ulna (2) and radius (2) (forearm).
ii. ***Hands (54 bones)*** - Left and right carpals (16) (wrist), metacarpals (10), proximal phalanges (10), intermediate phalanges (8) and distal phalanges (10).

Humerus

This is the bone of the upper arm. The head articulates with the glenoid cavity of the scapula, forming the shoulder joint.

Distal to the head there are two roughened projections of bone, the greater and lesser tubercles, and between them there is a deep groove, the bicipital groove or intertubercular sulcus, occupied by one of the tendons of the biceps muscle.

The distal end of the bone presents two surfaces that articulate with the radius and ulna to form the elbow joint

Ulna and radius

These are the two bones of the forearm. The ulna is longer than and medial to the radius and when the arm is in the anatomical position, i.e. with the palm of the hand facing forward, the two bones are parallel.

They articulate with the humerus at the elbow joint, the carpal bones at the wrist joint and with each other at the proximal and distal radioulnar joints.

Carpal or wrist bones

There are eight carpal bones arranged in two rows of four. From outside inwards they are:

- Proximal row: scaphoid, lunate, triquetral, pisiform
- Distal row: trapezium, trapezoid, capitate, hamate

These bones are closely fitted together and held in position by ligaments which allow a certain amount of movement between them.

The bones of the proximal row are associated with the wrist joint and those of the distal row form joints with the metacarpal bones.

Tendons of muscles lying in the forearm cross the wrist and are held close to the bones by strong fibrous bands, called retinacula

Metacarpal bones or the bones of the hand

These five bones form the palm of the hand. They are numbered from the thumb side inwards.

The proximal ends articulate with the carpal bones and the distal ends with the phalanges

Phalanges or finger bones

There are 14 phalanges, three in each finger and two in the thumb. They articulate with the metacarpal bones and with each other.

3. Pelvic girdle

The bones of the pelvic girdle are:

- 2 innominate bones or hip
- 1 sacrum

\

Hip bones or Innominate: Composed of three pair of fused bones

- Ilium, Ischium&Pubic bone

- Each hip bone consists of three fused bones, the ilium, ischium and pubis.
- On its outer surface there is a deep depression, the acetabulum, which forms the hip joint with the almost-spherical head of femur.
- The ilium is the upper flattened part of the bone and it presents the iliac crest, the anterior point of which is called the anterior superior iliac spine.
- The pubis is the anterior part of the bone and it articulates with the pubis of the other hip bone at a cartilaginous joint, the symphysis pubis.
- The ischium is the inferior and posterior part. The union of the three parts takes place in the acetabulum.

It is divided into two parts by the brim of the pelvis, consisting of the promontory of the sacrum and the iliopectineal lines of the innominate bones.

The greater or false pelvis is above the brim and the lesser or true pelvis is below.

The total weight of the upper body rests on the pelvis

Protects several organs:

- Reproductive organs, Urinary bladder and Part of the large intestine

Differences between male and female pelvis

The shape of the female pelvis allows for the passage of the baby during childbirth.

In comparison with the male pelvis, the female pelvis has lighter bones, is more shallow and rounded and is generally more roomy.

4. Lower limbs

The lower limbs consist of:

i. **Thighs and legs (8 bones) -** Left and right femur (2) (thigh), patella (2) (knee), tibia (2) and fibula (2) (leg).

ii. **Feet and ankles (52 bones) -** Left and right tarsals (14) (ankle), metatarsals (10), proximal phalanges (10), intermediate phalanges (8) and distal phalanges (10).

Femur or thigh bone

The femur is the longest and strongest bone of the body. The head is almost spherical and fits into the acetabulum of the hip bone to form the hip joint.

In the center of the head there is a small depression for the attachment of the ligament of the head of the femur. This extends from the acetabulum to the femur and contains a blood vessel that supplies blood to an area of the head of the bone.

The neck extends outwards and slightly downwards from the head to the shaft and most of it is within the capsule of the hip joint.

The posterior surface of the lower third forms a flat triangular area called the popliteal surface.

The distal extremity has two articular condyles which, with the tibia and patella, form the knee joint.

Tibia or shin bone

The tibia is the medial of the two bones of the lower leg. The proximal extremity is broad and flat and presents two condoles for articulation with the femur at the knee joint.

The head of the fibula articulates with the inferior aspect of the lateral condyle, forming theproximal tibiofibular joint.

The distal extremity of the tibia forms the ankle joint with the talus and the fibula. The medial malleolus is a downward projection of bone medial to the ankle joint.

Fibula

The fibula is the long slender lateral bone in the leg. The head or upper extremity articulates with the lateral condyle of the tibia forming the proximal tibiofibular joint and the lower extremity articulates with the tibia then projects beyond it to form the lateral malleolus

Patella or knee cap

This is a roughly triangular-shaped sesamoid bone associated with the knee joint. Its posterior surface articulates with the patellar surface of the femur in the knee joint and its anterior surface is in the patellar tendon, i.e. the tendon of the quadriceps femoris muscle.

Tarsal or ankle bones

There are seven tarsal bones which form the posterior part of the foot. They are:

- 1 talus, 3 cuneiform, 1 calcaneus, 1 cuboid& 1 navicular

The talus articulates with the tibia and fibula at the ankle joint. The calcaneus forms the heel of the foot.

The other bones articulate with each other and with the metatarsal bones.

Metatarsal bones of the foot

These are five bones, numbered from within outwards, which form the greater part of the dorsum of the foot.

At their proximal ends they articulate with the tarsal bones and at their distal ends, with the phalanges. The enlarged distal head of the 1st metatarsal bone forms the 'ball' of the foot.

Phalanges of the toes

There are 14 phalanges arranged in a similar manner to those in the fingers, i.e. two in the great toe (the halux) and three in each of the other toes

Arches of the foot

The bones have a bridge-like arrangement and are supported by muscles and ligaments so that four arches are formed, a medial and lateral longitudinal arch and two transverse arches.

Medial longitudinal arch. This is the highest of the arches and is formed by the calcaneus, talus, navicular, three cuneiform and first three metatarsal bones.

Only the calcaneus and the distal end of the metatarsal bones should touch the ground.

Lateral longitudinal arch. The lateral arch is much less marked than its medial counterpart. The bony components are the calcaneus, cuboid and the two lateral metatarsal bones. Again only the calcaneus and metatarsal bones should touch the ground.

Transverse arches. These run across the foot and can be more easily seen by examining the skeleton than the live model. They are most marked at the level of the three cuneiform and cuboid bones.

REPORT:

HEMATOLOGY

EX. NO: **DATE:**

INTRODUCTIONS TO HEMOCYTOMETRY

Aim:

To study the working and uses of hemocytometer

Principle:

- The formed elements of blood are counted by hemocytometry. The apparatus used is called the hemocytometer, consisting of diluting pipettes and counting (Neubauer's) chambers.
- The procedures used for enumeration of blood cells include the manual method of hemocytometry and the use of electronic counting devices.
- The cells counted in routine practice are red cells, white cells and platelets
- Manual techniques lend themselves to enumeration of all small separate bodies such as spermatozoa, eosinophils and cells in the cerebrospinal fluid
- Since the enumerated constituents are to be reported in units per litre of blood, the number of the cells actually counted must be converted to the number present per litre of blood
- The alternative method is units of cells per cubic millimeter (mm^3) or microliter (µl), Since 1 µl is essentially equal to 1 mm^3
- Through the report is usually expressed per cubic millimeter of blood , the reporting unit of choice is cells per litre of blood
    - $1mm^3 = 1\ \mu l = 10^{-6}$ litre
    - $1\ \mu l \times 10^6 = 1$ litre

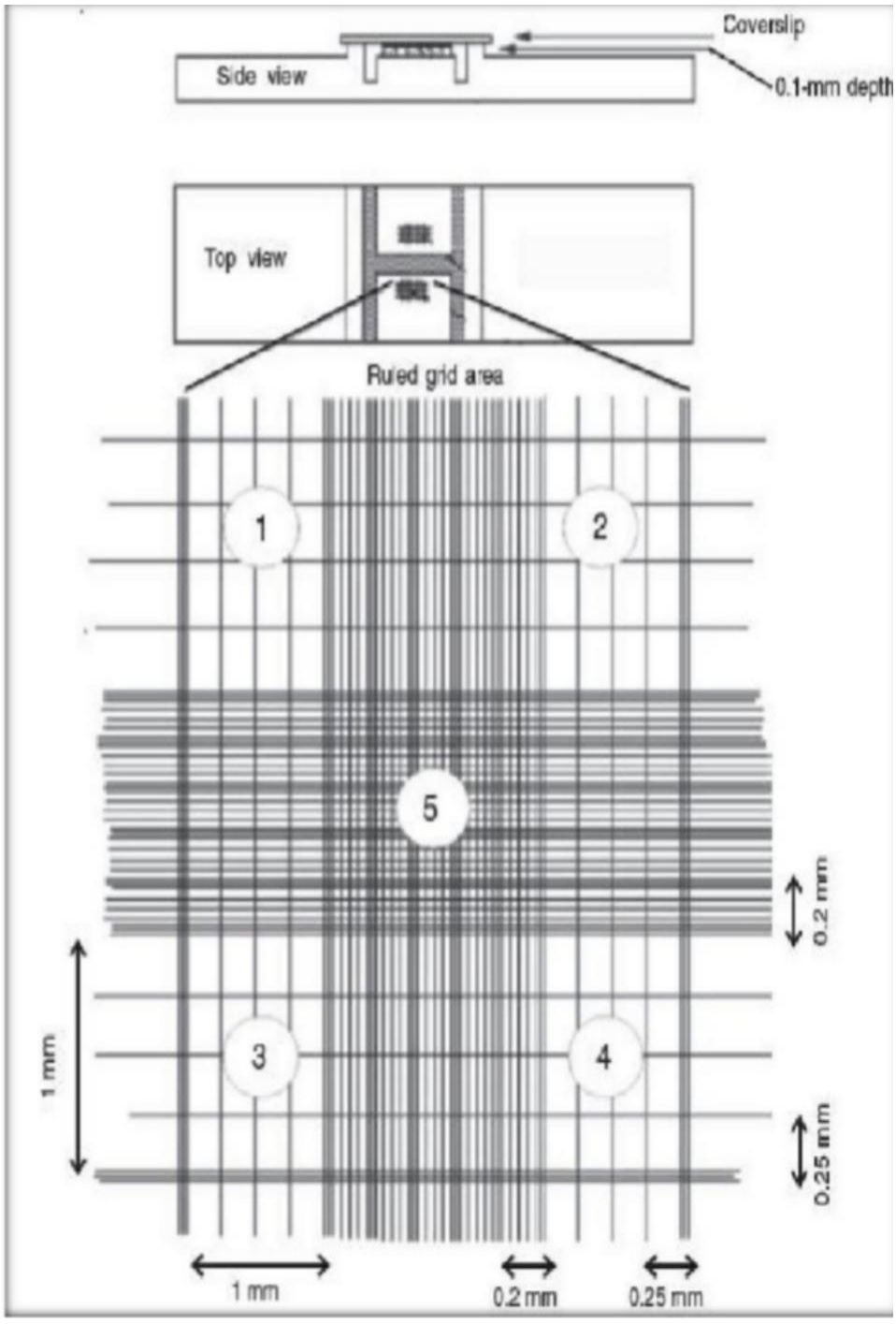
Methods:

Hemocytometry includes pipetting (diluting the blood in the pipette) and charging (charging the Neubauer's chamber with diluted blood)

Pipetting:

To ensure proper dilution of the blood of the sample to be used for counting blood cells, the blood can be precisely measured and diluted with specially designed pipettes.

For counting the blood cells, two types of pipettes are used: RBC &WBC pipettes



Neubauer's Counting Chamber

RBC pipettes: This used for counting red blood cells. It has a stem, bulb, rubber tube and mouthpiece

The stem has two markings, 0.5 and 1. The stem widen into a bulb with a red bead in it.

The bead helps identifying the pipette and mixing the fluid with the blood in the bulb of the pipette

The capacity of the bulb is 100 parts (from 1 to 101 marks). The bulb narrow above (101 is marked just above the bulb) and to this end a rubber tubing which end in a mouthpiece is attached the mouth is in red in color

WBC pipette: This is used for counting white blood cells. It is similar to the RBC pipette exceptthat the capacity of the bulb is less (10 parts) and the bulb contains a white bead

The marking above the bulb is 11. The mouthpiece in the stem is more than that of RBC pipettes.

Diluting fluids: Different varieties of diluting fluids are used for counting different types of cells. Diluting fluids are described with each cell count.

PROCEDURE:

Clean the pipette and ensure that is dry.

Collect the diluting fluid in a watch glass from a stock bottle

Prick the fingertip with all aseptic precaution to obtained free flow of blood

Let a large drop of blood accumulate on the finger tip

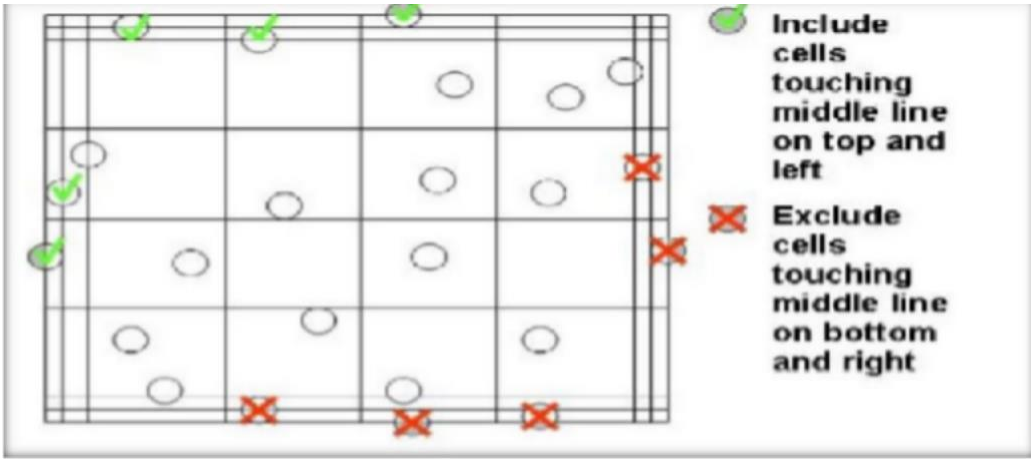
Hold the pipette horizontally and dip its end into the drop of blood. Suck gently on the mouthpiece to draw blood up to the required mark (0.5) on the stem of the pipette

If the more than the required amount of blood is drawn into the pipette, tap gently onto the finger nail or palm.

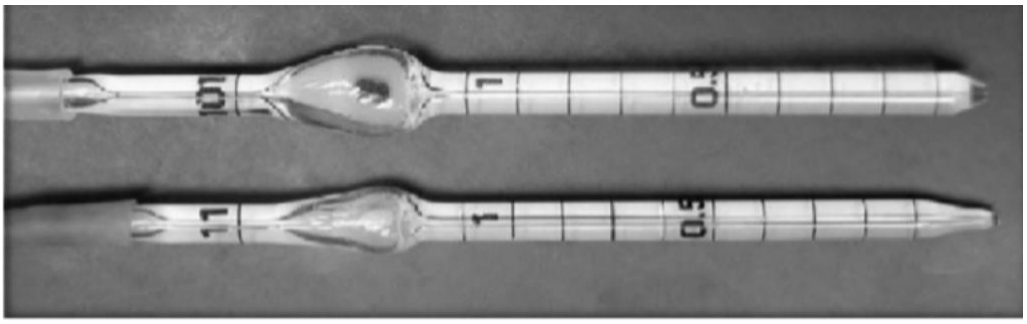
Wipe off the tip of the pipette to remove the extra blood sticking to it.

Maintain the blood level at the 0.5 mark and place the tip of the pipette in the diluting fluid well below the surface of the liquid

Do not directly draw the fluids from the stock bottle as it may contaminate the solution with the cells



Counting system to ensure accuracy and consistency



RBC & WBC Pipette

Difference between RBC and WBC pipettes

S. NO	RBC PIPETTE	WBC PIPETTE
1	RBC pipette is having a red bead	WBC pipette is having a white bead.
2	Mark on the capillary portion above the bulb is 101.	Mark on the capillary portion above the bulb is 11.
3	Bulb of RBC pipette is bigger than the WBC pipette.	Bulb of WBC pipette is smaller than the RBC pipette.
4	Stem of the RBC pipette is shorter than the WBC pipette.	Stem of the WBC pipette is longer than the RBC pipette.

Draw the mixture exactly to the top mark (101 or 11) above the blub. While the bulb is being filled, you may tap the pipette with the finger to knock the bead down below the surface of the solution in the bulb to prevent the formation of bubbles.

Maintain the level of the mixture exactly to the mark by closing the pipette tip with the index.

Hold the pipette horizontal position is also important.

Mix the content of the bulb thoroughly for 2-3 minutes by rotating the pipette with it tip pressing against the palm of the left hand

Place the pipette on a horizontal surface. The pipette is now ready for charging.

Charging the chamber:

The counting chamber in common use is the improved Neubauer's counting chamber.

This is consists of a thick glass slide divided into two central platforms by an H- shaped groove. The central platforms is slightly lower than the sides when a cover-slip is placed covering the central platform and resting on the side platforms.

The depth under the cover-slip and the central platform is 1/10 mm. when the chamber is charged with diluted blood, a thin film of fluid of known volume is spread on the central platform, and this is used for performing the cell counts.

On the central platforms are engraved ruled squares used for various cell counts. The ruled area is a square measuring 3 mm × 3 mm.

This area is divided into nine large squares, each having an area of 1 mm^2 (1 mm × 1 mm). the four large corner squares are used for WBC count, while the large 1 mm square in the centre is used for RBC count.

Each WBC square is further subdivided into 16 small squares, each measuring 1/16 1 mm^2. The 1 mm^2 central RBC squares is divided into 25 medium sized squares by means of the triple lines.

Each medium sized square measures 1/5 mm in length and has area of 1/5 × 1/5 =1/25 mm^2.

The four corner medium sized and the central medium sized squares are used for the RBC count. The area of five medium squares is 1/25 × 5 = 1/5 mm^2.

Since the depth of chamber is 1/10 mm, the volume of the five medium sized squares is 1/5 ×1/10= 1/50 mm^3. Each medium sized square is further divided into 16 small Squares, that is, 25 × 16 = 400 small squares.

The side of the smallest RBC square measures 1/20 mm. Therefore, the area of the smallest RBC square measures 1/20 × 1/20 = 1/ 400 mm^2.

Since the depth under the cover-slip is 1/10 mm, the volume of the smallest RBS square is 1/ 400 × 1/10 = 1/ 4000 mm^3

The red cells are counted in 80 small squares (5 medium sized squares); The volume of 80 small squares is 1/400 × 80 =1/50 mm^3.

Procedure:

Clean the Neubauer's chamber and the cover-slip.

Place the cover-slip on the central platform of the chamber.

Mix the content of the bulb thoroughly.

Discard the first two drops of fluid from the pipette as it is contains only diluting fluid.

Place the tip of the pipette on the surface of the chamber touching the edge of the cover-slip at an angle of 45º.

Allow the diluted blood to flow under the cover-slip by capillary action. Remove the pipette quickly from the edge of cover-slip as soon as the counting platform is filled with the diluted blood.

Take care to avoid entry of air bubbles and overflow of the fluid into the gutters. The mixture should completely cover the platform, but should not enter the gutters. If air bubbles enter the platform or fluid enters the gutters, discard the specimen and recharge.

Allow the cells to settle for 2-3 minutes before placing the charged chamber on the stage of the microscope for counting.

Report:

EX. NO: **DATE:**

ENUMERATION OF RED BLOOD CELL COUNT

Aim:

To enumerate of Red Blood Cell Count on your own blood by manual method

Requirements:

Hemocytometer

Microscope

Cover slips

Hayem's fluid

Cotton

Surgical spirit

Sterile Lancet

Principle:

The blood is diluted (usually 200 times) with the diluting fluid, which does not remove the white cells, but allow the red cells to be counted in a known volume of fluid.

Finally, the number of cells in undiluted blood is calculated and reported as the number of red blood cells per mm^3 of whole blood.

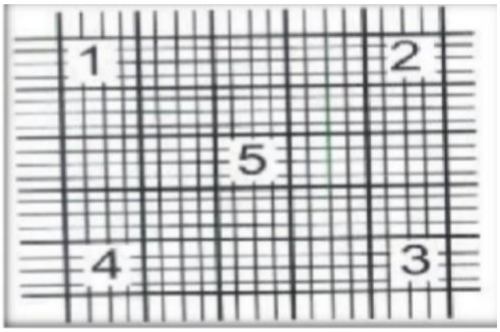
Normal value:

Male : 4.5– 6.0 million /cu.mm

Female : 4.0– 5.5 million /cu.mm

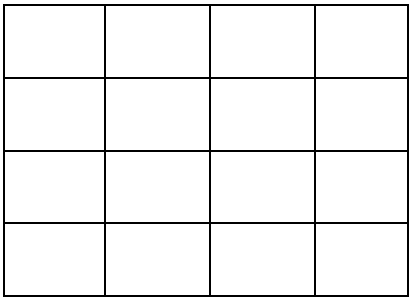
Composition & Significance of RBC (Hayem's)fluid

S. No	COMPOSITION	QTY	SIGNIFICANTS
1	Sodium chloride	0.5 g	To maintain osmolarity
2	Sodium sulfate	2.5 g	To prevent aggregation (rouleaux formation) RBC
3	Mercuric chloride	0.25 g	To fix the cells and act as a preservative
4	Distilled water	100 ml	Act as a solvent



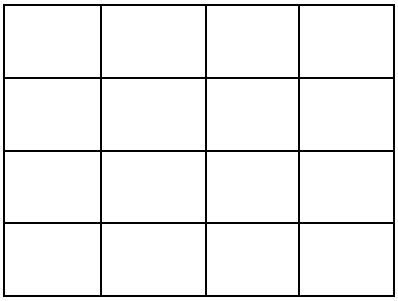
Hemocytometer square for RBC

Observation



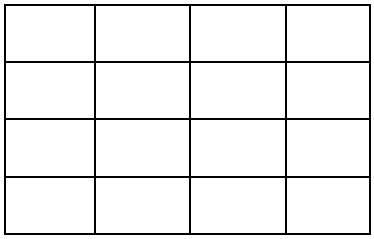
R1=

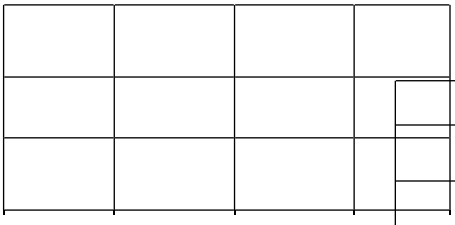
R3=



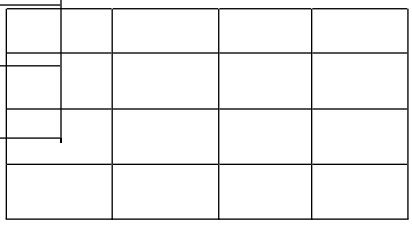
R3=

R4=





R5=



N =R1 + R2 + R3+ R4 + R5=

PROCEDURE:

Assemble all equipment needed for the practical and ensure the pipette, cover-slip and Neubauer's chamber are thoroughly clean and dry.

Take adequate RBC diluting fluid in a watch glass

Prick the finger under the aseptic conditions and suck the blood into the pipette and dilute it following step by step as described in introduction of hemocytometry (Procedure of pipetting)

Hold the pipette horizontally and close both ends, then gently mix the content of the bulb. For mixing, shake the pipette at right angle to its long axis for a few second. The red bead in the pipette should move from one side to other side during the mixing.

After the mixing, keep the pipette in a horizontal position to prevent any loss of its content until the cell count is performed. Discard the first two drops of fluid from the pipette.

Charge the Neubauer's chamber to allow two minutes so that the cells settle down.

Place the charged chamber on the stage of the microscope and adjust the microscope for observation under low power (10^x).

Focus the central square of the Neubauer's chamber under the low power objective, and check uniform distribution of cell. If the cells are not uniformly distributed, clean the Neubauer's chamber and charge it.

Focus the RBC square under the high power objective (45^x).

Count the cells in five medium RBC square in sequence (four corner and the central medium-sized RBC squares) that is 16 x 5 = 80 small RBC squares.

Care should be taken not to count the same cell again. To avoid this count the red cells present in the square and those present on its left and lower lines. Ignore those on its right and upper lines.

Draw the RBC squares in your note book and enter the observation. Calculate the final result.

CALCULATION

Area of 5 medium sized squares = 1/25 x 5 = 1/25 mm^2

Volume of 5 medium sized square = 1/5 x 1/10 = 1/50 mm^3 (1/10 is the depth)

Dilution factor = 1: 200

The cells in 1/50 mm^3volume of diluted blood is n

Cells in 1mm^3volume of diluted blood = n x 50

Cells in 1 mm^3volume of undiluted blood = n x 50 x 200

= n x 10, 000 where n is total number of cells is counted in 5 medium sized RBC squares.

EX. NO: **DATE:**

ENUMERATION OF WHITE BLOOD CELL COUNT

Aim:

To enumerate of White Blood Cell count on your own blood by manual method

Requirements:

Hemocytometer

Microscope

Cover slips

Turk's fluid

Cotton,Surgical spirit &Sterile Lancet

Principle:

- Blood is diluted with acid solution that removes red blood cells by the hemolysis and accentuates the nuclei of white cells
- The counting of the white cells then becomes easy. Counting is done using a microscope under low power objective (10 x) and with knowledge of the volume of fluid examined and the dilution of the blood obtained.
- The number of white cells per mm^3 of undiluted whole bold is calculated.

Normal value:

Adult : 4,000 – 11,000 / mm3 (cc. mm)

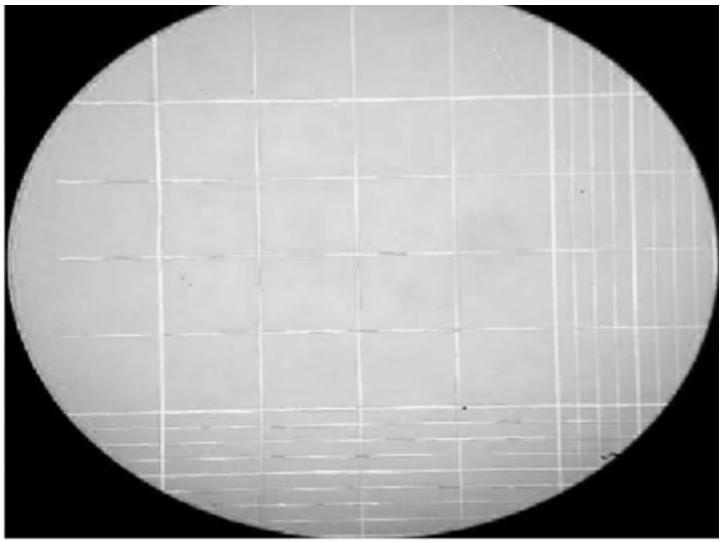
Children : 5,000 – 15,000/ mm3 (cc. mm)

Infants : 6,000 – 18,000/ mm3 (cc. mm)

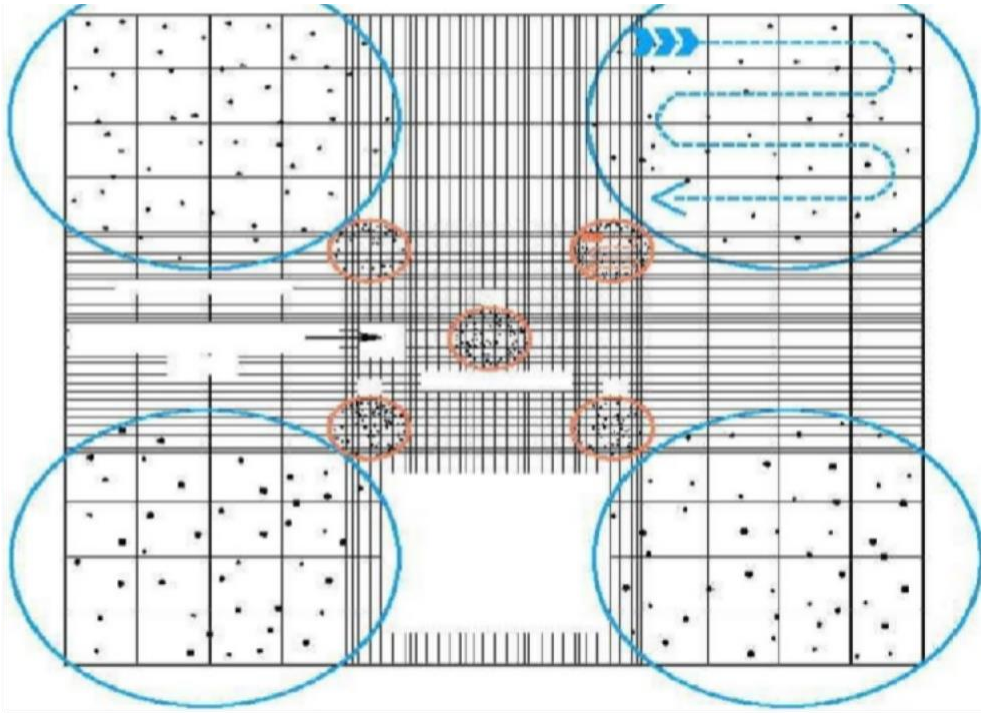
Newborns : 10,000- 25,000/ mm3 (cc. mm)

Composition& Significanceof WBC (Turk's) fluid

S. NO	COMPOSITION	QUANTITY	USES
1	Glacial acetic acid	2 ml (1 %)	Destroys RBC
2	Gentian violet	(0.3 %0	Stains the nuclei of WBC
3	Distilled water	100 ml	Act as a solvent



WBC Counting Square

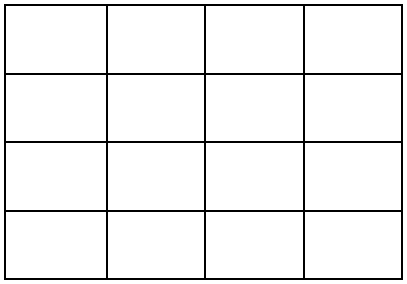


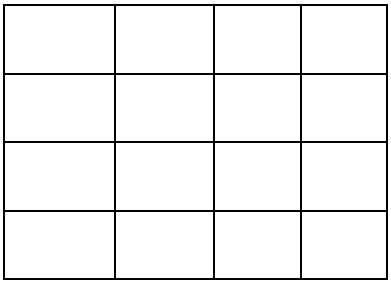
Counting Procedure

PROCEDURE:

- Clean the pipette and ensure that is dry and collect the diluting fluid in a watch glass from a stock bottle
- Prick the fingertip with all aseptic precaution to obtained free flow of blood
- Let a large drop of blood accumulate on the finger tip
- Hold the pipette horizontally and dip its end into the drop of blood. Suck gently on the mouthpiece to draw blood up to the required mark (0.5) on the stem of the pipette
- If the more than the required amount of blood is drawn into the pipette, tap gently onto the finger nail or palm. Wipe off the tip of the pipette to remove the extra blood sticking to it.
- Maintain the blood level at the 0.5 mark and place the tip of the pipette in the diluting fluid well below the surface of the liquid
- Do not directly draw the fluids from the stock bottle as it may contaminate the solution with the cells
- Draw the mixture exactly to the top mark (11) above the blub. While the bulb is being filled, you may tap the pipette with the finger to knock the bead down below the surface of the solution in the bulb to prevent the formation of bubbles.
- Maintain the level of the mixture exactly to the mark by closing the pipette tip with the index. Hold the pipette horizontal position is also important.
- Mix the content of the bulb thoroughly for 2-3 minutes by rotating the pipette with it tip pressing against the palm of the left hand. Place the pipette on a horizontal surface. The pipette is now ready for charging.
- Place the charged chamber on the stage of the microscope and adjust the microscope for observation under low power (10^x).
- Focus the Neubauer's chamber under the low power objective, and check uniform distribution of cell in the WBC squares. If the cells are not uniformly distributed, clean the Neubauer's chamber and charge it. Count the total number of WBCs in the four corners square under the low power objective (10x)

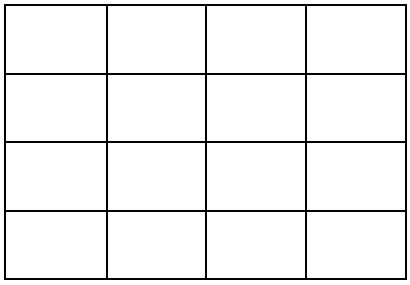
Observation

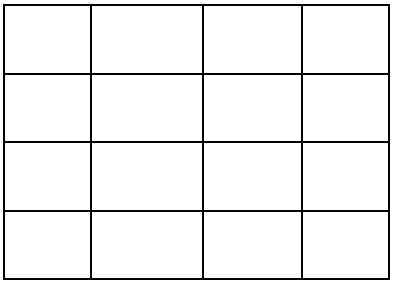




W1 =

W2 =





W4 =

W3 =

N = W1 + W2 + W3 + W4 =

CALCULATION:

Area of 4 WBC squares = 4x 1 =4 mm^2

Volume of the 4 WBC square = 4 x 1/10 = 4/10 mm3

Dilution factor = 1:20

Cells in 4/10 mm3 volume of diluted blood = n

Therefore the cells in 1 mm3 volume of diluted blood N x 10/4

Cell in 1mm3 volume of the undiluted bold = n x 10/4 x20 = n x50

- To avoid this count the same again, count the white cells present in the squareand those present on its left and lower lines. Ignore those on its right and upper lines
- WBC appears similar to clumped red cell debris or stained particles. They areidentified as clear, nucleated and retractile bodies.

Report:

EX. NO: **DATE:**

DETERMINATION OF BLEEDING TIME

Aim: To determine bleeding time of your own blood by Duke Method

Requirements:

Blotting or Filter paper

Stopwatch

Sterile LancetCotton

Surgical spirit

Principle:

- A deep skin puncture is made and the length of time required for bleeding to stop is recorded.
- It determines the functions of platelets and integrity of capillaries.
- The duke method is the most frequently used method to determine bleeding time in clinical laboratories at it is easy to perform and requires minimal equipment and laboratory skill.

Normal value:

1-5 minutes (Duke Method)

Procedure:

- Clean your ring finger tip with alcohol (Surgical spirit), and allow the skin to dry completely.
- Make a deep puncture about 3- 4 mm (blood should flow freely without squeezing)with the help of sterile lancet.
- Immediately start the stop watch.
- Blot the drop of the blood coming out of the incision every 15 seconds by using the blotting paper (filter paper).
- Place each subsequent drop a little further along the side of the filter paper.
- Do not allow the filter paper to press on the bleeding spot. Note that the drops become progressively smaller.

Stop the stop watch as soon as bleeding ceases.

- Count the number of drops on the filter paper and multiple it by 15 seconds
- If the bleeding does not stop in 10 minutes, discontinue the test and apply the pressure to the spot to stop bleeding.

REPORT:

The bleeding time of my own blood was found to be ____

EX. NO: **DATE:**

DETERMINATION OF CLOTTING TIME

Aim:

To determine the clotting time of your own blood by Wright's method (Capillary tube method)

Requirements:

- Stop watch
- Sterile lancet
- Cotton
- Surgical spirit
- Capillary tube (10-15 cm length and 1.5 mm in diameter)

Principle:

A standard incision is made in the skin and blood is taken into a capillary tube

- The length of time that it takes for blood to clot (as detected by the appearance of fibrin string) is reported as Clotting time
- The clotting time is usually determined by two methods:

Capillary tube method

Lee White (vein puncture) method

- The capillary tube method is routinely used in clinical laboratories to determine clotting time.

Normal value:

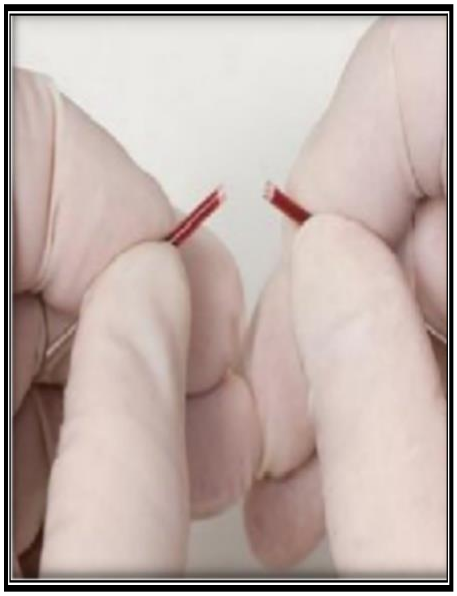
Wright's Capillary tube method: 2- 8 minutes

Lee White Method: 5-12 minutes

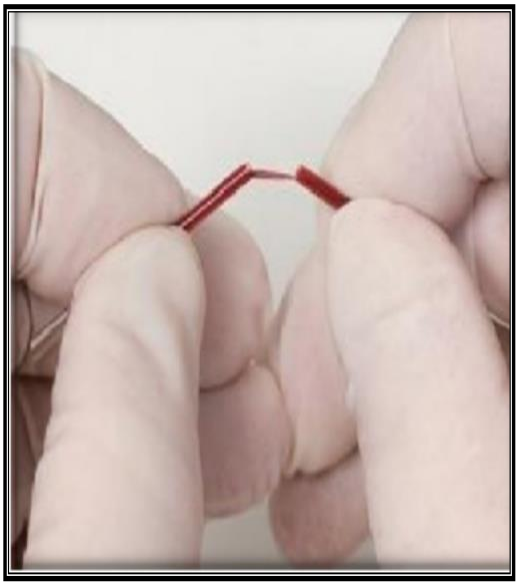
Drop Method: 2- 4 minutes

Procedure:

- Make the sterile finger puncture by using the lancet to depth of 3 mm
- As soon as blood is visible, start the stopwatch.



No Fibrin formation



Fibrin Formation- End point of Clotting

Calculation:

Clotting time = Number of broken pieces x 30 sec
=

- A standard incision is made in the skin and blood is taken into a capillary tube
- The length of time that it takes for blood to clot (as detected by the appearance of fibrin string) is reported as Clotting time
- The clotting time is usually determined by two methods:

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Normal value:

Wright's Capillary tube method: 2- 8 minutes

Lee White Method: 5-12 minutes

Drop Method: 2- 4 minutes

Procedure:

- Make the sterile finger puncture by using the lancet to depth of 3 mm
- As soon as blood is visible, start the stopwatch.
- Wipe off the first drop of the blood and allow the next drop of blood to flow into the drop and holding the other end at lower end at lower level
- Hold the capillary tube filled with blood between the palms so as to maintain body temperature
- After 2 minutes, break off the capillary tubing 1-2 cm from the one end every 30 seconds and look for appearance of a thread of fibrin
- When a string of fibrin is seen between the broken ends, stop the watch and note the time in the broken

REPORT:

The Clotting time of my own blood was found to be _____

EX. NO: **DATE:**

ESTIMATION OF HEMOGLOBIN CONTENT

Aim: To estimate the hemoglobin content on your own blood

Requirements:

Sahli's hemoglobinometer (Hemometer) which consists of

- Comparator – a rectangular plastic box with a slot which accommodates the Hb tube and

  non-fading standard fixed on either side in front of a opaque white glass
- Hemoglobin tube – graduated in g % (2-24 g %) on one side and percentage (20 – 40 %) on the other
- Hemoglobin pipette with a 20 mm mark and rubber tubing with a mouth piece
- A glass rod to stirrer- It is a thin glass rod used for stirring the solution
- N/10 hydrochloric acid
- Distilled water and dropper
- Lancet, Cotton and Surgical spirit

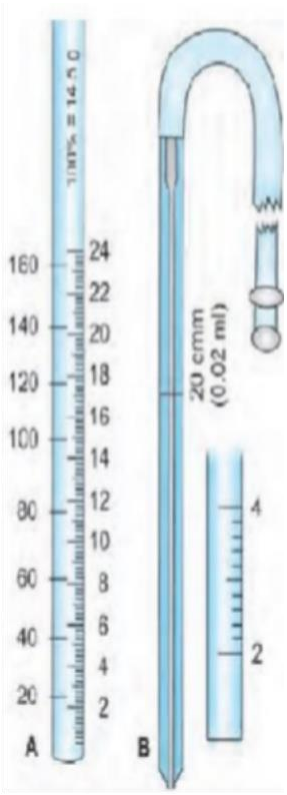
Principle:

- Hemoglobin is a conjugated protein present present in the red blood cells.
- It carries the oxygen from the lungs to the tissues and carbondioxide from the tissue to the lungs.
- Hemoglobin is converted in to acid hematin by the actions of Hydrochloric acid.
- The acid hematin solution further diluted until the color match exactly with that of the permanent standard of the comparator block.
- The hemoglobin concentration is read directly from the calibration tube.

Normal value:

- **Adult males : 14 - 18 (16 ± 2) g / dl**
- **Adult female : 12-16 (14 ± 2) g / dl**
- **Newborns : 16 – 22 g /dl.**

- It is decreases to 9-14 g / dl by about two months of age.



Hemoglobin Comparator **Hemoglobin pipette**

Procedure:

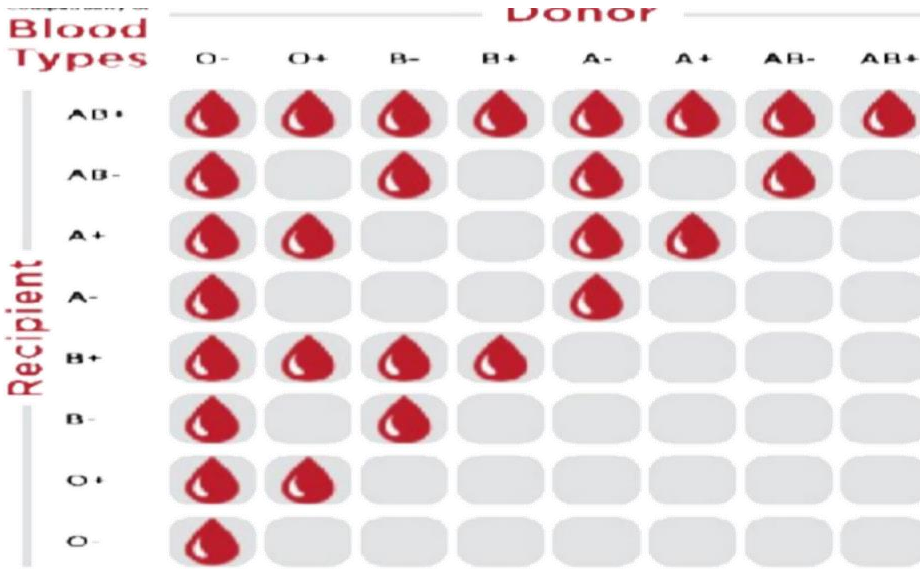
- Clean the hemoglobininometer tube and pipette and ensure that they are dry
- Fill the hemoglobinometer tube with N/10 Hydrochloric acid up to its lowest mark (10 % or 2gm) with the help of dropper.
- Prick the finger, observing all aseptic precautions, and discard the first drop of the blood
- The prick should be deep enough to enable spontaneous flow of blood.
- Do not squeeze the finger to bring out of the drop of blood.
- Allow a large drop of blood to form on the finger tip, then the tip of the hemoglobinometer pipette into the drop and suck blood up to the 20 cu mm mark of the pipette.
- While sucking blood into the pipette care should be taken to prevent entry of air bubbles.
- This is done by not lifting the pipette out of the blood drop during pipetting.
- If any air bubble enters, remove and discard the blood and obtain another drop of blood to re- pipette.
- If blood is sucked above the 20 mm^3 markof the pipette, bring the blood column down to the mark by tapping the pipette against the finger, but not by using any absorbent material like cotton
- Wipe the tip of the pipette. Immediately transfer the 0.02 ml of the pipette into the hemoglobinometer tube containing N/10 Hydrochloric acid by the immersing the tip of pipette in the acid solution and blowing out blood from the pipette.
- Rinse the pipette two to three times by drawing up and blowing out of the acid solution. With draw the pipette from the tube. Make the sure that no solution remains in the pipette.
- Leave the solution the tube in the hemoglobinometer, for about 10 minutes
- After the ten minutes, dilute the acid hematin by adding distilled water drop by drop
- Mix it with the stirrer. Match the color of the solution in the tube with the standard of the comparator.

- After the addition of every drop of the distilled water, the solution should be mixed and color of the solution be compared with standard. While matching, take care to hold the stirrer above the level of the solution.
- If the color of the test solution is darker, continue dilution till it matches with that of the standard.
- Note reading when the color of the solution exactly matches with the standard and express the hemoglobin content as g %.
- The reading of the lower meniscus of the solution should be added and the color should be noted as the result.

Report:

	GROUP A	GROUP B	GROUP AB	GROUP 0
Erythrocytes	A	B	AB	0
Antibodies	Anti-B	Anti-A	none	Anti-A Anti-B
Antigenes	A antigen	B antigen	A and B antigen	none

ABO BLOOD GROUP SYSTEM



EX. NO: **DATE:**

DETERMINATION OF BLOOD GROUP

Aim: To determination of blood group of your own blood

Requirements:

Sterile lancet, Cotton swab
Surgical sprit, Toothpicks
Porcelain tile, Sodium citrate in normal
salineAnti-A serum (Blue)
Anti-B (Yellow)
Anti-D serum

Principle:

- The RBCs contain a series of antigens known as agglutinogens on their cell membrane while the plasma contains antibodies known as agglutinins.
- The red cells of the subject are allowed to react with commercially made agglutinins.
- The presence or absence of the clumping of the red cells in different agglutinins and hemolysis of RBCs determines the blood groups.
- Clinically, the important blood groups are 1. The ABO system and (2) the Rh system
- The MN system is important from the medicolegal point of view.
- The system was first discovered in Rhesus monkeys; hence it is called the Rh system.

The ABO system

- The current system of Blood grouping was discovered by Landsteiner in 1900 and is known as the Landsteiner's ABO system

  Based on the presence or absence of these antigens, blood groups are classified as:

  Group A : Antigen A is present & Antibody B is present

  Group B : Antigen B is present & Antibody A is present

  Group AB : Both A and B Antigen are present & Antibody A and B absent

  Group O : Both A and B Antibody present & both A and B Antigen absent

Observation:

If any agglutination occurs it is usually visible to the naked eye as dark red clumps of different sizes.

The presence or absence of agglutination indicates the blood group of the subject as shown in the following table:

Anti A serum	Anti B serum	Anti D serum	Agglutinogen (in RBC)	Blood group
-	-	+	D	O +Ve
-	-	-	NIL	O -Ve
+	-	+	A	A+ Ve
+	-	-	A	A -Ve
-	+	+	B	B +Ve
-	+	-	B	B -Ve
+	+	+	ABD	AB +Ve
+ : Agglutination - : No agglutination				

- The A antigen is of two types, A and A1. Therefore, group A is further divided into two subgroups:
- **Group A1 : Containing A and A1 Antigens**
- **Group A2 : Containing A antigen only**
- Similarly, the AB blood group is subdivided into the A1B and A2B blood groups.

The Rh system.

- In this system, there are six antigens, but there are no naturally occurring antibodies
- The antigens areC,D,E,c,d and e
- Of these six antigen, immunologically D is the most significant
- Therefore, the Rh system has two blood groups:

Rh positive : D antigen present

Rh negative : D antigen absent

- The antibody in this system is called anti –D antibody and is produced only when Rh-negative individual receives the Rh-positive blood
- In Indian population 95-98 % is Rh -positive and 2-5 % is Rh- negative

Procedure:

- With a glass marking pencil the porcelain tile is divided into 3 portions.
- Under aseptic conditions the subject whose blood group is to be determined is asked tostick out his finger which is pricked and 3-4 drops of blood is then obtained.
- Following this the blood is diluted with 1 ml of sodium citrate.
- A drop of anti-A, anti-B and anti-D sera is placed on each of the 3 portions containingthe sera.
- This is mixed with 3separate toothpicks.
- After 5-10 minutes each portion is examined and observes the serum-cell mixture for agglutination (clumping).

REPORT:

My blood group was found to be ----------

EX. NO: **DATE:**

DETERMINATION OF ERYTHROCYTE SEDIMENTATION RATE

Aim:

To find out the erythrocyte sedimentation rate (ESR) of the given sample of blood

Requirements:

Westergren's tube &stand,

3.8 %sodium citrate solution

3ml Disposable syringe with needle

Surgical Spirit and cotton

Principle:

- If blood containing an anticoagulant is allowed to stand in a tube placed vertically, the redcellssettledowngraduallytothebottom,sincetheirspecificgravityisgreaterthanthat of plasma.
- The level of the column of red cells is noted in the beginning (0 h) and after one and two hours
- The distance (mm) the column moves noted as the ESR (mm/h).

Normal values:

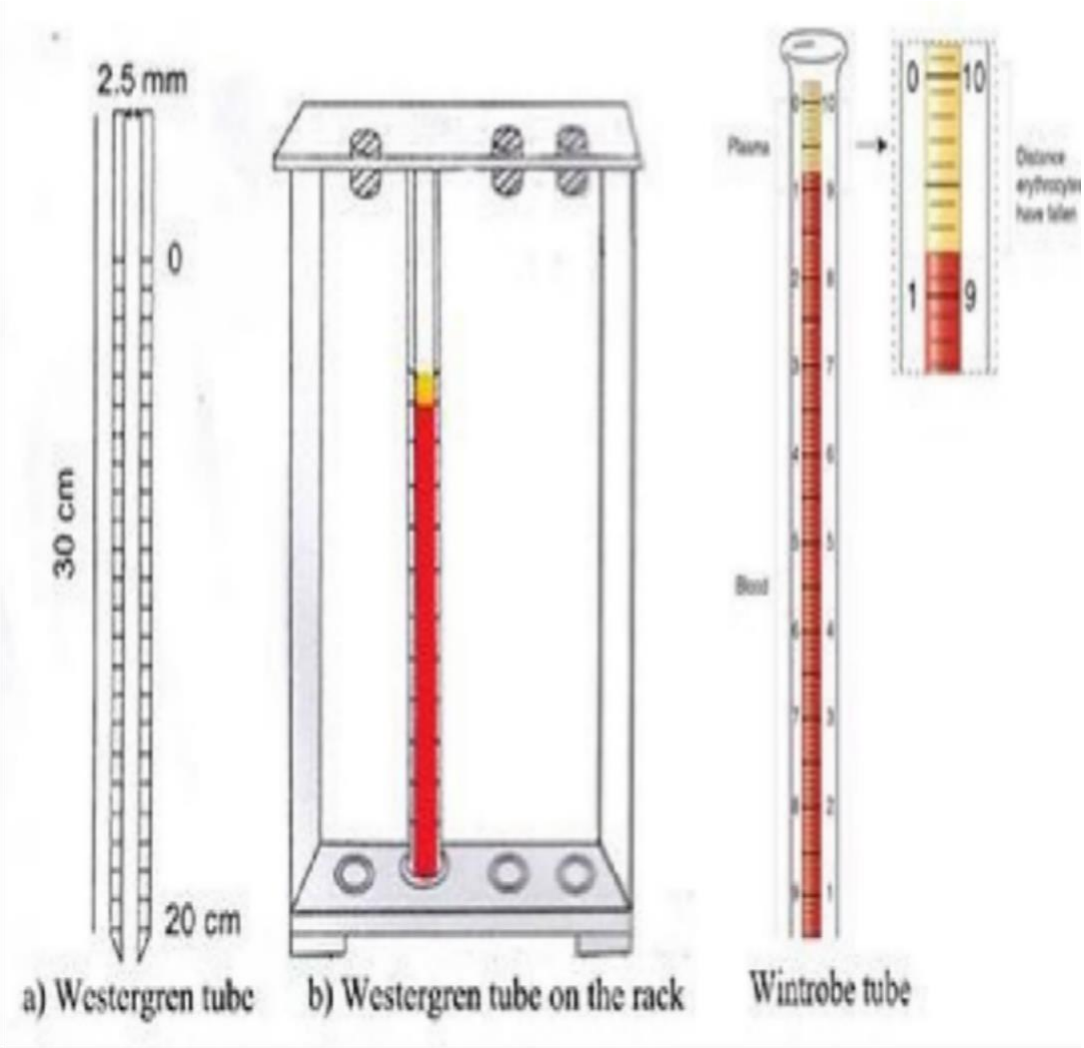
- **Male =3-5 mm / hour**
- **Female and children=5-12 mm/ hour**

Methods:

Two methods are used to determine ESR

1. Westergren's method
2. Wintrobe's method

Westergren'smethod



Erythrocyte Sedimentation Rate (ESR) apparatus

Westergren'smethod

Procedure:

1.6 ml of blood from the antecubital vein is diluted with 0.4 ml of 3.8 % sodiumcitrate solution.It is drawn into the Westergren'stube upto zero mark and fixed vertically in the Westergren's stand. The cells can settle down and upper level of the cells is noted at the end of1/2 hour and 1 hour.

Discussion:

This test measures the rate at which red cells settle spontaneously in a standing column of blood. This sedimentation process is divided into 3 stages for convenience

Report:

The Erythrocyte sedimentation rate was found to be----------

EX. NO: **DATE:**

DETERMINATION OF HEART RATE / PULSE RATE

Aim: To determine the heart rate and pulse rate of given subject

Principle:

Pulse/heart rate is the wave of blood in the artery created by contraction of the left ventricle during a cardiac cycle. The strength or amplitude of the pulse reflects the amount of blood ejected with myocardial contraction (stroke volume).

Types of Pulse Rate

- **Peripheral pulses:** that can be felt at the periphery of the body by palpating an artery over a bony prominence. Examples are carotid, radial and popliteal pulses
- **Apical pulses:** which is a central pulse located on the apex of the heart that is monitored using a stethoscope.

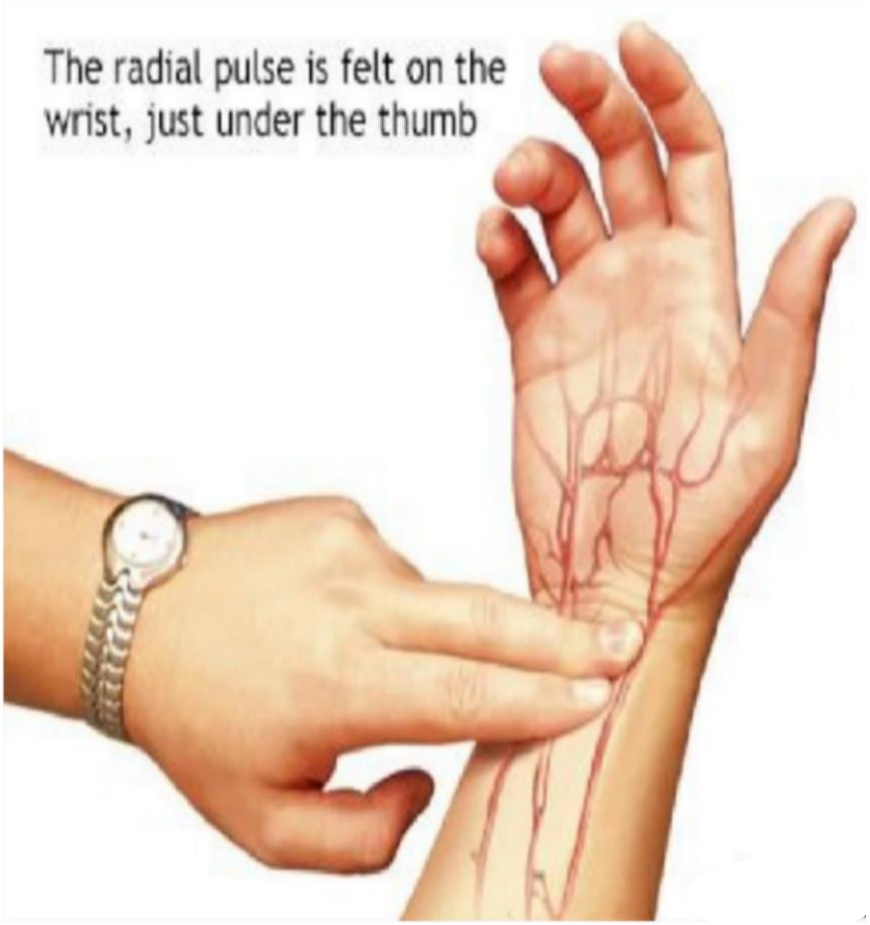
Normal Value: American Heart Association (AHA)

Children (ages 6 - 15) : 70 – 100 beats per minute

Adults (age 18 and over) : 60 – 100 beats per minute

Procedure:

The subject is seated and at resting for a while Using the first and second fingertips, press firmly but gently on the arteries until you feel a pulse. Begin counting the pulse when the clock's second hand is on the 12.Count your pulse for 60 seconds. When counting, do not watch the clock continuously, but concentrate on the beats of the pulse. If unsure about yourresults, ask another person to count for you. Stethoscope can also be used to check heart beat and pulse. Record the pulsations felt about 10 seconds and multiply by 6 or the pulsation can be recorded for 1 whole minute to calculate the heart rate of the subject.



Determination of Heart Rate / Pulse Rate

Conclusion:

The measurement of the heart provide a correct diagnosis of the heart muscle function and helps in diagnosis of cardio vascular disease

Report:

The heart rate and pulse rate was found to be ----------

CARDIOLOGY

EX NO: **DATE**

RECORDING BLOOD PRESSURE

Aim: To determine the blood pressure of given subject

Requirements:

Sphygmomanometer and Stethoscope

Principle:

The blood pressure (BP) is the lateral pressure excreted by the column of blood on the walls of the artery. The pressure of blood in the artery (brachial artery) is balanced against the pressure of air in a rubber cuff surrounding the artery. The pressure of air in the cuff is then measured by means of a mercury manometer.

Methods:

1. Palpatory method
2. Auscultatory method
3. Oscillatory method

Normal value:

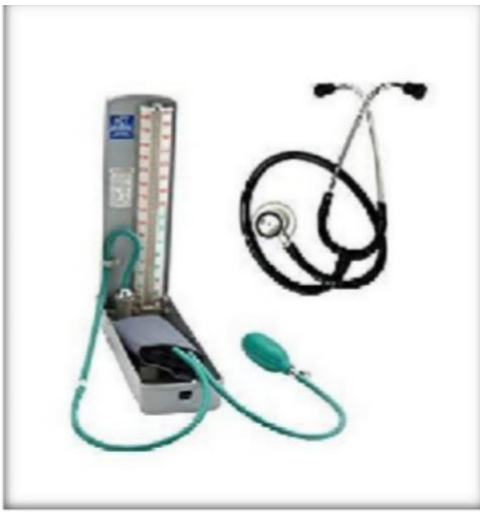
Procedure

Palpatory method: The subject is asked to sit on the stool. The cuff is tied around the upper arm with the lower border of the cuff not less than 2.5 cm above the cubital fossa.

The outlet valve of the bulb is closed. The radial pulse is palpated while the cuff is being inflated to a pressure slightly above the level at which the radial pulsation is no longer felt.

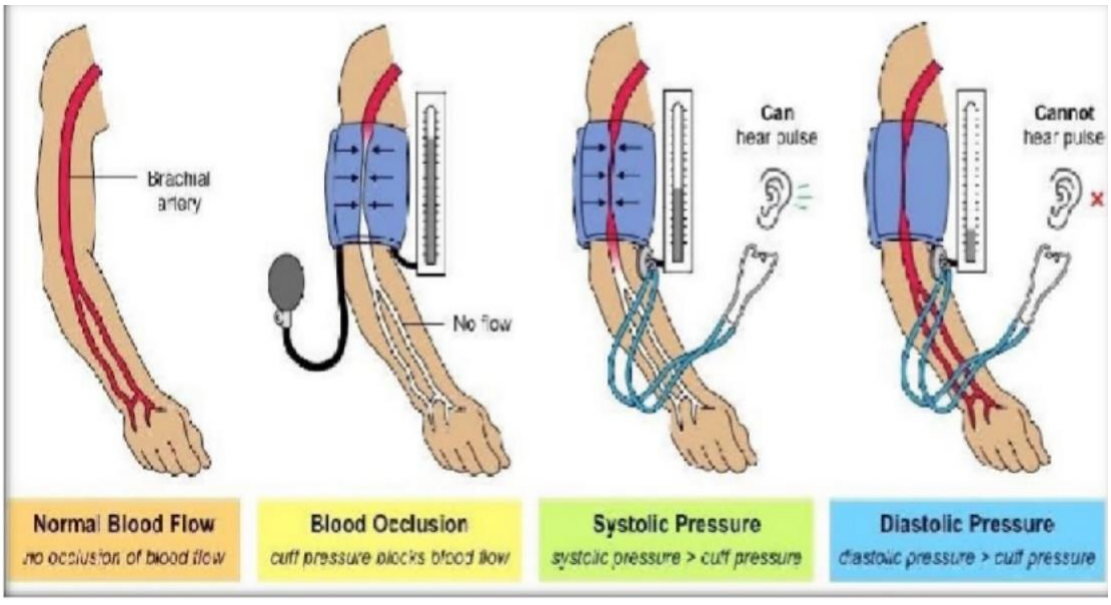
The pressure at which the pulsation was obliterated is read in the mercury manometer. The outlet valve is opened. The manometric reading is noted at the point where the pulsation reappeared.

The average of the two readings gives the systolic pressure. The diastolic pressure cannot be determined by this method.



Manual **Digital**

Blood Pressure Apparatus



Auscultatory method: By palpatory method, only systolic blood pressure could be measured. By auscultatory method, both the systolic and diastolic blood pressure can be measured.

The chest piece of the stethoscope of the stethoscope is placed over the brachial artery. The pressure in the cuff is raised above the systolic pressure (by about 30 mm Hg) previously determined by the palpatory method.

The pressure is then lowered gradually (2-3 mm per second). The sounds that are heard are the Korotkoff's sounds.

The first sound (phase 1) that occurs is a sharp tapping sound, indicates the peak systolic pressure, the second and third phases, initially murmurish in quality and then louder and more banging, are due to turbulent flow of blood through a partially occluded vessel.

In the fourth phase, the sound becomes muffled and dull and the fifth phase accurately gives truly diastolic pressure, which is disappearance of the sound.

Oscillatory method: This is another method of determining blood pressure. By this method, both the systolic and diastolic blood pressure is determined.

The pressure which oscillations appear in the mercury manometer gives the systolic pressure and the pressure at which it disappears gives the diastolic pressure.

However, this method is not accurate. Important precautions in the use of sphygmomanometer:

1. The manometer should be placed at the level of the heart
2. The lower border of the cuff should be 2.5 cm above the cubital fossa. For children, a narrow cuff should be used
3. Blood pressure should be preferably taken in the left arm

REPORT: The blood pressure of give subject was found to be----------

PHARMACEUTICAL ANALYSIS

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16		Assay of sodium chloride by precipitation titration			
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COMMON ANALYTICAL APPARATUS

To avoid impurities during analysis, apparatus made from resistance glass are used. A borosilicate glass is preferred for most purposes.

Some of the glass wares that can be used are:

1. **Beaker:** The most satisfactory beakers for general use are beakers with a spout, which have the advantage of pouring, making an outlet for steam or gases, etc. The most useful sizes are 250 and 500 mL.

2. **Conical flask:** Conical flasks of 150, 250 and 500 mL capacity are used frequently.

3. **Pipettes:** There are three kinds of pipettes:

a. *Transfer pipettes:* These have one mark and deliver a constant volume of liquid. It is made up of long glass tube with large central cylindrical bulb. A calibration mark is present around the upper tube while the lower delivery tube is drawn out to a fine tip. Transfer pipettes are constructed with capacities of 1, 2, 5, 10, 20, 25, 50 and 100 mL.

b. *Graduated pipettes or measuring pipettes:* These are graduated or marked and used to deliver small volume as required. They are made up of straight, fairly narrow tubes with no central bulb. They deliver a measured volume from a top zero to a selected graduation mark or to the jet, i.e. the zero is at the set. Three different types are available:

- ✓ **Type 1** delivers a measured volume from a top zero to a selected graduation mark.
- ✓ **Type 2** delivers a measured volume from a selected graduation mark to the jet, i.e. the zero is at the jet.
- ✓ **Type 3** is calibrated to contain a given capacity from the jet to a selected graduation mark and thus to remove a selected volume of solution.

c. *Syringe pipettes:* These have a fixed or variable volume and are usually employed for dispensing large numbers of identical volumes very quickly. They have a push button design in which the syringe is operated by pressing a button on the top of the pipette; the plunger travels between two fixed stops and a reliable constant volume of liquid is delivered.

4. **Burettes:** Burettes are long cylindrical tubes with uniform bore throughout the graduated length, a narrow lower end with a glass stopcock and a jet. Before using, it is thoroughly cleaned with a cleansing agent, rinsed well with distilled water and the solution is filled with the help of a funnel up to zero mark. Burettes are constructed with capacities of 25 and 50 mL.

5. **Graduated cylinder:** A **graduated cylinder**, **measuring cylinder** or **mixing cylinder** is a pieceof laboratory equipment used to measure the volume of a liquid. Graduated cylinders are generally more accurate and precise than laboratory flasks and beakers. However, they are less accurate and precise thanvolumetric glassware, such as a volumetric flask or volumetric pipette. For these reasons, graduated cylinders should not be used to perform volumetric. A traditional graduated cylinder (A in the image) is usually narrow and high (so as to increase the accuracy and precision of volume measurement) and has aplastic or glass bottom and a "spout" for easy pouring from the measured liquid. A graduated cylinder is meant to be read with the surface of the liquid at eye level, where the center of the meniscus shows the measurement line. Typical capacities of graduated cylinders are between 5 ml and 2000 ml.

6. **Nessler cylinders: Nessler cylinders** (also named **Colour comparison cylinders**, **Colour comparing cylinders**) are laboratory tubes with a fixed volume, made of glass with optically plane bottom. On the walls there are marks of the nominal stroke volume (usually 100 ml) and possibly one half-way mark (then usually 50 ml) To minimize differences in the subjective impression of the colour of the solution of the substance to be analysed, cylinders of a series should have the same characteristics - height, diameter and thickness of glass. The colour of the substance contained in a Nessler cylinder visually compared with the model.

Weighing bottle: Weighing bottles are glass laboratory equipment used for precise weighing of solids. Most of the glass used in the bottles is thin and fragile glass, but sometimes they are also madeof ceramics or plastics. The most chemicals are weighed by difference through placing the material insidethe stoppered weighing bottle, which is then weighed. The substance is transferred to a suitable vessel andthe weight of the substance taken is determined by weighing the empty weighing bottle

ANALYTICAL BALANCE

To measure the mass of a substance a balance or laboratory balance is used. It consists of a pivoted horizontal lever of equal length arms, called the beam, with a weighing pan, also called **scale** or **scale pan** or **bason** suspended from each arm. The unknown mass is placed in one pan, and standard masses are added to the other pan until the beam is as close to equilibrium as possible.

An analytical balance is an instrument used to determine mass to a very high degree of precision. It consists of a transparent enclosure with doors so dust does not collect and also any air currents in the room do not affect the delicate balance.

The main components are the beam supported on a pillar of metal and the pans. The beam is a rigid piece of metal which rests through a knife edge of agate on a plate of agate attached to the pillar top. On each end of the beam, at equal distance from the central knife edge, are two terminal knife edges of agate facing upwards. Each terminal agate supports a suspension from which a pan is hung. To the centre of the beam a long pointer is attached which moves over a scale at the foot of the pillar. The top of the beam is divided accurately for the use of a rider. The two adjusting screws at each end of the beam are used for adjusting the equilibrium position, a state when the pointer rests at the centre of the scale and the beam is horizontal when unloaded. The pillar is fixed on a rectangular stable base. The balance is fixed with levelling screws at the bottom and the plumb line suspension. On both sides of the top of the pillar a horizontal metallic piece extends, which is monitored by a key placed centrally in front of the base. This is called as **beam arrest**. When the key is moved to the left the beam oscillates. It has a maximum load capacity of 100- 200 g and a sensitivity of 0.1 mg.

Weight Box

The weights are used to determine the mass of an object. The unit of mass that is employed in the laboratory work is gram. The weights are made of heavy metal alloy and are cylindrical in shape, each with a knob at the top. An ordinary set has 100, 50, 20, 10, 5, 2 and 1 g weights. The set contains a pair each of 20 and 2 g weights. Weights less than 1 g are called as milligram (mg) weights or fractional weights. They are leaf shaped foils of Aluminium or other suitable metal with one of the sides turned up for picking up with forceps. The fractional weight set has 500, 200, 100, 50, 20, 10, 5, 2 and 1 mg weights. In a weight box, 200, 20 and 2 mg weights are present in a pair. Weights smaller than 10 mg are generally not used, instead the use of rider is recommended for this purpose. All the weights are kept in a wooden box lined with velvet. The weights should be calibrated.

Rider

A rider is a piece of suitable wire appropriately shaped to ride on the beam of the balance. The commonly used rider weighs 10 mg, when the beam is graduated. The beam scale is divided into 10 equal parts on each side from the centre, each part being subdivided into five parts. The right hand section of the beam is normally used and the left hand section is seldom used. The rider is placed at division 0 before adjusting the balance.

If the rider is placed at marking 10 on the right hand section of the scale the effect is equal to that of 0.01 g weight placed on the right hand pan of the balance; if it is hung at marking 4 the effective weight is equal to

0.004 g. The distance between two small divisions represents a weight of 0.002 g. Thus, if the rider is placed on the second small division between the markings 6 and 7, the weight is 0.0064 g. The placing of the rider on the beam scale is manipulated by means of a sliding hook.

Care and Use of the Balance

While using a balance care should be taken about the following:

1. The balance should be placed on a firm platform. The balance and pan should be clean.
2. The balance should be tested and adjusted so that the pointer swings freely and to equal distance on either side of the zero point of the pointer scale.
3. The object to be weighed is placed on the left hand pan and the weights on the right hand pan.
4. The chemicals to be weighed must not be placed directly on the balance pan. This should be placed and weighed in a weighing bottle or over a watch glass.
5. The object should be cooled to room temperature, if it is hot before weighing.
6. When the final weighing is made the doors of the balance must be closed.
7. During weighing when the objects are being placed or removed from the pans, the beam and pan must be arrested.
8. All the weights should be handled with forceps and not with fingers.
9. The balance must not be overloaded.
10. When the weighing has been completed, the object and weights should be removed from the pans. Any spilled material should be immediately removed with the help of a camel hair brush.
11. If the balance is in disorder, do not try to rectify the defect yourself. Bring it to the notice of an instructor.

1. **Weighing by difference**: This is the standard procedure of weighing in analytical work.

a. Take a thoroughly clean and dry stoppered weighing bottle.

b. Place the sample to be weighed in it, and weigh accurately (x g).

c. Take out the bottle from the balance.

d. Pour out the required amount of the sample into the flask or other vessel by rotating and gently tapping the weighing bottle.

e. Weigh the bottle and its contents again (y g).

f. The difference between the weights represents the weight of the sample transferred.

Record the observation in the following manner:

Weight of the weighing bottle and the substance = x g

Weight of the weighing bottle and the substance after transfer = y g

Weight of the substance taken out = (x - y) g

Weighing by addition: This method is generally adopted for preparing standard solutions. The weighing container can be a clock glass, watch glass, weighing bottle or a small beaker.

a. The clean and dry container is weighed.

b. Introduce the sample into the container in small portions until the correct weight has been added.

c. Weigh the container and the contents.

d. Take out the container and hold it over the receiving vessel (beaker or funnel on the mouth of a flask) and pour the contents carefully into the receiving vessel.

e. Wash the container thoroughly with a jet of water, washings being collected into the receiving vessel.

LIMIT TEST

EX NO **DATE**

LIMIT TEST FOR CHLORIDE

Aim

To perform the limit test for chloride in the given sample.

Apparatus and Reagents

Nesslers cylinders, glass rod, dilute nitric acid, silver nitrate etc.

Principle

The limit test for chloride is based on the reaction between silver nitrate and soluble chloride forming the precipitate of silver chloride which is insoluble in dilute nitric acid. The opalescence produced depends upon the amount of chloride present in the sample.It is compared with the opalescence produced in a standard solution containing the prescribed quantity of chloride similarly treated. If the opalescence in the test sample is more than that of the standard, the test fails.If the opalescence of test sample is less than that of the standard, the test passes. The test is done in Nessler cylinders.

$$Cl^- + AgNO_3 \; AgCl \rightarrow NO_3^{2-}$$

Procedure

Take 50ml Nessler cylinders. Label one as “Test” and the other as “Standard”

TEST	STANDARD
1. Pipette out 10 ml of test solution into a Nessler cylinder	1. Pipette out 10ml of chloride standard solution into a Nessler cylinder
2. Add 10ml of dilute nitric acid	2. Add 10ml of dilute nitric acid
3. Dilute to 50ml with water	3. Dilute to 50ml with water
4. Add 1ml of 0.1M silver nitrate solution	4. Add 1ml of 0.1M silver nitrate solution

5. Stir immediately with a glass	5. Stir immediately with a glass
6. rod for five minutes protected from light	6. rod for five minutes protected from light

Compare the opalescence produced in the test with that of standard.

Observation

Report

Reference

1. Practical Pharmaceutical Chemistry by Harkishan Singh and V. K. Kapoor: page no.56.
2. Textbook of Pharmaceutical Inorganic Chemistry by V N Rajasekaran Page no: 399-400

EX NO DATE

LIMIT TEST FOR SULPHATE

Aim

To perform the limit test for sulphate in the given sample.

Apparatus and reagents

Nessler cylinders, glass rod, ethanolic sulphate solution, Barium chloride, Dilute acetic acid

Principle

The test is based on precipitation of sulphate as barium sulphate by barium sulphate reagent in presence of dilute acetic acid

$$SO^{2-}_4 + BaCl_2 \rightarrow BaSo_4 + 2Cl^-_4$$

Barium Sulphate reagent which contains barium chloride, sulphate free alcohol and a small quantity of potassium sulphate is used as the reagent. The inclusion of small quantity of potassium sulphate in the reagent increases the sensitivity of the test by giving the ionic concentration in the reagent which just exceeds the solubility product of barium sulphate. Alcohol prevents supersaturation. The turbidity produced depends upon the amount of sulphate present in the sample.It is compared with the turbidity produced in a standard solution containing the prescribed quantity of sulphate similarly treated. If the turbidity in the test sample is more than that of the standard, the test fails.If the turbidity of test sample is less than that of the standard, the test passes. The test is done in Nessler cylinders.

Procedure

Take 50ml Nessler cylinders. Label one as "Test" and the other as "Standard"

TEST	STANDARD
1. Take 1.5ml of ethanolic sulphate solution into a Nessler cylinder (10ppm SO4)	1. Take 1.5ml of ethanolic sulphate solution into a Nessler cylinder (10ppm SO4)
2. Add 1ml of 25% w/v of barium chloride. Mix well allow to stand for 1 min	2. Add 1ml of 25% w/v of barium chloride. Mix well allow to stand for 1 min
3. 15 ml of the solution of the substance prepared as directed in the monograph is pipette into the above solution	3. 15 ml of the sulphate standard solution is pipette into the above solution
4. Add sufficient distilled water to produce 50ml	4. Add sufficient distilled water to produce 50ml

Compare the opalescence produced in the test with that of standard.

Observation

Report

Reference

1. Practical Pharmaceutical Chemistry by Harikishan Singh and V. K. Kapoor: page no.57-58.
2. Textbook of Pharmaceutical Inorganic Chemistry by V N Rajasekaran Page no: 401-402

EX NO DATE

LIMIT TEST FOR IRON

Aim

To carry out the limit test for iron in the given sample.

Apparatus

Nessler cylinders, Glass rod, Beaker

Reagents

1. Standard Iron Solution:0.1726 gm of Ferric ammonium sulphate and dissolve in 10ml of 0.1 N H_2SO_4 and sufficient water to produce 1000 ml.
2. 0.1 N H_2SO_4:4.904 gm in 1000ml of water.
3. 20% Iron free citric acid.
4. Thioglycollic acid
5. Ammonia solution.

Principle

The test depends upon the reaction between ferrous ion and thioglycollic acid in the presence of ammonia when a pale pink to deep reddish purple colour is produced. Ferric ion is reduced to ferrous ion by the thioglycollic acid and the compound produced is ferrous thioglycollate. Citric acid forms a soluble complex with iron and prevents its precipitation by ammonia as ferrous hydroxide. Ferrous thioglycollate is colourless in neutral or acid solutions. The colour develops only in the presence of alkali. It is stable in the absence of air but fades when exposed to air due to oxidation to the ferric compound. Therefore the colour should be compared immediately after the time allowed for full development of colour is over.

Procedure

Take 50ml Nessler cylinders. Label one as "Test" and the other as "Standard"

TEST	STANDARD
1. Dissolve a specified quantity of the substance (2g of sodium chloride) in 20ml of water in a Nessler cylinder	1. Dilute 2ml of standard iron solution (20ppm Fe) with 20ml water in a Nessler cylinder.
2. Add 2ml of 20% w/v solution of iron free citric acid and 0.1ml of thioglycollic acid.	2. Add 2ml of 20% w/v solution of iron free citric acid and 0.1ml of thioglycollic acid.
3. Makealkaline by the addition of iron free ammonia solution	3. Makealkaline by the addition of iron free ammonia solution
4. Add sufficient distilled water to produce 50ml	4. Add sufficient distilled water to produce 50ml
5. Allow to stand for five minutes	5. Allow to stand for five minutes

Compare the colour produced in the test with that of standard

Observation

Report

Reference

1. Practical Pharmaceutical Chemistry by Harkishan Singh and V. K. Kapoor: page no.58-59.
2. Textbook of Pharmaceutical Inorganic Chemistry by V N Rajasekaran Page no: 404-405

EX NO DATE

LIMIT TEST FOR ARSENIC

Aim

To perform the limit test for arsenic in the given sample.

Apparatus

Gutzeit apparatus, glass rod, measuring cylinder etc.

Reagents

Stannated hydrochloric acid AsT, 1M potassium iodide AsT, Galvanated Zinc AsT, Stannated Arsenic solution (10ppm).

Principle

Arsenic is present in two forms. Pentavalent arsenic (As^{5+}) and trivalent arsenic (As^{3+}). As^{5+} will be converted to arsenic acid and As^{3+} is converted to arsenious acid by action of HCl.

$$As^{5+} + HCl \longrightarrow H3AsO4 \text{ (Arsenic acid)}$$

$$As^{3+} + HCl \longrightarrow H3AsO3 \text{ (Arsenious acid)}$$

Arsenic acid is reduced to arsenious acid by the action of strong reducing agents like stannous chloride and potassium iodide.

$$H3AsO4 + SnCl2\ /KI \longrightarrow H3AsO3$$

Arsenious acid produces arsine gas by the reaction of nascent hydrogen which is produced by action of granulated zinc on hydrochloric acid.

$$3Zn + 6HCl \longrightarrow 3ZnCl2 + 6H^{+}$$

$$H3AsO3 + 6[H] \longrightarrow AsH3 + 3H2O$$

(Arsine gas)

The arsine gas produced passes through the narrow tube and reacts with the mercuric chloride paper ($HgCl_2$) to form mercuric arsenide and gives yellow or brown stain.

$$2AsH_3 + HgCl_2 \longrightarrow Hg(AsH_2)+HCl$$

(Mercuric arsenide)

Hydrogen sulphide gas formed from sulphide impurities may interfere with the test. The interference of hydrogen sulphide is removed by inserting cotton moistened with lead acetate in the narrow glass tube.

$$Pb(CH_3COO)_2+H_2S \longrightarrow 2\ CH_3COOH+PbS$$

Apparatus

Limit test of arsenic is done in a special apparatus called Gutzeitapparatus. It is a wide mouth bottle with about 120 ml capacity. It is fitted with rubber cork through which passes a narrow glass tube of 20 cm length and 6.5 mm internal diameter; it is constricted towards the bottom. A side hole of 2mm diameter is blown near the constricted part. The upper part of the tube is cut and surface made smooth. The parts arefitted in a hole of two different rubber bungs and two parts are held tightly with the help of a spring clip.

Procedure

Simultaneously carry out test and standard operations in 2 separate cylinders.

Test	**Standard**
1. Weigh accurately 5g of $MgSO_4$ into the bottle and add 50 ml of distilled water to dissolve the sample and label it as test.	1. Transfer accurately 1ml of arsenic standard solution (10ppm) into the assembly and dilute it with 50 ml of distilled water and label it as standard.
2. Add 10ml of stannated hydrochloric acid with AsT.	2. Add 10ml of stannated hydrochloric acid with AsT.
3. Add 5ml of 1M potassium iodide solution	3. Add 5ml of 1M potassium iodide solution
4. Add 10g of granulated zinc AsT	4. Add 10g of granulated zinc AsT

5. Place the cork immediately over the bottle with the attachments and immerse the bottle in a water bath at a suitable temperature.	5. Place the cork immediately over the bottle with the attachments and immerse the bottle in a water bath at a suitable temperature.
6. Allow the reaction to proceed for 40 minutes and remove the mercury chloride paper.	6. Allow the reaction to proceed for 40 minutes and remove the mercury chloride paper.

Compare the yellow stain produced in the test with the standard in daylight without delay.

Observation

Report

Reference

Indian Pharmacopoeia 1996, Volume.I, Page no: 451- 452, Appendix- 3.9.

PREPARATION AND STANDARDIZATION OF TITRANTS

EX NO DATE

PREPARATION AND STANDARDIZATION OF 0.1N SODIUM HYDROXIDE

Aim

To carry out the preparation and standardization of 0.1N Sodium hydroxide solution by using 0.1N oxalic acid.

Apparatus Required

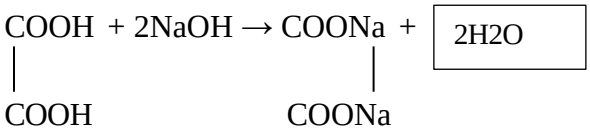
Conical Flask,Pipette, Burette, Volumetric flask, Weighing bottle.

Reagents Required

Oxalic acid, Sodium hydroxide, Phenolphthalein indicator.

Principle

It is an example of alkalimetry. When a weak acid is titrated with strong base, the salt produced in the reaction is completely hydrolyzed and the pH of the resultant solution at the endpoint is more than 7 (i.e., alkaline). Sodium hydroxide, a strong base, is standardized by titration with a weak acid, Oxalic acid (primary standard). The following reaction takes place when oxalic acid is titrated with sodium hydroxide.



(Oxalic acid) (Sodium oxalate)

In this titration, phenolphthalein is used as an indicator for detecting the end-point.

Procedure

a) Preparation of 0.1N Sodium Hydroxide:

Dissolve 4g of sodium hydroxide in sufficient carbon dioxide-free water to produce 1000 ml.

b) Standardization of 0.1NSodium Hydroxide:

Weigh accurately about 0.63g of Oxalic acid and transfer it into 100ml volumetric flask. Dissolve it with distilled water and make up the volume to 100ml. Pipette out 20ml of the solution into a conical flask and add 2 drops of phenolphthalein to it. Titrate it against sodium hydroxide taken in the burette until a permanent pale pink colour is obtained. Repeat the procedure till get a concordant value.

Report

The actual normality of the given solution of sodium hydroxide was found to be

Reference

Text book of Practical Pharmaceutical Analysis by Dr.G.Devala Rao; Page.No. 73.

EX NO DATE

PREPARATION AND STANDARDIZATION OF 0.5M SULPHURIC ACID

Aim

Tocarry out the preparation and standardization of 0.5MSulphuric acid

Apparatus Required

Burette stand, burette, standard flask, pipette, funnel, conical flask, weight box, weighing bottle.

Reagents Required

Sulphuric acid, Methyl red indicator, anhydrous sodium carbonate

Principle

This is an example of acidimetry. When a strong acid is titrated with a strong base, the salt produced in the reaction is not hydrolyzed and therefore, the pH of the resultant solution at the end point is exactly 7. Sulphuric acid, a strong acid, is standardized by titrating with a strong base, i.e. sodium carbonate (primary standard). In this reaction, methyl red is used as an indicator. At the end point a permanent pink colour will produce.

$$Na2CO3 + H2SO4 \rightarrow Na2SO4 + CO2 + H2O$$

Procedure

a) Preparation of 0.5 M Sulphuric Acid Solution

Take 27 ml sulphuric acid in a volumetric flask shake with approximately 50ml water and make volume up to 1000ml.

b) Standardization of 0.5M Sulphuric Acid Solution

Weigh accurately about 1.5g sodium carbonate into a clean conical flask, dissolve in 100ml of water add 2 -3 drops of methyl red indicator. Titrate the contents of the flask with sulphuric acid until pink colour is obtained. Heat the solution and titrate until pink colour is no longer affected by continuous boiling. Each ml of 0.5M Sulphuric acid is equivalent to 0.05299g of Na2CO3.

Report

The actual Molarity of given sample of sulphuric acid solution was found to be

Reference

Text book of Practical Pharmaceutical Analysis by Dr.G.Devala Rao; PageNo. 75

EX NO **DATE**

PREPARATION AND STANDARDIZATION OF 0.1 M SODIUM THIOSULPHATE

Aim

To carry out the preparation and standardization of 0.1M Sodium thiosulphate solution.

Apparatus

Burette, Pipette, Conical Flask, Volumetric flask, funnel etc.

Reagents

Potassium Bromate, Distilled Water, Potassium Iodide, 2M Hydrochloric Acid, Sodium Thiosulphate Solution and Starch Indicator.

Principle

The principle of standardization of sodium thiosulphate is based on redox iodometric titration with potassium bromate as primary standard compound. Potassium bromate, a strong oxidizing agent is treated with excess potassium iodide in acidic media which liberates iodine which is back titrated with sodium thiosulphate solution.

Chemical Reaction:

BrO - + 6H+ + 6e-à Br- + 3 H O 2 Br-à Br2 + 2e- ×3

BrO - + 5 Br- + 6H+à 3 Br + 3H O Br2 + 2KI à 2KBr +I2

I2 +2Na2S2O3à 2NaI+ Na2S4O6(sodium tetra thionate)

Procedure

a) Preparation of 0.1M Sodium thiosulphate solution:

Dissolve 25g of sodium thiosulphate and 0.2g of sodium carbonate in carbon dioxide-free water and dilute to 1000ml with the same solvent. Standardize the solution in the following manner.

b) Standardization of sodium thiosulphate solution:

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

1 ml of 0.1 M sodium thiosulphate is equivalent to 0.002784 g of $KBrO_3$.

Report

The actual molarity of the given solution of sodium thiosulphate was found to be

Reference

1. Indian Pharmacopoeia 2007, Volume 2, Page No: 316
2. Practicals in Pharmaceutical Analysis, Bhoomika R. Goyal, Hardik G. Bhatt and MayurM.Patel, Page No: 50-52

EX NO **DATE**

PREPARATION AND STANDARDIZATION OF 0.1N POTASSIUM PERMANGANATE

Aim

To carry out the preparation and standardization of the given solution of potassium permanganate

Apparatus

Burette, Pipette, Conical Flask, Volumetric flask, funnel etc.

Reagents

Potassium permanganate, Dilute Sulphuric acid, Oxalic acid.

Principle

In redox titrations, strength of an oxidizing agent is estimated by titrating with a reducing agent and vice - versa. Potassium permanganate acts as a strong oxidizing agent in acidic medium that oxidizes Oxalic acid into carbon dioxide. Known strength of oxalic acid is titrated directly with Potassium permanganate. End point can be detected with the appearance of permanent pink colour, Potassium permanganate acts as self- indicator. Sufficient acid must be present, otherwise formation of a brown colour during titration may be observed. Similar brown coloration can also be observed by using too high temperature or by using a dirty flask. To avoid such anomalies always rinse the flask with solution of hydrogen peroxide and dilute Sulphuric acid before performing titration.

$$2KMnO_4 + 3H_2SO_4 + 5H_2C_2O_4 \rightarrow K_2SO_4 + 2MnSO_4 + 8H_2O + 10CO_2$$

Procedure

a) Preparation of 0.1N potassium permanganate solution:

Weigh accurately about 3.16 g of Potassium permanganate and dissolve in 1000ml of distilled water. Cover the mouth of the flask with a watch glass and boil the solution for 15 -30 min. Allow the solution to cool and filter that through a funnel containing a plug of glass wool or asbestos. Collect the filtrate and store it in dark coloured bottle.

b) Standardization of 0.1N Potassium Permanganate Solution

Weigh accurately about 0.63g of oxalic acid into a standard flask. Dissolve in distilled water and make up to 100ml with distilled water. Pipette out 20ml and add 5ml of dil. Sulphuric acid. Warm the contents of the flask to 70^{o}C and titrate with $KMnO_4$ solution. Continue the titration until a faint pink colour is produced. Repeat the titration for concordant values.

Report

The actual normality of given solution of potassium permanganate was found to be

Reference

1. Indian Pharmacopoeia 2007, Volume 1, Page No: 315

EX NO DATE

PREPARATION AND STANDARDIZATION OF 0.1M CERIC AMMONIUM SULPHATE

Aim

To carry out the preparation and standardization of the given solution of 0.1M Ceric ammonium sulphate.

Apparatus required

Conical flask, Volumetric flask, Pipette, Burette, Weighing bottle.

Chemicals required

Ceric ammonium sulphate, Ferrous ammonium sulphate, Sulphuric acid, Ferroin indicator.

Principle

Ceric Ammonium Sulphate in sulphuric acid medium can function as strong oxidizing agent and considerably more stable than potassium permanganate solution and have a high oxidation potential. Sufficient sulphuric acid is to be used to prevent the hydrolysis and precipitation of basic salts. Instead of sulphuric acid, hydrochloric acid may also be used.

It has only one oxidation state as follows:

$$Ce^{4+} + e^{-} \rightarrow Ce^{3+}$$

In the presence of reducing agents, it undergoes reduction to the cerrous state.

$$2Ce(SO_4)_2 + 2FeSO_4 \rightarrow Ce_2(SO_4)_3 + Fe_2(SO_4)_3$$

Procedure

a) Preparation of 0.1MCeric ammonium sulphatesolution:

Weigh out 65g of Ceric Ammonium Sulphate with the aid of gentle heat in a mixture of 30ml of sulphuric acid and 500ml of water. Cool, filter the solution if turbid, and dilute to 1000ml with water.

b) Standardization of 0.1M Ceric ammonium sulphate solution:

Weigh accurately 3.921g of ferrous ammonium sulphate into a 100ml standard flask. Add dilute sulphuric acid, water mixture in the ratio 4:20and made up to 100ml with water. Pipette out 10ml and add 2 drops of ferroinindicator. Titrate the contents in the flask with 0.1M Ceric ammonium sulphate until the colour changed from orange to green.

Report

Theactual Molarity of the given Ceric Ammonium Sulphate was found to be

Reference

Pharmaceutical Titrimetric Analysis by A.A.Napoleon; Page No: 11.48.

ASSAY

EX NO DATE

ASSAY OF AMMONIUM CHLORIDE BY ACID BASE TITRATION

Aim

To determine the percentage purity (% w/w) of Ammonium chloride.

Apparatus

Conical flask, weighing bottle, Burette, Pipette, Weighing balance

Reagents

Ammonium chloride, Sodium hydroxide, Formaldehyde, Phenolphthalein, Distilled water

Principle

It is estimated by alkalimetric method. An accurately weighed quantity of the sample is dissolved in water to liberate hydrochloric acid and ammonia.The liberated hydrochloric acid is directly titrated with 0.1M NaOH, using phenolphthalein as indicator.Addition of neutralized formaldehyde is added to fix the ammonia as hexamine otherwise it will interfere with alkali. End point is colourless to permanent pale pink colour.

$$NH_4Cl + H_2O \rightarrow NH_4OH + HCl$$

(Ammonium chloride) (Ammonium hydroxide)

$$4NH_4OH + 6HCHO \rightarrow (CH_2)_6N_4 + 10H_2O$$

(Formaldehyde) (Hexamine)

$$HCl + NaOH \rightarrow Na^+ + Cl^- + H_2O$$

Procedure

Preparation of 0.1M sodium hydroxide

Dissolve 4g of sodium hydroxide in sufficient carbon dioxide-free water to produce 1000 ml.

Standardization of sodium hydroxide

Weigh accurately about 0.63g of Oxalic acid and transfer it into 100ml volumetric flask. Dissolve it with distilled water and make up the volume to 100ml. Pipette out 20ml of the solution into a conical flask and added 2 drops of phenolphthalein to it. Titrate it against sodium hydroxide taken in the burette. Repeat the procedure till get a concordant value.

Assay of ammonium chloride

Weigh accurately about 0.1g ammonium chloride add a mixture of 5ml of formaldehyde solution,previously neutralized to dilute phenolphthalein solution, and 20ml of water. After 2 minutes, titrate slowly with 0.1Msodium hydroxide using a further 0.2 ml of dilutephenolphthalein solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.005349 g ofNH_4Cl.

Report

The percentage purity of given sample of ammonium chloride was found to be

Reference

1. Pharmaceutical analysis volume 1 by P.C. Kamboj; Page No:299
2. Indian Pharmacopoeia 1996, Volume I Page No:47
3. Indian Pharmacopoeia 1996, Volume II Page No:A-213

EX NO DATE

ASSAY OF FERROUS SULPHATE BY CERIMETRY

Aim

To determine the percentage purity (% w/w) of Ferrous sulphate.

Apparatus

Burette, Pipette, Conical Flask, Volumetric flask, funnel etc.

Reagents

Ferrous sulphate, Sulphuric acid, Cerric ammonium sulphate, Sodium bicarbonate and Ferroin indicator.

Principle

The principle involved in the determination of ferrous sulphate is redox titration involving Ceric ammonium sulphate. Ferrous sulphate, a reducing agent in presence of sulphuric acid, is titrated with Ceric ammonium sulphate using ferroin as indicator which gives colour change from orange to green. Ceric ammonium sulphate is powerful oxidizing agent and is used only in acidic media because if the solution is neutral, Ceric hydroxide precipitates out. Ceric ion solution itself has intense yellow colour so it can be used as self-indicator, but to increase the sensitivity of end-point detection, internal redox indicator like ferroin is added.

Chemical Reaction:

$$2FeSO_4 + 2(NH_4)_2 Ce(SO_4)_3 \rightarrow Fe_2(SO_4)_3 + 2(NH_4)_2 SO_4 + Ce_2(SO_4)_3$$

$$Fe^{+2} \rightarrow Fe^{+3} + e^- \text{ (Oxidation)}$$

$$Ce^{+4} \rightarrow e^- Ce^{+3} \text{ (Reduction)}$$

$$Fe^{+2} + Ce^{+4} \rightarrow Fe^{+3} + Ce^{+3}$$

Procedure

a) Preparation of 0.1M Ammonium Cerric Sulphate Solution

Weigh out 65g of Cerric Ammonium Sulphate with the aid of gentle heat in a mixture of 30ml of sulphuric acid and 500ml of water. Cool, filter the solution if turbid, and dilute to 1000ml with water.

b) Standardization of 0.1M Ceric ammonium sulphate solution

Weigh accurately 3.921g of Ferrous ammonium sulphate. Dissolve in a mixture of 4ml of 1M sulphuric acid and 20ml water and make upto 100ml with water in a standard flask. Pipette out 10ml solution and add 2 drops of ferroin indicator. Titrate the contents in the flask with 0.1M ceric ammonium sulphate until the colour changed from orange to green.

c) Assay of Ferrous sulphate

Weigh accurately about 0.5g ferrous sulphate, dissolve in a mixture of 30ml of water and 20ml of 1M sulphuric acid and titrate with 0.1M Ceric ammonium sulphate using ferroin solution as indicator, until the colour changes from orange to green.

1ml of 0.1M Ceric ammonium sulphate is equivalent to 0.02780g of $FeSO_4.7H_2O$.

Report

The percentage purity of given sample of Ferrous sulphate was found to be

Reference

1. Practicals in Pharmaceutical Analysis, Bhoomika R. Goyal, Hardik G. Bhatt and Mayur M. Patel, Page No: 50-52
2. Indian Pharmacopoeia 2007, Volume 1, Page No: 506 and 507

EX NO DATE

ASSAY OF COPPER SULPHATE BY IODOMETRY

Aim

To determine the percentage purity (% w/w) of Copper sulphate.

Apparatus

Burette, Pipette, Conical Flask, Volumetric flask, funnel etc.

Reagents

Copper Sulphate, Dilute Acetic Acid, Sodium Carbonate and Potassium Iodide.

Principle

This is an iodometric type of titration. It depends upon the instability of cupric iodide which is formed in the reaction between $CuSO_4$ and potassium iodide with the liberation of free iodine. When $CuSO_4$is allowed to react with KI in the presence of acetic acid, cupric iodide is formed.

$$2CuSO_4 + 4KI \rightarrow 2CuI_2 + 2K_2SO_4$$

The cupric iodide (CuI_2) formed in the above reaction is unstable, so it decomposes to give cuprous iodide (Cu_2I_2) with the liberation of free iodine.

$$2CuI_2 \rightarrow 2Cu_2I_2 + I_2$$

The liberated iodine is titrated with 0.1Msodium thiosulphate ($Na_2S_2O_3$) using starch indicator.

$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$$

(Sodium tetrathionate)

Procedure

a) Preparation of 0.1M Sodium thiosulphate solution

Dissolve 25g of sodium thiosulphate and 0.2g of sodium carbonate in carbon dioxide-free water and dilute to 1000ml with the same solvent. Standardize the solution in the following manner.

b) Standardization of sodium thiosulphate solution

Dissolve 0.200g of potassium bromate, in sufficient water to produce 250.0ml. To 50.0ml of this solution add 2g of potassium iodide and 3ml of 2M hydrochloric acid and titrate with the sodium thiosulphate solution using starch solution, added towards the end of the titration, as indicator until the discharge of blue colour.

1ml of 0.1M sodium thiosulphate is equivalent to 0.002784g of $KBrO_3$.

c) Assay of copper sulphate

Weigh accurately 1g of the sample and dissolve in 50ml of water. Add 4ml of acetic acid and 3g of potassium iodide, and titrate the liberated iodine with 0.1M sodium thiosulphate, using starch as the indicator. Perform a blank determination, and make any necessary correction. Each ml of 0.1M sodium thiosulphate is equivalent to 0.02497g of $CuSO_4 \cdot 5H_2O$.

Report

The percentage purity of given sample of Copper Sulphate was found to be

Reference

1. Practical Pharmaceutical Analysis, Dr. G. DevalaRao, Page No: 51
2. Practicals in Pharmaceutical Analysis, Bhoomika R. Goyal, Hardik G. Bhatt and MayurM. Patel, Page No: 68 & 69

EX NO DATE

ASSAY OF CALCIUM GLUCONATE BY COMPLEXOMETRIC TITRATION

Aim

To determine the percentage purity of given sample of calcium gluconate.

Apparatus

Burette, Pipette, Conical Flask, Volumetric flask, funnel etc.

Reagents

Calcium Gluconate, Disodium Edetate, Ammonia buffer, strong ammonia solution, Magnesium Sulphate and Eriochrome Black T

Principle

The principle involved in the estimation of calcium gluconate is substitution Complexometric titration. Substitution titrations are carried out for metal ions that do not react or react satisfactorily with metal indicator. Further they are used for metal ions which form EDTA complexes more stable than that of magnesium or zinc ion. The metal ion to be determined is treated with magnesium edetate complex, hence replacement of magnesium ion from complex ion occurs and the amount of magnesium ions set freeis titrated with EDTA solution using suitable indicator.

Calcium ion is determined by this method because, Eriochrome black T does not react satisfactorily, resulting in poor end point and calcium forms more stable complex with EDTA. In estimation of calcium gluconate, magnesium ions are added in small amount which forms stable magnesium indicator complex required for indicator action. Here magnesium-indicator complex is more stable than calcium-indicator complex but less stable than magnesium-edetate complex. Hence during titration of calcium ion, in alkaline media with disodium edetate using Eriochrome black T indicator, EDTA reacts with first with free calcium ions and magnesium ions. Calcium edetate complex is more stable than magnesium edetate complex hence, calcium ions causes replacement of magnesium ions from magnesium edetate complex. These free magnesium ions forms red magnesium indicator complex which reacts with excess EDTA at the end point to liberate free indicator (blue). Volume of disodium edetate hence used is corrected for the slight amount of magnesium ions, to give net amount required for calcium. Ammonia buffer pH 10 solution is added to provide alkaline media.

Procedure

Preparation of 0.05M Disodium EDTA

Dissolve 18.6g of disodium edetate in sufficient water to produce 1000ml.

Standardization of 0.05M Disodium EDTA

Pipette out 20ml of the 0.05M Magnesium sulphate solution. Add 5ml of Ammonia-ammonium chloride buffer solution and titrate with 0.05M edetate using Erichrome black-T as indicator. End-point is a change from wine red to blue.

Assay of Calcium Gluconate

Weigh accurately about 0.5 g and dissolve in 50 ml of warm water; cool, add 5.0 ml of 0.05 M magnesium sulphate and 10 ml of strong ammonia solution and titrate with 0.05 M disodium edetate using mordant black II mixture as indicator. From the volume of 0.05 M disodium edetate required subtract the volume of the magnesium sulphate solution added.

1 ml of the remainder of 0.05M disodium edetate is equivalent to 0.02242 g of calcium gluconate ($C_{12}H_{22}CaO_{14}.H_2O$).

Report

The percentage purity of given sample of Calcium Gluconate was found to be

Reference

Pharmaceutical Analysis – I (Practical) by SonaliSheoroy and MeeraHonrao; Page No: 105.

EX NO DATE

ASSAY OF HYDROGEN PEROXIDE BY PERMANGANOMETRIC TITRATION

Aim

To determine the percentage purity of given hydrogen peroxide.

Apparatus

Burette, Pipette, Conical Flask, Volumetric flask, funnel etc.

Reagents

Hydrogen Peroxide, 0.02 M Potassium Permanganate and 1M Sulphuric Acid

Principle

Hydrogen peroxide is usually treated as a strong oxidizer, but in the presence of even stronger oxidizer it can become a reducing agent.

Permanganate in low pH is strong enough to quantitatively oxidize hydrogen peroxide to oxygen. This reaction is used for the determination of hydrogen peroxide concentration.

Chemical reaction:

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

$$2KMnO_4 + 5H_2O_2 + 3H_2SO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 5O_2 + 8H_2O$$

Procedure

a) Preparation of 0.1N potassium permanganate solution

Weigh accurately about 3.16 g of Potassium permanganate and dissolve in 1000ml of distilled water. Cover the mouth of the flask with a watch glass and boil the solution for 15-30 min. Allow the solution to cool and filter that through a funnel containing a plug of glass wool or asbestos. Collect the filtrate and store it in dark coloured bottle.

b) Standardization of 0.1N Potassium Permanganate Solution

Weigh accurately about 0.63g of oxalic acid into a standard flask. Dissolve in distilled water and make up to 100ml with distilled water. Pipette out 20ml and add 5ml of dil. Sulphuric acid (1M). Warm the contents of the flask to 70^{o}C and titrate with $KMnO_4$ solution. Continue the titration until a faint pink colour is produced. Repeat the titration for concordant values.

c) Assay of hydrogen peroxide

To 1.0 ml add 20 ml of 1M sulphuric acid and titrate with 0.1N potassium permanganate.1 ml of 0.1N potassium permanganate is equivalent to 0.001701 g of H_2O_2

Report

The percentage purity of given sample of hydrogen peroxide was found to be

Reference

Indian Pharmacopoeia 2007, Volume 2, Page No: 585

EX NO DATE

ASSAY OF SODIUM BENZOATE BY NON AQUEOUS TITRATION

Aim

To prepare and standardize the given solution of Perchloric acid

Apparatus Required

Burette, Pipette, Conical Flask, Volumetric flask, funnel

Reagents Required

Perchloric acid, glacial acetic acid, acetic anhydride, 0.1N Potassium Hydrogen Phthalate, crystal violet indicator (0.5% w/v in glacial acetic acid), 1-Naphtholbenzein, 0.1M Perchloric Acid, Sodium Benzoate and Anhydrous Glacial Acetic Acid

Principle

Sodium benzoateis a weakly basic drugs assayed by non-aqueous titration.Weak bases such as sodium benzoate are dissolved in acetic acid and are titrated with acetous perchloric acid using crystal violet as indicator. Acetic acid behaves as a weak acid because of poor dissociation into H^+ ion.

$$CH3COOH \rightarrow CH3COO^- + H^+$$

When a strong acid such as perchloric acid is added to acetic acid, it gets protonated and results in the formation of onium ion which can readily give up H^+ ion to a base.

$$HClO4 \rightarrow H^+ + ClO4^-$$

$$CH3COOH + H^+ \rightarrow CH3COOH2^+$$

When a weak base like sodium benzoate dissolved in acetic acid, an equivalent amount of acetate are produced which have more tendency to accept H^+ions?This basic property of weak base will enhance when titrated against perchloric acid using acetic acid as solvent in case of sodium benzoate.

$$C6H5COO^- + CH3COOH \rightarrow C6H5COOH + CH3COO^-$$

Ultimately, the reaction of weakly basic drug in acetic acid against acetous perchloric acid yields accurate endpoint. The weak acid functions as strong bases due to following reactions.

$$HClO4 + CH3COOH \rightarrow CH3COOH2^{+} + ClO4^{-}$$

$$C6H5COO^{-} + CH3COOH \rightarrow C6H5COOH +$$

$$CH3COO^{-}\ CH3COOH2^{+} + CH3COO^{-} \rightarrow$$

$$2CH3COOH$$

Therefore, the net reaction is:

$$HClO4 + C6H5COO^{-} \rightarrow C6H5COOH + ClO4^{-}$$

Procedure

Preparation of 0.1M perchloric acid in acetic acid

Mix 8.5 ml of perchloric acid with 500 ml of anhydrous glacial acetic acid and 25 ml of acetic anhydride, cool and add anhydrous glacial acetic acid to produce 1000 ml. Allow the repaired solution to stand for 1 day for the excess acetic anhydride to be combined and carry out the determination of water (2.3.43). If the water content exceeds 0.05percent, add more acetic anhydride. If the solution contains no titra table water, add sufficient water to obtain a content of water between 0.02 per cent and 0.05 per cent. Allow the solution to stand for 1 day and again titrate the water content. The solution so obtained should contain between 0.02 per cent and 0.05 per cent of water.

Standardization of perchloric acid:

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

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

Assay of Sodium Benzoate

Weigh accurately about 0.25g dissolve in 20ml of anhydrous glacial acetic acid, warming to 50º if necessary, cool. Titrate with 0.1M Perchloric acid, using 0.05ml of 1 -naphtholbenzein solution as indicator (crystal violet can also be used). Carry out a blank titration.

1 ml of 0.1 M Perchloric acid is equivalent to 0.01441g of (sodium benzoate) $C_7H_5NaO_2$.

Report

The Percentage purity of given sample of sodium benzoate was found to be

Reference

1. Indian Pharmacopoeia 2007, Volume 1, Page No: 314 and 315

2. Practical pharmaceutical analysis, Dr. G. DevalaRao, Page No: 76

3. Indian Pharmacopoeia 2007, Volume 3, Page No: 1080 & 1081

EX NO **DATE**

ASSAY OF SODIUM CHLORIDE BY PRECIPITATION TITRATION

Aim

To determine the percentage purity of given sodium chloride.

Apparatus

Burette, Pipette, Conical Flask, Volumetric flask, funnel etc.

Reagents

Sodium Chloride, Silver Nitrate, Ammonium Thiocyanate and Ferric

Ammonium Sulphate

Principle

This assay is based on the precipitation type of reaction. It is assayed by modified Volhard's method of chloride estimation. Sodium chloride is first reacted with excess of acidified silver nitrate using nitric acid which results in the formation of a white precipitate of silver chloride. The excess of silver nitrate is back titrated with 0.1M Ammonium thiocyanate solution. This is called Volhard' s method.Chloride precipitate is coagulated by means of nitrobenzene, because silver chloride reacts slowly with ammonium thiocyanate and makes the endpoint flat. The excess of silver nitrate is back titrated with ammonium thiocyanate using ferric ammonium sulphate as indicator. The determination depends upon the reactions expressed by the following equations:

$$NaCl + AgNO3 \rightarrow AgCl\downarrow + NaNO3$$

Sodium Chloride Silver Nitrate Silver Chloride Sodium Nitrate

$$\text{Excess } AgNO3 + NH4SCN \rightarrow 3AgSCN\downarrow + NH4NO3$$

Silver Nitrate Ammonium Thiocyanate Silver Thiocyanate Ammonium Nitrate

$$Fe(NH4)(SO4)2 + 3NH4SCN \rightarrow Fe(SCN)3 + 2(NH4)2SO4$$

Ferric Ammonium Sulphate Ammonium Thiocyanate Ferric Thiocyanate Ammonium Sulphate

Procedure

Preparation of 0.1M Ammonium Thiocyanate

Dissolve 7.612 g of ammonium thiocyanate in sufficient water to produce 1000ml.

a) Standardization of Ammonium Thiocyanate

Pipette 30.0ml of 0.1M silver nitrate into a glass-stoppered flask, dilute with 50ml of water, add 2ml of nitric acid and 2ml of ferric ammonium sulphate solution and titrate with the ammonium thiocyanate solution to the first appearance of a red-brown colour.

1 ml of 0.1 M silver nitrate is equivalent 0.007612g of NH_4SCN.

b) Assay of sodium chloride

Weigh accurately about 0.1g sample and dissolve in 50ml of water in a glass-stoppered flask. Add 50.0ml of 0.1M silver nitrate, 5ml of 2M nitric acid and 2ml of nitrobenzene, shake well and titrate with 0.1M ammonium thiocyanate using 2ml of ferric ammonium sulphate solution as indicator, until the colour becomes reddish yellow.

1 ml of 0.1 M silver nitrate is equivalent to 0.005844g of NaCl.

Report

The percentage purity of given sample of Sodium Chloride was found to be

Reference

1. Indian Pharmacopoeia 2007, Volume 2, Page No: 585
2. Practicals in Pharmaceutical Analysis, Bhoomika R. Goyal, Hardik G. Bhatt and Mayur M. Patel, Page No: 86-89
3. Indian Pharmacopoeia 2007, Volume 1, Page No: 312

ELECTROANALYTICAL METHODS

EX NO DATE

CONDUCTOMETRIC TITRATION OF STRONG ACID WITH STRONG BASE

AIM

To perform conductometric titration of hydrochloric acid with sodium hydroxide and to determine the end point.

REFERENCE

1. Bhoomika R Gpoal. Hardik G Bhatt. Mayur M Patel. Pharmaceutical analysis –I. Page No: 99-100.

REQUIREMENTS

Apparatus : Beaker, Pipette, Volumetric flask, conductivity cell

Reagents : 0.01 M Hydrochloric acid, 0.01 M Sodium hydroxide

PRINCIPLE

The solution of HCl shows fairly significant conductivity mainly due to two species H+ and Cl-. For each amount of NaOH added, an equivalent amount of H+ ion is consumed according to the following reaction.

$H^+ + NaOH == Na^+ + H_2O$

The highly conducting H+ cations are effectively replaced by the relatively poorly conducting Na+ ions and consequently the conductance decreases. This continues until sufficient NaOH has been added to react with all the H+ ions present and the mixture contains only Na+ and Cl- ions in water

$HCl + NaOH == H_2O + NaCl$

This is called equivalence point. Further addition of NaOH simply augments the quantity of Na+ and OH- ion in the mixture and increase the conductance due to higher OH- anions. As a result, the conductomeric titration curve (conductance vs. volume of NaOH) has a minimum at the equivalence point. The position of the equivalence Point can be localized as the point of inter section of two straight lines, a descending segment before and an ascending segment after the minimum observed. Usually in this experiment, the concentration of solution in the burette should be approximately 10 times than that of the other solution, under these conditions the change in concentration

due to dilution of the solution during the titration , will have minimum effect on the conductance measurements.

PROCEDURE

Transfer 50 ml 0.01M hydrochloric acid solution in a 100 ml beaker. Immerse the conductivity cell in the solution in the beaker such that the tip of the electrode remains dipped in the solution. Measure the conductance for the pure HCl solution. Titrate with
0.1 M NaOH solution using conductometer, adding aliquot of 0.5ml of
0.1 M NaOH solution each time. Remember to mix well after each addition of titrant before measuring the conductance. Continue in this way until you have measured about six points beyond the endpoint. Plot the graph of volume of titrant against conductance.

From graph, endpoint of the conductometric titration is found to be.....ml

EX NO DATE

POTENTIOMETRIC TITRATION OF A STRONG ACID WITH A STRONG BASE

AIM

To perform the titration of 0.1 N HCl with 0.1 N NaOH by potentiometry and to locate the end point.

REFERRENCE

1. G.devala rao. Pharmaceutical analysis. Page No: 136

REQUIREMENTS

Apparatus : pH meter, Magnetic stirrer, Beaker

Reagent : 0.1 N HCl, 0.1 N NaOH, Distilled water.

PRINCIPLE

Potentiometric determination of the end point depends on the fact that the potential across the two electrodes (reference and indicator) immersed in the solution changes sharply at the equivalence or end [point. This change is similar to the colour change by an indicator in usual method. But the potentiometric method is more accurate. These titrations are useful when no suitable colour indicators are available. Equivalence point can be accurately found out after plotting normal plot (volume of titrrant vs. potential)

PROCEDURE

In a 250 ml beaker add 10 ml of 0.1N HCl and add around 100 ml of waater of dip the electrodes properly. Keep the beaker on magnetic stirrer. Note the potential without adding any alkali slowly add with stirring, known volumes of 0.1 N NaOH solution, and note the potentials. enter the values in a tabular form. from the data (volume of titrant vs. potential). Plot the graph and calculate the end point.

REPORT

The end pont in the titration of 0.1 N HCl with 0.1 N NaOH by potentiometry is

PHARMACEUTICS I

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SYRUPS

EX NO **DATE**

SIMPLE SYRUP I.P. 1956

Aim: Prepare and submit Simple Syrup I.P.1966,20 ml

Requirements;

Apparatus: Weighing balance, beaker, stirrer, measuring cylinder etc.

Chemicals: sugar and purified water

Formula:

Ingredient	**Standard forumula**	**Working Formula**	**Role of Ingredient**
Sugar (sucrose)	667 g	13.34 g	Sweetening agent
Purified water q.s. to produce	1000 g	20 g	Vehicle

Theory:

Simple Syrup I.P is a concentrated nearly saturated, viscous solution of sucrose in purified water. If contains 66.7% w/w sucrose in purified water. Simple Syrup I.P differ from Syrup USP in terms of concentration of sucrose used to prepare it. Syrup USP Contains 85% w/v sucrose in purified water. Simple syrup act as self- preservative, because it is concentrated solution of sucrose and no additional water is available for growth of micro-organism. Sucrose at 66.7% w/w strength in syrup exerts high osmotic pressure which it withdraws water from micro-organism and causes their dehydration followed by their death.Simple Syrup I.P is prepared by solution by heat method.

$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$

Sucrose — Dextrose (glucose) — Levulose (fructose)

Procedure:

1. Clean all the glassware and dry them properly as per Standard Operating Procedure (SOP)
2. Weight accurately required of sucrose and dissolve into 3/4th of total volume of purified water in beaker.
3. Heat this solution on water bath with occasional stirring and add remaining amount of purified water to dissolve sucrose completely.
4. Allow to cool the solution to room temperature and filter through muslin cloth if necessary to remove any foreign particle.
5. Transfer preparation in to narrow mouth, light resistant (amber coloured) container
6. Cap the bottle, label and submit

Category: Pharmaceutical aid. It is used as sweetening agent and vehicle for preparation of many liquid dosage forms.

Storage: Store in tightly closed container at cool temperature not exceeding 25º C in dark place.

Report:

EX NO **DATE**

COMPOUND SYRUP OF FERROUS PHOSPHATE BPC 1968

Aim: Prepare and submit Compound Syrup of Ferrous Phosphate BPC 1968, 20mL.

Requirements:

Apparatus: Weighing balance, acid, calcium carbonate, potassium bicarbonate, sodium phosphate, cochineal, sucrose, orange-flower water of commerce, undiluted and purified water.

Formula:

Ingredient	**Standard Formula**	**Working Formula**	**Role of Ingredient**
Iron	4.3 g	0.086 g	Iron supplement
Phosphoric acid	48 mL	0.96 mL	Acid
Calcium carbonate	13.6 g	0.272 g	Calcium supplement
Potassium bicarbonate		0.02 g	Potassium supplement
Sodium Phosphate	1.0 g	0.02 g	Sodium supplement
Cochineal	3.5 g	0.07 g	Colouring agent
Sucrose	700 g	14 g	Sweetening agent
Orange-flower water of commerce, undiluted	50 mL	1mL	Flavouring agent
Purified water q.s. to produce	1000 mL	20 mL	Vehicle

Theory:

Synonyms: Ferrish‟s Food, Ferrish Syrup, Chemical Food.

A low iron level is known as iron deficiency anaemia. Iron is a critical part of a red blood cell protein, haemoglobin, which carries oxygen throughout the body Compound Syrup of Ferrous Phosphate is used as iron and electrolyte (calcium, potassium and sodium) supplement in anemic patient.In this formulation, phosphoric acid reacts with iron and form ferrousacid phosphate.

$$Fe + 2H_3PO_4 \longrightarrow Fe(H_2PO_4)_2 + H_2O$$

Iron Phosphoric acid Ferrous acid phosphate

Phosphoric acid also reacts with calcium carbonate, potassium bicarbonate and sodium phosphate and form calcium dihydrogen phosphate, potassium dihydrogen phosphate and sodium dihydrogen phosphate, respectively.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Dilute half the quantity of phosphoric acid with little amount of water and add iron to it. Gently heat this solution on a water –bath until iron gets dissolved.
3. In a separate beaker transfer remaining portion of phosphoric acid and add calcium carbonate, potassium bicarbonate and sodium phosphate with continuous stirring. At this step CO_2 .will be released. Allow CO_2 to evolve completely
4. Mix both the solutions with stirring and allow to stand for few minutes to complete reaction.
5. In a conical flask boil cochineal in water for 15 minutes, cool, strain, and pass sufficient water through the strainer. To this add sucrose and again boil for 15 minutes to form coloured syrup.
6. Filter filters iron solution into the coloured syrup. Add orange flower water and pass sufficient water through the filter to produce the required volume, mix, allow standing for cooling and filter if necessary.
7. Transfer preparation to narrow mouth, amber coloured glass bottle, close with cap, label and submit.

Category: Iron and electrolyte (calcium, potassium and sodium) supplement in anemic patient.

Storage: Store in well closed container in cool and dark place.

Report:

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,Apractical hand book of pharmaceutics – I,Niraliprakashan, Page No: 1.1-1.9

ELIXIRS

EX NO **DATE**

PIPERAZINE CITRATE ELIXIR I.P.

Aim: Prepare and submit Piperazine citrate Elixir I.P. 1966, 30mL

Requirements:

Apparatus: Weighing balance, Beaker, stirrer, Measuring cylinder, pipette, Porcelain dish, Water bath etc.

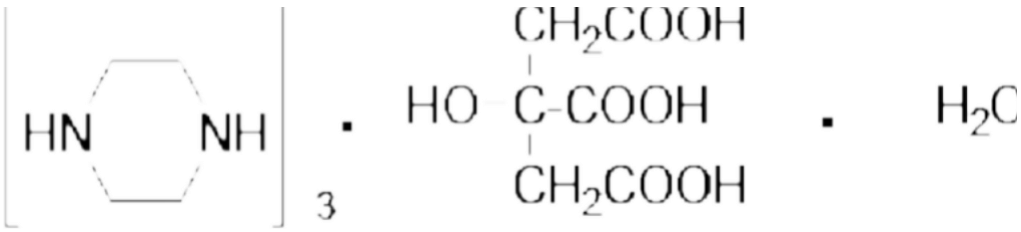
Chemicals: Piperazine citrate I.P., Chloroform Spirit I.P., glycerine, Orange oil, Simple Syrup I.P. and purified water.

Formula:

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Piperazine citrate I.P	180 g	5.4g	Anthelmintic
Chloroform Spirit I.P	5ml	0.15ml	Preservative
Glycerine	100ml	3.0ml	Solubilizing agent
Orange oil	0.25 ml	q.s.	Flavouring agent
Simple Syrup I.P	500 ml	15 ml	Sweetening agent
purified water q. s to produce	1000 ml	30ml	vechile

Theory:

Piperazine citrate Elixir is used for pinworm infections, threadworm infections, roundworm infections and other conditions. This formulation is an oral solution containing 15.3 to 17.7% w/v of Piperazine citrate as an active ingredient in a suitable flavoured vehicle. Piperazine is rapidely absorbed from the gastrointestinal tract. It is a white or almost white granular crystalline powder that melts at 190^{o} C. Approximately 25% is metabolized in the liver. Piperazine is nitrosated to form N- mononitrosopiperazine (MNPz) in gastric juice and further metabolized to N-nitroso-3hydroxypyrrolidine (NHPYR).Plasma half life is highly variable.Time to peak concentration 2 to 4 hours. Approximately 20% is renally excreted unchanged in the urine within 24 hours.



Piperazine is indicated as alternative treatment for ascariasis caused by *Ascarislumbricoides* (round worm) and enterobiasis(oxyuriasis) caused by *Enterobiasisvermicularis* (pinworm). Piperazine belongs to class of drugs called anthelmintic. The molecular formula of piperazine citrate is $(C_4\ H_{10}\ N_2)_3$, $2C_6\ H_8\ O7$.Piperazine blocks the response of the worm muscle to acetylcholine, presumably by causing hyper polarization of nerves endings, resulting in flaccid paralysis of the worm. Thus, produces a neuromuscular block that leads to muscle paralysis of the worms making them to pass in the stool.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Weigh the required quantity of piperazine citrate and dissolve is $3/4^{th}$ amount of water.
3. Dissolve orange oil in chloroform spirit. Add this solution of piperazine citrate.
4. Add simple syrup and glycerine to above solution and mix uniformly.
5. Using measuring cylinder adjust final volume to 50 ml with purified water.
6. Transfer content to amber coloured (light resistant) narrow mouth plastic or glass bottle and close tightly with screw cap, polish and label.

Category:

As an anthelmintic for human and farm animals against intestinal obstruction by the common roundworms,a condition primarily occurring in children.

Storage: Store in cool and dark place from light.

Auxiliary label: For oral use.

Dose: For children

9-12 months : 2.5 ml

2 to 3 years : 5 ml

4 to 6 years : 7.5ml

7 to 12 years : 10 ml

To treat thread infection a dose 4 to 15 ml in daily in divided doses is recommended.

Report:

EX NO **DATE**

PAEDIATRIC PARACETAMOL ELIXIR B.P.

Aim: Prepareand submit Paediatric paracetamol Elixir B.P.1993 30ml.

Requirments:

Apparatus: Weighing balance, Beaker, stirrer, Measuring cylinder, pipette, Porcelain dish, Water bath etc

Chemicals: Paracetamol B.P., amaranth solution, chloroform spirit, concentrated raspberry juice, alcohol (95%v/v), propylene glycol,invert syrup and glycerol.

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Paracetamol	24g	0.72g	Analgesicand antipyretic
Amaranth solution	2 ml	0.06 ml	Colouring agent
chloroform spirit	20 ml	0.6ml	Flavouring agent
concentrated raspberry juice	25 ml	0.75 ml	Colouring and flavouring agent
alcohol (95%v/v)	100 ml	3 ml	Co-solvent
propylene glycol	100 ml	3 ml	Co-solvent
invert syrup	275 ml	8.25ml	Sweetening agent
Glycerol q.s to produce.	1000ml	30ml	vehicle

Theory :

Pediatric Paracetamol elixir is a solution that contain 2.4% w/v of paracetamol in a suitable flavoured vehicle. Invert syrup is prepared by hydrolyzing a 66.7%w/w solution of sucrose with suitable mineral acid, and neutralizing the solution with sodium carbonate. Paracetamol is analgesic and antipyretic. Antipyretics are substances that reduce fever. Analgisics are those drugs that mainly provide pain relief .Chloroform spirit is used as flavouring agent. Alcohol and propylene glycol are used as colouring and flavouring agent. Alcohol and propylene glycol are co-solvents. Invert syrup and glycerol are sweetening agents and vehicles, respectively.Paracetamol inhibit prostaglandin synthesis, more centrally than peripherally.

Paracetamol is rapidly absorbed from the upper gastrointestinal tract after oral administration. Adverse effects of paracetamol are rare but hypersensitivity including skin rash may occur.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Dissolve the weighed amount of paracetamol in a mixture of the alcohol and the chloroform spirit.
3. Dilute the raspberry juice with the invert syrup,the amaranth solution,propylene glycol and about half of the glycerol.
4. Add the solution prepared in step (3) to the paracetamol solution and mix well.
5. Transfer to a measuring cylinder and adjust to the final volume with glycerol.
6. Transfer contents to amber coloured (light resistant)narrow mouth plastic or glass bottle and close it tightly with screw cap, polish and label.

Category: To treat mild to moderate pain,as an anti-pyretic and in post immunisation pyrexia.

Storage: Store in cool and dark place.

Direction: „SHAKE THE BOTTLE FOR AT LEAST" 10 seconds before use.

Auxiliary label: For oral use.

Dose: As directed by physician.

If necessary, after 4-6 hours,give a second dose of 2.5 mL.

Contraindications: Hypersensitivity to paracetamol and/or other constituents.

Report:

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,A practical hand book of pharmaceutics –I, Niraliprakashan , Page No: 2.1-2.10

LINCTUS

EX NO **DATE:**

TERPIN HYDRATE LINCTUS I.P. 1966

Aim: Prepare and submit Terpin Hydrate Linctus I.P.1966, 20 ml.

Requirements:

Apparatus: Weighing balance, beaker, stirrer, measuring cylinder etc.

Chemicals: Terpin hydrate, glycerine, alcohol (30 % v/v), orange oil, purified water.

Formula:

Ingredient	**Standard formula**	**Working formula**	**Role of ingredient**
Terpin hydrate	5 g	1 g	Expectorant
Glycerin	40 ml	8 ml	Demulcent
Alcohol (30% v/v)	40 ml	8 ml	Solubilizer
Syrup	10 ml	2 ml	Sweetening agent
Orange oil	0.02 ml (q.s)	0.004 ml (q.s)	Flavouring agent
Purified water q.s. to produce	100 ml	20 ml	Vehicle

Theory:

Terpin hydrate is an expectorant, commonly used to loosen mucus in patients presenting with acute or chronic bronchitis, and related conditions. It is derived from sources such as oil of turpentine, oregano, thyme and eucalyptus. Terpin hydrate is commonly used in the treatment of acute and chronic bronchitis. It is commonly formulated with antitussives (for example, codeine) as a combined preparation.Terpin hydrate is slightly soluble in water and hence alcohol (30 %v/v) is used to dissolve it. Glycerin increases viscosity of linctus and is used as demulcent as it gives soothing effect.Syrup is used as sweetening agent. Orange oil acts as a flavouring agent. Alcohol and orange oil are volatile in nature hence; terpin hydrate linctus is stored in tightly closed container. Terpin hydrate is sensitive to light and hence it should be packed in amber colour narrow mouth container.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Weigh required quantity of terpin hydrate and dissolve into alcohol.
3. Add glycerine, syrup and orange oil with continuous stirring.
4. Pour content into measuring cylinder and make-up volume up to 20 ml by addition of water.
5. Transfer contents to amber colored narrow mouth container and close it tightly with screw cap, label and submit.

Category: Expectorant

Storage: Store in tightly closed container in cool and dark place.

Dose: As directed by physician.

Direction: DO NOT DILUTE WITH WATER BEFORE USE

Report:

EX NO **DATE**

IODINE THROAT PAINT (MANDL'S PAINT) B.P.C. 1968

Aim: Prepare and submit Iodine Throat Paint (Mandl"s Paint) B.P.C. 1968, 20 ml

Requirements;

Apparatus: Weighing balance, beaker, stirrer, glass mortar and pestle, measuring cylinder etc

Chemicals: Potassium iodide, iodine, water, alcohol (90 %), peppermint oil, glycerin.

Formula:

Ingredient	**Standard formula**	**Working formula**	**Role of Ingredient**
Potassium iodide	24 g	0.7 g	Solubility enhancer
Iodine	12g	0.36 g	Antibacterial agent and anti-inflammatory
Water	24 ml	0.72 ml	Solvent
Alcohol (90%)	40 ml	1.2 ml	Co-solvent
Peppermint oil	4 ml	0.12 ml	Flavouring agent
Glycerinq.s. to produce	1000 ml	30 ml	Vehicle, humectant

Theory:

Throat paints are viscous solutions or dispersions of one or more active ingredients intended for application to the mucosa of the throat or mouth. Iodine can reduce pain of the throat, eliminate throat infections; boosts overall immune function and help with conditions such as tonsillitis, pharyngitis, laryngitis and more. Iodine is a naturally occurring mineral that the body needs to produce hormones and control the body"s metabolism. In a liquid form, it is also used as an antibacterial agent and anti-inflammatory.Iodine is slightly soluble in water hence potassium iodide is used. When potassium iodide it reacts with iodine it forms polyiodides which are soluble in water and solution becomes monophasic. Reaction between iodine and potassium iodide takes place as follows:

$$I_2 + KI \longrightarrow I_2KI$$

Iodine		Potassium iodide		Potassium mono iodide
$2I_2$	+	KI	⟶	$2I_2KI$
Iodine		Potassium iodide		Potassium di-iodide
$3I_2$	+	KI		$3I_2KI$
Iodine		Potassium iodide		Potassium tri iodide

Glycerin is commonly used, because it increases viscosity of preparation and hence preparation adheres well to the mucous membrane of mouth and throat. It also gives a sweet taste and warm sensation to the inflamed area. Alcohol (90% v/v) is used as co-solvent which increases solubility of iodine in water. It also help to dissolve peppermint oil in it. Peppermint oil is used as flavouring agent.

Procedure:

1. Clean all apparatus and glassware and dry them properly as per SOP.
2. Weigh required quantity of iodine and transfer into glass mortar and triturate to get fine powder.
3. Weight and dissolve required quantity of potassium iodide in water.
4. To this solution add fine powdered iodine with continuous stirring.
5. In another beaker containing alcohol (90 % v/v) add required quantity of peppermint oil and half the quantity of glycerine. Add this solution to above iodine and potassium iodide solution with continuous stirring.
6. Pour content into measuring cylinder and make-up volume to 20 ml by addition of glycerin.
7. Transfer contents to amber colored wide mouth glass bottle, close it tightly with plastic screw cap, label and submit.

Category: In treatment of pharyngitis laryngitis and follicular tonsillitis.

Storage: Store in tightly closed container in cool and dark place.

Direction: Apply with the help of soft brush or a cotton swab. Food and water before and after application of throat paint should be avoided for 1 hour.

Auxiliary Label:

For local application.

Shake well before use.

Not to be swallowed in large amount.

Keep container tightly closed.

Marketed products: MANDL"S PAINT BPC"68 (K. Pharmaceutical Works), Mandl"s Throat Paint BPC (Agrawal Pharmaceuticals).

Report:

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,A practical hand book of pharmaceutics –I, Niraliprakashan , Page No:3.1-3.6

SOLUTIONS

EX NO **DATE**

STRONG SOLUTION OF AMMONIUM ACETATEI.P.1996

Aim: Prepare and submit Strong solution of Ammonium acetate I.P.1996,30mL

Requirements:

Apparatus: Weighing balance, measuring cylinder,beaker ,stirrer,test tube etc.

Chemicals: Glacial acetic acid, ammonium bicarbonate, ammonia solution strong, purified water, etc

Formula:

Ingredients	Standard formula	Working formula	Role of ingredient
Glacial acetic acid	453g	1359mL	Acid
Ammonium bicarbonate	470g	14.1 g	Alkali
Ammonia solution strong (q.s)	100 mL	0.3 mL	Alkali
Purified water q.s to produce	1000 mL	30 mL	Solvent

Theory:

Strong solution of ammonium acetate is also called as „*Liquor Ammonia Acetalis Fortis*". It contains 57.5%w/v of ammonium acetate and used as diaphoretic, diuretic and mild expectorant. It is prepared by acid – base (neutralization) reaction between glacial acetic acid, ammonium bicarbonate and ammonia solution strong.Glacial acetic acid reacts with ammonium bicarbonate and produce ammonium acetate. The reaction takes place as follows:

$CH_3COOH + NH_4HCO_3 \rightarrow CH_3COONH_4 + H_2O + CO_2$

Glacial acetic acid — Ammonium bicarbonate — Ammonium acetate

If ammonium bicarbonate is used alone as alkali in excess amount it does not neutralize glacial acetic acid completely and excess ammonium bicarbonate remainsin the solution which settles at the bottom of container. If strong ammonium solution is used alone as alkali to neutralize glacial acetic acid, it increases bulk (volume) of the solution. Hence in this preparation two alkalies namely; ammonium bicarbonate and ammonia solution strong are used to complete neutralization of glacial acetic acid. The neutralization point lies between pH 7.6 and 8.Strong Ammonium Acetate Solution should be kept in lead – free glass containers.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Mix glacial acetic acid with $1/4^{th}$ of total volume of water.
3. To dilute glacial acetic acid, add ammonium bicarbonate with continuous stirring to allow escape carbon dioxide formed in this reaction.
4. Take required quantity of strong ammonia solution and add drop wise with stirring in above solution.(Here one can check neutralization point by taking resulting solution diluted with water in test tube. Add strong ammonia solution drop wise until this diluted solution becomes blue after strong ammonia solution drop wise until this diluted solution becomes blue after addition of bromothymol blue and becomes yellow after addition of thymol blue).
5. Make – up volume up to 30 ml by addition of purified water.
6. Transfer solution in lead-free narrow mouth glass container. Label and submit.

Category: Diaphoretic, diuretic and mild expectorant.

Storage: Store in tightly closed container in cool place.

Direction: Take solution (1 to 4 ml) as directed by physician.

Report:

EX NO **DATE**

CRESOL WITH SOAP SOLUTION I.P.1966

Aim: Prepare and submit cresol soap solution i.p.1966,30 ml

Requirements

Apparatus: Weighing balance, measuring cylinder, conical flask, beaker, stirrer, water bath etc

Chemicals: Cresol, vegetables oil (Linseed oil), Potassium hydroxide,purified water.

Formula:

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Cresol	50 ml	15 ml	Disinfectant
Vegetable oil (Linseed oil)	18 ml	5.4 ml	Source of free fatty acid
Potassium hydroxide	4.2g	1.26g	Alkali for soap formation
Purified water q.s to produce	100 ml	30 ml	Solvent/vehicle

Theory:

Disinfectant are antimicrobial agents that are applied to the surface of non – living (in animate) objects to destroy micro organisms that are living on the objects and the process is called as „disinfection". Cresol is a mixture of ortho, meta and para cresol and it is used as disinfectant. Cresol (Substituted phenol) is a cytoplasmic poison to micro-organisms. It disrupts cell wall precipitates cytoplasmic proteins and inactivates essential enzyme systems in microbial cell and eliminates microbes. Cresols are less toxic and less caustic than phenols. But solubility of cresol in water is only 2% v/v. Hence to increase solubility of cresol solubilising agent such as soap is used and thus solution becomes is called as cresol soap solution. It is also called as „Lysol". Lysol contains 50%v/v of cresol. Vegetable oil contain free fatty acid. When alkali such as potassium hydroxide or sodium hydroxide reacts with few fatty acid; it will form sodium/potassium saltof free fatty acid i.e. soap and this soap act as solubilising agent (surfactant).Which increase solubility of cresol in water from 2%v/v to 50%v/v. This saponification is carried out by heating mixture of alkali solution with vegetable oil.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Dissolve required quantity of sodium hydroxide in 1/4th portion of purified water.
3. Transfer required quantity of linseed oil in conical flask and add above alkali mixture in to it.
4. Heat this solution on water bath with continuous stirring until complete saponification take place. Completion of saponification reaction can be tested by adding 1-2 drops of mixture into water. Absence of oil globules in water is indication of completed saponification reaction. If oil globules are seen in the water the saponification reaction is not yet completed and needs continue heating.
5. Once saponification is completed add required quantity of cresol to above mixture with continuous stirring.
6. Transfer this solution to measuring cylinder and adjust final volume to 30 ml with purified water and mix it well.
7. Transfer final solution into narrow mouth, coloured plastic bottle. Close thoroughly with plastic screw cap. Label and submit.

Category: Disinfectant.

Storage: Store in cool and dry place.

Direction: "For external use only"

Precautions:

Not to be used near infants such as nursery, children wards etc.

Not to be used in places where food is prepared and served.

Store in cool and dry place, keep away from the children.

Marketed products: Lysol® (Reckitt Benckiser Inc) Lysol (Aarsha chemicals P Ltd).

Report:

EX NO **DATE:**

LUGOL'S SOLUTION I.P 1966

Aim: Prepare and submit Lugol"s Solution I.P. 1966,30 ml(Aqueous iodine Solution I.P)

Requirements :

Apparatus: Weighing balance, measuring cylinder, glass mortar and pestle, beaker, stirrer etc.

Chemical: Iodine, potassium iodide, Purified water.

Formula:

Ingredients	Standard formula	Working formula	Role of ingredient
Iodine	50 g	1.5g	Antiseptic
Potassium iodide	100G	3g	Solubilizer
Purified water q.s to produce	1000ml	30ml	Vehicle

Theory:

- Lugol,s solution is one of the first antiseptic iodine preparation to be used in wound care which contain elemental iodine and potassium in water, which was developed in 1829. Iodine prevents growth of micro- organism by oxidation or halogenations of bacterial cell protein. Hence externally it is used as antiseptic. Elemental iodine is slightly soluble in water, It is soluble in water containing potassium iodide. When iodine reacts with potassium iodide it forms polyiodide complex and which increase solubility of iodine in water .Reaction between iodine and potassium iodide is as follows:

  $KI + I_2$ $KI.I_2$ (Potassium mono-iodide)

  $KI + 2I_2$ $KI.2I_2$ (Potassium di-iodide)

  $KI + 3I_2$ $KI.3I_2$ (Potassiumtri-iodide)

  potassium iodide iodide polyiodides

- Iodine stains apparatus made-up of steel and iodine act as oxidizing agent which oxidizes iron into ferrous iodide. As iodine is light sensitive its solution should be packed unto amber coloured narrow mouth, plastic screw cap container.

Procedure:

1. Clean all the glassware and dry them properly as pr SOP
2. Weigh required quantity of iodine and transfer to glass mortar and triturate into fine powder.
3. In a beaker dissolve potassium iodide in small amount of purified water.
4. Add fine powdered iodine in small amount of purified water.
5. Once iodine dissolves completely; add sufficient quantity of purified water and transfer into measuring cylinder.
6. Make-up the volume up to 30 ml using purified water.
7. Transfer solution to narrow mouth amber glass bottle, close tightly with plastic screw cap. Label and submit.

Category: For internal use in the treatment of simple goiter. For example use an antiseptic.

Storage: Store in cool and dark place.

Direction: For internal use: Take as directed by physician. If used as antiseptic "For external use only" should be mentioned on label.

Marketed products: Lugol‟s Solution (Xenex Laboratories),Lugol‟s iodine solution (Art Pharmaceutical Ltd.), Lugol‟s iodine 5% Solution (Sisco Research Laboratories Pvt.Ltd),Solution Aqueous iodine 20 mL (Agarwal Pharmaceuticals).

Report:

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,A practical hand book of pharmaceutics –I, Niraliprakashan , Page No: 41-4.

SUSPENSIONS

EX NO **DATE:**

CALAMINE LOTION I.P. 1966

Aim: Prepare and submit Calamine Lotion I.P. 1966, 20mL.

Requirements;

Apparatus: Weighing balance, beaker, stirrer, measuring cylinder, mortar and pestle, etc.

Chemicals: Calamine, zinc oxide, bentonite, sodium citrate, liquefied phenol, glycerin, rose water.

Formula:

Ingredient	**Standard Formula**	**Working formula**	**Role of Ingredient**
Calamine	150 g	3 g	Astringent
Zinc oxide	50 g	1 g	Protective
Bentonite	30 g	0.6 g	Suspending agent
Sodium citrate	5 g	0.1 g	Chelating agent
Liquefied Phenol	5 mL	0.1 Ml	Antiseptic / preservative
Glycerin	50 mL	1 mL	Humectant
Rose water q.s. to produce	1000 mL	20 mL	Vehicle and flavouring agent

Theory:

Calamine is a basic zinc carbonate suitably colored with ferric acid. It is used as mild astringent. It is combination zinc oxide and small portion (0.5%) of ferric oxide (Fe_2O_3). Zinc oxide acts as astringent and ferric oxide acts as antipruritic (anti-itch). Calamine lotion is a liquid preparation meant for external application without friction. They are directly applied with the help of cotton. Calamine lotion falls in the category of antiseptic and protective.

Bentonite is a colloidal hydrated ,aluminium silicate and acts as a suspending agent which swells in presence of water and increases viscosity of preparation.Sodium citrare acts as chelating agent which forms complex with free iron of ferric oxide.In Calamine lotion is

stored in a well-closed container because zinc oxide gradually converts into zinc carbonate due to atmospheric carbon dioxide.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Prepare solution of sodium citrate with 50 % of total volume of water.
3. Triturate calamine, zinc oxide and bentonite with solution of sodium citrate in mortar to form a smooth cream.
4. Add liquid phenol, glycerin and some water to it with continuous stirring to make preparation in pourable form.
5. Pour this preparation in measuring cylinder and make-up the volume up to 20 ml with remaining quantity of water.
6. Transfer this lotion narrow mouth, coloured plastic container. Close it tightly with screw cap. Label and submit.

Category: Protective and astringent.

Storage: Store in well closed container in cool place.

Direction: Apply to affected part without rubbing whenever necessary.

Auxiliary label: "For external use only"

"Shake well before use"

Marketed products: Aquaminol (Skin) (Pasteur Laboratories Pvt Ltd), Calamine IP (Arora Pharmaceuticals Pvt Ltd.), Calak (Shalaks Pharmaceuticals Pvt Ltd.), Calosoft (Micro Gratia), Dermocalm (GSK).

Report:

EXPERIMENT NO 11 **DATE:**

MAGNESIUM HYDROXIDE

MIXTUREB.P. 1993

Aim: Prepare and submitMagnesium Hydroxide Mixture B.P. 1993,20 ml.

Requirements
Apparatus: Weighing balance, beaker, stirrer, measuring cylinder, mortar and pestle etc.

Chemicals: Magnesium sulphate, sodium hydroxide, light magnesium oxide, chloroform, citric acid, purified water.

Formula:

Ingredient	**Standard formula**	**Working formula**	**Role of Ingredient**
Magnesium sulphate	47.5 g	0.95 g	To prepare magnesium hydroxid by precipitation and hydratio reaction
Sodium hydroxide	15 g	0.3 g	
Light magnesium oxide	52.5 g	1.05 g	
Chloroform	2.5 ml	0.05 ml	Preservative
Citric acid	0.01 g	0.002 g	Flavouring agent
Purified water q.s. to produce	1000 ml	20 ml	Vehicle

Theory

Magnesium hydroxide mixture is an aqueous oral suspension of hydrated magnesium oxide. It is also called as Milk of Magnesia or Cream of Magnesia or Magnesium Hydroxide Oral Suspension. Milk of Magnesium is used for its antacid and laxative properties. Magnesium hydroxide is prepared during preparation (in-situ) by precipitation reaction between magnesium sulphate and sodium hydroxide and by hydration of light magnesium oxide with water. Magnesium hydroxide is prepared by both precipitation and hydration mechanism to uniform distribution of prepared magnesium hydroxide in the suspension. In this preparation, large amount of water is used to get magnesium hydroxide of very fine particle size. The reactions of both these mechanisms are as follows

Preparation of magnesium hydroxide by precipitation:

$$MgSO_4 + 2NaOH \rightarrow Mg(OH)_2 + Na_2SO_4$$

Magnesium sulphate Sodium hydroxide Magnesium hydroxide Sodium sulphate

Preparation of magnesium hydroxide by hydration:

$$MgO + H_2O \rightarrow Mg(OH)_2$$

Light magnesium oxide Water Magnesium hydroxide

Upon formation of magnesium hydroxide and once it gets settled the mixture is filtered and washed with large amount of water to remove sulphate (SO4) ions of sodium sulphate formed during precipitation reaction from preparation because it may give purgative action.

Procedure

1. Clean all the glassware and dry them properly as per SOP.
   Dissolve required quantity of magnesium sulphate in 200 ml of water in one beaker.
2. Take required quantity of sodium hydroxide in small amount of water in mortar. Add light magnesium oxide to this solution and triturate to get smooth cream.
3. To the initially prepared magnesium sulphate solution pour dispersion of sodium hydroxide and light magnesium oxide with continuous stirring to form magnesium hydroxide.
4. Stand for some time to settle magnesium hydroxide.
5. Once magnesium hydroxide is settled, remove clear supernatant and wash precipitate of magnesium hydroxide repeatedly with water to remove sulphate.
6. Confirm removal of sulphate by carrying out sulphate test with barium chloride solution. (In brief, the test solution is acidified using a few drops of dilute hydrochloric acid, and then a few drops of barium chloride solution are added. A white precipitate of barium sulphate forms if sulphate ions are present).
7. Once filtrate pass sulphate test, mix washed precipitate with water and add required quantity of chloroform and citric acid and pour this dispersion in measuring cylinder.
8. Make-up the volume up to 20 ml using purified water and transfer suspension in narrow mouth glass or plastic container. Close thoroughly with metallic screw cap Label and submit.

Category: Antacid at lower dose (5 to 10 ml) and laxative in larger dose.

Storage: Store in well closed container in cool place or at room temperature not exceeding 25ºc.

Direction: Take dose as directed by physician.

Auxiliary Label:

"Shake well before use"

"Do not store in refrigerator"

Marketed products: ACME℠S Milk of Magnesia (Acme Laboratories Ltd.)

Report:

EX NO **DATE:**

ALUMINIUM HYDROXIDE GEL U.S.P. 2006

Aim: Prepare and submit Mixture of Aluminium Hydroxide Gel U.S.P. 2006, 20 ml.

Requirements

Apparatus: Weighing balance, beaker, stirrer, measuring cylinder, mortar and pestle, etc.

Chemicals: Aluminium hydroxide gel, sorbitol, methyl paraben, propyl paraben, alcohol purified water.

Formula:

Ingredient	Standard formula	Working formula	Role of Ingredient
Aluminium hydroxide gel	36 g	7.2 g	Antacid
Sorbitol	7 g	1.4	Sweetening agent
Methyl paraben	0.2 g	0.04 g	Preservative
Propyl paraben	0.2 g	0.04 g	Preservative
Peppermint oil (q.s.)	0.005 ml	0.001 ml	Flavouring agent
Alcohol	1 ml	0.2 ml	Solvent for flavouring agent
Purified water q.s. to produce	100 ml	20 ml	Vehicle

Theory

Aluminium hydroxide is an inorganic salt used as an antacid. It is a basic compound that acts by neutralizing hydrochloric acid in gastric secretions. Subsequent increases in pH may inhibit the action of pepsin.Aluminium hydroxide gel $Al(OH)_3$ is prepared by dissolving separately 100 g potassium alum and 100 g sodium carbonate in 150 ml water in two different beakers. Both solutions are filtered and heated to boiling. With constant stirring hot solution of alum is poured into the hot solution of sodium carbonate (Na_2CO_3) and not vice- versa (If sodium carbonate solution is added to potash alum solution, then it is difficult to wash out the sulphate completely), additionally add 100 ml hot water. The formed precipitate of aluminium hydroxide is decanted and mixed with 200 ml hot water. The precipitated is decanted again and washed with hot water until the washings give no positive reaction for sulphate ions with barium chloride. Aluminium hydroxide is filtered and dried at a temperature not exceeding 40ºC yielding white, light, amorphous, odorless and tasteless powder, insoluble in water and in alcohol. Reaction between sodium carbonate and potassium alum is takes place as follows:

$3Na_2CO_3 + 2KAl(SO_4)_2 + 3H_20 \quad 3Na_2SO4 + K_2SO_4 + 2AI(OH)_3 + 3CO_2$

Sodium carbonate Potassium alum

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Weigh required of aluminium hydroxide gel, sorbitol and transfer to half the portion of water in mortar and triturate to form smooth dispersion.
3. Add some water and methyl paraben and propyl paraben with continuous stirring.
4. Add peppermint oil dissolved in required quantity of alcohol to above dispersion with continuous stirring.
5. Pour formed suspension in measuring cylinder and make-up the volume of to 20 ml using purified water
6. Transfer this suspension to narrow mouth plastic container. Close thoroughly with metallic screw cap. Label and submit.

Category: Antacid

Storage: Store in well closed container in cool place.

Direction: As directed by physician.

Auxiliary Label: "Shake well before use".

Marketed products: Alternagel® (Johnson and Johnson), Acigon (NICHOLAS PIRAMAL), Alucinol (FRANCO-INDIAN), ENCID-MPS (H.L. HEALTHCARE).

Report:

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,A practical hand book of pharmaceutics –I, Niraliprakashan , Page No: 5.1-5.13

EMULSION

EXP NO **DATE:**

TURPENTINE LINIMENT I.P. 1966

Aim: Prepare and submit Turpentine Liniment I.P. 1966, 20 ml

Requirements:

Apparatus: Weighing balance, beaker, stirrer, measuring cylinder, mortar and pestle etc.

Chemicals: Soft soap, camphor, turpentine oil and purified water.

Formula:

Ingredient	**Standard formula**	**Working formula**	**Role of Ingredient**
Soft soap	90 g	1.8 g	Emulsifying agent
Camphor	50 g	1 g	Mild analgesic and rubefacient
Turpentine oil	690 ml	13.8 ml	Counter irritant and rubefacient
Purified water q.s. to	1000 ml	20 ml	Dispersion medium

Theory

Liniments are alcohol-or-oil-based solutions/emulsions that are applied externally to unbroken skin with gentle rubbing. Liniments with an alcoholic or hydro-alcoholic vehicle are useful when rubefacient, counterirritant, or penetrating action is desired. Turpentine oil (also called spirit of turpentine, oil of turpentine and wood turpentine) is a fluid obtained by the distillation of resin obtained from live trees, mainly pines. Turpentine liniment is o/w type of emulsion in which turpentine oil is dispersed in water. As Camphor is not soluble in water, but it is soluble in turpentine oil hence its gets dissolved.All liniments should bear a label indicating that they are suitable „Only for external use‟ and must „Never be taken internally‟. Preparations should be labelled shake well, before use liniments should be stored in air tight containers.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Weigh required quantity of camphor and transfer to measured quantity of turpentine oil in a beaker.
3. Transfer required quantity of soft soap in mortar followed by addition of some water to it and triturate to form soap solution.
4. To soap solution add camphor turpentine oil solution drop wise with trituration until thick creamy emulsion is formed.
5. Add further quantity of water to it. Transfer contents to measuring cylinder and make-up the volume up to 20 ml using purified water.
6. Transfer prepared liniment into narrow mouth amber glass bottle and close tightly with plastic screw cap because both camphor and turpentine oil are volatile in nature. Label and submit.

Category: Counter irritant and rubefacient.

Storage: Store in tightly closed container in dry place, protect from sunlight.

Direction: To be rubbed on affected parts of body if pain is severe.

Auxiliary Label: "For external use only"

"Not to be applied on broken skin"

"Shake well before use"

Marketed products: Turpentine Liniment (Glister Pharmaceuticals), Turpentine Liniment BP (Garima Healthcare Pvt ltd.), Turpentine Liniment 100 ml (Deepti Pharmaceuticals).

Report:

Experiment No. 14 **Date:**

Liquid Paraffin Emulsion B.P.

Aim: Prepare and submit Liquid paraffin emulsion B.P. 20 ml

Requirements:

Apparatus: Weighing balance, beaker, stirrer, measuring cylinder, mortar and pestle etc.

Chemicals: Liquid paraffin, methyl cellulose 20, benzoic acid solution, chloroform, vanillin, saccharin sodium and purified water.

Formula:

Ingredient	Standard formula	Working formula	Role of Ingredient
Liquid paraffin	50 ml	10 ml	Laxative
Methyl cellulose	2 g	0.4 g	Emulsifying agent
Benzoic acid solution	2 ml	0.4 ml	Preservative
chloroform	0.25 ml	0.05 ml	Preservative
vanillin	50 mg	10 mg	Flavouring agent
Saccharin sodium	5 mg	1 mg	Sweetening agent
Purified water q.s. to	100 ml	20 ml	Dispersion medium

Theory:

Liquid paraffin mineral oil which is a transparent, colourless, odourless, or almost odourless, only liquid composed of saturated hydrocarbons obtained from petroleum. Liquid paraffin emulsion is o/w type of emulsion is used as lubricant laxative for chronic constipation. It acts by softening and lubricating thee stools. This helps the stools to move more easily through the bowel. It relieves constipation, making stools easier to pass. Can also be used to reduce pain associated with passing tools in people with conditions affecting the anus, such as piles (haemorrhoids) or anal fissure. This emulsion is prepared by making methyl cellulose mucilage solution first followed by mixing with equal volume of liquid paraffin.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. First prepare mucilage by mixing the required quantity of methylcellulose 20 in about six times its weight of boiling water and allow stand for 30 minutes to hydrate. Add an

equal weight of ice and stir mechanically until homogenous mucilage is formed.

3. Dissolve vanillin in the benzoic acid solution and chloroform. Add this mixture to above mucilage and stir for 5 minutes.
4. Prepare saccharin sodium solution by triturating it water and add this solution to above mucilage.
5. Make the volume of the mucilage up to 10 ml, taking care to ensure that there is no entrapped air in the mucilage.
6. Make the emulsion by mixing together 10 ml of liquid paraffin and 10 ml of the prepared mucilage with constant stirring.
7. As liquid paraffin emulsion is very coarse in nature, the emulsion is more stable if passed through a hand homogenizer.
8. Transfer prepared emulsion into narrow mouth amber coloured bottle and close tightly with child resistant closure. Label and Submit.

Category: As a lubricant laxative for chronic constipation.

Storage: Store in a cool, dry place. Do not allow to freezer.

Direction: One 15 ml to be taken twice a day or as directed by physician.

Auxiliary Label: "Shake well before use". The emulsion should not be taken within 30 minutes of meal times and preferably on an empty stomach.

Marketed products: LAXIMA Oral Emulsion (Silicon Pharma.), DUOLAXIN (Glenmark Pharmaceuticals Ltd.), Magfin® (Incepta Pharmaceuticals Limited). These marketed products of emulsion contain liquid paraffin in combination with magnesium hydroxide.

Report:

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,A practical hand book of pharmaceutics –I, Niraliprakashan , Page No: 61-6.12

POWDERS AND GRANULES

EX NO **DATE**

ORS POWDER (WHO) I.P. 2007

Aim: Prepare and submit Oral Rehydration Salt (ORS) powder I.P. 2007,(WHO2005) for oral rehydration solution 1L.

Requirements:

Apparatus: weighing balance, mortar and pestle, etc

Chemicals: Sodium chloride, glucose, anhydrous, potassium chloride, trisodium citrate, dihydrate.

Formula:

Ingredient	Standard formula	Working formula	Role of Ingredient
Sodium chloride	12.683%	2.6 g/L	Sodium supplement
Glucose, anhydrous	65.854%	13.5 g/L	Facilitate absorption of sodium
Potassium chloride	7.317%	1.5 g/L	Potassium supplement
Trisodium citrate, dihydrate	14.146%	2.9 g/L	Corrects the acidosis

Theory:

Administration of fluid and electrolytes by mouth to prevent or treat dehydration due to accurate diarrhea diseases is known as oral rehydration therapy. Acute diarrhea leads to loss essential water and salts unless these are adequately replaced, dehydration will develop. Oral rehydration therapy does not stop diarrhea but the diarrhea continues for only a limited time. The universal oral rehydration salts are recommended by the WHO (World Health Organization) and UNICEF (The United Nations Children‟s Fund is a United Nations programme) as prescribed in this experiment.

ORS is dry mixture of sodium chloride, potassium chloride, glucose, anhydrous and trisodium citrate, dehydrate or sodium bicarbonate. Glucose facilitates the absorption of sodium (and hence water) on a 1:1 molar basis in the small intestine; sodium and potassium

are needed to replace the body losses of these essential on ions during diarrhoea (and vomiting); citrate corrects the acidosis that occurs because of diarrhoea and dehydration.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Separately weigh each ingredient properly.
3. Incorporate the powders in order of bulk starting with potassium chloride, followed by trisodium citrate, dehydrate, then sodium chloride and finally add anhydrous glucose in portions adding at each addition a quantity that approximately doubles the bulk already in the mortar.
4. Triturate powder mixture properly.
5. Transfer the powder into the packet/sachet. Label and submit.

Category: Oral rehydration therapy in diarrhea.

Storage: Store in cool and dry place.

Marketed products: Oral Rehydration Salts-Citrate I.P. (Micron Pharmaceuticals), ELECTRAL (FDC Limited), PUNARJAL (FDC Limited).

Report:

EX NO **DATE**

EFFERVESCENT GRANULES

Aim: Prepare and submit Sodium Phosphate Effervescent Granules USP 20 g.

Requirements:

Apparatus: Weighing balance, porcelain dish, stirrer, water bath, mortar and pestle, etc.

Chemicals: Sodium phosphate (dried and powdered), sodium bicarbonate, citric acid, tartaric acid.

Formula:

Ingredient	**Standard formula**	**Working formula**	**Role of Ingredient**
Sodium phosphate (dry powder)	200 g	5 g	Cathartic
Sodium bicarbonate	477 g	11.925 g	Form effervescence in water
Citric acid	162 g	4.05 g	
Tartaric acid	252 g	6.3 g	

Theory:

Sodium phosphate is delivered in the form of effervescent granules in this formulation; because effervescence formed by sodium bicarbonate, citric acid and tartaric acid when comes in contact with water mask unpleasant saline test of sodium bicarbonate. As granules have large particle size than powders, less effective surface area is available for water and will effervescent slowly and in controlled manner.

Generally; effervescent formulations are prepared using combinations of citric acid and tartaric acid.Combination will give a balance and obtain better granulation mixture. Following reactions takes place between sodium bicarbonate, citric acid and tartaric acid in presence of water which liberates carbon dioxide causes effervescences.

Citric acid sodium bicarbonate sodium citrate water carbon dioxide(effervescence).

$H_2C_4H_4O_6 + 2\ NaHCO_3 \rightarrow Na_2C_4H_4O_6 + 2H_2O + 2CO_2$

Tartaric acid sodium bicarbonate sodium tartrate water carbon dioxide

Effervescent granules are generally prepared by two methods:

(a) Dry or Fusion method
(b) Wet method

(a) Dry or fusion method

Tartaric acid is anhydrous but citric acid used in this preparation has one molecule of water of crystallization which liberates upon heating. This liberated water act as granulating fluid for making granules. Hence while preparing effervescent granules by this method all powdered ingredients are mixed in preheated porcelain dish. Use of preheated porcelain dish is most important while preparing this formulation because heating after mixing of all powdered ingredient may cause evaporation of liberated water of crystallization from citric acid and will not be available for granulation as granulating agent.In this method loss of weight of material due to liberation of carbon dioxide and evaporation of moisture from damp mass of powder should be considered.

(b) Wet method

Wet method differs from the fusion method. In this method the source of binding agent is not necessarily the water of crystallization from citric acid but may be water added to the non-solvent such as alcohol which is employed as the moistening agent to form damp mass of material for granulation.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Weigh accurately all ingredients and mix in ascending order of their weights by trituration.
3. Place porcelain dish on water bath and heat water bath till boiling the water on water bath.
4. Place powder mixture in pre-heated porcelain dish and mix it thoroughly to form damp or coherent mass of powder.

5. Pass this coherent mass of powder through sieve 20 by pressing and collect the wet granules on butter paper.
6. Dry these wet granules in hot air oven at temperature not exceeding 60ºC.
7. Fill granules into air tight wide mouth container. Label and submit.

Category: Cathartic (Purgative)

Storage: Store in dry place.

Direction: Add two spoonfuls of granules to a glass of water with stirring and drink the solution while effervescing.

Marketed products: Effervescent Sodium Phosphate USP (Mckesson and Robbins), Addphos (Steadfast MediShieldPvt. Ltd.)

Report:

EX NO **DATE**

DUSTING POWDER

Aim: Prepare and submit Zinc Oxide – Salicylic Acid Dusting Powder 20 g.

Requirements

Apparatus: weighting balance, mortar and pestle, beaker etc.

Chemicals: Zinc oxide, salicylic acid, starch powder.

Formula:

Ingredient	Standard formula	Working formula	Role of Ingredient
Zinc oxide	20 g	5 g	Astringent
Salicylic acid	2 g	0.5 g	Local antiseptic
Starch powder	78 g	19.5 g	Absorbency and covering

Theory:

Dusting powders are externally used bulky powders for local application not intended for systemic action. They are free flowing very fine powders containing antiseptic, antipruritics, astringents, antiperspirants, absorbents, lubricants etc. dusting powders are two types: a) Medical dusting powder and b) Surgical dusting powder.

Medical dusting powders are used mainly for superficial skin conditions,whereas surgical dusting powder is used in body cavities and on major wounds because of burns and umbilical cords of infants. Surgical dusting powders must be sterilized before their use, whereas medical dusting powders must be free from pathogenic micro-organisms.The dusting powders are dispensed in sifter-top containers or aerosol containers. Zinc oxide – Salicylic acid dusting powder is medicated dusting powder and intended for application to open wound. Zinc oxide in this preparation acts as astringent and salicylic acid acts as local antiseptic starch has very good absorbency power which absorb watery portion of wound and get struck on affected area for longer period.

Dusting powder is passed through 80 mesh sieve because small particles are less likely to irritate the affected area.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Mix salicylic acid with zinc oxide followed by starch powder by light trituration in mortar.
3. Mix above powder and pass through sieve 80.
4. Weigh required quantity of powder and fill into a screw cap wide mouthed glass bottle or self dispensing plastic container. Label and submit.

Category: Astringent and local antiseptic

Storage: Store in well closed container

Auxiliary label:"FOR EXTERNAL USE ONLY".

Marketed products: Betzee-S (Apex Laboratories Limited), Funginil (Unicure (India) Pvt. Ltd.), Zincoderm-S (Apex Laboratories Limited).

Report:

EX NO **DATE**

DIVIDED POWDER

Aim: Prepare and submit 3 packets of divided powder each weighing 600 mg of Aspirin Powder.

Requirements:

Apparatus: Weighing balance, mortar and pestle etc.

Chemicals: Aspirin.
Formula:

Ingredient	Standard formula	Working formula	Role of ingredient
Aspirin	600 mg	2.4 g	Analgesic and Antipyretic

Theory

Divided powders (or charta) are single doses of powdered drugs individually wrapped in cellophane, metallic foil, or paper.

(a) Simple powder: It consists of only one active ingredient and suitable inert substances. If powder is in crystalline form, then it is reduced to fine.
For example: Aspirin, Calcium Gluconate Powder etc.

(b) Compound powder: It consists of mixture of more than one active ingredient and other constituents.
For example: Aspirin, Paracetamol and Caffeine Powder.

(c) Powders enclosed in cachet: Cachets consists of a dry powder enclosed in a shell, usually prepared from a mixture of rice flour and water by moulding into a suitable shape and drying. For example: Sodium Amino Salicylate cachets, Sodium Amino Salicylate with Isoniazid Cachets.

(d) Tablet triturates: Tablet triturates are powders moulded into tablets. Tablet triturates are generally prepared by mixing the active drug with lactose, dextrose, sucrose, mannitol, or some other appropriate diluent that can serve as the base. For example: Propranolol Scopolamine Tablet Triturate

Aspirin i.e. acetyl salicylic acid is non-steroidal anti-inflammatory drug (NSAID) used as analgesic and antipyretic. Aspirin is crystalline drug and hence need to be finely divided before packaging. As aspirin has large dose; this divided powder is dispensed as it is without adding

any diluents and other additives. Aspirin divided powder is simple divided powder intended for internal use.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Weigh accurately aspirin and triturate in dry mortar.
3. Divide this finely divided powder into 3 equal parts each containing 600 mg of aspirin on butter paper.
4. Fold butter paper as per folding procedure generally used for pharmaceutical powders.
5. Transfer these folded packets into suitable container or envelop. Label and submit.

Category: Analgesic and Antipyretic

Storage: Store in dry place.

Direction: Take one packet as directed by physician.

Marketed products:BC®powder for fast pain relief.

Report:

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,A practical hand book of pharmaceutics –I, Niraliprakashan , Page No: 7.1-713

SUPPOSITORIES

EX NO **DATE**

GLYCEROGELATIN SUPPOSITORY

Aim: Prepare and submit GlyceroGelatinsuppository of 1g capacity,quantity 6.

Requirements:

Apparatus: Weighing balance, beaker, stirrer, measuring cylinder, pipette, porcelain dish, water bath etc.

Chemicals: Glycerin, gelatine and purified water.

Formula:

Ingredient	**BP and EP**	**USP**	**BPC**
Glycerin	20g	2.4g	Laxative
Gelatin	70ml	84ml	Stiffness
Purified water q.s to produce	100ml	12ml	Vehicle

Theory:

Glycero- gelatine base is mixture of glycerine and water is made thick/ a stiff jelly by addition of gelatine. The base dose not melt at body temperature, but rather dissolve in the secretions of the cavity in which they are inserted. Solution time is regulated by proportion of gelatine: glycerin: water used, the nature of gelatine used, and the chemical reaction of the drug with gelatine.

Procedure:

1. Clean all the glassware and dry them properly as per SOP
2. Weigh required quantity of gelatine and soak it in enough water until thoroughly softened.

3. Take calculated amount of glycerine in a tarred porcelain dish and heat on water bath maintained at 100°C temperature.
4. Put soaked gelatine to hot glycerine to dissolve gelatine to exhibit a constant weight (During evaporation the liquid mass should be gently stirred. Rapid stirring produce air bubbles which may appear in the finished suppositories).
5. Remove any skin formed on the surface before pouring.
6. Pour the mass while it is hot, into cavities of suppository mould which is chilled and are pre-lubricated with liquid paraffin. (Do not let the melted mass to overflow as glycerol- gelatin base contract very less on cooling compared to cocoa butter base).

Category: Glycero- gelatine suppository is used as laxative in paediatrics during constipation.

Storage: Store in cool place.

Auxiliary label: For rectal use only.

Direction: Insert one suppository as directed by the physician.

Marketed products: GLAX-1: Glycerin suppositories USP 1 g(Galan pharmaceutical Ltd), Glycerin suppositories(Mastrowin life line Pvt.Ltd, Oregon pharma services Pvt.Ltd) Glycerin suppositories USP XVI (Bhartia Pharmaceutical).

Report:

EX NO **DATE**

Cocoa butter suppository

Aim: Prepare and submit Boric acid suppository of 1 g capacity containing cocoa butter as base, quantity 8.

Requirements:

Apparatus: Weighing balance, beaker, stirrer, measuring cylinder, pipette, porcelain dish, water bath etc

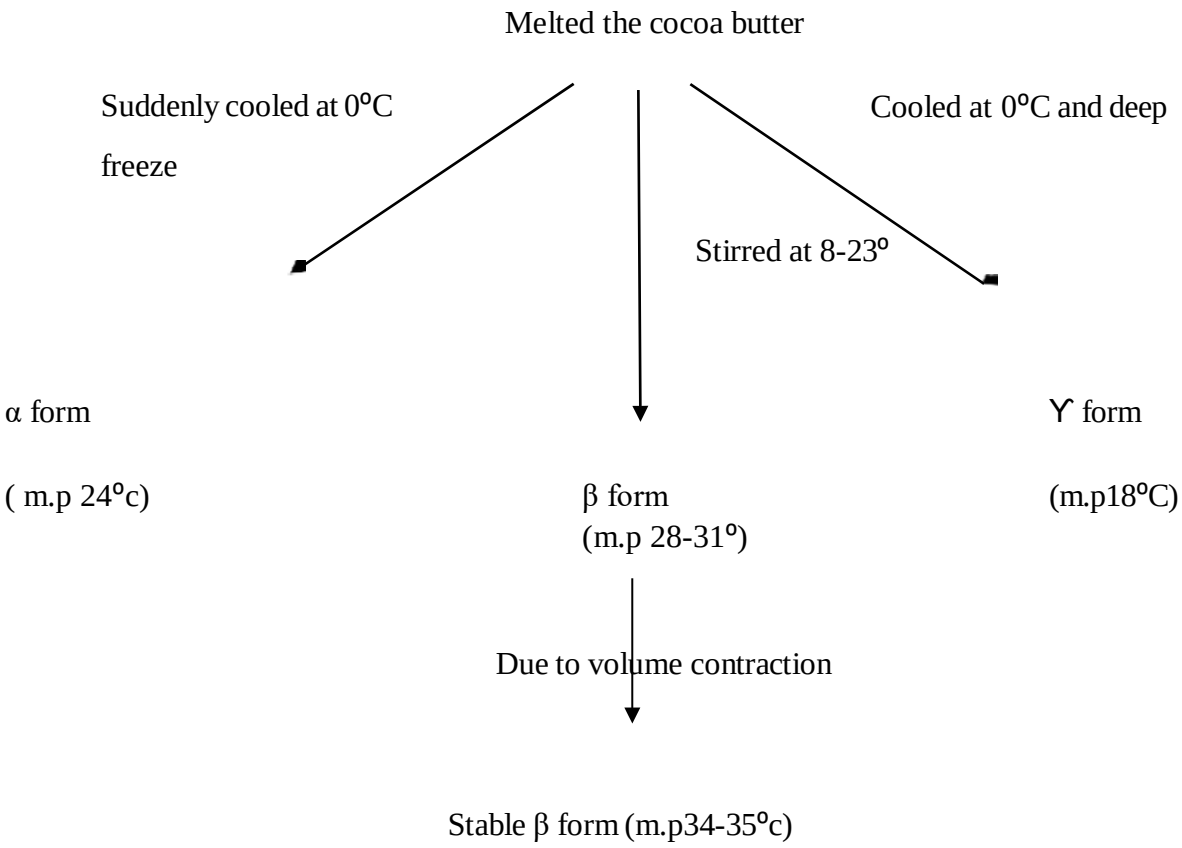
Chemicals: Boric acid, cocoa butter, liquid paraffin.

Formula:

Ingredient	Standard formula	Working formula	Role of Ingredient
Boric acid	120mg	1.2g	Local anti-infective
Cocoa butter q.s to produce	1g	9.2g	Suppository base

Theory:

- Boric acid suppository is example of cocoa butter suppository containing insoluble solid. Boric acid in this preparation is used as local anti-infective. As boric acid is insoluble in cocoa butter it is dispersed in molten cocoa butter by fusion method.
- Cocoa butter is a fat obtained from roasted seed of *Theobroma cacao* belonging to family *Sterculiaceae*. It is yellowish white solid which becomes white on storage. It has butter like consistency and chocolate like odour. Cocoa butter is widely used rectal suppository base because it is solid at room temperature but melts readily on contact with the skin. Its melting point is 30-36°C.



Procedure:

1. Wash and clean suppository mould using detergent (if required) and water. Lubricate it with liquid paraffin and place it in inverted position on ice to drain excess lubricant and to cool suppository moulds.
2. Weigh required quantity of cocoa butter by considering displacement value of boric acid and transfer to porcelain dish.
3. Heat porcelain dish on water bath until 2/3rd portion of cocoa butter melts. Avoid over heating of cocoa butter in porcelain dish.
4. Remove porcelain dish containing partly molten cocoa butter from water bath and stir thoroughly to melt remaining portion of cocoa butter.
5. Transfer required quantity of boric acid on warmed slab and pour half portion of molten cocoa butter on it and levigate it thoroughly to disperse boric acid in cocoa butter.
6. Pour this dispersion in cavities of suppository mould in excess quantity till overfills and place mould on ice for half an hour.
7. Trim or scarp excess quantity after solidification using knife or blade and open mould to remove s suppositories.
8. Wrap individual suppository in wax paper or butter paper and put into partitioned box

or wide mouth container. Label container properly and submit.

Category: Boric acid suppository is used as local anti – infective.

Storage : Store in cool place.

Auxiliary label: For rectal use only.

Direction: Insert one suppository as directed by physician.

Marketed products: Boric -cap®:Boric Acid vaginal suppositories (KE Health llc),Ph-D ®: Boric acid vaginal suppositories(Feminine Health Support)

Report:

EX NO **DATE**

ZINC OXIDE SUPPOSITORY

Aim: Prepare and submit Zinc oxide suppositories of 1 g capacity containing cocoa butter as base, quantity 8.

Requirements:

Apparatus: Weighing balance, beaker,stirrer, measuring cylinder, pipette, porcelain dish, water bath, etc.

Chemicals: Zinc liquid paraffin oxide, cocoa butter.

Formula:

Ingredients	Standard formula	Working formula	Role of ingredients
Zinc oxide	400mg	4g	Local anti-infective
Cocoa butter q.s. to produce	1g	9.2g	Suppository base

Theory:

Zinc oxide suppository is used as an antiseptic, astringent and skin protectant. it is used to temporarily relieve burning, pain, and itching caused by haemorrhoids. It forms a protective barrier to prevent too much irritating contact with stool. This barrier helps protect inflamed, irritated skin and helps to make bowel movements less painful, they are effective in providing temporary relief from itching irritation and burning.

Procedure:

1. Wash and clean the suppository mould using detergent (if required) and water. Lubricate it with liquid paraffin and place it in inverted position on ice to drain excess lubricant to cool suppository mould.
2. Weigh required quantity of cocoa butter by considering displacement value of zinc oxide and transfer to porcelain dish.
3. Heat porcelain dish on water bath until 2/3rd portion of cocoa butter melts. Avoid over heating of cocoa butter in porcelain dish.

4. Remove porcelain dish containing partly molten cocoa butter from water bath and stir thoroughly to melt remaining portion of cocoa butter.
5. Transfer required quantity of zinc oxide on warmed slab and pour half portion of molten cocoa butter on it levigate it thoroughly to disperse zinc oxide in cocoa butter.
6. Pour this dispersion in cavities of suppository mould in excess quantity till overfills and place mould on ice for half an hour.
7. Trim or scrap excess quantity after solidification using knife or blade and open mould to remove suppositories.
8. Wrap individual suppository is used in wax paper or butter paper and put into partitioned box wide mouth container. Label container properly and submit.

Category: Zinc oxide suppository is used as an antiseptic, astringent and skin protectant.

Storage: Store in cool place.

Auxiliary label: For rectal use only.

Direction: Asdirected by physician. Insert one suppository.

Marketed products: Calmol 4 (ResiCalinc), Corect(Meridian enterprises Pvt.Ltd.)

Report:

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,A practical hand book of pharmaceutics –I, Niraliprakashan , Page No: 8.1-8.13

SEMISOLIDS

EX NO **DATE**

SULPHUR OINTMENT I.P.1996

Aim: Prepare and submit Sulphur ointment I.P.1996,20g

Requirments:

Apparatus: Weighing balance, porcelain dish, water bath, ointment slab, ointment spatula etc

Chemicals: Sulphur (precipitatied), wool fat, hard paraffin, cetostearyl alcohol, white soft paraffin.

Formula:

For simple ointment

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Wool fat	5g	1.25g	o/w emulsifying agent
Hard paraffin	5g	1.25g	Stiffening agent
Cetostearyl alcohol	5g	1.25g	Improve stability
White soft paraffin	85g	21.25g	Emollient

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Sulphur(Precipitated)	10g	2.2g	Antiseptic,parasitic and scabicide
Simple ointment	90g	19.8g	Ointment base

Ointments are homogenous, semi-solid preparations intended for external application to the skin or mucous membranes. They are used as emollients or for the application of active ingredients to the skin for protective, therapeutic, or prophylactic purposes and where a degree of occlusion is desired.Sulphur is used for several conditions and available in the form

of ointment, Cream and lotions. Sulphur ointments has antiseptic, parasitic and scabicide activity and is used in treatments of scabies and acne.

Simple ointment is prepared by fusion method.It is absorption base composed of wool fat, hard paraffin, cetostearyl alcohol and yellow or white soft paraffin. Wool fat in simple ointment act as o/w type of emulsifying agent. Hard paraffin act as stiffening agent in simple ointment which stiffens ointment base. Cetrostearyl alcohol enhances stability of ointment and gives emollients effect. White soft paraffin exerts emollient effect and mask colour of sulphur in this preparation.

Procedure:

For preparation of simple ointment by fusion method

1. Clean all the glassware and dry them properly as per SOP.
2. Weigh all ingredients properly.
3. Melt hard paraffin and cetrostearyl alcohol in porcelain dish kept on water bath.
4. To above molten mixtures add wool fat and white soft paraffin and stir it well.
5. After melting all ingredients remove porcelain dish from water bath and stir it continuously until semisolid base in obtained.
6. Use this base for preparation of sulphur ointment.

For preparation of sulphur ointment by levigation

1. Weigh required quantity of sulphur and simple ointment properly.
2. Place sulphur on one part of clean ointment slab and simple ointment on other part of ointment slab.
3. Take small quantity of sulphur and about three times more volume of simple ointment at the center of ointment slab and levigate using ointment spatula until sulphur is mixed(geometric mixing) thoroughly in taken volume of simple ointment. Continue this process until all quantity of sulphur is mixed with simple ointment so as to get homogenous and amooth ointment.
4. Transfer required quantity of sulphur ointment in wide mouth light resistant container or collapsible tube. Lable and submit.

Category: In treatment of scabies and acne.

Storage: Store in cool and dark place.

Direction: Apply on affected area as directed by physician

Auxiliary label: For external use only

Marketed products: San Marcos Sulphur Ointments (Brannfels Lab),Sulphur Ointment USP (Anhui Medipharm Co. Ltd. China)

Report:

EX NO **DATE**

Non staining – iodine ointment with methyl salicylate BPC

Aim: Prepare and submit Non staining – iodine ointment with methyl salicylate BPC 10g

Requirements:

Apparatus: Weighing balance, beaker, stirrer, glass mortar and pestle, glass stopper bottle, porcelain dish ,water bath etc.

Chemicals: Iodine, methyl salicylate, oleic acid, arachis oil, yellow soft paraffin.

Formula

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Iodine	1.5g	0.48g	Counter irritant
Mehyl salicylate	1.5g	0.48g	Anti-inflammatory
Arachis oil	4.5g	1.8g	Source of unsaturated fatty acid
Yellow soft paraffin q.s. to	30g	12g	Ointment base

Theory:

Non staining – iodine ointment with methyl salicylate BPC is 5% iodine in yellow soft paraffin and arachis oil. This is example of ointments containing combined iodine and it is prepared by chemical reaction. This fixed oil and may fats obtained from vegetable and animal sources contain unsaturated constituents, These unsaturated fatty acids contain double and triple bond linkage. Arachis oil contain fatty acids such as oleic acid(46.8% as olein), linoleic acid (33.4% as linolein) and palmitic acid (10.0% as palmitin. In this preparation iodine is combined with double bonds of oleic acid (unsaturated fatty acid) and form di - iodo stearic acid(saturated fatty acid).Thus ,free iodine is not available. Since free iodine is not available, this ointment is dark, greenish black in colour. It does not leave stain when rubbed on to the skin. Hence it is called as non-staining iodine ointment.

$CH_3(CH_2)_7$-CH=CH-$(CH_2)_7$-COOH + I_2 ⟶ $CH_3(CH_2)_7$-CHI-CHI-$(CH_2)_7$-COOH

Oleic acid (unsaturated fatty acid)Iodine di-iodo stearic acid (saturated fatty acid)

Procedure:

1. Clean all the glassware and dry them properly as per SOP
2. Accurately weigh iodine and triturate in glass mortar.
3. Place triturated iodine to glass stopper bottle containing arachis oil.
4. Heat above mixture on water bath at 50^{o}C until colour changes from brown to greenish black.
5. Melt separately yellow soft paraffin and mix into above mixture with continuous stirring.
6. Add required quantity of methyl salicylate to above mentioned iodine ointment at room temperature and mix well.
7. Transfer ointment into wide mouth amber or greenish coloured glass containers and close with plastic screw cap, label and submit.

Category: Local analgesic and anti-inflammatory.

Storage: Store in cool and dry place.

Direction: Apply on affected area as per requirement.

Auxillary label: For external use only

Report:

Marketed products: Iodex®Methylsalicylate skin ointment(Lee pharmaceuticals),Medrum®(Medi products Pvt. Ltd)

EX NO **DATE**

CARBOPOL GEL

Aim: Prepare and submit Diclofenac Sodium gel 10g using Carbopol 934P

Requirments:

Apparatus: Weighing balance,beaker,stirrer etc.

Chemicals: Diclofenac sodium, carbopol 934P NF, isopropyl alcohol, Propylene glycol, butylated hydroxyl toluene, purified water.

Formula:

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Diclofenac sodium	1g	0.12g	Anti-inflammatory
Carbopol 934 P	4g	0.48g	Gelling agent
Isopropyl alcohol	5g	0.6g	Solvent for drug
Propylene glycol	5g	0.6g	Co-Solvent for drug
Butylated hydroxyl toluene (BHA)	0.1g	0.012g	Antioxidant
Purified water q.s. to produce	100g	12g	Solvent for gelling agent

Theroy:

Gels are usually homogenous, clear, semi-solid preparations consisting of a liquid phase within a three dimensional polymeric matrix with physical or sometimes chemical cross- linkage by means of suitable gelling agent. There are three methods of preparing gel namely; fusion method, cold method and dispersion method. Diclofenac gel using Carbopol 943P NF is prepared by dispersion method in which gelling agent is dispersed in water with stirring.

Drug is dissolved in another solvent with preservative. This solution is then added in above gel with continuous stirring.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Weight accurately diclofenac sodium and dissolve in 5g of isopropyl alcohol.
3. Add required quantity of propylene glycol with continuous stirring to above solution. Name this solution as Solution – I
4. In separate beaker take required quantity of Carbopol 934P NF and BHA dissolved in 9ml of water and stir continuously. Name this as Solution-II
5. Mix solution-I into Solution-II thoroughly with continuous stirring and make-up the final weight with water.
6. Transfer gel into plastic tube or wide mouth container, close with screw cap, lable and submit.

Category: Analgesic and anti-inflammatory.

Storage : Store in cool and dry place.

Direction : Apply on affected area as per requirement.

Auxiliary label: For external use only

Marketed products: Nacgel(Systopic), Naclo Gel (Sun pharma)

Report:

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,A practical hand book of pharmaceutics –I, Niraliprakashan , Page No: 91-9.12

GARGLES AND MOUTHWASHES

EX NO **DATE**

Povidone Iodine Gargle

Aim: Prepare and submit Povidone Iodine Gargles 100 ml

Requirments

Apparatus: Weighing balance, measuring cylinder, pipette, beaker, stirrer etc

Chemicals: Povidone iodine I.P, ethanol, glycerine, methyl salicylate, methnol, saccharin sodium, purified water etc.

Formula:

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Povidone iodine I.P (0.1% w/v available iodine)	1% w/v	1mL	Antiseptic
Ethanol	8.38% v/v	8.38mL	Solvent
Glycerin	4%	4mL	Make preparation viscous
Methyl salicylate	006%(q.s)	006mL	Local analgesic
Methnol	004% (q.s)	0.04mL	Local analgesic and flavouring agent
Saccharin sodium	0.1%	0.1g	Sweetening agent
Purified water q.s to produce	100%	100mL	Vehicle

Theory

Povidone-iodine is iodine based product; a complex of iodine and povidone which is used as an antiseptic solution. Povidone iodine gargle is clear red-brown solution. It ia used as antiseptic in the management of local infections of mouth and oroharynx is dental surgery.

Betadine gargle contains povidone-iodine,a complex of iodine which shows all the broad spectrum germicidal activity is maintained in the presence of blood, pus, serum and necrotic tissue. Betadine gargle kills bacteria, viruses, fungi, spores and protozoa. This preparation is contraindicated in patient with hypersensitivity to iodine, polyvinyl pyrrolidone or to any excipient. Betadine gargle can permanently discolour white gold jewellery and it is recommended that this type of jewellery should be removed before using Betadine gargle.

Procedure:

1. Clean all the glass wares and dry them properly as per SOP.
2. Dissolve required quantity of povidone iodine in some portion of purified water in one beaker. Add glycerine, methanol and saccharin sodium in it with continuous stirring.
3. Dissolve methyl salicylate in required quantity of ethanol in a separate beaker.
4. Mix both the solution and transfer into measuring cylinder.
5. Mix both volume up to 100 ml by addition of purified water.
6. Transfer solution in narrow mouth amber coloured bottle and close thoroughly with polypropylene screw cap. Label properly and submit.

Category: Antiseptic in the management of local infections of the mouth and oropharynx in dental surgery.

Storage: Store below 25^0C.Protect from light.

Direction: "Diluted with an equal volume of warm water"

"For external use only"

"Not to be swallowed in large amounts"

Marketed products: Arodin (Aristopharma Ltd), Betadine (Win medicare)

EX NO **DATE**

CHLORHEXIDINE MOUTHWASH

Aim: Prepare and submit Chlorhexidine Mouthwash 30Ml.

Requirements

Apparatus: Weighing balance, measuring cylinder, pipette, beaker, stirrer etc.

Chemicals: Chlorhexidine gluconate ,ethanol 96% v/v, glycerine, citric acid, sodium citrate, sodium metabisulfite, sodium benzoate, brilliant Blue, tartrazine yellow, mint flavour, Purified water.

FORMULA

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Chlorhexidine	0.21%	0.12g	Antiseptic,anti-plaque
Ethanol96% v/v	3.5%	3.5 ml	Solubilising agent
Glycerin	3.0%	3.0ml	Humectant
Citric acid	022%	0.22g	pH regulator
Sodium metabisulfite	0.15%	0.15g	Neutralizing the pigmentation activity of chlorhexidine
Sodium benzoate	0.1%	0.1g	Preservative
Brilliant blue and Tartarazine yellow	0.001%	0.001g	Colouring agent
Mint flavour	0.01%	0.01g	Flavouring agent
Purified water q.s. to produce	100%	100ml	Vehicle

Theory:

Chlorhexidine gluconate is a bisguanide antiseptic and disinfectant which is bactericidal or bacteriostatic against a wide range of gram negative and gram positive vegetative bacteria, yeast, dermatophyte fungi and lipophylic viruses. The antimicrobial activity covers most of the important species occurring in the oral micro flora. Chlorhexidine gluconate mouthwash is an antimicrobial solution which inhibits the formation of dental plaque. It is indicated as an

aid to the treatment and prevention of gingivitis and in the maintenance of oral hygiene, particularly in situations where tooth brushing cannot be adequately employed(for example, following oral surgery or in physically handicapped patients).In this preparation chlorhexidine gluconate is used as antiseptic and anti-plaque.

Procedure:

1. Clean all the glassware and dry them properly as per SOP.
2. Dissolve required quantity of chlorhexidine gluconate, citric acid and sodium citrate in some portion of purified water with continuous stirring until clear solution is not obtained. Add required quantity of ethanol to this solution.
3. Add measured quantity of glycerine, sodium metabisulfite, sodium benzoate, colouring agent and mint flavour gradually with continuous stirring.
4. Pour this solution to measuring cylinder. Make-up volume up to 100 ml using purified water.
5. Transfer prepared mouthwash to amber coloured bottle and close thoroughly with polypropylene screw cap. This cap is conical in shape and has “10ml” and a level line engraved on the inside. Label properly and submit.

Category: In treatment and prevention of gingivitis and in the maintenance of oral hygiene.

Storage: Store in cool and dry place.

Dose: 10 ml, twice a day.

For treatment and prevention, swish in your mouth for one minute. Do not rinse, eat or drink thereafter for 20 minutes. Recommended only under professional guidance.

Direction: “NOT TO BE SWALLOWED”,”FOR ORAL USE ONLY”.

Marketed products: Clohex (Dr Reddy Laboratories Ltd), Dentcare (Nova scotia)

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,A practical hand book of pharmaceutics –I, Niraliprakashan , Page No: 10.1-1

SUPPOSITORIES

EX NO **DATE**

GLYCEROGELATIN SUPPOSITORY

Aim: Prepare and submit GlyceroGelatinsuppository of 1g capacity,quantity 6.

Requirements:

Apparatus: Weighing balance, beaker, stirrer, measuring cylinder, pipette, porcelain dish, water bath etc.

Chemicals: Glycerin, gelatine and purified water.

Formula:

Ingredient	**BP and EP**	**USP**	**BPC**
Glycerin	20g	2.4g	Laxative
Gelatin	70ml	84ml	Stiffness
Purified water q.s to produce	100ml	12ml	Vehicle

Theory:

Glycero- gelatine base is mixture of glycerine and water is made thick/ a stiff jelly by addition of gelatine. The base dose not melt at body temperature, but rather dissolve in the secretions of the cavity in which they are inserted. Solution time is regulated by proportion of gelatine: glycerin: water used, the nature of gelatine used, and the chemical reaction of the drug with gelatine.

Procedure:

1. Clean all the glassware and dry them properly as per SOP
2. Weigh required quantity of gelatine and soak it in enough water until thoroughly softened.

3. Take calculated amount of glycerine in a tarred porcelain dish and heat on water bath maintained at 100°C temperature.
4. Put soaked gelatine to hot glycerine to dissolve gelatine to exhibit a constant weight (During evaporation the liquid mass should be gently stirred. Rapid stirring produce air bubbles which may appear in the finished suppositories).
5. Remove any skin formed on the surface before pouring.
6. Pour the mass while it is hot, into cavities of suppository mould which is chilled and are pre-lubricated with liquid paraffin. (Do not let the melted mass to overflow as glycerol- gelatin base contract very less on cooling compared to cocoa butter base).

Category: Glycero- gelatine suppository is used as laxative in paediatrics during constipation.

Storage: Store in cool place.

Auxiliary label: For rectal use only.

Direction: Insert one suppository as directed by the physician.

Marketed products: GLAX-1: Glycerin suppositories USP 1 g(Galan pharmaceutical Ltd), Glycerin suppositories(Mastrowin life line Pvt.Ltd, Oregon pharma services Pvt.Ltd) Glycerin suppositories USP XVI (Bhartia Pharmaceutical).

Report:

EX NO **DATE**

COCOA BUTTER SUPPOSITORY

Aim: Prepare and submit Boric acid suppository of 1 g capacity containing cocoa butter as base, quantity 8.

Requirements:

Apparatus: Weighing balance, beaker, stirrer, measuring cylinder, pipette, porcelain dish, water bath etc

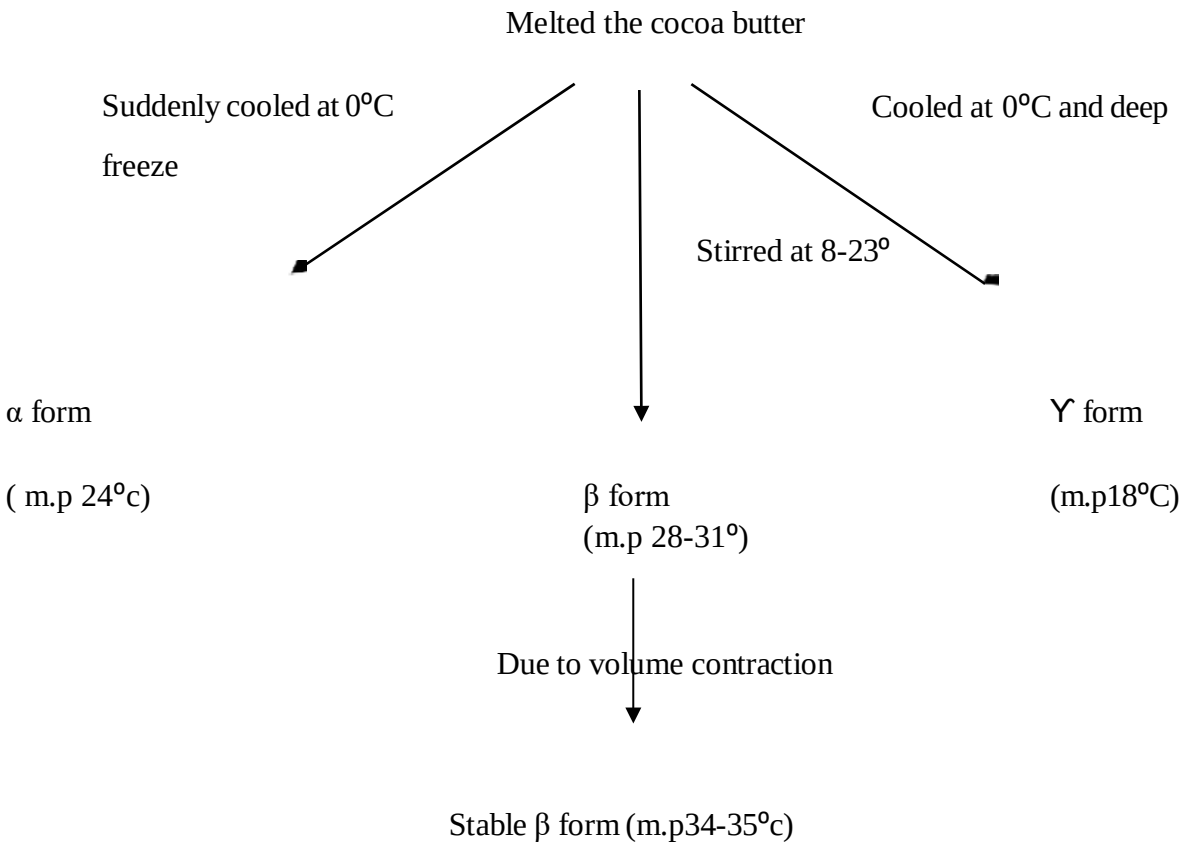
Chemicals: Boric acid, cocoa butter, liquid paraffin.

Formula:

Ingredient	**Standard formula**	**Working formula**	**Role of Ingredient**
Boric acid	120mg	1.2g	Local anti-infective
Cocoa butter q.s to produce	1g	9.2g	Suppository base

Theory:

- Boric acid suppository is example of cocoa butter suppository containing insoluble solid. Boric acid in this preparation is used as local anti-infective. As boric acid is insoluble in cocoa butter it is dispersed in molten cocoa butter by fusion method.
- Cocoa butter is a fat obtained from roasted seed of *Theobroma cacao* belonging to family *Sterculiaceae*. It is yellowish white solid which becomes white on storage. It has butter like consistency and chocolate like odour. Cocoa butter is widely used rectal suppository base because it is solid at room temperature but melts readily on contact with the skin. Its melting point is 30-36°C.



Procedure:

9. Wash and clean suppository mould using detergent (if required) and water. Lubricate it with liquid paraffin and place it in inverted position on ice to drain excess lubricant and to cool suppository moulds.
10. Weigh required quantity of cocoa butter by considering displacement value of boric acid and transfer to porcelain dish.
11. Heat porcelain dish on water bath until 2/3rd portion of cocoa butter melts. Avoid over heating of cocoa butter in porcelain dish.
12. Remove porcelain dish containing partly molten cocoa butter from water bath and stir thoroughly to melt remaining portion of cocoa butter.
13. Transfer required quantity of boric acid on warmed slab and pour half portion of molten cocoa butter on it and levigate it thoroughly to disperse boric acid in cocoa butter.
14. Pour this dispersion in cavities of suppository mould in excess quantity till overfills and place mould on ice for half an hour.
15. Trim or scarp excess quantity after solidification using knife or blade and open mould to remove s suppositories.
16. Wrap individual suppository in wax paper or butter paper and put into partitioned box

or wide mouth container. Label container properly and submit.

Category: Boric acid suppository is used as local anti – infective.

Storage : Store in cool place.

Auxiliary label: For rectal use only.

Direction: Insert one suppository as directed by physician.

Marketed products: Boric -cap®:Boric Acid vaginal suppositories (KE Health llc),Ph-D ®: Boric acid vaginal suppositories(Feminine Health Support)

Report:

EX NO **DATE**

ZINC OXIDE SUPPOSITORY

Aim: Prepare and submit Zinc oxide suppositories of 1 g capacity containing cocoa butter as base, quantity 8.

Requirements:

Apparatus: Weighing balance, beaker,stirrer, measuring cylinder, pipette, porcelain dish, water bath, etc.

Chemicals: Zinc liquid paraffin oxide, cocoa butter.

Formula:

Ingredients	Standard formula	Working formula	Role of ingredients
Zinc oxide	400mg	4g	Local anti-infective
Cocoa butter q.s. to produce	1g	9.2g	Suppository base

Theory:

Zinc oxide suppository is used as an antiseptic, astringent and skin protectant. it is used to temporarily relieve burning, pain, and itching caused by haemorrhoids. It forms a protective barrier to prevent too much irritating contact with stool. This barrier helps protect inflamed, irritated skin and helps to make bowel movements less painful, they are effective in providing temporary relief from itching irritation and burning.

Procedure:

1. Wash and clean the suppository mould using detergent (if required) and water. Lubricate it with liquid paraffin and place it in inverted position on ice to drain excess lubricant to cool suppository mould.
2. Weigh required quantity of cocoa butter by considering displacement value of zinc oxide and transfer to porcelain dish.
3. Heat porcelain dish on water bath until 2/3rd portion of cocoa butter melts. Avoid over heating of cocoa butter in porcelain dish.

4. Remove porcelain dish containing partly molten cocoa butter from water bath and stir thoroughly to melt remaining portion of cocoa butter.
5. Transfer required quantity of zinc oxide on warmed slab and pour half portion of molten cocoa butter on it levigate it thoroughly to disperse zinc oxide in cocoa butter.
6. Pour this dispersion in cavities of suppository mould in excess quantity till overfills and place mould on ice for half an hour.
7. Trim or scrap excess quantity after solidification using knife or blade and open mould to remove suppositories.
8. Wrap individual suppository is used in wax paper or butter paper and put into partitioned box wide mouth container. Label container properly and submit.

Category: Zinc oxide suppository is used as an antiseptic, astringent and skin protectant.

Storage: Store in cool place.

Auxiliary label: For rectal use only.

Direction: Asdirected by physician. Insert one suppository.

Marketed products: Calmol 4 (ResiCalinc), Corect(Meridian enterprises Pvt.Ltd.)

Report:

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,A practical hand book of pharmaceutics –I, Niraliprakashan , Page No: 8.1-8.13

SEMISOLIDS

EX NO **DATE**

SULPHUR OINTMENT I.P.1996

Aim: Prepare and submit Sulphur ointment I.P.1996,20g

Requirments:

Apparatus: Weighing balance, porcelain dish, water bath, ointment slab, ointment spatula etc

Chemicals: Sulphur (precipitatied), wool fat, hard paraffin, cetostearyl alcohol, white soft paraffin.

Formula:

For simple ointment

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Wool fat	5g	1.25g	o/w emulsifying agent
Hard paraffin	5g	1.25g	Stiffening agent
Cetostearyl alcohol	5g	1.25g	Improve stability
White soft paraffin	85g	21.25g	Emollient

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Sulphur(Precipitated)	10g	2.2g	Antiseptic,parasitic and scabicide
Simple ointment	90g	19.8g	Ointment base

Ointments are homogenous, semi-solid preparations intended for external application to the skin or mucous membranes. They are used as emollients or for the application of active ingredients to the skin for protective, therapeutic, or prophylactic purposes and where a degree of occlusion is desired.Sulphur is used for several conditions and available in the form

of ointment, Cream and lotions. Sulphur ointments has antiseptic, parasitic and scabicide activity and is used in treatments of scabies and acne.

Simple ointment is prepared by fusion method.It is absorption base composed of wool fat, hard paraffin, cetostearyl alcohol and yellow or white soft paraffin. Wool fat in simple ointment act as o/w type of emulsifying agent. Hard paraffin act as stiffening agent in simple ointment which stiffens ointment base. Cetrostearyl alcohol enhances stability of ointment and gives emollients effect. White soft paraffin exerts emollient effect and mask colour of sulphur in this preparation.

Procedure:

For preparation of simple ointment by fusion method

7. Clean all the glassware and dry them properly as per SOP.
8. Weigh all ingredients properly.
9. Melt hard paraffin and cetrostearyl alcohol in porcelain dish kept on water bath.
10. To above molten mixtures add wool fat and white soft paraffin and stir it well.
11. After melting all ingredients remove porcelain dish from water bath and stir it continuously until semisolid base in obtained.
12. Use this base for preparation of sulphur ointment.

For preparation of sulphur ointment by levigation

5. Weigh required quantity of sulphur and simple ointment properly.
6. Place sulphur on one part of clean ointment slab and simple ointment on other part of ointment slab.
7. Take small quantity of sulphur and about three times more volume of simple ointment at the center of ointment slab and levigate using ointment spatula until sulphur is mixed(geometric mixing) thoroughly in taken volume of simple ointment. Continue this process until all quantity of sulphur is mixed with simple ointment so as to get homogenous and amooth ointment.
8. Transfer required quantity of sulphur ointment in wide mouth light resistant container or collapsible tube. Lable and submit.

Category: In treatment of scabies and acne.

Storage: Store in cool and dark place.

Direction: Apply on affected area as directed by physician

Auxiliary label: For external use only

Marketed products: San Marcos Sulphur Ointments (Brannfels Lab),Sulphur Ointment USP (Anhui Medipharm Co. Ltd. China)

Report:

EX NO **DATE**

NON STAINING – IODINE OINTMENT WITH METHYL SALICYLATE BPC

Aim: Prepare and submit Non staining – iodine ointment with methyl salicylate BPC 10g

Requirements:

Apparatus: Weighing balance, beaker, stirrer, glass mortar and pestle, glass stopper bottle, porcelain dish ,water bath etc.

Chemicals: Iodine, methyl salicylate, oleic acid, arachis oil, yellow soft paraffin.

Formula

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Iodine	1.5g	0.48g	Counter irritant
Mehyl salicylate	1.5g	0.48g	Anti-inflammatory
Arachis oil	4.5g	1.8g	Source of unsaturated fatty acid
Yellow soft paraffin q.s. to	30g	12g	Ointment base

Theory:

Non staining – iodine ointment with methyl salicylate BPC is 5% iodine in yellow soft paraffin and arachis oil. This is example of ointments containing combined iodine and it is prepared by chemical reaction. This fixed oil and may fats obtained from vegetable and animal sources contain unsaturated constituents, These unsaturated fatty acids contain double and triple bond linkage. Arachis oil contain fatty acids such as oleic acid(46.8% as olein), linoleic acid (33.4% as linolein) and palmitic acid (10.0% as palmitin. In this preparation iodine is combined with double bonds of oleic acid (unsaturated fatty acid) and form di - iodo stearic acid(saturated fatty acid).Thus ,free iodine is not available. Since free iodine is not available, this ointment is dark, greenish black in colour. It does not leave stain when rubbed on to the skin. Hence it is called as non-staining iodine ointment.

$CH_3(CH_2)_7$-CH=CH-$(CH_2)_7$-COOH + I_2 ⟶ $CH_3(CH_2)_7$-CHI-CHI-$(CH_2)_7$-COOH

Oleic acid (unsaturated fatty acid)Iodine di-iodo stearic acid (saturated fatty acid)

Procedure:

8. Clean all the glassware and dry them properly as per SOP
9. Accurately weigh iodine and triturate in glass mortar.
10. Place triturated iodine to glass stopper bottle containing arachis oil.
11. Heat above mixture on water bath at 50^{o}C until colour changes from brown to greenish black.
12. Melt separately yellow soft paraffin and mix into above mixture with continuous stirring.
13. Add required quantity of methyl salicylate to above mentioned iodine ointment at room temperature and mix well.
14. Transfer ointment into wide mouth amber or greenish coloured glass containers and close with plastic screw cap, label and submit.

Category: Local analgesic and anti-inflammatory.

Storage: Store in cool and dry place.

Direction: Apply on affected area as per requirement.

Auxillary label: For external use only

Report:

Marketed products: Iodex®Methylsalicylate skin ointment(Lee pharmaceuticals),Medrum®(Medi products Pvt. Ltd)

EX NO **DATE**

CARBOPOL GEL

Aim: Prepare and submit Diclofenac Sodium gel 10g using Carbopol 934P

Requirments:

Apparatus: Weighing balance,beaker,stirrer etc.

Chemicals: Diclofenac sodium, carbopol 934P NF, isopropyl alcohol, Propylene glycol, butylated hydroxyl toluene, purified water.

Formula:

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Diclofenac sodium	1g	0.12g	Anti-inflammatory
Carbopol 934 P	4g	0.48g	Gelling agent
Isopropyl alcohol	5g	0.6g	Solvent for drug
Propylene glycol	5g	0.6g	Co-Solvent for drug
Butylated hydroxyl toluene (BHA)	0.1g	0.012g	Antioxidant
Purified water q.s. to produce	100g	12g	Solvent for gelling agent

Theroy:

Gels are usually homogenous, clear, semi-solid preparations consisting of a liquid phase within a three dimensional polymeric matrix with physical or sometimes chemical cross- linkage by means of suitable gelling agent. There are three methods of preparing gel namely; fusion method, cold method and dispersion method. Diclofenac gel using Carbopol 943P NF is prepared by dispersion method in which gelling agent is dispersed in water with stirring.

Drug is dissolved in another solvent with preservative. This solution is then added in above gel with continuous stirring.

Procedure:

7. Clean all the glassware and dry them properly as per SOP.
8. Weight accurately diclofenac sodium and dissolve in 5g of isopropyl alcohol.
9. Add required quantity of propylene glycol with continuous stirring to above solution. Name this solution as Solution – I
10. In separate beaker take required quantity of Carbopol 934P NF and BHA dissolved in 9ml of water and stir continuously. Name this as Solution-II
11. Mix solution-I into Solution-II thoroughly with continuous stirring and make-up the final weight with water.
12. Transfer gel into plastic tube or wide mouth container, close with screw cap, lable and submit.

Category: Analgesic and anti-inflammatory.

Storage : Store in cool and dry place.

Direction : Apply on affected area as per requirement.

Auxiliary label: For external use only

Marketed products: Nacgel(Systopic), Naclo Gel (Sun pharma)

Report:

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,A practical hand book of pharmaceutics –I, Niraliprakashan , Page No: 91-9.12

GARGLES AND MOUTHWASHES

EX NO **DATE**

POVIDONE IODINE GARGLE

Aim: Prepare and submit Povidone Iodine Gargles 100 ml

Requirments

Apparatus: Weighing balance, measuring cylinder, pipette, beaker, stirrer etc

Chemicals: Povidone iodine I.P, ethanol, glycerine, methyl salicylate, methnol, saccharin sodium, purified water etc.

Formula:

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Povidone iodine I.P (0.1% w/v available iodine)	1% w/v	1mL	Antiseptic
Ethanol	8.38% v/v	8.38mL	Solvent
Glycerin	4%	4mL	Make preparation viscous
Methyl salicylate	006%(q.s)	006mL	Local analgesic
Methnol	004% (q.s)	0.04mL	Local analgesic and flavouring agent
Saccharin sodium	0.1%	0.1g	Sweetening agent
Purified water q.s to produce	100%	100mL	Vehicle

Theory

Povidone-iodine is iodine based product; a complex of iodine and povidone which is used as an antiseptic solution. Povidone iodine gargle is clear red-brown solution. It ia used as antiseptic in the management of local infections of mouth and oroharynx is dental surgery.

Betadine gargle contains povidone-iodine,a complex of iodine which shows all the broad spectrum germicidal activity is maintained in the presence of blood, pus, serum and necrotic tissue. Betadine gargle kills bacteria, viruses, fungi, spores and protozoa. This preparation is contraindicated in patient with hypersensitivity to iodine, polyvinyl pyrrolidone or to any excipient. Betadine gargle can permanently discolour white gold jewellery and it is recommended that this type of jewellery should be removed before using Betadine gargle.

Procedure:

7. Clean all the glass wares and dry them properly as per SOP.
8. Dissolve required quantity of povidone iodine in some portion of purified water in one beaker. Add glycerine, methanol and saccharin sodium in it with continuous stirring.
9. Dissolve methyl salicylate in required quantity of ethanol in a separate beaker.
10. Mix both the solution and transfer into measuring cylinder.
11. Mix both volume up to 100 ml by addition of purified water.
12. Transfer solution in narrow mouth amber coloured bottle and close thoroughly with polypropylene screw cap. Label properly and submit.

Category: Antiseptic in the management of local infections of the mouth and oropharynx in dental surgery.

Storage: Store below 25^0C.Protect from light.

Direction: "Diluted with an equal volume of warm water"

"For external use only"

"Not to be swallowed in large amounts"

Marketed products: Arodin (Aristopharma Ltd), Betadine (Win medicare)

EX NO **DATE**

CHLORHEXIDINE MOUTHWASH

Aim: Prepare and submit Chlorhexidine Mouthwash 30Ml.

Requirements

Apparatus: Weighing balance, measuring cylinder, pipette, beaker, stirrer etc.

Chemicals: Chlorhexidine gluconate ,ethanol 96% v/v, glycerine, citric acid, sodium citrate, sodium metabisulfite, sodium benzoate, brilliant Blue, tartrazine yellow, mint flavour, Purified water.

FORMULA

Ingredients	**Standard formula**	**Working formula**	**Role of ingredient**
Chlorhexidine	0.21%	0.12g	Antiseptic,anti-plaque
Ethanol96% v/v	3.5%	3.5 ml	Solubilising agent
Glycerin	3.0%	3.0ml	Humectant
Citric acid	022%	0.22g	pH regulator
Sodium metabisulfite	0.15%	0.15g	Neutralizing the pigmentation activity of chlorhexidine
Sodium benzoate	0.1%	0.1g	Preservative
Brilliant blue and Tartarazine yellow	0.001%	0.001g	Colouring agent
Mint flavour	0.01%	0.01g	Flavouring agent
Purified water q.s. to produce	100%	100ml	Vehicle

Theory:

Chlorhexidine gluconate is a bisguanide antiseptic and disinfectant which is bactericidal or bacteriostatic against a wide range of gram negative and gram positive vegetative bacteria, yeast, dermatophyte fungi and lipophylic viruses. The antimicrobial activity covers most of the important species occurring in the oral micro flora. Chlorhexidine gluconate mouthwash is an antimicrobial solution which inhibits the formation of dental plaque. It is indicated as an

aid to the treatment and prevention of gingivitis and in the maintenance of oral hygiene, particularly in situations where tooth brushing cannot be adequately employed(for example, following oral surgery or in physically handicapped patients).In this preparation chlorhexidine gluconate is used as antiseptic and anti-plaque.

Procedure:

6. Clean all the glassware and dry them properly as per SOP.
7. Dissolve required quantity of chlorhexidine gluconate, citric acid and sodium citrate in some portion of purified water with continuous stirring until clear solution is not obtained. Add required quantity of ethanol to this solution.
8. Add measured quantity of glycerine, sodium metabisulfite, sodium benzoate, colouring agent and mint flavour gradually with continuous stirring.
9. Pour this solution to measuring cylinder. Make-up volume up to 100 ml using purified water.
10. Transfer prepared mouthwash to amber coloured bottle and close thoroughly with polypropylene screw cap. This cap is conical in shape and has “10ml” and a level line engraved on the inside. Label properly and submit.

Category: In treatment and prevention of gingivitis and in the maintenance of oral hygiene.

Storage: Store in cool and dry place.

Dose: 10 ml, twice a day.

For treatment and prevention, swish in your mouth for one minute. Do not rinse, eat or drink thereafter for 20 minutes. Recommended only under professional guidance.

Direction: “NOT TO BE SWALLOWED”,”FOR ORAL USE ONLY”.

Marketed products: Clohex (Dr Reddy Laboratories Ltd), Dentcare (Nova scotia)

Reference: Dr.A.A.Hjare, Dr.D.A.Bhagwat,A practical hand book of pharmaceutics –I, Niraliprakashan , Page No: 10.1-1

PHARMACEUTICAL INORGANIC CHEMISTRY

CONTENTS


INTRODUCTION

INTRODUCTION

PHARMACEUTICAL CHEMISTRY

Pharmaceutical Chemistry is a branch of chemistry that deals with the chemical, biochemical and pharmacological aspects of drugs. It includes synthesis/isolation, identification, structural elucidation, structural modification, Structural Activity Relationship (SAR) studies, study of the chemical characteristics, biochemical changes after drug administration and their pharmacological effects.

Inorganic Chemistry

Inorganic chemistry is the study of all the elements and their compounds except carbon and its compounds (which is studied under organic chemistry). Inorganic chemistry describes the characteristics of substances such as nonliving matter and minerals which are found in the earth except the class of organic compounds. Branches of inorganic chemistry include coordination chemistry, bioinorganic chemistry, organometallic compounds and synthetic inorganic chemistry. The distinction between the organic and inorganic are not absolute, and there is much overlap, especially in the organometallic chemistry, which has applications in every aspect of the pharmacy, chemical industry–including catalysis in drug synthesis, pigments, surfactants and agriculture. In short, Inorganic chemistry is the branch of chemistry that deals with inorganic compounds. In other words, it is the chemistry of compounds that do not contain hydrocarbon radicals.

Inorganic Compounds:

These are traditionally viewed as compounds being synthesized by the geological systems and lack hydrocarbon (carbon-hydrogen). In contrast, organic compounds are those found in biological systems. In general organic chemists say any molecule containing carbon as an organic compound and hence this means that inorganic chemistry deals with the compounds or molecules which lack carbon atom. Berzelius, the 19th century chemist, described inorganic compounds as inanimate. The first important synthetic inorganic compound was ammonium nitrate for soil fertilization. Inorganic compounds are found in nature as minerals. Soil contain iron

sulfide as pyrite or calcium sulfate as gypsum. They are also found multitasking as biomolecules: As electrolytes (sodium chloride), in energy storage (ATP) or in construction (the polyphosphate backbone in DNA). Inorganic compounds are synthesized for use as drugs such as cisplatin, magnesium hydroxide, catalysts such as vanadium (V) oxide and titanium (III) chloride, or as reagents in organic chemistry such as lithium aluminium hydride.

Medicinally useful substances are derived from either organic or inorganic sources. Naturally obtained compounds attracted the attention of humans always, in which inorganic chemicals contributing significantly in some of the ailments, even after the development of many drugs from synthetic and plant sources. Many of the inorganic salts (antimony, arsenic and mercury) are known to be poison, still they are used in medicine cautiously. Some of them are replaced by the organic medicines.

Importance of Inorganic Pharmaceuticals:

Inorganic pharmaceuticals are useful in any of the following ways.

1. Useful medicinally for their therapeutic purpose. Example: Astringents and antimicrobials etc.

2. Useful as pharmaceutical aids. Example: Bentonite, talc etc.

3. To change the reaction of body fluid. To acidify or alkalise. Example: Antacids, alkalis, mineral acids.

4. Replacing or replenishing the normal content of body fluids. Example: Sodium, potassium, calcium, chloride, phosphate etc.

5. Useful as reagents to carry out the reactions. Example: Catalysts (platinum, nickel) oxidizing and reducing agents (lithium aluminium hydride).

6. Useful in Pharmaceutical analysis. Example: Titrants such as potassium permanganate etc.

LIMIT TEST:

Limit tests are quantitative or semi-quantitative tests designed to identify and control small quantities of impurity, which are likely to be present in the substance. The quantity of any one impurity in an official substance is often small, and consequently the visible reaction response to any test for that impurity is also small. The design of individual tests is therefore important if errors are to be avoided in the hands of different operators.

This is accomplished by giving attention to a number of factors, which are discussed below.

SPECIFICITY OF THE TESTS

Any test used as a limit test must, of necessity, give some form of selective reaction with the trace impurity. Many tests used for the detection of inorganic impurities in official inorganic chemicals are based upon the separations involved in inorganic qualitative analysis. A test may be demanded which will exclude one specific impurity, but highly specific tests are not always the best; a less specific test, which limits several likely impurities, at once, is obviously advantageous, and in fact can often be accomplished. An example of such a test is the heavy metals test applied to alum, which not only limits contamination by lead, but also other heavy metal contaminants precipitated by thioacetamide as sulphide at pH 3.5.

SENSITIVITY

The degree of sensitivity required in a limit test varies enormously according to the standard of purity demanded by the monograph. The sensitivity of most tests is dependent upon a number of variable factors all capable of strict definition, and all favorable towards the production of reproducible results. Thus the precipitation of an insoluble substance from solution is governed by such factors as concentration of the solute and of the precipitating reagent, duration of the reaction, reaction temperature, and the nature and concentration of other substances unavoidably present in solution. As a general rule, cold dilute solutions give light precipitates, whereas more granular ones are obtained from hot concentrated solutions. Many of the limit tests, however, are concerned with very dilute solutions, which are often slow to react, and here sensitivity of the reaction can often be increased by extending the duration of the reaction or by raising the reaction temperature. Similar considerations apply in the design of colour and other tests

employed as limit tests. With suitable control of the factors described the same degree of reproductivity can be guaranteeed in all cases.

CONTROL OF PERSONAL ERRORS

It is essential to exclude all possible sources of ambiguity in the description of a test. Vague terms such as 'slight precipitate,' should be avoided as far as possible. The extent of the visible reaction to be expected under the specified test conditions should be clearly and precisely defined. This is usually accomplished in one of three ways.

(a) Tests in which there is no visible reaction: A definite statement is incorporated in the wording of the test, which states that there shall be no colour, opalescence or precipitate, whichever is appropriate to the particular test. One example of this type of requirement is the test for barium, and calcium in Dilute Hypophosphorus. Acid (BP Appendix I), where the additions of dilute sulphuric acid under precisely controlled condition shall produce 'no turbidity, or precipitate' within one hour. The time factor is used here as a means of increasing the sensitivity of the test. Tests such as these which give negative results do not necessarily imply the complete absence of the impurity, the test as laid down merely indicating the absence of an undesirably large amount of the impurity

(b) Comparison methods: Tests of this type require a standard containing a definite amount of impurity, to be set up at the same time and under the same conditions as the test experiment. In this way the extent of the reaction is readily determined by direct comparison of the test solution with a standard of known concentration. The official limit tests for chlorides, sulphate iron and heavy metals are based on this principle. The limit tests for lead and arsenic are, in practice, also comparison methods. They are, however, so designed that they can be readily applied as quantitative determinations.

(c) Quantitative determinations: Quantitative determination of impurities is only applied in special circumstances, usually in those cases where the limit is not readily susceptible to simple and more direct chemical determination.

The method is used in the following different types of tests:

(i) Limits of insoluble matter

(ii) Limits of soluble matter

(iii) Limits of moisture, volatile matter, and residual solvents

(iv) Limits of non-volatile matters

(v) Limits of residue on ignition

(vi) Loss on ignition

(vii) Ash values

(viii) Precipitation methods.

IDENTIFICATION TEST

The purpose of identification test is to ensure the correct labeling of the substances. Identification tests are specific but they are not necessary sufficient in establishing the absolute proof of identity of the substances. If an articles taken from a labeled container do not meet to the requirements of a prescribed identification test indicates that the articles is either mislabeled or substituted. In same monographs, more than one identification tests are given. In such cases, if the articles complies with the either one or the other identification test, in sufficient to verify the identity of the article.

Identification tests are generally based upon the combination of simple chemical test and measurement of the appropriate physical constants. There is considerable overlap between identification tests and the limit tests. Limit tests are designed to ensure that the undesirable impurities are within the prescribed limits. Identification tests whether physical or chemical, provided they are sufficiently specific, can be used as the basis of a quantitative estimation or in the design of specific limit tests. Practically, a single identification test may contribute to identification as well as standardization of the substance.

Chemical tests, used for identification are basically qualitative confirming to the presence of the substance under investigation. They may be far too general or lack specificity but can be considered sufficiently specific when used in conjugation with the other requirement of the monograph.

Physical constants such as melting point, boiling point, solubility, weight per ml, refractive index, optical rotation, viscosity etc., have characteristic values for a given substance. They can be used in identification, checking quality and maintaining standard of purity.

TEST FOR PURITY

Test for purity' for substances have been prescribed by the pharmacopoeias of the various countries in order to ensure reasonable freedom from the undesirable impurities. The socalled 'Test for purity' are infact the tests for the presence of impurities in the substance and fix the limits of tolerance for these undesirable impurities. Test for purity are not framed to guard against all the possible impurity rather they provide appropriate limitation of the potential impurities only.

The guiding factor for fixing a limit of tolerance for the various impurities is the amount of impurity that is likely to be harm. Arsenic and lead are quite dangerous even in trace amounts therefore very small limits of tolerance have been fixed for their presence in all pharmaceutical substances. Another factor is the practicability of the commercial method of production of the substance meeting the requirements of a particular standard of purity. It would be useless to fix the limits of tolerance which can only be attained at a very high cost. There are cases in which the limits fixed in the pharmacopoeia were later relaxed because they were found to be too difficult to attain by the available methods of manufacturing.

The ultimate objective is that the pharmaceutical substances if not completely free from the undesirable or toxic impurities should be of reasonable good purity ensuring therapeutic safety. The presence of sodium bromide (NaBr) in the more expensive potassium bromide (KBr) is not likely to cause any harm to the patient but at the same time the KBr should be of sufficiently good pharmaceutical quality and purity not containing excess amount of sodium bromide. Some of the tests which may be undertaken to ascertain the purity of a substance are:

(a) Clarity of solution: The degree of clarity or opalescence of solution is measured by direct comparison with a reference solution having standard opalescence. The comparison is against a black

background by

viewing vertically downward under diffused light. A solution is considered clear if its clarity is the same as that of water or of the solvent employed in the preparation of the solution being examined.

(b) Colour of solution: In Indian Pharmacopoeia, the colour standards are based on three primary colorimetric solutions: yellow, red and blue prepared from ferric chloride, cobaltous chloride and cupric sulphate respectively. These primary solutions are mixed in various proportions with or without 1% w/v hydrochloric acid to give five reference colour solutions which are yellow (YS), greenish yellow (GYS), brownish yellow (BYS), brown (BS), and red (RS). The colour of the solution is compared with reference colour solution by viewing vertically downwards through the columns of liquids in diffused light. A solution may be considered as colourless if it has the same appearance as water or as the solvent employed in the preparation of the solution being examined.

(c) Acidity or alkalinity: Pharmaceutical substances prepared using chemical reactions involving acids and alkalies may possess some degree of acidity or alkalinity resulting from improper purification by inadequate washings after their separation. The limits for acid or alkali impurities are fixed for various pharmaceutical substances and the test for acidity and alkalinity is of great help in determining the extent of such impurities.

(d) Loss on ignition: It is the loss of weight in % w/w resulting from a volatile part of any test material that is driven off under specified conditions. It is applied to thermostable substances which contain thermolabile impurities that decompose and lose a volatile product e.g., zinc carbonate decomposes losing carbon dioxide. The substance is heated, cooled and weighed repetitively until a constant weight is attained. The loss on ignition in this case should not be more than 2% w/w.

(e) Loss on drying: It is the loss of weight in % w/w resulting from water and volatile matter that is lost under specified conditions. The temperature to which the substance is subjected varies considerably according to the nature of the substance. The temperature applied should not be so high as to cause decomposition of the substance but at the same time it should be sufficiently high to produce the desired results within a reasonable time. It is usually applied by drying the substance to constant weight at 105°C.

(f) Moisture content: Sometimes the determination of the moisture content of the substance is a good measure of the purity of the substance especially in case of crude drugs.

(g) Ash values: The determination of ash values in crude drugs, organic compounds and certain inorganic compounds provides valuable information regarding the extent of heavy metals and minerals impurities.

LIMIT TEST

LIMIT TEST FOR CHLORIDE

AIM:

To perform the limit test for chloride in the given sample of magnesium sulphate. ($MgSO^4$)

APPARATUS USED:

- Nesslers cylinder,
- Measuring cylinder,
- Glass rod,
- Beaker.

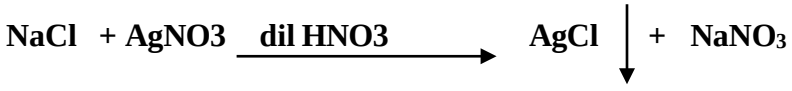
REAGENTS USED:

- Silver nitrate ($AgNO_3$)
- Dil.Nitric acid (HNO_3)
- Sodium chloride solution (NaCl).& Magnesium sulphate

PRINCIPLE:

The limit test for chloride is based on a well known reaction between silver nitrate and soluble chloride forming a precipitate of silver chloride which is insoluble in dilute nitric acid.The silver chloride produced in the presence of dilute nitric acid makes the solution turbid.The extend of turbidity depends upon the amount of chloride present in the test solution. The opalescence or turbidity so obtained is compared with the opalescence obtained in the standard solution containing a known quantity of chloride. Dilute nitric acid is added to prevent the precipitation of other acid radicals.

If the opalescence produced in the test is less than that of standard opalescence, the samples will pass the limit test for chloride. If the opalescence produced in the test is more than that of standard opalescence the sample fails the limit test for chloride.



PROCEDURE:

S.NO	TEST	STANDARD
1.	Dissolve 1gm of magnesium sulphate in about 10 ml of distilled water and transfer it to Nesslers cylinder and label It as "test".	Place 1 ml of 0.0585% w/v solution of sodium chloride in a Nesslers cylinder and label it as "standard" Add 9 ml of distilled water and mix.
2.	Add 10 ml of dil. Nitric acid.	Add 10 ml of dil. Nitric acid
3.	Dilute to 50 ml mark with distilled water.	Dilute to 50 ml mark with distilled water.
4.	Add 1 ml of 50% silver nitrate solution.	Add 1 ml of 50% silver nitrate solution

Allow to stand for 5minutes protected from light. Any opalescence produced when viewed transversely against a black background.

INFERENCE:

REPORT:

REFERENCE:

Indian Pharmacopoeia Volume- I

Bently and drivers textbook of pharmaceutical chemistry.

LIMIT TEST FOR SULPHATE

AIM:

To perform the limit test for sulphate in the given sample of sodium chloride.(NaCl)

APPARATUS USED:

- Nesslers cylinder,
- Measuring cylinder,
- Glass rod,
- Beaker.

REAGENTS USED:

- Sodium chloride (NaCl)
- Dil.HCl
- $BaSO_4$ Reagents
- K_2SO_4

PRINCIPLE:

The limit test for sulphate depends upon the reaction of sulphate with barium chloride in the presence of dilute hydrochloric acid. This result in the precipitation of sulphate as barium sulphate.

$$SO_4^{2-} + BaCl_2 \xrightarrow{\text{dil HCl}} BaSO_4 + 2Cl^-$$

Dilute hydrochloric acid is added to prevent precipitation of other acid radicals. In the limit test for sulphate, barium sulphate reagents is used. This barium sulphate reagents contains barium chloride and a very small quantity of potassium sulphate and also alcohol. The presence of small amount of potassium sulphate in barium sulphate reagent increases the sensitivity of the test.

If the turbidity produced in the test solution is less than the standard turbidity, the sample passes the limit test for sulphate.

If the turbidity produced in the test solution is more than the standard turbidity, the sample fails the limit test for sulphate.

PROCEDURE:

S.NO	TEST	STANDARD
1.	Dissolve 2g of NaCl in about 10ml of distilled water and transfer it to Nesslers cylinder and label it as "test".	Place 1ml of 0.1089%w/v solution of potassium sulphate in Nesslers cylinder and about 9ml of distilled water label it as "standard".
2.	Add 2ml of dilute Hcl acid.	Add 2ml of dilute Hcl acid.
3.	Dilute to 25ml with distilled water.	Dilute to 25ml with distilled water.
4.	Add 5ml of barium sulphate reagent.	Add 5ml of barium sulphate reagent
5.	Dilute to 50ml with distilled water.	Dilute to 50ml with distilled water.

Allow to stand for 5minutes protected from light. Any opalescence produced when viewed transversely against a black background.

INFERENCE:

REPORT:

REFERENCE:

- Indian pharmacopoeia - page no A44
- Bently and Drivers Text book of pharmaceutical chemistry - page no -112
- Pharmaceutical chemistry I by N.C. Choudhary and NK. Gurbani - page no-226.

MODIFIED PROCEDURE FOR THE LIMIT TEST TEST FOR CHLORIDE

AIM:

To perform the limit test for chloride in the given sample of sodium bicarbonate and potassium permanganate.

APPARATUS USED:

- Nesslers cylinder
- Glass rod
- Measuring cylinder

REAGENTS USED:

- Sodium bicarbonate
- Potassium permanganate
- 0.1M Silver nitrate
- Dilute nitric acid
- Sodium chloride
- Ethanol
- Distilled water.

PRINCIPLE:

The limit test for chloride based on a well known reaction between silver nitrate and soluble chloride forming a precipitate of silver chloride which is insoluble in dilute nitric acid. The silver chloride produced in the presence of dilute nitric acid makes the solution turbid. The extend of turbidity depends upon the amount of chloride present in the test solution. The opalescene or turbidity obtained in the standard solution containing a known quantity of chloride. Dilute nitric acid is added to prevent the precipitation of other acid radicals. If the opalescence produced in the test is less than that of standard opalescence, the samples will passes the limit test for chloride. If the opalescence produced in the test is more than that of standard opalescence, the samples will the limit test for chloride.

PROCEDURE:

STANDARD SOLUTION	TEST SOLUTION (SODIUM BICARBONATE)	TEST SOLUTION (POTASSIUM PERMANGANATE)
Take 10ml of chloride standard solution in labeled nesslers cylinder.	Weigh accurately about 1.25gm of sodium bicarbonate.	Weigh accurately about 1.5gm of potassium permanganate and transferred it in250ml conical flask.
Added 5ml of distilled water.	Dissolve it in 15ml of water, in a labeled nesslers cylinder.	Added the mixture of 4ml of nitric acid and 4ml of distilled water,5ml of ethanol and heated to boiling, shaking frequently.
Add 10ml of dilute nitric acid and mix well.	Add 2ml of dilute nitric acid and mix well	Add 12ml of distilled water allowed to cool, filtered or centrifuged to obtain a clear solution
Dilute to 50ml with distilled water.	Dilute to 50ml with distilled water.	Dilute the filtrate to20ml with distilled water.
Add 1ml of 0.1M silver nitrate solution.	Add 1ml of 0.1M silver nitrate solution.	Taken 0.5ml of above solution in a labeled
stirred immediately with glass rod and allowed to stand for 2 min, protected from direct sun light.	stirred immediately with glass rod and allowed to stand for 2 min, protected from direct sun light.	stirred immediately with glass rod and allowed to stand for 2 min, protected from direct sun light.

Allow to stand for 5minutes protected from light. Any opalescence produced when viewed transversely against a black background.

OBSERVATION:

REPORT:

STANDARD SOLUTION	TEST SOLUTION (SODIUM BENZOATE)	TEST SOLUTION (SODIUM SALICYLATE)
Take 10ml of chloride standard solution in labeled nesslers cylinder.	Weigh accurately about 0.25gm of sodium benzoate.	Weigh accurately about 0.25gm of sodium salicylate.
Added 5ml of distilled water.	Dissolve it in 15ml of water, filtered and the test is applied as filtrate.	Dissolve it in 15ml of water, filtered and the test is applied as filtrate.
Add 1ml of 2M acetic acid and mix well.	Add 1ml of 2M acetic acid and mix well.	Add 1ml of 2M acetic acid and mix well.
Add 1ml of 0.1M silver nitrate solution.	Add 1ml of 0.1M silver nitrate solution.	Add 1ml of 0.1M silver nitrate solution.
Dilute to 50ml with distilled water.	Dilute to 50ml with distilled water.	Dilute to 50ml with distilled water.
stirred immediately with glass rod and allowed to stand for 2 min, protected from direct sun light.	stirred immediately with glass rod and allowed to stand for 2 min, protected from direct sun light.	stirred immediately with glass rod and allowed to stand for 2 min, protected from direct sun light.

Allow to stand for 5minutes protected from light. Any opalescence produced when viewed transversely against a black background.

OBSERVATION:

REPORT:

EX NO **DATE**

MODIFIDED PROCEDURE FOR THE LIMIT TEST FOR SULPHATE

AIM:

To perform the limit test for sulphate in the given sample of sodium bicarbonate and potassium permanganate.

APPARATUS USED:

- Nesslers cylinder,
- Measuring cylinder,
- Glass rod,
- Beaker.

REAGENTS USED:

- Sodium bicarbonate
- Potassium permanganate
- Dil.HCl
- $BaSO_4$ Reagents
- K_2SO_4
- Ethanol
- Distilled water.

PRINCIPLE:

The limit test for sulphate depends upon the reaction of sulphate with barium chloride in the presence of dilute hydrochloric acid. This result in the precipitation of sulphate as barium sulphate.

$$SO_4^{2-} + BaCl_2 \xrightarrow{\text{dil HCl}} BaSO_4 + 2Cl^-$$

Dilute hydrochloric acid is added to prevent precipitation of other acid radicals. In the limit test for sulphate, barium sulphate reagents is used. This barium sulphate reagents contains barium chloride and a very small quantity of potassium sulphate and also alcohol. The presence of small amount of potassium sulphate in barium sulphate reagent increases the sensitivity of the test.

If the turbidity produced in the test solution is less than the standard turbidity, the sample passes the limit test for sulphate.

If the turbidity produced in the test solution is more than the standard turbidity, the sample fails the limit test for sulphate.

PROCEDURE:

STANDARD	TEST (SODIUM BICARBONATE)	TEST(POTASSIUM PERMANGANATE)
Take 1ml of 25% w/w solution of barium chloride in the nesslers cylinder.	Weighed accurately 1gm of sodium bi carbonate and added to labeled nesslers cylinder.	Weighed accurately 1gm of potassium permanganate and added to labeled nesslers cylinder.
Added 1.5ml of the ethanolic solution (10ppm SO4) mixed and allowed to stand for 1min.	Added 10ml of distilled water, neutralize with HCl and diluted to 5ml withdistilled water	Dissolve it by adding 15ml of distilled water
Add 0.15 ml of 5M acetic acid	Add 0.15 ml of 5M acetic acid	Add 0.15 ml of 5M acetic acid and 5ml of alcohol. Heat the above mixture to boiling, shaking frequently add 12 ml of distilled water. Allowed to cool, filtered or centrifuged for obtaining clear solution. Dilute the filtrate to 20ml with water. Take 0.5ml of the solution.

Added sufficient water to produce 50ml, stirred immediately with glass rod and allowed to stand for 5min.	Added sufficient water to produce 50ml, stirred immediately with glass rod and allowed to stand for 5 min.	Added sufficient water to produce 50ml, stirred immediately with glass rod and allowed to stand for 5 min.
	View transversely against a black background compare the opalescence with that of standard solution.	View transversely against a black background compare the opalescence with that of standard solution.

Allow to stand for 5minutes protected from light. Any opalescence produced when viewed transversely against a black background.

OBSERVATIONS:

REPORT:

PROCEDURE:

STANDARD	TEST (SODIUM BENZOATE)	TEST(SODIUM SALICYLATE)
Take 1ml of 25% w/w solution of barium chloride in the nesslers cylinder.	Weighed accurately 0.75gm of sodium benzoate and added to labeled nesslers cylinder.	Weighed accurately 0.75gm of sodium salicylate and added to labeled nesslers cylinder.
Added 1.5ml of the ethanolic solution (10ppm SO4) mixed and allowed to stand for 1min.	Added 10ml of distilled water, neutralize with HCl and diluted to 5ml with distilled water, filtered and the test is applied to the filtrate.	Dissolve it by adding 15ml of distilled water, fitered and the test is applied to the filtrate.
Add 0.15 ml of 5M acetic acid	Add 0.15 ml of 5M acetic acid	Add 0.15 ml of 5M acetic acid.
Added sufficient water to produce 50ml, stirred immediately with glass rod and allowed to stand for 5min.	Added sufficient water to produce 50ml, stirred immediately with glass rod and allowed to stand for 5 min.	Added sufficient water to produce 50ml, stirred immediately with glass rod and allowed to stand for 5 min.

	View transversely against a black background compare the opalescence with that of standard solution.	View transversely against a black background compare the opalescence with that of standard solution.

Allow to stand for 5minutes protected from light. Any opalescence produced when viewed transversely against a black background.

OBSERVATIONS:

REPORT:

EX NO **DATE**

LIMIT TEST FOR IRON

AIM:

To perform the limit test for iron in the given sample of sodium chloride. (NaCl)

APPARATUS USED:

- Nesslers cylinder,
- Measuring cylinder,
- Glass rod,
- Beaker.

REAGENTS USED:

- Sodium chloride (NaCl)
- Iron free citric acid
- Thioglycollic acid
- Iron free ammonia
- Standard iron solution.

PRINCIPLE:

Limit test for iron depends upon the interaction of thioglycollic acid with iron in the presence of citric acid and ammonical alkaline medium.The results in the formation of purple coloured ferrous thioglycollate complex. If the intensity of the purple colour produced in the test solution is less than that of standard the sample passes the limit test for iron.

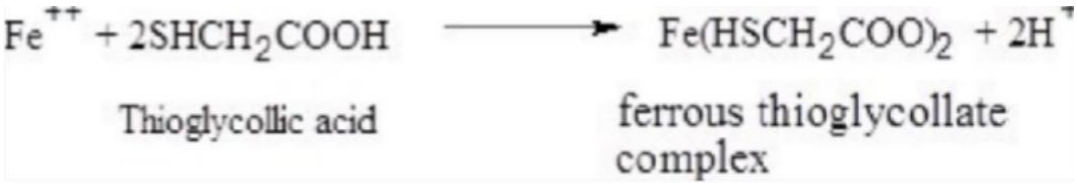
Thioglycollic acid performs the following functions:

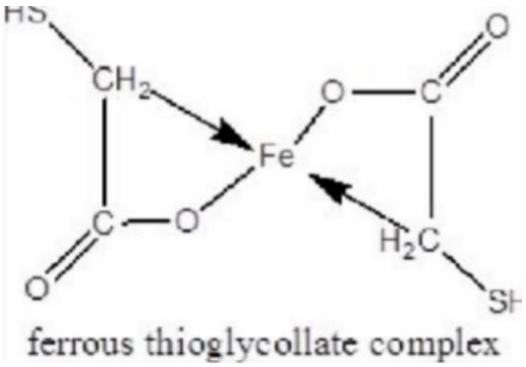
- Iron impurity may be present in the trivalent ferric form or divalent ferros form. If it is present in the ferric form, the thioglycollic acid reduces it to ferrous (Fe^{2+}) form.
- Thioglycollic acid produces purple colour with the ferrous iron in the ammonical alkaline medium.
- Citric acid prevents precipitation of iron with ammonia all the reagent used for this test must be iron free.

PRINCIPLE*: REACTION*

$Fe^{+++} + 2SHCH_2COOH \rightarrow Fe^{++} + S\text{-}CH_2COOH + 2H^+$

Ferric Ferrous





ferrous thioglycollate complex

PROCEDURE:

S.NO	TEST	STANDARD
1.	Place 1g of NaCl in Nesslers cylinder and label it as test. Dissolve it with 40ml of distilled water.	Place 2ml of standard iron solution in a Nesslers cylinder and label it as standard. Add 40ml of distilled water.
2.	Add 2ml of 20% iron free citric acid.	Add 2ml of 20% iron free citric acid.
3.	Add 0.1 ml of thioglycollic acid.	Add 0.1 ml of thioglycollic acid.
4.	Make Solution alkaline using iron free ammonia.	Make Solution alkaline using iron free ammonia
5.	Dilute to 50 ml with distilled water.	Dilute to 50 ml with distilled water.

Allow to stand for 5minutes protected from light. Any opalescence produced when viewed transversely against a white background.

INFERENCE:

REPORT:

REFERENCE:

- Indian pharmacopoeia - page no A44
- Bently and Drivers Text book of pharmaceutical chemistry - page no -112
- Pharmaceutical chemistry I by N.C. Choudhary and NK. Gurbani - page no-220

EX NO **DATE**

LIMIT TEST FOR HEAVY METALS

AIM:

To perform the limit test for heavy metals in the given sample of boric acid.(H_3BO_3)

APPARATUS USED:

- Nesslers cylinder,
- Measuring cylinder,
- Glass rod,
- Beaker.

REAGENTS USED:

- Stannated lead solution
- Sample solution (Boric acid)
- Sodium hydroxide
- Hydrogen sulphide
- Distilled water

PRINCIPLE:

Indian pharmacopoeia prescribes 4 methods A,B,C,D for the limit test for heavy metals.

METHOD A & B:

Method A is used for colourless substances and Method Bis used for coloured substances. Both the methods are based on the reaction between freshly prepared saturated solution of hydrogen sulphide in water and heavy metals in an acidic medium to produce the metal sulphide. This remains distributed in a colloidal state and produce brownish colouration. The test is compared against the standard which is prepared by using lead nitrate solution. Method B is similar to method A except that in this case the coloured substance is given special treatment to make it colorless before preparing its solution.

METHOD C & D:

Method C is used for those substances which give clear and colorless solutions when they are dissolved in sodium hydroxide. While the precipitation of metallic sulphide is carried out in Method A **&** B in moderatively acidic condition, in method C it is done only alkaline condition. Method D is applied to substance in which other impurities may interfere in the usual sulphide test.

Metal + H2S ' Metal sulphide

PROCEDURE:

S.NO	TEST	STANDARD
1.	1g of boric acid in a Nesslers cylinder and add 2ml of acetic acid and label it as test.Dissolve it with 25ml of distilled water.	Place 2ml of standard solution in a Nesslers cylinder label it as standard and dilute to 25 ml with distilled water.
2.	Add 5ml of NaOH solution.	Add 5ml of NaOH solution.
3.	Add 5 drops of H_2S and mix well	Add 5 drops of H_2S and mix well
4.	Add distilled water to make up to 50ml.	Add distilled water to make up to 50ml.

Allow to stand for 5minutes protected from light. Any opalescence produced when viewed transversely against a white background.

INFERENCE:

REPORT:

REFERENCE:

- Indian pharmacopoeia - page no A 43,44.
- Pharmaceutical chemistry I by N.C. Choudhary and NK. Gurbani - page no-224,225.

EX NO **DATE**

LIMIT TEST FOR LEAD

AIM:

To perform the limit test for lead in the given sample.

APPARATUS USED:

- Nesslers cylinder,
- Measuring cylinder,
- Glass rod,
- Beaker.
- Separating funnel.

REAGENTS USED:

- Stannated lead solution
- Sample solution (ammonium chloride)
- Potassium cyanide
- Sodium sulphide.
- Dithizone
- Choloroform
- Distilled water.

Preparation of Reagents:

Ammonia-cyanide solution:

Dissolve 2 g of potassium cyanide in 15 mL of ammonium hydroxide, and dilute with water to 100ml

Ammonium citrate solution:

Dissolve 40 g of citric acid in 90 mL of water. Add 2 or 3 drops phenol red TS, and then cautiously add ammonium hydroxide until the solution acquires a reddish color. Remove any lead that may be present by extracting the solution with 20 mL portions of dithizone extraction solution; until the dithizone solution retains its orange-green color.

Diluted standard lead solution:

Dilute an accurately measured volume of standard lead solution (containing 10 µg of lead per mL), with 9 volumes of dilute nitric acid (1 in 100) to obtain a solution that contains 1 µg of lead per mL.

Dithizone extraction solution:

Dissolve 30 mg of dithizone in 1000 mL of chloroform, and add 5 mL of alcohol. Store the solution in a refrigerator. Before use, shake a suitable volume of the dithizone extraction solution with about half its volume of dilute nitric acid (1 in 100) discarding the nitric Acid.

Hydroxylamine hydrochloride solution:

Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make approximately 65 mL. Transfer to a separator, add 5 drops of thymol blue TS, then add ammonium hydroxide until the solution assumes a yellow color. Add 10 mL of sodium diethyldithiocarbamate solution (1 in 25), mix, and allow to stand for 5 minutes. Extract this solution with successive 10- to 15- mL portions of chloroform until a 5-mL portion of the chloroform extract does not assume a yellow color when shaken with cupric sulphate TS. Add 3 N hydrochloric acid until the solution is pink (if necessary, add 1 or 2 drops more of thymol blue TS), and then dilute with water to 100 mL.

Potassium cyanide solution:

Dissolve 50 g of potassium cyanide in sufficient water to make 100 mL. Remove the lead from this solution by extraction with successive portions of dithizone extraction solution, as described under ammonium citrate solution above, then extract any dithizone remaining in the

cyanide solution by shaking with chloroform. Finally dilute the cyanide solution with sufficient water so that each 100 mL contains 10 g of potassium cyanide.

Standard dithizone solution:

Dissolve 10 mg of dithizone in 1000 mL of chloroform. Keep the solution in a glass-stoppered, lead-free bottle, suitably wrapped to protect it from light, and store in a refrigerator.

Phenol red TS (Phenolsulfonphthalein TS):

Dissolve 100 mg of phenolsulfonphthalein in 100 mL of alcohol, and filter if necessary.

Thymol blue TS:

Dissolve 100 mg of thymol blue in 100 mL of alcohol, and filter if necessary.

Cupric sulphate TS:

Dissolve 12.5 g of cupric sulfate in water to make 100 mL.

Test preparation: If, in the following preparation, the substance under test reacts too rapidly and begins charring with 5 mL of sulfuric acid before heating, use instead 10 mL of cooled dilute sulfuric acid (1 in 2), and add a few drops of the hydrogen peroxide before heating.

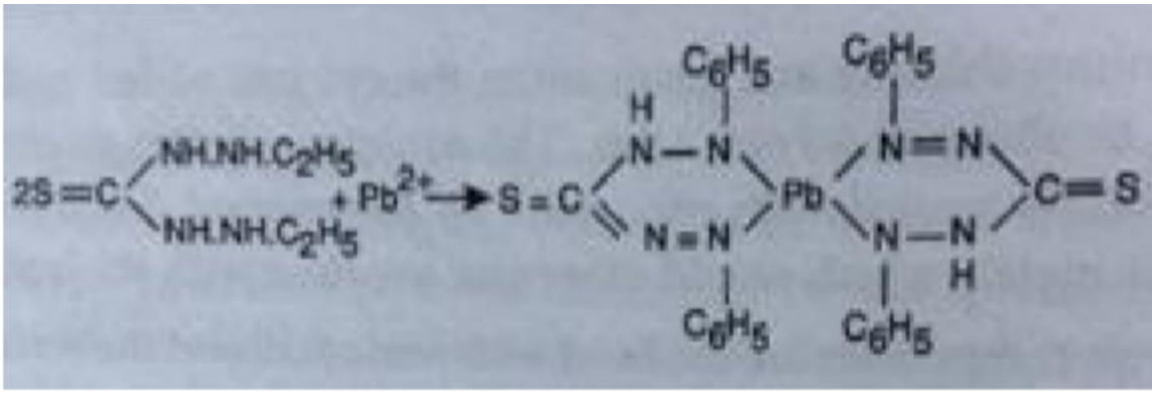
Transfer 1.0 g of the substance under test to a suitable flask, add 5 mL of sulfuric acid and a few glass beads, and digest on a hot plate in a hood until charring begins. Other suitable means of heating may be substituted. (Add additional sulfuric acid, if necessary, to wet the substance completely, but do not add more than a total of 10 mL). Add, dropwise and with caution, 30 percent hydrogen peroxide, allowing the reaction to subside and again heating between drops. Add the first few drops very slowly, mix carefully to prevent a rapid reaction, and discontinue heating if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on the walls of the flask. [NOTE: Add peroxide whenever the mixture turns brown or darkens.] Continue the digestion until the substance is completely destroyed, copious fumes of sulfur trioxide are evolved, and the solution is colourless. Cool, cautiously add 10 mL of water, evaporate until sulfur trioxide again is evolved, and cool. Repeat this procedure with another 10 mL of Water to remove any traces of hydrogen peroxide. Cautiously dilute with 10 mL of Water, and cool.

PRINCIPLE:

Limit test of lead as per IP& USP

The limit test for lead as per I.P. and U.S.P. has been based upon the reaction between lead and diphenyl thiocarbazone (dithizone)

Dithizone in chloroform is able to extract lead from alkaline aqueous solution as a lead dithizone complex (red in colour).



The original dithizone is having a green colour in chloroform while the leaddithizone is having a violet colour. The intensity of the colour of complex is dependent upon the amount of lead in the solution. The colour of the lead-dithizone complex in chloroform has been compared with a standard volume of lead solution, treated in the same manner.

In this method the lead present as an impunity in the substances, gets separated by extracting an alkaline solution with a dithizone extraction solution. The interference and influence of oilier metal ions etc. have been eliminated by adjusting the optimum pH for the extraction by employing ammonium citrate, potassium cyanide, hydroxylamine hydrochloride reagents, etc.

PROCEDURE

TEST SAMPLE	STANDARD COMPOUND
A known quantity of sample solution is transferred in a separating funnel	A standard lead solution is prepared equivalent to the amount of lead permitted in the sample under examination
Add 6ml of ammonium citrate	Add 6ml of ammonium citrate
Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride	Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride
Add 2 drops of phenol red	Add 2 drops of phenol red
Make solution alkaline by adding ammonia solution.	Make solution alkaline by adding ammonia solution.
Extract with 5 ml of dithizone until it becomes green	Extract with 5 ml of dithizone until it becomes green
Combine dithizone extracts are shaken for 30 mins with 30 ml of nitric acid and the chloroform layer is discarded	Combine dithizone extracts are shaken for 30 mins with 30 ml of nitric acid and the chloroform layer is discarded
To the acid solution add 5 ml of standard dithizone solution	To the acid solution add 5 ml of standard dithizone solution
Add 4 ml of ammonium cyanide	Add 4 ml of ammonium cyanide
Shake for 30 mins	Shake for 30 mins
Observe the color	Observe the color

Reasons:

Ammonium citrate, potassium cyanide, hydroxylamine hydrochloride is used to make pH optimum so interference and influence of other impurities have been eliminated.

Phenol red is used as indicator to develop the color at the end of process Lead present as an impurities in the substance, gets separated by extracting an alkaline solution with a dithizone extraction solution.

INFRENCE:

REPORT:

REFERENCE:

- **Indian pharmacopoeia**
- **Pharmaceutical chemistry I by N.C. Choudhary and NK. Gurbani - page no-221-224.**
- Pharmaceutical chemistry – Inorganic by G.R Chatwal -55-57

Limit Test for Lead as per B.P.

Principle:

The test described in B.P. is dependent upon the formation of brownish 6colouration when sodium sulphide is added to dilute solution of lead salts, the intensity of the coloration varying with the quantity of lead present. If the lead has been present in more than traces, a colloidal brownish black precipitate of lead sulphide has been formed instead of the colour. The colour so obtained is matched against standard colour produced from a known amount, of lead and thereby, the exact quantity of lead present in the sample determined.

The comparison is done in two similar 50 ml Nessler cylinder made of thin lead free glass. In order to carry out this test, two solutions called primary and auxiliary prepared from the sample.

Procedure:

For the test, two standard solutions of the substance have to be prepared, a primary solution and an auxiliary solution, the primary solution containing a definite but greater amount of the substance than the auxiliary.

Two solutions of the substance under test are prepared; with hot water and acetic acid. The primary solution, containing a definite but greater amount of substance, is placed in a 50 ml. Nessler cylinder. The auxiliary solution, containing a known amount of the test substance is taken in another 50 ml Nessler cylinder. To this auxiliary solution, a definite amount of a dilute solution of lead nitrate is added. Ammonia and potassium cyanide solutions are added to the both solutions in the Nessler cylinders. If they are coloured, then small amount of burnt sugar solution is added to both solutions, to correct any difference of colour and the volume is made up to 50 ml. If the solutions appear ' turbid, then they are filtered, and the volume made up to 50 ml. Both solutions are treated with sodium sulphide solution, and a colour is developed. If the colour in the auxiliary solution becomes darker than that in the primary, then the substance is having lead within limits.

The method may be used for determining approximate amount of lead in the substance by preparing a number of auxiliary solutions and adding varying amounts of dilute lead solutions to each. The one nearest in colour to the primary will give the required value. If more than 15 ml of dilute lead

solution PbT is needed, a smaller quantity of substance is to be taken.

S.NO	PRIMARY (TEST)	AUXILLARY (STANDARD)
1.	4 g of substance dissolved in water having 10 ml of Acetic Acid PbT.	1 g substance dissolved in water having 7 ml of Acetic Acid PbT is added. 2 ml of dilute solution of lead PbT is added
2.	The solution is made just alkaline with solution of Ammonia PbT and then 1 ml of solutionof potassium Cyanide PbTis added.	The solution is made just alkaline with solution of Ammonia PbT and then 1 ml of solution of potassium Cyanide PbTis added.
	Filter both the solution if they get turbid. And insert colouring matter if colours are different	
3.	Dilute the solution to 50 ml with water, add 2 drops of Solution of Sodium Sulfide PbT, and stir.	Dilute the solution to 50 ml with water, add 2 drops of Solution of Sodium Sulfide PbT, and stir.
	Compare the two colours	

If the two solutions are having the same tint, it implies that 2 ml of dilute solution of lead PbT is having the same amount of lead as is contained in the difference of weights of substance in the two solutions, primary and the secondary. In the above case, the difference has been 2 g in the weights of the substance in the auxiliary and the primary solutions. 2 ml of dilute solution of lead PbT is having 10 p.p.m. of lead.

INFERENCE:

REPORT:

REFERENCE:

- **Indian pharmacopoeia**
- **Pharmaceutical chemistry I by N.C. Choudhary and NK. Gurbani - page no-221-224.**
- Pharmaceutical chemistry – Inorganic by G.R Chatwal -55-57

EX NO **DATE**

LIMIT TEST FOR ARSENIC

AIM:

To perform the limit test for arsenic in the given sample of ammonium chloride.

APPARATUS USED:

- Gut- Zeit apparatus
- Glass rod,
- Beaker.

REAGENTS USED:

- Stannated HCl
- Potassium iodide
- Ast,granulated zinc
- Ast dilute arsenic solution.

GUT-ZEIT APPARATUS:

The apparatus consist of a wide- mouthed glass bottle fitted with rubber bung through which passes a glass tube. The length of the tube is 20mm, outer diameter 8mm,inner diameter 6.5mm.The tube is open at its upper end but the lower end where the tube gets narrower. The purpose of providing a small aperture at the lower end is for smooth and slow passage of arsine gas, through the bottle to the tube.

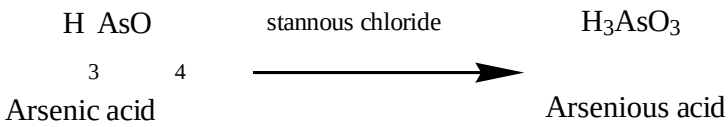
The glass tube is slightly packed with cotton wool which has been previously moistened with lead acetate solution and dried.The purpose of lead acetate cotton wool is to trap any hydrogen sulphide gas which would otherwise interfere with this test as it also give stain with **mercury chloride**

paper. A piece of dry mercury chloride paper is placed flat on the top of the bung and other bung is placed over it,and is served by means of clips. The clips will be placed in such a manner that boring of the 2 bungs meet to form a tube of the same diameter.

PRINCIPLE:

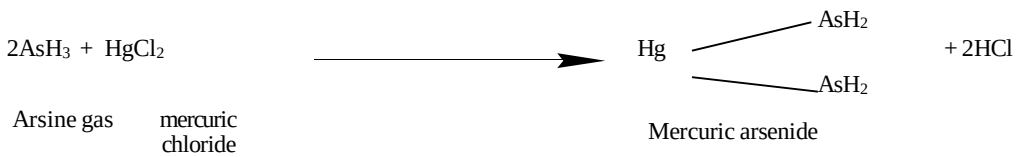
The sample is dissolved in acid which converts the arsenic impurity to arsenious acid or arsenic acid depending upon the valancy state of arsenic in the given sample.

The solution is treated with a reducing agent such as stannous chloride to convert the pentavalent arsenic acid into trivalent arsenious acid.



Arsenious acid is converted to arsine gas with the help of nascent hydrogen**.**

Arsine gas is passed through the tube by the steam of hydrogen and out through the mercury chloride paper. A reaction occurs between arsine and mercuric chloride which may be represented as



This results in the formation of yellow or brown stain on the mercuric chloride paper. The intensity of the color is proportional to the quantity of arsenic if the presence of sulphur is detected a special reagent lead acetate is used in AsT hydrogen sulphate may be formed during the reaction. Lead acetate is placed on to prevent the reaction between hydrogen sulphide and mercurous chloride. Hydrogen sulphide is trapped out.

H_2S + $Pb(CH_3COO)_2$ → $2CH_3COOH$ + PbS

Hydrogen sulphide — lead acetate — Acetic acid — Lead sulphide

PROCEDURE:

S.NO	TEST	STANDARD
1.	Place 50ml of distilled water in the arsine test apparatus and label it as test.	Place 50ml of distilled water in the arsine test apparatus and label it as standard.
2.	Add 2.5 g of sample and dissolve it.	Add 2.5 g of sample and dissolve it.
3.	Addition of stannated hydrochloric acid (10ml)	Addition of stannated hydrochloric acid (10ml)
4.	Add 1g of potassium iodide	Add 1g of potassium iodide
5.	Add 10g of granulated zinc.	Add 10g of granulated zinc.

Allow the reaction to proceed for 40 min in the dark compare the yellow stain produced in the test and standard.

INFERENCE:

REPORT:

REFERENCE:

- Indian pharmacopoeia 1996 - page no A-42
- Bently and Drivers Text book of pharmaceutical chemistry - page no -113.


PREPARATION

EX NO

DATE

AIM: **PREPARATION OF BORIC ACID**

To prepare boric acid from borax and find out its percentage yield.

REQUIREMENTS:

Apparatus required:

- Beaker
- Funnel
- Glass rod
- Conical flask

Reagent required:

- Borax
- Concentrated HCl
- Distilled water.

PRINCIPLE:

Boric acid is prepared by adding a mixture of concentrated.HCl and adds water to the boling solution of borax in water.

Boric acid is also obtained by the action of sulphuric acid on borax. On cooling the reaction mixture, white flakes of boric acid are obtained.

PROCEDURE:

- A solution of 4gm of Borax in 10ml of distilled water is prepared in a beaker.
- The above contents in the beaker are boiled and filtered. While hot , add 4.2ml of conc. HCl it is mixed well and set aside for crystallization.
- The crystals are filtered off and drained until free from mother liquor.
- Washed with very small amount of water and drained completely.
- Recrystallised from water and dried.ss

DESCRIPTION:

- A white crystalline powder or colourless shiny plates, unctuous to touch or white crystals.
- It is soluble in water and glycerin.
- It is odourless and tasteless.

STORAGE:

It is stored in a tightly closed container in a cool place.

IDENTIFICATION TEST:

- Solution of boric acid in alcohol on igniting in a porcelain dish burns with green tinged flame.
- An aqueous solution of boric acid on addition of HCl turn the turmeric paper to pink or brownish red, which upon being moistened with ammonia solution changes to blue or green colour.

USES:

- Used as anti infective.
- Used as bacteriostatic & fungistatics

REPORT:

The boric acid was prepared and submitted.

- ➢ Theoretical yield of boric acid:
- ➢ Practical yield of boric acid:
- ➢ Percentage yield of boric acid:

REFERENCES:

- ❖ Indian pharmacopoeia 1996 volume I.
- ❖ The merck index 13th edition.

EX NO **DATE**

PREPARATION OF POTASH ALUM $K_2SO_4.Al_2(SO_4)_3.24H_2O$

AIM:

To prepare potash alum from aluminium sulphate and find out its percentage yield.

REQUIREMENTS:

Apparatus required:

- Beaker
- China dish
- Funnel
- Glass rod
- Tripod stand
- Wire gauze
- Burner
- Wash bottle
- Measuring jar

Reagent required:

- Potassium sulphate
- Aluminium sulphate
- Dil. Sulphuric acid
- Distilled water

PRINCIPLE

Potash alum, a double salt, commonly known as fitkari has the formula K2SO4.Al2(SO4)3.24H2O. It can be prepared by making equimolar solution of potassium sulphate and aluminium sulphate in minimum amount of water. A few ml of dil. H2SO4 is added to prevent the hydrolysis of Al2(SO4)3.18H2O. Cooling of the hot saturated solution yields colourless crystals of Potash alum.

$$K2SO4 + Al2(SO4)3.18H2O + 6H2O \rightarrow K2SO4.Al2(SO4)3.24H2O$$

PROCEDURE:

- Take 2.5g potassium sulphate crystals in a clean beaker.
- To this add 20ml of distilled water and stir using a glass rod until the crystals completely dissolve.
- Take 10g aluminium sulphate in another beaker.
- Add about 20ml of distilled water and 1ml of dil.sulphuric acid to it.
- Heat the contents of the beaker for about 5 minutes.
- Mix the two solutions in a china dish.
- Heat the solution in the china dish for some time to concentrate it to the crystallisation point.
- Transfer the solution into a crystallising dish and do not disturb it.
- On cooling crystals of potash alum separate.
- Decant the mother liquor and wash the crystals with a small quantity of ice-cold water.
- Dry the crystals by placing them between filter paper pads.
- Find the weight of the crystals.

SYNONYMS :

- Potassium alum, potash alum Burnt alum (anhydrous)
- $K_2SO_4.Al_2(SO_4)_3.24H_2O$

DESCRIPTION:

- Large, transparent crystals or crystalline fragments, or white crystalline powder;
- Odourless
- Freely soluble in water; insoluble in ethanol
- pH 3.0 - 4.0 (10% solution)

STORAGE:

It is stored in a tightly closed container.

IDENTIFICATION TEST:

- Add 1 N sodium hydroxide drop wise to a solution of it (1 in 20): a precipitate is formed that dissolves in an excess of the reagent. Ammonia is not evolved (distinction from Ammonium Alum).
- Hold it in a non-luminous flame: a violet color is imparted to the flame.
- Add 10 mL of sodium bi tartrate TS to 5 mL of a saturated solution of it: a white, crystalline precipitate is formed within 30 minutes.
- A solution (1 in 20) responds to the tests for Aluminum and for Sulfate.
- **Sulfate (SO_4^{2-}) :** Add a few drops of barium chloride solution (Toxic) Barium chloride followed by a few drops of dilute hydrochloric acid. A white precipitate of barium sulfate is formed.
- **Aluminium (Al^{3+}) :** Add sodium hydroxide solution (Irritant). Colourless precipitate, $Al(OH)_3$

  Add ammonia solution. Colourless precipitate, $Al(OH)_3$

USES:

- Acidity regulator, firming agent, raising agent
- Astringent.

REFERENCES:

- The merck index 13th edition. - 356

EX NO **DATE**

PREPARATION OF FERROUS SULPHATE

AIM:

To prepare ferrous sulphate from ferrous carbonate and find out its percentage yield.

REQUIREMENTS:

Apparatus required:

- Beaker
- Funnel
- Glass rod
- Conical flask

Reagent required:

- Ferrous carbonate
- 30-40% sulphuric acid
- Distilled water.

PRINCIPLE:

Ferrous sulphate is obtained by the addition of 30-40% of sulphuric acid with ferrous carbonate. The resulting solution is filtered. The filtrate is evaporated to crytallization

PROCEDURE:

- Ferrous sulphate is prepared by dissolving 2gm of ferrous carbonate in sufficient quantity of 30-40% sulphuric acid.
- The resulting solution is filtered off.
- The solution of ferrous sulphate is concentrated to yield crystals.

DESCRIPTION:

- ➢ It is blue green colourless crystals or granules.
- ➢ Efflorescent in dry air, oxidize in moist air forming brown coating of basic ferrous sulphate.
- ➢ It is soluble in water, practically insoluble in alcohol.

STORAGE:

It is stored in a tightly closed container.

IDENTIFICATION TEST:

Prepare a solution (1 in 20) of the solution given sample and perform the following tests.

- ➢ To 5ml of the solution, add 1ml of dil. HCl and 1ml of dil. HCl solution. A white precipitate is formed.
- ➢ To 5ml of the solution add 2ml of lead acetate solution. A white precipitate is formed. The precipitate is soluble in ammonium acetate solution and in sodium hydroxide solution.

USES:

- ➢ Used as haematinic for iron deficiency anemia.
- ➢ Used as food and feed supplement.
- ➢ Used as reducing agents.
- ➢ Used as wood preservative and weed killer.

REPORT:

The ferrous sulphate was prepared and submitted.

- Theoretical yield of ferrous sulphate :
- Practical yield of ferrous sulphate:
- Percentage yield of ferrous sulphate:

REFERENCES:

- Indian pharmacopoeia 2007 volume II Page-1124.
- The merck index 13th edition page-717, 4091.
- Remington volume II 21st edition page: 1345.

TEST FOR PURITY

EX NO **DATE**

TEST FOR PURITY OF BENTONITE

AIM:

To find out the swelling property of given sample of bentonite.

POSSIBLE IMPURITIES:

Gritty particles

PROCEDURE:

SWELLING POWER

Add 2gm in twenty portions at intervals of 2 min to 100ml of a 1% w/v solution of sodium lauryl sulphate in 100ml graduated cylinder about 3cm in diameter. Allow each portion to settle before adding the next and let it stand for 2 hours. The apparent volume of the sediment at the bottom of the cylinder is not less than 24ml.

OBSERVATION:

REPORT:

REFERENCE:

Indian pharmacopoeia vol II page: 776.

EX NO **DATE**

TEST FOR PURITY OF ALUMINIUM HYDROXIDE GEL

AIM:

To find out the neutralizing capacity present in the given sample of aluminium hydroxide gel.

POSSIBLE IMPURITIES:

Neutralizing capacity

PROCEDURE:

(Pass a sufficient quantity , triturated if necessary through a sieve of normal mesh aperture of 150 µm.)

Weigh accurately 0.5gm of the shifted material and add to 200 ml of 0. 5M HCl previously heated to 37ºC and stir continuously, maintaining the temperature at 37ºC. The pH of the solution at 37ºC after 10, 15 & 20 min is not less than 1.8, 2.3 & 3.0 respectively. And at no time is more than 4.5. Add 10ml of 0.5M HCl previously heated to 37ºC and stir continuously for 1 hour maintaining the temperature at 37ºC and titrate with 0.1M NaOH to pH 3.5.

Not more than 35 ml of 0.1 M sodium hydroxide is required and the pH of the solution 37ºC then at no time is more than 4.5.

OBSERVATION:

REPORT:

REFERENCE:

Indian pharmacopoeia 2007 vol III page: 1575- 1576.

EX NO **DATE**

TEST FOR PURITY OF POTASSIUM IODIDE

AIM:

To find out the iodates present in the given sample of potassium iodide.

POSSIBLE IMPURITIES:

Iodates

PROCEDURE:

Dissolve 0.5 gm of potassium iodide in 10ml of carbon dioxide free water and add 0.15 ml of dilute sulphuric acid and a drop of iodide- free starch solution, no blue colour is produced with in 2 min. if the iodide is present the it reacts with the potassium iodide to liberate iodine which will give blue colour with starch.

OBSERVATION:

REPORT:

REFERENCE:

Indian pharmacopoeia 2007 vol III page: 1575- 1576.

IDENTIFICATION TEST

EX NO **DATE**

IDENTIFICATION TEST FOR FERROUS SULPHATE

AIM:

To perform the identification test for ferrous sulphate

REQUIREMENTS:

Apparatus required:

- Beaker
- Funnel
- Glass rod
- Conical flask

Reagent required:

- Hydrochloric acid
- Barium chloride
- Lead acetate
- Ammonium acetate
- Sodium hydroxide.
- dilute sulphuric acid
- 1, 10 phenanthroline& of 0.1 N ceric ammonium sulphate.
- potassium ferrocyanide

DESCRIPTION:

- It is blue green colourless crystals or granules.
- Efflorescent in dry air, oxidize in moist air forming brown coating of basic ferrous sulphate.
- It is soluble in water, practically insoluble in alcohol.

STORAGE:

It is stored in a tightly closed container because on exposure to moist air, ferrous sulphate crystals undergoes slow oxidation and becomes coated with brown basic ferric sulphate.

IDENTIFICATION TEST:

S.NO	TEST	OBSERVATION	INFERENCE
	Responds to the tests for ferrous salt and sulphate.- Prepare a solution (1 in 20) of the solution given sample and perform the following tests.		
1.	**Sulphates (SO_4^{2-})** (i) To 5ml of the solution, add 1ml of dil. HCl and 1ml of barium chloride solution.	A white precipitate is formed.	Presence of sulphate.
	(ii) To 5ml of the solution add 2ml of lead acetate solution.	A white precipitate is formed. The precipitate is soluble in ammonium acetate solution and in sodium hydroxide solution.	Presence of sulphate.
2.	**Ferrous salts (Fe^{2+}):** ***(i)*** A quantity of the substance being examined corresponding to about 10mg of iron is dissolved in 2ml of water. To this 2ml of dilute sulphuric acid and 1ml of a 0.1 % w/v solution of 1, 10 phenanthroline are added.	A intense blue colour is produced which is discharged by adding a slight excess of 0.1 N ceric ammonium sulphate. (Red colouration is because of formation of complx cation)	Presence of ferrous salt.
	(ii) To 1ml of a solution having not less than 1 mg of iron or 1ml of prescribed solution, 1ml of potassium ferricyanide is added.	A dark blue ppt is formed. Which is insoluble in dil.HCl, but is decomposed by NaOH solution.	Presence of ferrous salt.
	(iii) To 1ml of a solution having not less than 1 mg of iron or 1ml of prescribed solution, 1ml of potassium ferrocyanide is added.	A white ppt is obtained which rapidly becomes blue because of atmospheric oxidation and is insoluble in dil.HCl.	Presence of ferrous salt.

REACTIONS:

(TEST1)

(i) $FeSO_4 + BaCl_2 \rightarrow BaSO_4 + FeCl_2$

(ii) $FeSO_4 + (CH3COO)_2\ Pb \rightarrow PbSO_4 + 2\ CH_3COOFe$

(TEST2)

(i)

(The colour is discharged by ceric ammonium sulphate because of oxidation of Fe^{2+} to Fe^{3+})

(ii) $\mathbf{FeSO_4 + K_3[Fe(CN)_6] \rightarrow KFe[Fe(CN)_6] + K_2SO_4}$

(iii) $\mathbf{FeSO_4 + K_4[Fe(CN)_6] \rightarrow K_2Fe[Fe(CN)_6] + K_2SO_4}$

(O) ↓

$\mathbf{KFe[Fe(CN)_6}$

USES:

- ➢ Used as haematinic for iron deficiency anemia.
- ➢ Used as food and feed supplement.
- ➢ Used as reducing agents.
- ➢ Used as wood preservative and weed killer.

REPORT:

REFERENCES:

- ❖ Pharmaceutical chemistry – Inorganic by G.R Chatwal -307,445 & 452.
- ❖ Pharmaceutical chemistry – I by N.C.Chaudhry – 161.
- ❖ The merck index 13th edition page-717, 4091
- ❖ Remington volume II 21st edition page: 1345

EX NO **DATE**

IDENTIFICATION TEST FOR MAGNSIUM HYDROXIDE

AIM:

To perform the identification test for magnesium hydroxide.

REQUIREMENTS:

Apparatus required:

- Beaker
- Funnel
- Glass rod
- Conical flask

Reagent required:

- Dilute ammonia
- Sodium hydroxide.
- 2M ammonium chloride
- 0.25M disodium hydrogen phosphate
- Titan yellow

DESCRIPTION:

- White fine amorphous powder.
- It is almost insoluble in water yielding a solution which is slightly alkaline.
- It dissolves in dilute mineral acids.

STORAGE:

It is stored in a tightly closed container.

IDENTIFICATION TEST:

S.NO	TEST	OBSERVATION	INFERENCE
	Responds to the tests for magnesium (Mg^{2+})		
1.	**Magnesium (Mg^{2+})** (i) 15mg of the substances is dissolved in2ml of water.to this 1ml of dil.ammonia solution is added. .	A white precipitate is formed. (which is redissolved by adding 1ml of 2M ammonium chloride. To this 1ml of 0.25M disodium hydrogen phosphate is added) A white crystalline ppt is obtained.	Presence of magnesium.
	(ii) To 0.5ml of a neutral or slightly acidic solution of the substance being examined. 0.2ml of 0.1% solution of titan yellow and 0.5 ml of 0.1N sodium hydroxide are added.	A bright red turbidity is developed which gradually settles to give a bright red ppt.	Presence of magnesium.
2.	**Alkali: Hydroxide ion** : Litmus	It turns litmus blue.	Presence of hydroxide.
	Add a little of an ammonium salts.	Ammonia released from salt. $NH_{4(aq)}^{+} + OH_{(aq)}^{-} \Longrightarrow NH_{3(g)} + H_2O_{(l)}$	Presence of hydroxide.

Reaction:

Test (i) :

$$Mg^{2+} + 2OH^- \xrightleftharpoons[NH_4Cl]{NH_3 \text{ solution } (NH_4OH)} Mg(OH)_2$$

USES:

- Antacid to relieve **indigestion**, sour stomach, and **heartburn**.
- It is used as a laxative to relieve occasional **constipation** (irregularity
- It is also reduces **stomach acid**, and increases water in the intestines which may induce defecation.

REPORT:

REFERENCES:

- Pharmaceutical chemistry – Inorganic by G.R Chatwal -167-168 & 453-454.
- Pharmaceutical chemistry – I by N.C.Chaudhry
- .The merck index 13th edition

EX NO **DATE**

IDENTIFICATION TEST FOR SODIUM BICARBONATE

AIM:

To perform the identification test for sodium bicarbonate.

REQUIREMENTS:

Apparatus required:

- Beaker
- Funnel
- Glass rod
- Conical flask

Reagent required:

- Hydrochloric acid
- Uranyl zinc acetate.
- Potassium carbonate
- Potassium antimonite
- 1N acetic acid
- Magnesium uranyl acetate & magnesium sulphate
- Mercury chloride&2N acetic acid
- Barium hydroxide

.

DESCRIPTION:

- White crystalline or amorphous powder.
- Saline taste
- It is freely soluble in water. Practically insoluble in alcohol.
- When heated 100^0 C it gets converted into sesquicarbonate.
- It gives effervescence with acids. Its solution is alkaline in nature.

STORAGE: is stored in a tightly closed container.

IDENTIFICATION TEST:

S.NO	TEST	OBSERVATION	INFERENCE
	Responds to the tests for sodium (Na^+) & Bicarbonate (HCO_3)		
1.	**Sodium (Na^+)** (i) Sodium compounds moistend with hydrochloric acid & introduced on a platinum wire into the flame of a bunsen burner.	It give a yellow colour to the flame.	Presence of Sodium.
	(ii) solution of sodium salt with a solution of uranyl zinc acetate.	A yellow crystalline ppt is obtained.	Presence of Sodium.
	(iii) 0.1 g of substance dissolved in 2ml of water. To this solution 2ml of 15% w/v solution of potassium carbonate is added. Add 4 ml of freshly prepared potassium antimonite. The solution is boiled. After this solution is cooled in ice water.	A white ppt is obtained.	Presence of Sodium.
	(iv) 0.1 g of substance dissolved in 2ml of water. This solution is acidified with 1N acetic acid followed by addition of a large excess of magnesium uranyl acetate solution.	A yellow crystalline ppt is obtained.	Presence of Sodium.
2.	**Bicarbonate: (HCO_3)** (i) when solutions of bicarbonate are boiled. substance add to dilute hydrochloric acid.	Effervescence produced. (CO_2 is evolved)	Presence of Bicarbonate

IDENTIFICATION TEST:

S.NO	TEST	OBSERVATION	INFERENCE
	(ii) A solution of substance is treated with a solution of magnesium sulphate.	No ppt is formed. If the mixture of solution is warmed, a white ppt is obtained.	Presence of Bicarbonate
	(iii) Aqueous solution of bicarbonates when treated with mercury chloride solution.	A white ppt of mercuric bicarbonate.	Presence of Bicarbonate
	(iv) 0.1g of substance is dissolved in 2ml of water and add 2ml of 2N acetic acid. This tube is closed immediatetly. Gently tube is heated and the gas is collected barium hydroxide solution.	A white ppt is formed. It dissolves on addition of an excess of dilute hydrochloric acid.	Presence of Bicarbonate

Reaction:

Bicarbonate: (HCO_3)

(i) $2\ NaHCO_3 \rightarrow Na_2CO_3 + H_2O + CO_2$

(ii) $NaHCO_3 + MgSO_4 \rightarrow$ No reaction (in cold)

$2\ NaHCO_3 \rightarrow Na_2CO_3 + H_2O + CO_2$

$Na_2CO_3 + H_2O \rightarrow 2Na^+ + 2OH^- + CO_2$

$MgSO_4 + 2OH^- \rightarrow Mg(OH)_2 + SO_4^{2-}$

(iii) $2\ NaHCO_3 + HgCl2 \rightarrow Hg\ (HCO3)2 + 2\ NaCl$

(iv) $NaHCO_3 + CH_3COOH \rightarrow CH_3\ COONa + CO_2 + H_2O$

$Ba(OH)_2 + CO_2 \rightarrow Ba\ CO_3 + H_2O$

$BaCO_3 + 2\ HCl \rightarrow BaCl2 + CO_2 + H_2O$

USES:

- Sodium bicarbonate reduces stomach acid. It is used as an antacid to treat heartburn, indigestion, and upset stomach.
- It is the active ingredient in baking soda.
- Sodium bicarbonate is an electrolyte. It act by neutralizing excess acid in the blood. It may also replace bicarbonate when there are excess losses from the body.

REPORT:

REFERENCES:

- Pharmaceutical chemistry – Inorganic by G.R Chatwal -171,438 & 456
- .The merck index 13th edition
- Concise inorganic pharmaceutical chemistry by Dr.K.R.Mahadik- 212 & 215.

EX NO **AIM:**

DATE

IDENTIFICATION TEST FOR CALCIUM GLUCONATE

To perform the identification test for calcium glugonate.

REQUIREMENTS:

Apparatus required:

- Beaker
- Funnel
- Glass rod
- Conical flask

Reagent required:

- Ammonium carbonate.
- Ammonium oxalate .
- Ammonium chloride
- Acetic acid
- Potassium ferrocyanide
- Potassium chromate
- Ferric chloride.

DESCRIPTION:

- White crystalline powder or granular powder.
- It is odorless and tasteless. .
- Soluble in water; insoluble in ethanol
- stable in air

STORAGE:

It is stored in a well- closed container

IDENTIFICATION TEST:

S.NO	TEST	OBSERVATION	INFERENCE
	Responds to the tests for Calcium (Ca^{2+})		
1.	**Calcium (Ca^{2+})** (i) Solution of calcium salt treated with ammonium carbonate.	A white ppt is obtained. (The ppt is after boiling & cooling is insoluble in ammonium chloride) .	Presence of Calcium.
	(ii) Solution of calcium salt, few drops of ammonium oxalate is added.	A white ppt is obtained. (sparingly soluble in dil.acetic acid but is soluble in hydrochloric acid).	Presence of Calcium.
	(iii) 20mg of the substance is dissolved in 5ml of 5M acetic acid. Then 0.5ml of potassium ferrocyanide solution is added when the solution remains clear. Add 50mg of ammonium chloride.	A white crystalline ppt is obtained.	Presence of Calcium.
	(iv) Concentrated solution of calcium salt with the solution of potassium chromate.	A yellow crystalline ppt is obtained.	Presence of Calcium.
	Calcium gluconate: To 1 ml of Calcium Gluconate solution (1 → 40), add 1 drop of ferric chloride solution (1 → 10).	A dark yellow color develops.	Presence of Calcium gluconate

Reaction:

Calcium (Ca^{2+}) :

(i) $Ca^{2+} + (NH_4)_2CO_3 \rightarrow CaCO_3 + 2NH_4^+$

(ii) $Ca^{2+} + (NH_4)_2C_2O_4 \rightarrow CaC_2O_4 + 2NH_4^+$

$CaC_2O_4 + 2HCl \rightarrow CaCl_2 + H_2C_2O_4$

(iii) $2Ca^{2+} + K_4[Fe(CN)_6] \rightarrow Ca_2[Fe(CN)_6] + 4K^+$

$Ca_2[Fe(CN)_6] + 2NH_4Cl \rightarrow (NH_4)_2Ca[Fe(CN)_6] + CaCl_2$

(iv) $Ca^{2+} + K_2CrO_4 \rightarrow CaCrO_4 + 2K^+$

USES:

- It is used as a calcium replenisher.
- It is an important source of calcium in the treatment of hypocalcaemic tetany and other calcium defiency.

REPORT:

REFERENCES:

- Pharmaceutical chemistry – Inorganic by G.R Chatwal -450
- .The merck index 13th edition
- Concise inorganic pharmaceutical chemistry by Dr.K.R.Mahadik- 211 & 212.
- Pharmaceutical chemistry – I by N.C.Chaudhry-166.

EX NO **DATE**

IDENTIFICATION TEST FOR COPPER SULPHATE

AIM:

To perform the identification test for copper sulphate.

REQUIREMENTS:

Apparatus required:

- Beaker
- Funnel
- Glass rod
- Conical flask

Reagent required:

- Hydrochloric acid
- Barium chloride
- Lead acetate
- Ammonium acetate
- Sodium hydroxide
- 2M NH_4OH
- $CuSO_4$ solution
- *Ammonia*

.

DESCRIPTION:

- A blue, crystalline powder or transparent, blue crystals.
- Freely soluble in water, soluble in methanol.
- Practically insoluble in alcohol.

STORAGE:

It is stored in a tightly closed container.

IDENTIFICATION TEST:

S.NO	TEST	OBSERVATION	INFERENCE
	Responds to the tests for sulphate & Copper Prepare a solution (1 in 20) of the solution given sample and perform the following tests.		
1.	**Sulphates (SO_4^{2-})** (i) To 5ml of the solution, add 1ml of dil. HCl and 1ml of barium chloride solution.	A white precipitate is formed.	Presence of sulphate.
	(ii) To 5ml of the solution add 2ml of lead acetate solution.	A white precipitate is formed. The precipitate is soluble in ammonium acetate solution and in sodium hydroxide solution.	Presence of sulphate.
2.	**Copper ion (Cu2+)** Add a few drops of 2M NH_4OH to 1 ml of $CuSO_4$ solution	The precipitate is white-bluish basic salt that solubilizes in an excess of ammonia, giving the dark-blue color product – a tetraamminecopper sulphate –	Presence of copper
3.	Add several drops of *dilute ammonia* to 1 ml of solution	A blue precipitate is formed on further addition of *dilute ammonia* the precipitate dissolves and a dark blue colour is produced.	Presence of copper sulphate.

REACTIONS:

(TEST1)

(i) $CuSO_4 + BaCl_2 \rightarrow BaSO_4 + CuCl_2$

(ii) $CuSO_4 + (CH3COO)_2 Pb \rightarrow PbSO_4 + 2\ CH_3COOCu$

(TEST2)

$2CuSO_4 + 2NH_3 \cdot H2O \rightarrow [CuOH]_2SO4 + (NH_4)_2SO4$

$[CuOH]_2SO_4 + 6NH_3 \cdot H2O + (NH_4)_2SO4 \rightarrow 2[Cu(NH_3)_4]SO_4 + 8H2O$

USES:

- Copper sulphate is used for the preparation of Bordeaux and Burgundy mixtures which are used as fungicides.
- It is also used for the manufacture of other copper fungicides such as copper-lime dust, tribasiccopper sulphate, copper carbonate and cuprous oxide.
- It is also used to prepare insecticides like copper arsenite and Paris green.
- Also used as a fungicide and herbicide
- Several chemical tests utilize copper sulfate. It is used in Fehling's solution and Benedict's solution to test for reducing sugars, which reduce the soluble blue copper(II) sulfate to insoluble red copper(I) oxide.
- Copper(II) sulfate is also used in the Biuret reagent to test for proteins.
- Copper sulfate is used to test blood for anemia.

REPORT:

REFERENCES:

- .The merck index 13th edition
- Wikipedia.

IDENTIFICATION TEST:

S.NO	TEST	OBSERVATION	INFERENCE
	Responds to the tests for sodium (Na^+) & Bicarbonate (HCO_3)		
1.	**Sodium (Na^+)** (i) Sodium compounds moistend with hydrochloric acid & introduced on a platinum wire into the flame of a bunsen burner.	It give a yellow colour to the flame.	Presence of Sodium.
	(ii) solution of sodium salt with a solution of uranyl zinc acetate.	A yellow crystalline ppt is obtained.	Presence of Sodium.
	(iii) 0.1 g of substance dissolved in 2ml of water. To this solution 2ml of 15% w/v solution of potassium carbonate is added. Add 4 ml of freshly prepared potassium antimonite. The solution is boiled. After this solution is cooled in ice water.	A white ppt is obtained.	Presence of Sodium.
	(iv) 0.1 g of substance dissolved in 2ml of water. This solution is acidified with 1N acetic acid followed by addition of a large excess of magnesium uranyl acetate solution.	A yellow crystalline ppt is obtained.	Presence of Sodium.
2.	**Bicarbonate: (HCO_3)** (i) when solutions of bicarbonate are boiled. substance add to dilute hydrochloric acid.	Effervescence produced. (CO_2 is evolved)	Presence of Bicarbonate

IDENTIFICATION TEST:

S.NO	TEST	OBSERVATION	INFERENCE
	(ii) A solution of substance is treated with a solution of magnesium sulphate.	No ppt is formed. If the mixture of solution is warmed, a white ppt is obtained.	Presence of Bicarbonate
	(iii) Aqueous solution of bicarbonates when treated with mercury chloride solution.	A white ppt of mercuric bicarbonate.	Presence of Bicarbonate
	(iv) 0.1g of substance is dissolved in 2ml of water and add 2ml of 2N acetic acid. This tube is closed immediatetly. Gently tube is heated and the gas is collected barium hydroxide solution.	A white ppt is formed. It dissolves on addition of an excess of dilute hydrochloric acid.	Presence of Bicarbonate

Reaction:

Bicarbonate: (HCO_3)

(v) $2\ NaHCO_3 \rightarrow Na_2CO_3 + H_2O + CO_2$

(vi) $NaHCO_3 + MgSO_4 \rightarrow$ No reaction (in cold)

$2\ NaHCO_3 \rightarrow Na_2CO_3 + H_2O + CO_2$

$Na_2CO_3 + H_2O \rightarrow 2Na^+ + 2OH^- + CO_2$

$MgSO_4 + 2OH^- \rightarrow Mg(OH)_2 + SO_4^{2-}$

(vii) $2\ NaHCO_3 + HgCl2 \rightarrow Hg\ (HCO3)2 + 2\ NaCl$

(viii) $NaHCO_3 + CH_3COOH \rightarrow CH_3\ COONa + CO_2 + H_2O$

$Ba(OH)_2 + CO_2 \rightarrow Ba\ CO_3 + H_2O$

$BaCO_3 + 2\ HCl$ $BaCl2 + CO_2 + H_2O$

IDENTIFICATION TEST:

S.NO	TEST	OBSERVATION	INFERENCE
	Responds to the tests for sodium (Na^+) & Bicarbonate (HCO_3)		
1.	**Sodium (Na^+)** (i) Sodium compounds moistend with hydrochloric acid & introduced on a platinum wire into the flame of a bunsen burner.	It give a yellow colour to the flame.	Presence of Sodium.
	(ii) solution of sodium salt with a solution of uranyl zinc acetate.	A yellow crystalline ppt is obtained.	Presence of Sodium.
	(iii) 0.1 g of substance dissolved in 2ml of water. To this solution 2ml of 15% w/v solution of potassium carbonate is added. Add 4 ml of freshly prepared potassium antimonite. The solution is boiled. After this solution is cooled in ice water.	A white ppt is obtained.	Presence of Sodium.
	(iv) 0.1 g of substance dissolved in 2ml of water. This solution is acidified with 1N acetic acid followed by addition of a large excess of magnesium uranyl acetate solution.	A yellow crystalline ppt is obtained.	Presence of Sodium.
2.	**Bicarbonate: (HCO_3)** (i) when solutions of bicarbonate are boiled. substance add to dilute hydrochloric acid.	Effervescence produced. (CO_2 is evolved)	Presence of Bicarbonate

IDENTIFICATION TEST:

S.NO	TEST	OBSERVATION	INFERENCE
	(ii) A solution of substance is treated with a solution of magnesium sulphate.	No ppt is formed. If the mixture of solution is warmed, a white ppt is obtained.	Presence of Bicarbonate
	(iii) Aqueous solution of bicarbonates when treated with mercury chloride solution.	A white ppt of mercuric bicarbonate.	Presence of Bicarbonate
	(iv) 0.1g of substance is dissolved in 2ml of water and add 2ml of 2N acetic acid. This tube is closed immediatetly. Gently tube is heated and the gas is collected barium hydroxide solution.	A white ppt is formed. It dissolves on addition of an excess of dilute hydrochloric acid.	Presence of Bicarbonate

Reaction:

Bicarbonate: (HCO_3)

(ix) $2\ NaHCO_3 \rightarrow Na_2CO_3 + H_2O + CO_2$

(x) $NaHCO_3 + MgSO_4 \rightarrow$ No reaction (in cold)

$2\ NaHCO_3 \rightarrow Na_2CO_3 + H_2O + CO_2$

$Na_2CO_3 + H_2O \rightarrow 2Na^+ + 2OH^- + CO_2$

$MgSO_4 + 2OH^- \rightarrow Mg(OH)_2 + SO_4^{2-}$

(xi) $2\ NaHCO_3 + HgCl2 \rightarrow Hg\ (HCO3)2 + 2\ NaCl$

(xii) $NaHCO_3 + CH_3COOH \rightarrow CH_3\ COONa + CO_2 + H_2O$

$Ba(OH)_2 + CO_2 \rightarrow Ba\ CO_3 + H_2O$

$BaCO_3 + 2\ HCl \rightarrow BaCl2 + CO_2 + H_2O$

USES:

- Sodium bicarbonate reduces stomach acid. It is used as an antacid to treat heartburn, indigestion, and upset stomach.
- It is the active ingredient in baking soda.
- Sodium bicarbonate is an electrolyte. It act by neutralizing excess acid in the blood. It may also replace bicarbonate when there are excess losses from the body.

REPORT:

REFERENCES:

- Pharmaceutical chemistry – Inorganic by G.R Chatwal -171,438 & 456
- .The merck index 13th edition
- Concise inorganic pharmaceutical chemistry by Dr.K.R.Mahadik- 212 & 215.

EX NO **DATE**

IDENTIFICATION TEST FOR CALCIUM GLUGONATE.

AIM

To perform the identification test for calcium glugonate.

REQUIREMENTS:

Apparatus required:

- Beaker
- Funnel
- Glass rod
- Conical flask

Reagent required:

- Ammonium carbonate.
- Ammonium oxalate .
- Ammonium chloride
- Acetic acid
- Potassium ferrocyanide
- Potassium chromate
- Ferric chloride.

DESCRIPTION:

- White crystalline powder or granular powder.
- It is odorless and tasteless. .
- Soluble in water; insoluble in ethanol
- stable in air

STORAGE:

It is stored in a well- closed container

IDENTIFICATION TEST:

S.NO	TEST	OBSERVATION	INFERENCE
	Responds to the tests for Calcium (Ca^{2+})		
1.	**Calcium (Ca^{2+})** (i) Solution of calcium salt treated with ammonium carbonate.	A white ppt is obtained. (The ppt is after boiling & cooling is insoluble in ammonium chloride) .	Presence of Calcium.
	(ii) Solution of calcium salt, few drops of ammonium oxalate is added.	A white ppt is obtained. (sparingly soluble in dil.acetic acid but is soluble in hydrochloric acid).	Presence of Calcium.
	(iii) 20mg of the substance is dissolved in 5ml of 5M acetic acid. Then 0.5ml of potassium ferrocyanide solution is added when the solution remains clear. Add 50mg of ammonium chloride.	A white crystalline ppt is obtained.	Presence of Calcium.
	(iv) Concentrated solution of calcium salt with the solution of potassium chromate.	A yellow crystalline ppt is obtained.	Presence of Calcium.
	Calcium gluconate: To 1 ml of Calcium Gluconate solution (1 → 40), add 1 drop of ferric chloride solution (1 → 10).	A dark yellow color develops.	Presence of Calcium gluconate

Reaction:

Calcium (Ca^{2+}) :

(v) $Ca^{2+} + (NH_4)_2CO_3 \rightarrow CaCO_3 + 2NH_4^+$

(vi) $Ca^{2+} + (NH_4)_2C_2O_4 \rightarrow CaC_2O_4 + 2NH_4^+$

$CaC_2O_4 + 2HCl \rightarrow CaCl_2 + H_2C_2O_4$

(vii) $2Ca^{2+} + K_4[Fe(CN)_6] \rightarrow Ca_2[Fe(CN)_6] + 4K^+$

$Ca_2[Fe(CN)_6] + 2NH_4Cl \rightarrow (NH_4)_2Ca[Fe(CN)_6] + CaCl_2$

(viii) $Ca^{2+} + K_2CrO_4 \rightarrow CaCrO_4 + 2K^+$

USES:

- It is used as a calcium replenisher.
- It is an important source of calcium in the treatment of hypocalcaemic tetany and other calcium defiency.

REPORT:

REFERENCES:

- Pharmaceutical chemistry – Inorganic by G.R Chatwal -450
- .The merck index 13th edition
- Concise inorganic pharmaceutical chemistry by Dr.K.R.Mahadik- 211 & 212.
- Pharmaceutical chemistry – I by N.C.Chaudhry-166.

EX NO **DATE**

IDENTIFICATION TEST FOR COPPER SULPHATE

AIM:

To perform the identification test for copper sulphate.

REQUIREMENTS:

Apparatus required:

- Beaker
- Funnel
- Glass rod
- Conical flask

Reagent required:

- Hydrochloric acid
- Barium chloride
- Lead acetate
- Ammonium acetate
- Sodium hydroxide
- 2M NH_4OH
- $CuSO_4$ solution
- *Ammonia*

.

DESCRIPTION:

- A blue, crystalline powder or transparent, blue crystals.
- Freely soluble in water, soluble in methanol.
- Practically insoluble in alcohol.

STORAGE:

It is stored in a tightly closed container.

IDENTIFICATION TEST:

S.NO	TEST	OBSERVATION	INFERENCE
	Responds to the tests for sulphate & Copper Prepare a solution (1 in 20) of the solution given sample and perform the following tests.		
1.	**Sulphates (SO_4^{2-})** (i) To 5ml of the solution, add 1ml of dil. HCl and 1ml of barium chloride solution.	A white precipitate is formed.	Presence of sulphate.
	(ii) To 5ml of the solution add 2ml of lead acetate solution.	A white precipitate is formed. The precipitate is soluble in ammonium acetate solution and in sodium hydroxide solution.	Presence of sulphate.
2.	**Copper ion (Cu2+)** Add a few drops of 2M NH_4OH to 1 ml of $CuSO_4$ solution	The precipitate is white-bluish basic salt that solubilizes in an excess of ammonia, giving the dark-blue color product – a tetraamminecopper sulphate –	Presence of copper
3.	Add several drops of *dilute ammonia* to 1 ml of solution	A blue precipitate is formed on further addition of *dilute ammonia* the precipitate dissolves and a dark blue colour is produced.	Presence of copper sulphate.

REACTIONS:

(TEST1)

(iii) $CuSO_4 + BaCl_2 \rightarrow BaSO_4 + CuCl_2$

(iv) $CuSO_4 + (CH3COO)_2\ Pb \rightarrow PbSO_4 + 2\ CH_3COOCu$

(TEST2)

$2CuSO_4 + 2NH_3 \cdot H2O \rightarrow [CuOH]_2SO4 + (NH_4)_2SO4$

$[CuOH]_2SO_4 + 6NH_3 \cdot H2O + (NH_4)_2SO4 \rightarrow 2[Cu(NH_3)_4]SO_4 + 8H2O$

USES:

- Copper sulphate is used for the preparation of Bordeaux and Burgundy mixtures which are used as fungicides.
- It is also used for the manufacture of other copper fungicides such as copper-lime dust, tribasiccopper sulphate, copper carbonate and cuprous oxide.
- It is also used to prepare insecticides like copper arsenite and Paris green.
- Also used as a fungicide and herbicide
- Several chemical tests utilize copper sulfate. It is used in Fehling's solution and Benedict's solution to test for reducing sugars, which reduce the soluble blue copper(II) sulfate to insoluble red copper(I) oxide.
- Copper(II) sulfate is also used in the Biuret reagent to test for proteins.
- Copper sulfate is used to test blood for anemia.

REPORT:

REFERENCES:

- .The merck index 13th edition
- Wikipedia.