MEDICAL LAB TECHNICIAN

Paper - I

BIOCHEMISTRY - 1

INDEX

Chapter-1.

INTRODUCTION TO BIOCHEMISTRY.

Structure

1.1 Introduction to Bio Chemistry

1.2 Code of ethics for Medical Lab Technicians

1.3 Medical Lab Organization.

Learning objectives:

1. Student should understand the perspective of Bio chemistry & clinical biochemistry.

2.Student should learn and follow the code of ethics

3. The pattern of division of a lab into different sections and their organization should be observed and understood.

1.1 Introduction to biochemistry

Bio chemistry is the science of study of chemical reactions taking place in living matter. It explains the complex processes of life from the simplest organisms to complex organism .As it explains the physiological process ,it is also called ***Physiological Chemistry***. **Alexander Neuberg** is often referred to as Father of Bio chemistry.

Basic Bio chemistry deals with carbohydrates,lipids,proteins, amino acids etc.

Medical Bio chemistry is the study of bio chemical, metabolic and physiological processes.

Chemical biology is a discipline at the interphase of life sciences and physical sciences.

Analytical chemistry is a subdivision of chemistry dealing with qualitative and quantitative determination of chemical components.

Clinical Bio chemistry is the application part of Bio chemistry for the diagnosis of the clinical condition by determining various constituents of the body like blood/plasma/serum, urine, C.S.F, Sputum ,stools ,semen etc.Bio chemistry has applications in various fields- Medicine, Phar macy , Medical lab technology ,Biomedical engineering, Biotechnology, Nursing, Veterrnary, Dentistry, Agriculture, Dairying etc.

1.2 Code of Ethics for Medical Lab Technicians

Lab technicians should follow certain ethics and abide by the code of conduct to discharge his duties perfectly.

Punctuality:Lab worker should maintain punctuality in attending the duties assigned to him. In emergencies,punctuality saves the life of patient.

Promptness: lab worker should be prompt in his work,postponement of the work of the work delays diagnosis and thus the treatment.

1. **Accuracy**:Lab worker should be accurate in giving report.Guessing and assumptions should not be done in reporting.
2. **Confidentiality**: Clinical revealing should be maintained confidentially between lab technicianand Doctor.It should not be disclosed to the patient attendants or his relatives.
3. **Courtesy**: Courteous,kind and sympathetic approach should be followed towards the patient and his attendants.
4. **Obedience:** Technicians should be loyal and obedient to the superiors like Superintendent,pathologist, Micro Biologist,blood bank medical officer.
5. **Cordiality:** Technicians should maintain good relation and work in harmony with colleagues.
6. **Generosity:**Technicians should be generous and kind to the subordinates so as to extract their services promptly.
7. **Sincerity:** Technicians should maintain sincerity in the profession.Unlawful method of earning should not be followed.
8. **Cautiousness and Alertness**: Technicians should always be cautious and be acquainted with the safety procedures to be followed in the lab for preventing lab accidents and first -aid measures in case of lab accidents.
9. **Awareness:** Technicians should always be aware of the advancements through journals,books and literature so as to perform advanced methods.
10. **Maintenance of standards**: Technicians should maintain high standards in the work to elevate the status of the profession and reputation.
11. **Discipline:** Smoking,eating, drinking beverages should not be done in lab premises.Persons not connected with lab work should not be allowed into the lab. A white, cotton apron has to be worn by technicians.

1.3 Medical Lab Organization:

Medical Laboratory in big hospitals is divided into different specialties'. They are:

1. Clinical Pathology: It includes examination of the body fluids.
2. Haematology: It is the examination of blood cells and constituents.
3. Bio chemistry: It is the qualitative and quantitative examination of body fluids.
4. Micro Biology: It includes microbiological cultures, assays, staining, antibiotic sensitivity tests etc.
5. Histo-Pathology and Cytology:It includes processing and preparation of tissue specimen for microscopic examination.
6. Blood Bank and Immuno Haematology: In this collection and donation of blood takes place. Compatibility tests are done here before the blood is collected.

All the technicians working in a lab are called Medical Lab Technicians.

The following chart shows different members involved in a medical laboratory.

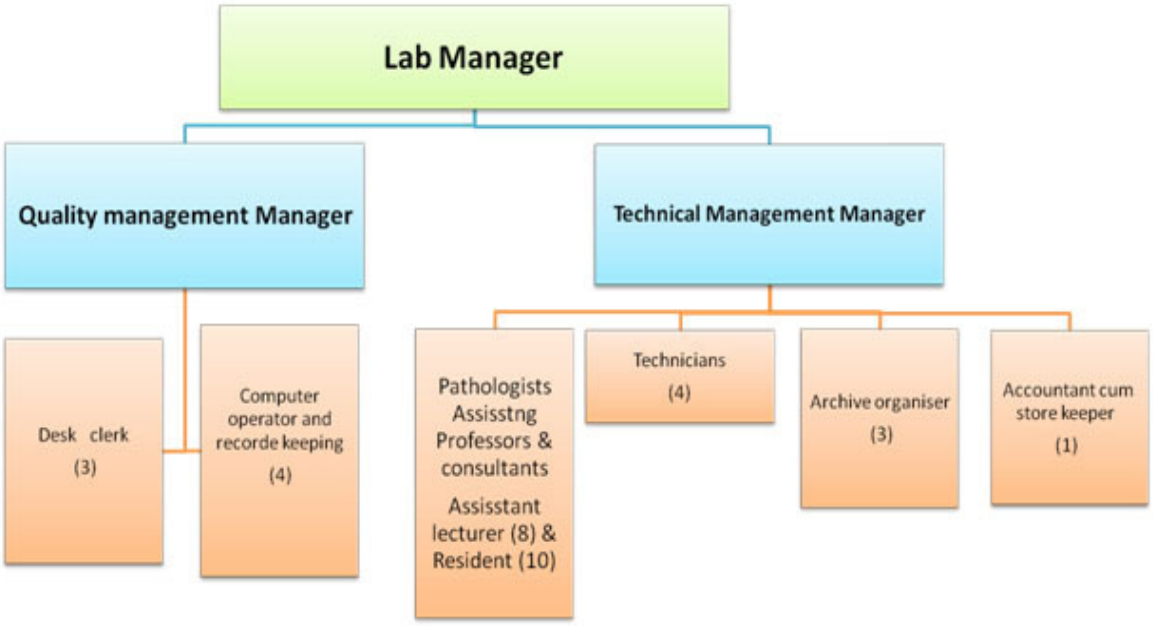

Fig 1.1 Different members of Medical Laboratory.

The following chart represents a medical laboratory lay out.

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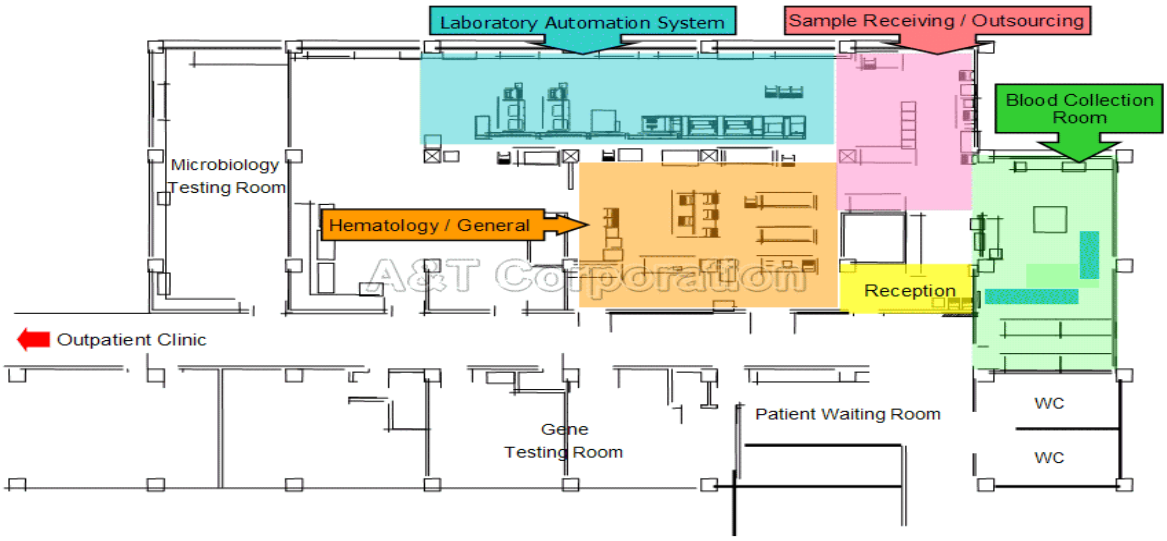

Fig 1.2 Medical Laboratory Layout

Many Accrediting Agencies like NABL,ISO,CRICIL,etc.are laboratories which provides formal recognition of competence of a laboratory with international criteria.

Conclusion:

Medical lab technicians should follow the code of ethics strictly.Medicine is the science of healing of body and mind.Medical lab technology is a part of it.Bio chemistry is needed in medical lab technology for the purpose of diagnosis.

Summary:

Medicine is the science of diagnosis,treatment or preventing thediseases that damage Body and mind.Bio chemistry is the science of study of bio chemical changes taking place in living beings.

A lab technician has to follow certain ethics and abide by the code of conduct.A lab technician should be punctual, prompt,accurate,courteous,loyal,sincere and maintain discipline.

A medical lab is divided into different specialities like Bio chemistry, Micro biology and serology, clinical pathology, blood bank and Immuno Haematology and histopathology and cytology.

Short answer type questions:

1. Define Biochemistry?
2. What are the fields of application Biochemistry?
3. Mention any two things of requisition in the code of conduct for lab technicians.

Long answer question:

1. Write an essay on the code of ethics for medical lab technicians.

Chapter -----2.

RECEPTION, REGISTRATION,MODELS OF STOCK REGISTERS, TYPES OF CHEMICALS AND LABORATORY SAFTY.

Structure:

2.1 Reception and Registration.

2.2 Different models of stock registers used laboratory.

2.3 Types of chemicals used in bio chemistry lab.

2.4 Safety measures to be taken in laboratory.

Learning objectives:

After studying this unit, the student is able to:

1. Understand the concept of reception and registration process in a laboratory and understand about lab registration form.
2. Student learn about the stock registers maintained in a lab.
3. Lab safety rules are understood.

2.1 Reception and Registration

When a person is ill, diagnosis begins with the physical examination by a doctor. There are various diagnostic tests to confirm a disease.Diagnostic tests ordered to be conducted are written over a format called lab order form.

Out- patient slip is given to out- patient and it contains the details of the patient which are entered into outpatient registration.

In patient is a patient whose clinical condition needs hospitalization and is given treatment by admitting the patient in the hospital ward.

Contents of lab order form:

Details of lab order form or lab request form should contain details such as hospital name and address , name of the patient , age, sex , date , O.P/ I.P. no; etc. are written on the upper column of the lab order form. Investigations to be done are written in the lower column of the lab order received.

Patient who receives lab order from doctor, hands over it to reception area lab technician in the reception. The technician will have the duties like entry of date and name ,sample collection, labelling and sorting of pathological samples like urine, blood, C S F, stools and other body fluids. In this form.

Reception:

Reception is the first point of contact with Laboratory Medicine where specimens requiring analysis are way they provide support to Bio Medical Scientists (BMS), clinical scientists and medical staff. Reception area lab assistant should possess good communication skills average computer skills and familiarity with medical terminology.

The lab order form is received in the lab, details are entered into lab register and samples are sent to different sections such as Haematology, Pathology, Biochemistry, Microbiology, immunology etc. Patient is instructed to collect the sample himself or technician collects the sample required from the patient. Collection container is labelled properly for identification. The specimen label contains details as name of the patient, O.P/I.P no. ,name of the tests, type of specimen etc.

Specimen reception is done not only in reception area, also in the doctor's chamber, O.P clinics, I.P clinics and other units of hospital , medical , surgical camps, epidemics investigation camps, patients home depending on the situation.

Fig 2.1 central specimen unit.

Specimen collection unit is also called central specimen unit (CSU) since it is the place where thepatient's samples from different wards,clinics, and other hospitals arrive, sorted and sent to related laboratories with relevant information.

Registration:

Registration is the process of entering the details of the order form into lab register. The data is entered into computer also. Separate lab software is also available for the purpose.

Lab register:

Register into which details such as date, I.P/ O.P.no.,name of the patient, age,sex, ward, nature of test,

name of the specimen, report findings are entered,is called a lab register.

After testing the specimen, findings of the tests are entered into the lab register, signed by the technician in the column concerned and report is submitted to the referring doctor confidentially.

Fig 2.2 Form for registration of in patient (case sheet)

Inner page of case sheet containing lab tests ordered is given below:

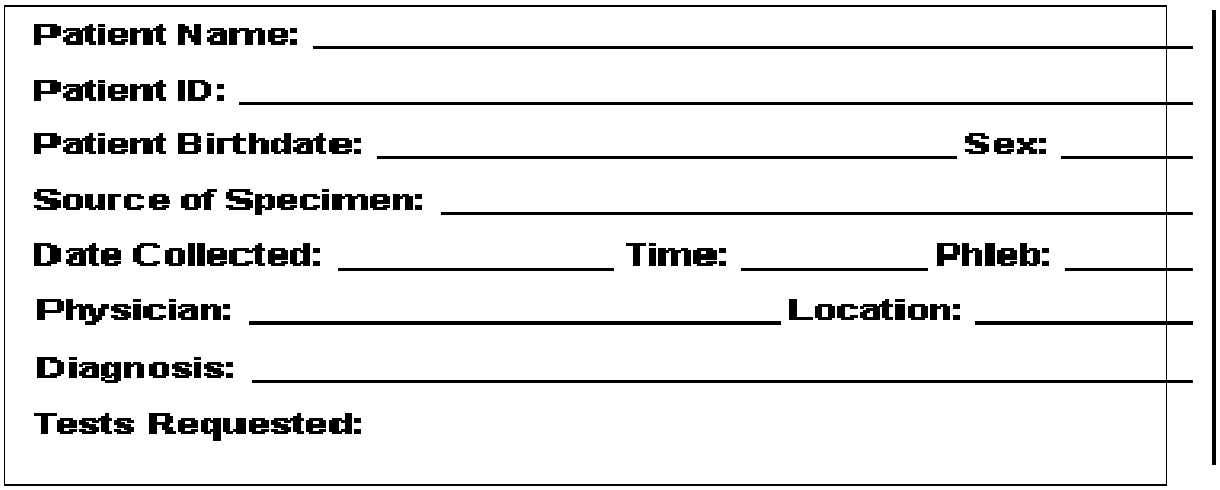

Fig 2.3Lab order form as the out- patient slip.

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2.2 Different stock registers used in laboratories:

Stock registers is a record of goods purchased and/or stored in a laboratory. It is updating of addition of incoming stock. Stock registers are mainly of two types: they are-

(a) **consumable stock register and Non-consumable stock register**.

Consumable stock register: In this register, the products or commodities which are used up regularly and quickly are mentioned. Ex. Spirit, cotton, syringes, etc.

Non-consumable stock register: In this register, the products or commodities which are unaltered for long time and do not deteriorate for long time are mentioned. Ex. Centrifuge, Micro-scope, glass ware in lab, etc

Stock Register

Date	Name of the item	Opening stock	Stock received	Batch No.	Expiry date	Consumed	Wastage	Closing stock	Remarks	Signature

Fig 2.4 Format of stock register.

2.3 Types of chemicals used in biochemistry laboratory:

Some chemicals used in laboratory are toxic and corrosive. Corrosives are substances which can cause destruction of living tissues. Proper handling storage of chemicals and is necessary to prevent hazards. Chemicals are stored according to their physical and chemical properties. Types of chemicals are.

1. **Flammable chemicals**. Ex: Acetone, Ether, Xylene, Alcohol, etc.
2. **Corrosive chemicals**. Ex: concentrated acids, alkalies, phenol etc.
3. **Oxidizing chemicals**. Ex: Potassium dichromate, chromic acid, chlorites, etc.
4. **Explosive chemicals.** Ex: picric acid.
5. **Radioactive chemicals**. Ex: I-125, I-131, H-3.
6. **Carcinogenic chemicals**. Ex: Benzidine, O-toludine, Selenite, etc.
7. **Toxic chemicals**. Ex: Potassium cyanide.

2.4 Safety measures:

Students working in laboratory must have two major concerns to avoid accidents. They are:

(1) Safety and
(2) Efficiency in the laboratory work.

Students working in a bio chemistry laboratory must always be aware that the chemicals used are potentially toxic. Students must be familiar with general safety practices, facilities and emergency action.

Safety rules to be followed in laboratories:

1. Do not work alone in the laboratory.
2. Eating, drinking and playing in the lab is strictly avoided.
3. Wear gloves, aprons and safety glasses while handling dangerous substances.
4. Foot ware must completely cover the foot.
5. Visitors are not allowed into the lab.
6. Keep your work area and the common work areas clean.
7. Clean up spill or inform the instructor immediately about the spill so that he takes some immediate action.
8. Dispose the waste properly and in a timely manner according to the instructions provided in the lab manual.
9. Do not use earphones while working in the lab.
10. Replace the chemicals in the specified racks after the experiment is finished.
11. Do not pipet chemicals with your mouth.
12. Do not taste the chemicals because they are poisonous.
13. Do not heat, measure or mix the chemicals in front of your face.
14. Make sure test tubes containing chemicals reactions are pointed away from people.
15. A laboratory must always be equipped with a first aid kit.
16. In a potentially life threatening emergencies inform the instructor immediately and the person is rushed to the hospital.

Conclusion:

Reception is the first point of laboratory medicine. Registration is recording the things of lab request form into register .A biomedical laboratory maintains certain stock

registers to be maintained in the laboratory with details of equipment. Some chemicals used in labs are corrosive. Laboratory safety measures are to be followed by a student to perform practical safely.

Summary:

When a patient approach a medical laboratory, a lab order form is received at the lab, its lab register and after testing process is completed, the test findings are entered into lab register. The process of receiving the lab order form and collection of specimen from the patient is called reception. Entering the details of patient and test recordings into register is called registration.

Different stock registers are register maintained ina laboratory which contain all the details of the consumable and non-consumable equipment present in the lab. Corrosives are the substances which can cause destruction of living tissue. Several safety measures are to be followed by a student to perform the lab practical safely.

Short answer type questions:

1. What is lab order form?
2. Define lab register.
3. What is lab registration?
4. What is a stock register? Mention the different types of stock registers?
5. Define (a) out- patient (b) in-patient.
6. Define (a) Strong acid (b) Weak acid.
7. Define (a) Strong alkali (b) Weak alkali.
8. Write the properties of Hydrochloric acid.
9. Write the properties of sodium hydroxide.
10. What are corrosives? Mention some examples.
11. Name some chemicals used in laboratory.
12. Mention types of chemicals used in Bio chemistry lab.
13. Write some examples of carcinogenic chemicals.
14. Mention examples of Flammable chemicals.

Long Answer Type questions:

1. Write about reception and registration.
2. Mention the safety measures to be followed while working in a laboratory.

Activities:

1. Visit a hospital and observe in patient and out- patient registration.
2. Go to a lab and observe the process of registration and reception.

Chapter-3

Glassware and plastic ware used in Biochemical laboratory

Structure:

3.1 Glassware

3.2 Plastic ware: Brief outline.

3.3 Cleaning and drying of glass ware and plastic ware.

Learning objectives*:*

Student should-

1. Visit a chemistry, pharmacy and medical labs and get acquainted to different lab glassware and their uses.
2. Prepare a museum of glass ware with the help of fellow students and lecturer.
3. Learn to clean and dry the glass ware after the use.

3.1 Glass ware:

Types and composition:

Different glass and plastic containers are used in a laboratory for various purposes as measuring, transfer, mixing, boiling, filtration, etc. During these processes, they are exposed to chemicals contained in them. Glass used for manufacture of these glass ware must resist the action of chemicals like acids, alkalies etc. They must withstand the mechanical rigors during handling, operation and the effect of sudden changesof temperature during boiling, refluxing, etc.

(a) Composition of glass ware: The composition of glass is--

Pure silica, Sodium carbonate, Brokenglass, Boron oxide, Lead and Alumina.

- Boron glass acts as fusion agent.
- Reduction in the quantity of sodium ions renders the glass chemical resistance

- Without alkalies, melting the glass is expensive and difficult.
- Boron oxide reduces the temperature required for melting the glass.
- Trace quantities of lead gives clarity and brilliance.
- Alumina imparts hardness and increased resistance to chemical action and durability.

(b) Types of glass:Different types of glass are—

1. Borosilicate glass.
2. Treated soda lime glass.
3. Regular soda lime glass and
4. General purpose soda lime glass.

1. **Borosilicate Glass:** The glass ware generally used in laboratory is manufactured with Borosilicate glass. It is resistant to the action of chemicals and effects of sudden changes of temperature during boiling, refluxing etc. However it is not resistant to the action of hydro fluoric acid. This highly resistant glass is made by replacing alkali and alkaline earth cations by Boron /Aluminium and Zinc. It is chemically more resistant than soda lime glass. It is composed of --
    - Silica- 80.6%
    - Boron oxide- 12.6%
    - Sodium oxide- 4.15% and
    - Alumina -2.2%.

    Addition of boron reduces leaching action.

2. **Treated Soda Lime Glass:** Soda - lime glass subjected to the treatment by an atmosphere containing water vapour and Sulphur dioxide at elevated temperature is called as Treated Soda Lime Glass. This type of treatment is called as Sulphur treatment and it protects the glass from blooming or weathering.
3. **Regular Soda Lime Glass:** This is commercial glass. It is not subjected to Sulphur treatment. It has average to better than average resistance.
4. **General purpose soda lime Glass:** This is soda-lime glass used for general purpose.

Types of glass ware, their identification, application and use:

Types of laboratory glass ware:

(a) Graduated glassware.

(b) General glassware.

Graduated glass ware:

Glassware which have graduations on them and intended for purpose as containing, delivering etc. and which are used in accurate measurements for use in volumetric analysis are called as graduated glassware. There are two types of graduated apparatus in Great Britain –(1) class A and (2) class B

Class A apparatus is used in work of highest accuracy. Class B apparatus is used in routine work.

Different graduated glassware are:

1. Graduated flasks
2. Pipettes
3. Burettes
4. Weight burettes
5. Measuring cylinders
6. Hb tube and
7. Syringes.

1. **Graduated flasks:** They are also known as volumetric flasks. They are of two types-
   (a) To contain (TC) type: They are more accurate for quantitative work.
   (b) To deliver(TD) type

The graduated flasks has-

- Its bottom flat
- Is pear shape
- Has a long narrow neck
- A thin line etched around the neck. It indicates the volume it holds at definite temperature –usually 20 degrees C.

Fig 3.1Different graduated flasks.

Uses: They are used in making up final volume of standard solutions.

2. **Pipettes**: pipettes are two kinds :
   (a) Transfer pipettes
   (b) Measuring pipettes and
   (c) Specialized pipettes.

- **Transfer pipettes:** They are also called volumetric pipettes. It consists of a central bulb joined at the both ends to narrow tube. It has three parts:

  ---Upper suction tube.

  ---Central cylindrical bulb.

  ---Lower delivery tube.:

Fig 3.2 Transfer pipettes

Upper suction contains a mark etched around. When a liquid is pipetted up to this mark, liquid will be of the volume, the pipette is specified to contain. Lower tube is drawn out to a fine tip. Transfer pipettes are constructed with capacities of 1, 2, 5, 10, 20, 25, 50 and 100ml.

Uses: These pipettes are useful for delivering specific quantity of liquid. They are very accurate in quantitative work.

- **Measuring pipettes:** They are also called as graduated pipette. They do not contain a central bulb. There are three types of graduated pipettes.
    (1) **Type 1 graduated pipette**: They deliver required volume from top zero to selected graduation.
    (2) **Type 2 graduated pipette**: They deliver measured volume from selected graduation to the tip of delivery.
    (3) **Type 3 graduated pipette**: They are designed to remove selected volume of solution, thus to contain the capacity from tip of delivery to the selected graduation.

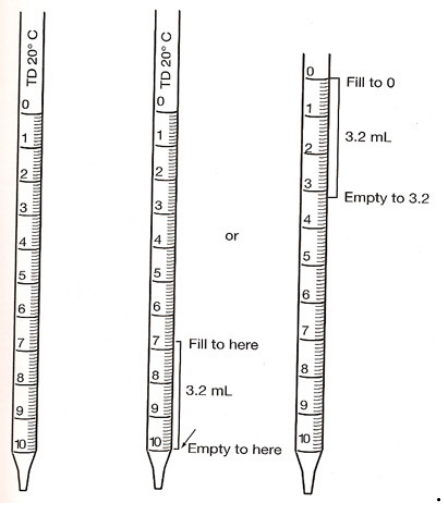


Fig 3.3 Image of measuring pipettes.

Uses: They can be used to deliver variable volume of liquid in the range of volume specified for the pipette. However they are not employed in accurate work where burette is preferred.

- **Specialised pipettes:**
  1. ***Serological pipettes***: serological pipettes are graduated pipettes used for pipetting serum, plasma, standard solutions, distilled water, etc. They are marked upto the tip 0.1 ml to 0.2 ml.
  2. ***Mohr pipettes***: Mohr pipettes are pipettes graduated above the tip, so that they can be used even when the tip is broken. They have similar application as serological pipettes but not that much accurate.
  3. ***Folin Oswald Measuring volumetric pipettes***: They are volumetric/transfer pipettes for measuring specific volume of liquid. They are useful in accurate quantitative works.
  4. ***Blood pipettes:*** They are:

(a) **R.B.C pipettes** used in Erythrocyte count. It contains a red bead in the central bulb. There are two marks etched around the lower tube. 0.5 to 1 below the bulb. 101 above the bulb. Rubber tube connected to the upper section of the tube aids in pipetting.

Blood is sucked up to 0.5 or 1 mark and diluted fluid is sucked up to 101 mark. Red bead helps in identification of the pipette and also in mixing of blood and diluting fluid. When clotting occurs in the lower delivery tube, a fuse wire is used to remove the clot.

Uses: It is used in R.B.C count.

(b) **W.B.C pipette** is T.C of Leucocytes. It is a pipette with a white bead in the central bulb. There are two marks etched around the lower delivery tube. 0.5 to 1 below the tube. 11 above the bulb. Rubber tube connected to the upper suction tube aids in pipetting.

Blood is sucked upto 0.5 or 1 mark .Diluting fluid is sucked up to 11 mark .White bead helps in identification and uniform mixing of blood with diluting fluid. Any clot in the lower delivery tube can be removed by a fuse wire.

Uses: It is used in 1.W.B.C. count 2.Sperm count

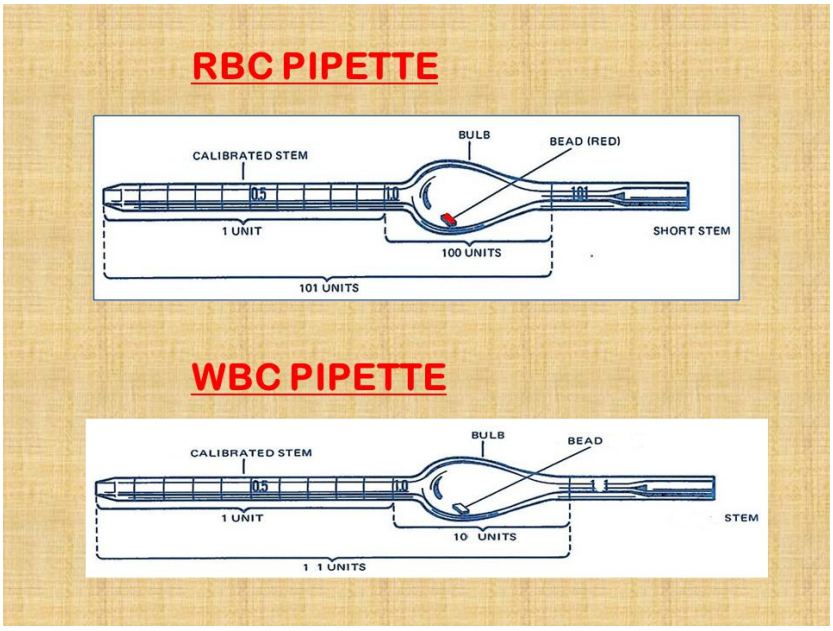


Fig 3.4 R.B.C pipette and W.B.C pipette.

(c) **Hb pipette**: This pipette is used in Hb estimation. It has no bulb. The mark etched around the tube is 20 micro liters (0.02ml) suction tube is attached at the top to aid in pipetting. Clot can be removed by a fuse wire.

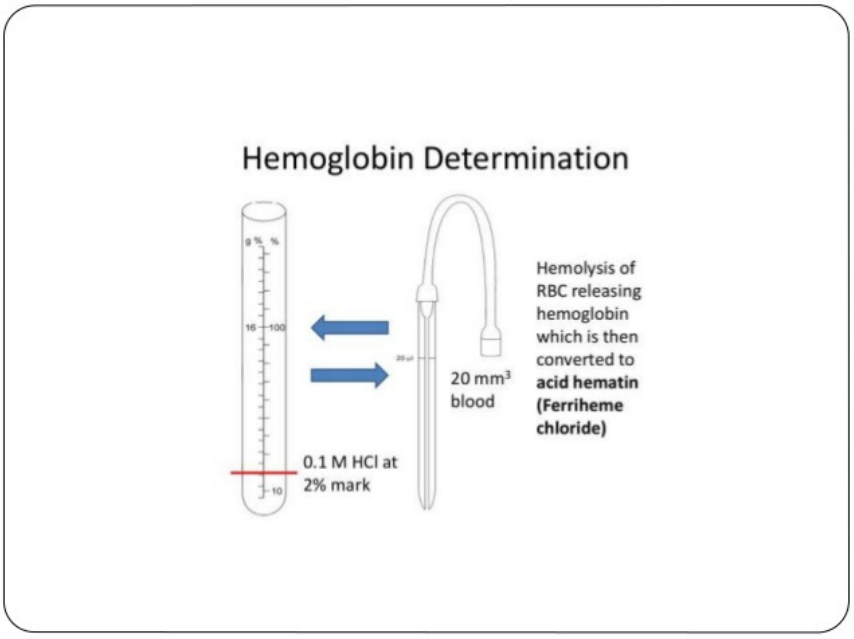


Fig 3.5 Diagram of a Hb pipette.

Uses: This pipette is useful in determining of Hb by Sahli's method and Cyanomethaenoglobin method.

(d) **E.S.R Pipette**: It is used in determination of erythrocyte sedimentation rate. It is a graduated with graduations from 0 to 200mm.

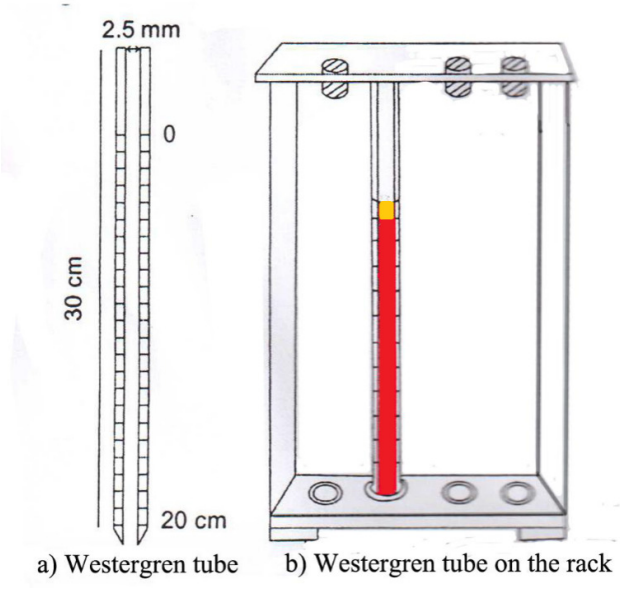


Fig 3.6 E.S.R pipette and E.S R stand.

Uses*:* It is used in E.S.R determination by Westergren's method.

3. **Burettes**: Burettes are long cylindrical tubes of uniform diameter. Bottom of the burettes is provided with stopcock. They are available from 5 to 100ml. There are two

classes of burettes-class A and class B.

Fig **3.7 Diagram of a burette.**

For smooth functioning of stopcock without friction or freezing, lubrication with pure Vaseline helps.

Uses: 1) They are used in titrations.

2) They are used in measuring and transfer of liquids.

4. **Weight burette**: They are used to measure the weight of the liquids, they transfer.

5. **Measuring Cylinders**: They are cylindrical in shape with flat bottom attached to provide base for resting vertically. They have pour points. There are graduations on the cylinder. They are available in capacities of 2 to 2000 ml. since the area of surface is more than in volumetric flasks, measuring is less accurate with these cylinders.

Fig 3.8Measuring cylinders.

(6) **Hb tube**: Hb tube is used in haemoglobin determination by Sahli's method. There 20 mark graduation on it up to 0.1N HCl is taken.

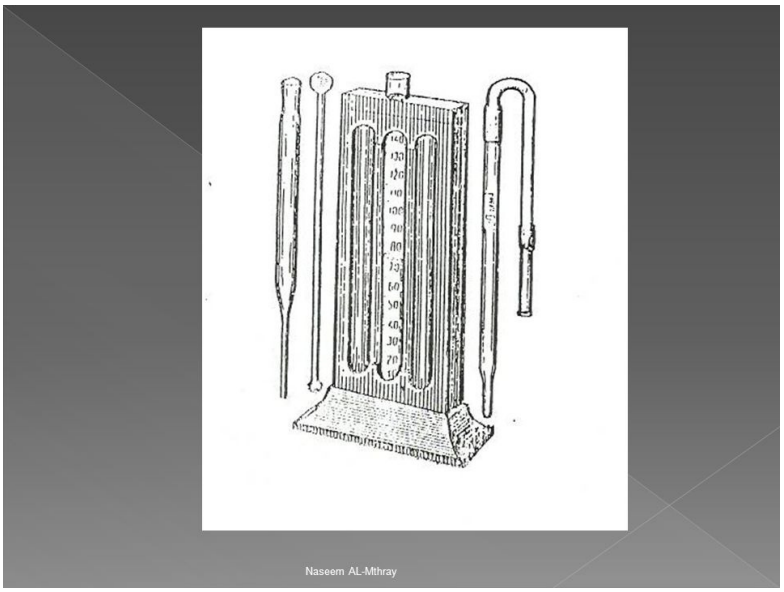


Fig 3.9 Hb tube in Hemocytometer.

(7) **Syringes:** Syringes are used in biochemical/ medical laboratories for the collection of venous and arterial blood specimens.

Fig 3.10 Image of a glass syringe.

General Glass Apparatus:

The general apparatus are:

1.Beaker 2.Flasks

3.Tubes 4.Separating funnels

5.Funnels 6. Condensers

7. Desiccators 8.Bottles

9.Wash bottles 10.Stirring rods

11.Weighing bottles 12.Watch glass

13.Clock glass 14.Weighing funnels

15.Glass slides

1. Beakers: Beakers are flat bottomed, broad based cylindrical vessels with a spout or pour point. They are available in capacities from 5 to 5000 ml. The most useful sizes are from 250 to 600 ml. pour point on the upper edge of beaker through which liquids can be transferred conveniently from the beaker into other container.

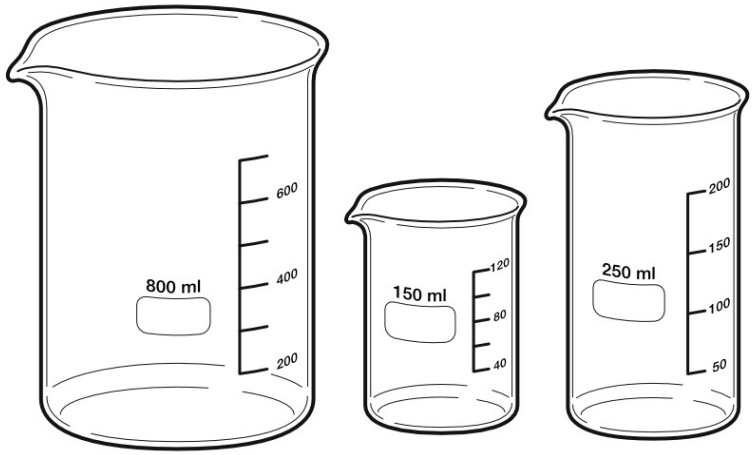


Fig 3.11 Different sizes of beaker.

Advantages of the pour point are:

- Convenience in pouring.
- Providing vent for steam during boiling.

- Providing convenience for protrusion of stirring rod when the beaker is covered.

Uses: 1.They are useful in preparation of solutions.

2. They are useful in boiling the solutions.

2.**Flasks:** Types of flasks – (a) Conical Flasks.

(b) Round bottomed flasks.

(c) Flat bottomed flasks.

(a) ***Conical flasks***: They are conical shaped with broad flat base. They are in different sizes ranging from 200-500 ml.

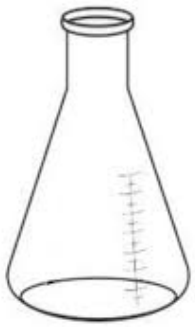


Fig 3.12 Diagram of a conical flask

Uses: 1.They are useful in conducting titrations.

2. They are also useful in boiling solutions. Their conical shape minimizes evaporation.

(b) ***Round bottomed flasks***: They have spherical bulb with a cylindrical neck. They can withstand high temperatures.

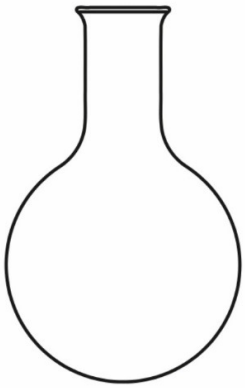


Fig 3.13 Diagram of round bottomed flask.

Uses: 1.They are used for making solutions.

2.They are used for boiling the solutions.

3. They are used in reflux condensations.

4. They are used for distillation purposes.

(c) ***Flat bottomed flask***: This is similar in shape to round bottomed flask except that it has flat bottom. It is used for boiling the solutions, liquids,etc.

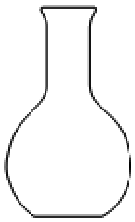
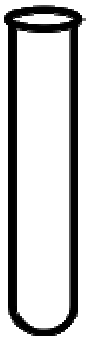


Fig 3.14Flat bottomed flask

3.***Tubes***: Different types of tubes are - (a) Test tubes (b) centrifuge tubes (c) Boiling tubes (d) Digestion tubes and (e) Nessler's tube.

(a) **Test tubes**: Test tube are two types--

1. *Test tubes with rim* are used when reagent is heated on a flame directly. Rim provides grim for holding with a test tube holder.



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2. *Test tubes without rim* are used for normal purpose.

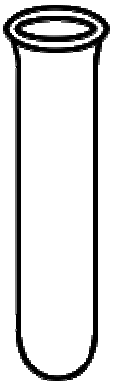


Fig **3.15(a) Test tubes without rim.** **Fig 3.15(b) Test tube with rim.**

They are available in different sizes. Commonly used test tubes are:

- Small size test tube have dimensions of 10 x 75 mm. They are used in qualitative testing procedures. They can also be used in place of centrifuge tubes.
- Medium size test tubes have dimensions of 15 x 125 mm. They find applications mainly in biochemical estimation.
- Big size test tubes have dimensions of 18 x 150 mm. They find application in heating directly on flame.

(b)**Centrifuge tubes:** They are similar in shape to the test tubes except that bottom portion is conical. They are useful for centrifugation and thus separation of solid from liquid in which it is insoluble.

Fig 3.16 Centrifuge tube.

(c)**Boiling Tubes:** They are bigger than tubes. They have similar shape as test tube. They are used for boiling small quantities of chemicals in qualitative testing procedures

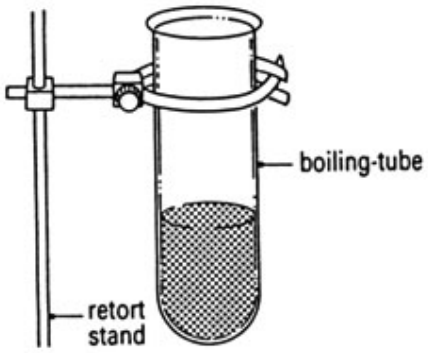


Fig **3.17 Boiling tube**.

(d)**Digestion Tube:** They are long narrow test tubes with slightly more than 50 ml capacity. They have graduations 25 ml. and 50 ml. etched around. They are used for converting organic matter into inorganic matter. This conversion is affected in the presence of digestion mixture which includes 50% sulphuric acid and 50% selenium dioxide.

Fig 3.18 Digestion tube

4. ***Separating Funnels***: It contains a conical chamber with a narrow neck into which a glass stopper fits. Conical chamber is attached to a narrow stem which has a stopcock. Stopcock helps in controlling delivery of the liquid contents taken in the chamber.

It is useful for the separation of immiscible liquids. For separation of immiscible liquids, stop cock is released until one liquid is delivered into. Another liquid remains in the chamber which can be delivered into another container.

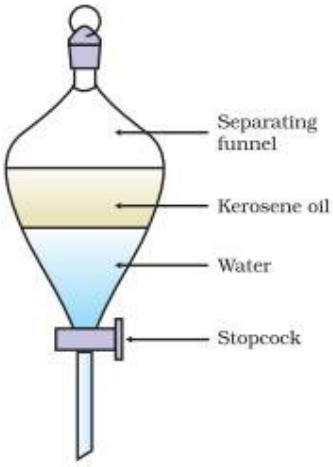


Fig 3.19 Diagram of a separating funnel

5. ***Funnels***: Funnels have conical top attached to a narrow stem. They are available in different sizes. Common sizes are 50, 65 and 75 ml. They useful in-
    a. Transfer of liquids from one container to another container.
    b. Separation of solid from liquid in which the solid is insoluble. This process is called filtration. Filtration is done with the help of a filter paper.

Fig 3.20 Drawing of a funnel

6. ***Condensers:*** condenser is a long narrow tube within a broad tube fused at the edges. It is arranged with inlet and outlet. It is useful in reflux condensation and distillation processes.

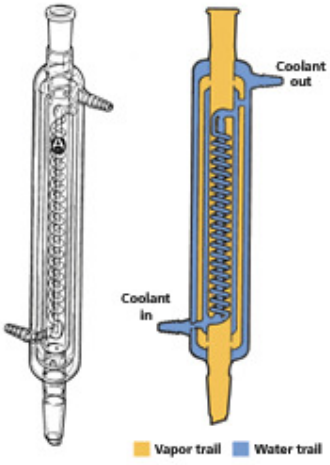


Fig 3.21Diagram of a condenser. **black and white diagram only**

7. ***Dessicators:*** Dessicator is a glass container. Dessicant substances are charged into the shallow dish at the bottom of the container. Substances to be protected from atmospheric moisture are taken in small containers and kept on a platform above the shallow dish and lid is closed. Examples of dessicants are Silicagel, Alumina, Calcium sulphate impregnated with cobalt salt.

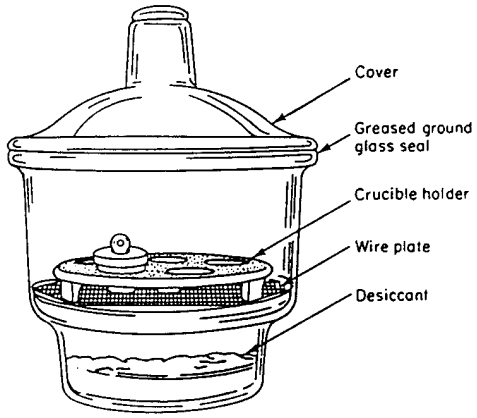


Fig **3.22 Diagram of a Dessicator.**

8. ***Bottles:*** Different types of bottles used in laboratory are—
   (a) **Reagent bottles**: They are cylindrical in shape with narrow neck fitted with stopper. They are available in different sizes ranging from 25-5000 ml. capacities. Amber colored bottles provide screening of U.V light present in the sunlight and prevent entry into the bottles. Thus photo sensitive chemicals like silver nitrate can be stored in such bottles.

Fig 3.23 Reagent bottle and Screw capped bottle.

(b)**Screw capped bottles**: They are available in 5-1000 ml. capacities. They are closed by screw caps made of metal or plastic. They store hygroscopic substances.

(c) **Winchester quart bottles**: They are cylindrical with narrow neck. They are fitted with glass stopper. They are white or amber colored available in 2 liters capacity. They are useful for stocking reagent.

Drop bottles are bottles containing slotted glass stoppers. Slotted glass stopper help in deliver of stains and indicators in the form of drops.

Fig 3.24 Dropper bottle

9. ***Wash bottles***: Wash bottle is a flat bottom flask that deliver distilled water or other liquid for use in the transfer and washing of precipitates. It is fitted with a rubber bung with two holes. Tubes are fitted into the bottle and air is blown into bottle. It used for washing precipitates since plastic wash bottles cannot with stand organic solvents.

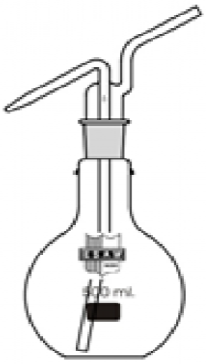


Fig 3.25 Glass wash bottle.

10. ***Stirring rods:*** They are made from 3-5 mm diameter glass rods cut into suitable length. It should be of such length that it should come 2-3 cm. out beyond the spout.

Fig 3.26 Glass stirring rod

Weighing Bottles: Bottles used for weighing chemicals are called as weighing bottles. They are used when substance is affected by atmosphere duringweighing. Chemical is placed in weighing bottles and weighed. Required quantity of substance is transferred into reaction vessel and then bottle is reweighed. Difference gives the weight of the chemical.

Fig 3.27 weighing bottle.

11. ***Watch glass***: Substance unaffected by atmosphere and which can affect balance pan are weighed using a watch glass.

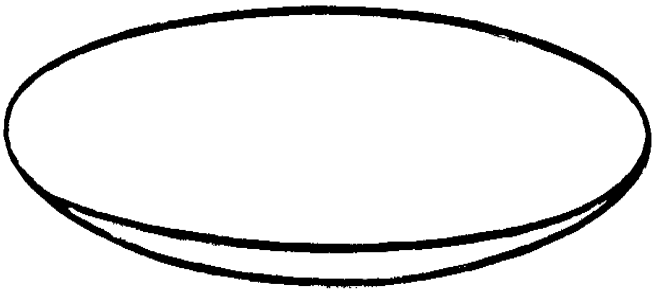


Fig 3.28 Watch glass.

12. ***Clock glass:*** clock glass is round flat piece of glass used for covering over a beaker can when a liquid and chemicals are heated in it. .

13. ***Weighing Funnel:*** Weighing funnel with scoop shaped end. After weighing on a balance, narrow stem of the funnel is inserted into the neck of the flask and the contents of the funnel are washed into flask with solvent/water from a wash.

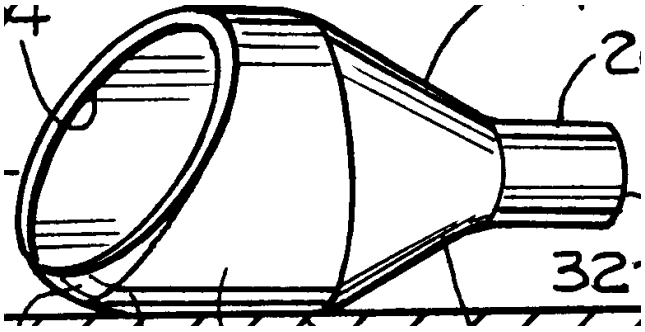


Fig 3.30 Weighing funnel. **Change fig no**

14. ***Glass slides***: Glass slides are flat rectangular pieces of glass. They are for microscopic examination of chemical substances in biochemistry.

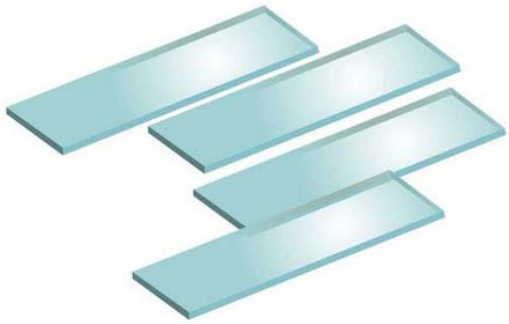


Fig 3.31 Glass slides

3.2 Plastic ware: Brief outline.

Materials used for making plastic lab ware: Different materials used for making plastic lab ware are:

--- Polyethylene.

---poly propylene.

---polyvinyl chloride

---polystyrene.

---poly amide(Nylon)

---poly carbonate and

---Tefflon.

Plastic is widely employed for lab ware like beakers, conical flasks, bottles, etc. They are cheaper than glass articles. Different plastic ware used in labs are:

1. ***Aspirator:*** It is also known as aspirator bottle which refers to a large cylindrical container used to store laboratory reagents. It is equipped with a tap or stopcock at the bottom for the delivery of the liquid.
2. ***Spatula:*** The term spatula refers to various small implements with a broad, flat, flexible blade used to mix. Spread, and lift materials from their containers. It has a long handle. Metallic spatulas also have been in use.

Fig 3.32 Plastic spatulas

3. ***Scoops:*** Scoops refers to small implements used to lift materials. Metallic scoops are also available.

Fig 3.33 plastic scoops

4. ***Lab wash bottles***: Also called Squeeze Bottles. A wash bottle has a screw-top lid with a nozzle and an internal dip to allow upright use. They are used to rinse various laboratory glass ware such as test tubes, flasks, etc. When hand pressure is applied to the bottle, the inside becomes pressurized and is forced out of the nozzle onto narrow stream of liquid. Wash bottles are used to fill with deionized water, distilled water, acetone, ethanol, etc.

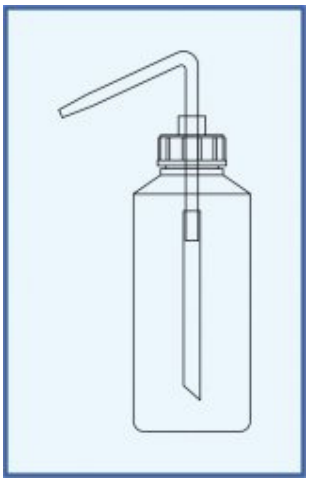


Fig 3.34 plastic wash bottle.

3.3(a)Cleaning, drying, maintenance and storage of glass ware:

All the glass ware must be thoroughly clean and dry before using, otherwise results will be unreliable.

Test for cleanliness:

1. Fill the apparatus with distilled water.
2. With draw the water
3. Observe for broken films of water.

   Presence of unbroken film of water is indicates thorough cleanliness. Collection of drops indicates presence of grease and dirt.

Aims of cleaning:

1. **Chemical cleanliness**: Glass ware should be free from all chemicals.
2. **Removal of particulate matter**: Glass ware should be free of particles and fibres.
3. **Removal of pyrogens**: Pyrogens are the products of bacterial metabolism capable of increasing body temperature above the normal. Glass ware should be free of pyrogens.
4. **Removal of grease**: Glass ware should be free from grease.

Cleaning agents:

1. **Organic cleaning agents**: They may be strongly adsorbed on to glass. For this reason, cationic detergents which are strongly anti -bacterial cannot be used for bacteriological glass ware.
2. **Soap flakes and powders**: Precipitation of calcium and magnesium soaps takes place in hard water. Precipitated deposits on glass ware impairs brilliance. Water softener such as sodium meta- phosphate eliminates this problem.
3. **Inorganic cleaning agents:** These are used alone or with soaps which gives brilliance to glass ware. They are also easily removed from the surface of the glass ware. Ex: sodium hexa-meta phosphate.
4. **Chromic acid:** Chromic acid cleaning solution is made by dissolving 70 grams of sodium or potassium dichromate in 40 ml. of water using heat. Then it is diluted to I litre with conc. Sulfuric acid with constant stirring. Solution must never be added to acid. Gloves and rubber apron should be worn while preparing this solution.
5. **Mixture of conc. Sulfuric acid and fuming nitric acid**: This acid mixture is more efficient cleaning liquid.it used when the vessel is greasy and dirty. It must be handled with extreme caution.
6. **10% KOH in methylated spirit**: 100 grams of KOH is dissolved on 50 ml. of water and diluted to 1 litre with industrial methylated spirit. It is very effective degreasing agent. **Teepol:** it is mild and inexpensive detergent. It is used in cleaning pipettes and burettes. 1ml.stock solution is diluted with 30 ml distilled water for routine use.

Basic steps in cleaning:

Different steps in cleaning are-

1**.Soaking:** Apparatus are filled with and immersed in hot cleaning solution and left overnight.

2**. Brushing**: Hand brushing can be done. For bottles, brushing machines may be used.

3**. Rinsing**: Rinsing has to be done with water followed by rinsing with distilled water.

4. **Draining:** water remaining on the surface of the glass ware should be drained. Properly cleaned glass ware should be dried before usage. It may be done in a hot air oven.

Maintenance and Storage:

- Glass ware should always be maintained clean and dry. Old glass ware should be cleaned before using.
- After use also, the glass ware should be cleaned again with required chemicals.
- They should be stored in a cupboard meant for their storage carefully to avoid breakages.
- List of articles are maintained in a register.
- Old articles are to be replaced by new ones.

3.3(b) Cleaning, maintenance and storage of plastic articles:

Plastic articles require one or more of the following treatments,

1. Boiling in week detergent. Concentration of detergent should be about 1%.
2. Boling in dilute alkali. 1%sodium carbonate is enough.
3. Boling in dilute acid.1%HCL is enough.
4. Boling in solution of sodium hexa-meta phosphate or EDTA.

15 minutes boiling in each case is enough. It should be followed by washing in running water. It should be followed by boiling in distilled water. Finally three more rinse in distilled water should be given.

5. After cleaning, they should be drained.
6. They can be air dried or dried in in oven at 65 degrees for 15 minutes with vent open. Oven should be used if the container material is thermostable enough.
7. After these steps, they can be used in different procedures.
8. After usage in different procedures, they have to be cleaned suitably.
9. Then they have to be kept in cup board and cleaned as above before using them again.

Conclusion

Different glassware is widely used in a lab. Though plastic ware has some disadvantages, it is also coming into usage due to non-breakability& low expensiveness.

Summary

Different glass and plastic apparatus are used in a laboratory for various purposes as measuring, transfer, filtration, storage etc. Glass used for lab purpose must be resistant to the action of chemicals. They must with stand mechanical rigors during handling, effect of high temperatures and sudden changes in temperature. Borosilicate glass is of choice in lab ware.

Laboratory glass ware can be classified in to 1) Graduated glassware 2) General glassware. Graduated glassware include flasks, pipettes, burettes, etc. General glassware include test tubes, syringes, funnels, etc.

Glassware and plastic ware used in lab must be thoroughly cleaned and dried before use. Several cleaning agents are available. Different steps in cleaning are 1) soaking 2) Brushing 3)Rinsing 4) draining 5) Drying. Lab ware is to be maintained and stored properly.

MODEL QUESTIONS:

Short Answer Type Questions:

1. Write the composition of glass.
2. Mention types of glass.
3. Give the composition of borosilicate glass.
4. What is sulfur treatment?
5. Mention the types of lab glassware.
6. Mention the types of graduated apparatus on the basis of accuracy.
7. What are the types of graduated flasks?
8. Give the uses of a) volumetric pipettes b) pipettes.
9. What are different types of graduated pipettes?
10. What are serological pipettes?
11. Mention different types of blood pipettes.
12. What are weight burettes?
13. Write the uses of a beaker.
14. What is pour point?
15. Write the uses if conical flasks.
16. Differentiate between round bottomed flask and flat bottomed flask.
17. Give the uses of funnel.
18. What is a condenser?

19. Mention the use of a dessicators.
20. Mention the different types of bottles.
21. What is a weighing bottle?
22. What is a weighing funnel?

Long answer Questions:

1. Give the composition of glass. Write about types of glass.
2. What are the types of glassware? Write about pipettes.
3. Mention the types of graduated apparatus. Write about graduated flasks.
4. Name different general apparatus used in the lab. Write about a) Flasks b) separating funnels.
5. What are the aims of cleaning? Write the steps involved in cleaning.
6. Write about plastic ware.

Chapter 4

Basic lab operations

Structure

4.1 Centrifuges: Separation of solids from liquids

4.2 Weighing: Types of balances used, care and maintenance

4.3 Evaporation

4.4 Distillation

Learning objectives

1. Student should know the basic lab operations like centrifugation, filtration, evaporation, distillation and refluxing to become a good technicians.
2. Student should become proficient in these operations in the process of acquiring skills.

Introduction

A lab technician should have a knowledge in basic lab operations like Separation of solids from liquids by centrifugation, filtration using funnels, weighing, evaporation and distillation.

4.1 Centrifugation

Centrifuges are used in a lab to separate solids from liquids in which solids are insoluble in liquids. It is also used in the separation of immiscible liquids. Centrifuges are designed to accelerate the process of sedimentation by centrifugal force. In a biochemical laboratory, they are useful in separation of cells from plasma, precipitate from liquid, etc.

Principle:centrifuges work on the principle of centrifugal force to separate insoluble solid from liquid. Centrifugal force needed to affect the separation process is provided by mechanical energy.

Types of centrifuges:

1. Hand centrifuge
2. Electrical centrifuge
3. High speed centrifuge
4. Ultra- centrifuge

5. Refrigerated centrifuge

Construction of Electrical centrifuge: Electrical centrifuge is a device operated by an electric motor which can give up to 5,000 RPM. Two types of centrifuges are used in laboratories. They are:

a) Swing out head type.
b) Angle head type.

Parts of electrical centrifuge:

1. Rotor with tubes
2. Motor
3. Chamber to enclose the internal parts
4. Lid on the top to close chamber during centrifugation
5. A multistage speed regulator to obtain desired speed
6. Timer
7. Tachometer to read the speed
8. Graphite brushes to provide electrical contact to the rotor
9. Power switch.

Fig 4.1 centrifuge

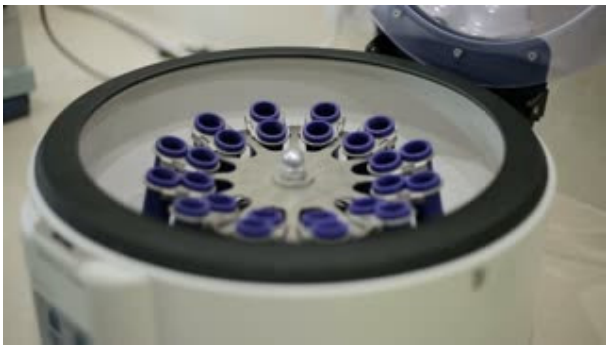


Fig 4.2 Rotor of centrifuge

Care and maintenance of electrical Centrifuge:

1. Place the centrifuge on a firm base.
2. Balance the tubes properly, otherwise tubes may be broken.
3. Increase or decrease the speed gradually.
4. Keep the lid closed during centrifugation.
5. Do not open the lid until the rotor comes to a stop.
6. Keep the chamber clean.
7. Cover the centrifuge with a cover, when not in use.
8. The graphite piece should be replaced by a new one, if it wears out.
9. Check the electrical connections at regular intervals of time.
10. Lubricate the moving parts at regular intervals of time.

Applications

It is useful in

1.Separation of insoluble solid from a liquid.

2.Separation of immiscible liquids.

In a medical laboratory, it has different applications. Some of them are-

1 .Separation of serum from clotted blood.

2 .Separation of plasma from cells.

3 .Separation of urinary sediment for predation for microscopic examination.

4 .Separation of precipitate or supernatant as required.

4.2 Weighing

Weighing is an important activity in a laboratory. Weighing of chemicals is required for preparation of reagents, standard solutions etc. Weighing for qualitative analysis can be approximate. Weighing requires balances. Accurate weighing can be done with analytical balances. Balance is one of the most important tools of an analytical chemist.

Different types of balances:

1. Simple balance.
2. Two knife single pan balance.
3. Top loading balance.
4. Electronic balance.
5. Miscellaneous:
   a) Torsion suspension balance
   b) Electro balance
   c) Torsion balance

Classification of balances according to capacity:

1 .Analytical balances:

Capacity: 150 to 200g. Accuracy upto 0.1 mg.

2 .Semimicro balances:

Capacity: 75 to 100g. Accuracy upto 0.01 mg.

3 .Micro balances:

Capacity: 10 to 30g. Accuracy upto 0,001 mg.

Simple Balance

It is also called as analytical balance, analytical 2 pan balance, chemical 2 pan balance.

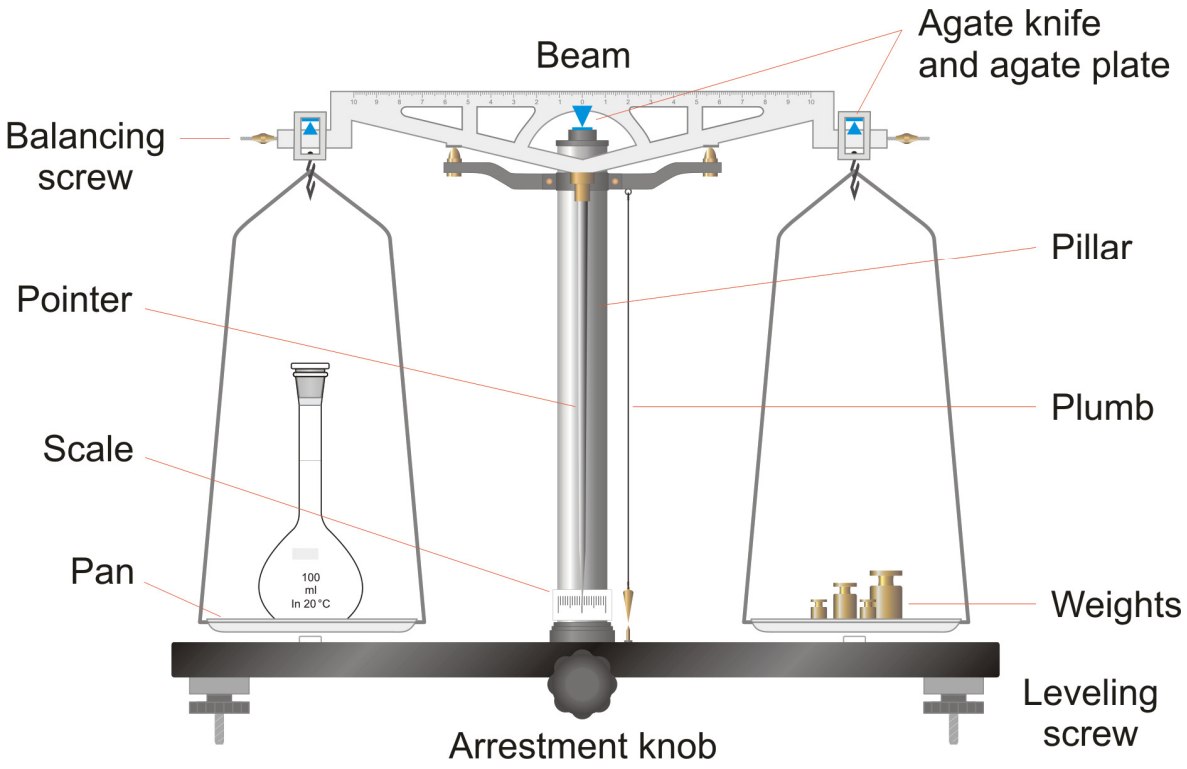


Fig 4.3 Simple balance

Components:

1. A beam
2. Knife edges and screw nuts
3. Stirrups
4. A pointer
5. Ivory scale
6. Rigid supports
7. Wooden platform with levelling screws
8. Handle
9. Central vertical pillar.

Operation:

1. Cleanthe pans of balance with camel brush.
2. Check the balance for equal sliding of the pointer on both sides by operating the handle lever.
3. Arrest the movement of the beam by operating the handle lever.
4. Place the sample to be weighed on the left pan and weights on the right pan.
5. Set the balance to move and check for equal movement of pointer on both sides of ivory scale. Arrest the movements of beam every time, a weight is removed or added to the pan.
6. Move the rider along the scale for adjustment of weight less than 10 mg.
7. When weighing is completed, arrest the beam and record the weight.
8. Clean any accidental spillage and close the balance case.

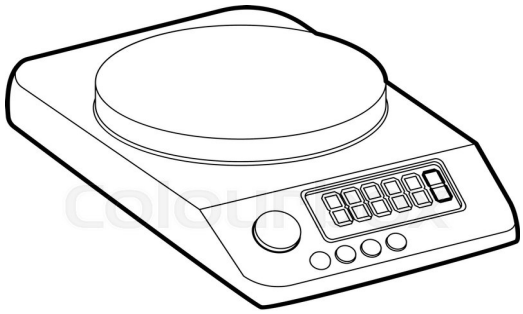
Care and maintenance:

1. The beam of the balance should be arrested when not in use, and also before adding or removing weights.
2. When not in use, the balance beam should be raised in order to protect the knife edges.
3. Doors of the balance must be closed whenever possible.
4. Pans should be maintained clean and dry.
5. The substance should not be weighed hot.
6. To release the balance, beam should be lowered gently.
7. Weights are to be kept in the pan with the help of a forceps.
8. The balance should be loaded with a weight greater than the maximum it is constructed to weigh.
9. Put a plastic cover over the wooden frame when not in use.

Uses: it is used to find out the mass of a substance in quantitative analysis.

Electronic balance

Electronic balance is modification of two knife single pan balance. In this type of balance, optical readout system is replaced by electrically operated measuring system.



balance

Fig 4.4 Electronic balance.

Components of electronic balance:

1. A null detector
2. Electromagnet
3. A read out device.

Operation:

1. Turn on its power supply.
2. Check digital display for zero reading.
3. If the reading is not zero, set it to zero.
4. Keep the substance to be weighed carefully on the balance.
5. Note the reading on the digital display.
6. If the substance being weighed is taken in weighing pan or watch glass, weight of empty pan or watch glass has to be substracted from total weight of substance along with pan or watch glass.

Uses: It is used for accurate weighing in quantitative determinations.

4.3 Evaporation

- Evaporation is the process of conversion of liquid into vapour at temperatures below boiling points.
- This technique can be used for purification of substances. Substances to be purified can be dissolved in a suitable solvent. Solvent on evaporation removes some of the impurities.
- Evaporation is allowed to take place by taking the solution in porcelain dish.

- Solvent on evaporation leaves purified substance in the dish.
- This process can be used for obtaining purest substances in volumetric analysis , recrystallization, etc.

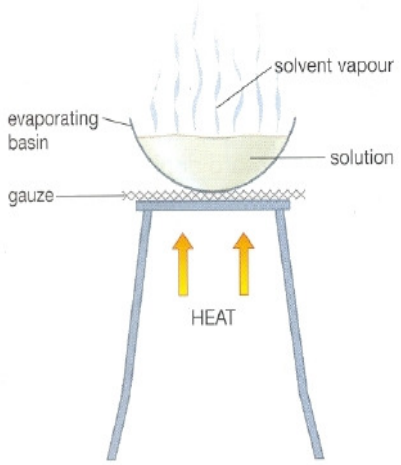


Fig 4.5 Process of evaporation

4.4 Distillation:

- Distillation is the process where in liquid is boiled and its vapors are condensed to collect the liquid. By this process, liquid is cleared off from the soluble impurities.
- Distillation is used in labs for the preparation of distilled water. It can be done in a using any of the following methods. a) Glass assembled apparatus.

b)All glass distillation apparatus

c)Stainless steel distillation apparatus.

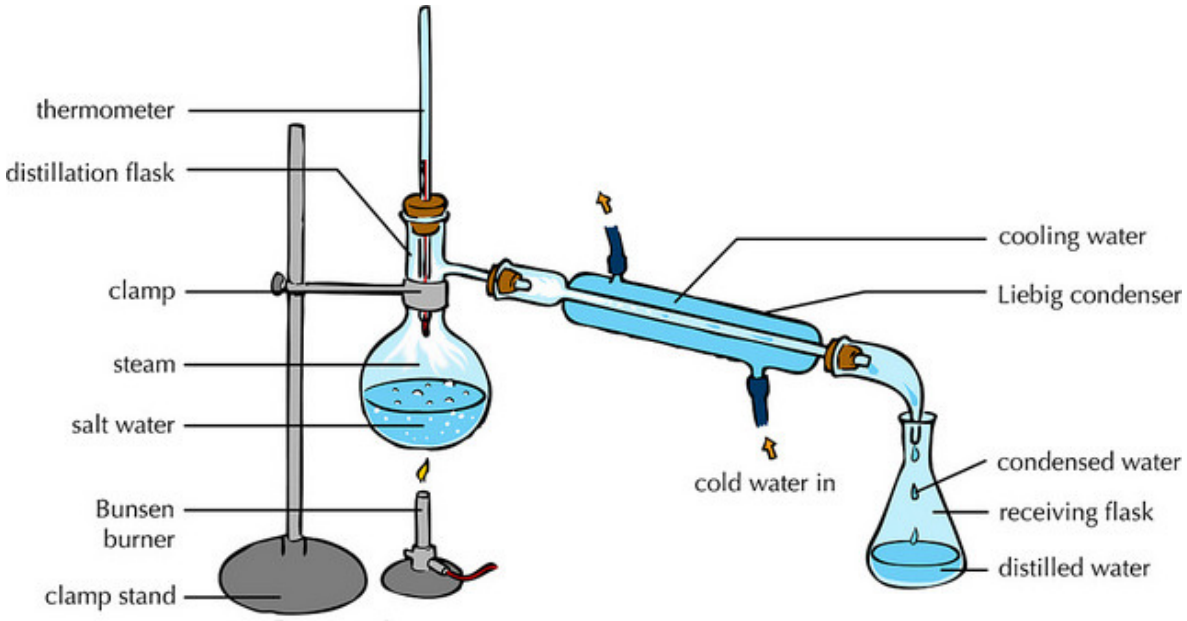


Fig 4.6 Distillation using glass assembled apparatus

Fig 4.7 All glass distillation apparatus

Process of distillation consists of:

1. Boiling of liquid forms vapors.
2. Condensation of the vapors in the condenser tube due to continuous cooling provided by continuous circulation of water.

3. Collecting the distillate. Distillate is the liquid formed by condensation of vapors of the boiled liquid.

Conclusion

A lab technician should have the basic knowledge of lab instruments such as centrifuge and weighing balance, their operation, care and maintenance.

Summary

Centrifugation, weighing, evaporation and distillation are some of the lab operations, a technician has to be perfect with. Centrifugation requires centrifuges, weighing is accomplished using different types of balances.

Evaporation helps purification. Distillation is useful for preparation of distilled water and purification. It requires distillation apparatus.

Model questions

Short Answer Type questions

1. Write the principle of centrifuge.
2. Mention different types of centrifuges.
3. What are the applications of centrifuges in a medical laboratory?
4. Define a) evaporation b) Distillation.
5. Name different types of weighing balances.

Long Answer Type Questions

1. What are the different types of centrifuges? Write about electrical centrifuge.
2. Write about simple balance and electronic balance.
3. Explain in detail about Evaporation and Distillation.

Chapter –5

Instrumental methods of Bio-chemical Analysis

Structure

5.1 Colorimeter

5.2 Spectrophotometry

Learning objective

1. Student should practice to operate the instrument
2. Student should know the application of these instruments.

Introduction

A lab technician should be well verse in basic lab operations like colorimeter and spectrophotometer, their working principal, care and maintenance.

5.1 Colorimetry

Colorimetryis the method of analysis of determination of concentrations by visual comparison of color intensities or by measurement of relative adsorption.

Colorimetersare the instruments used in colorimetric analysis for determination of concentrations by visual comparison of color intensities or by measurement of relative absorption.

Essential components of colorimeter:

1. Light source.
2. Wave length selector.
3. Filters.
4. Cuvette holders.
5. Photo cell or detectors.
6. Galvanometer.

There are two types of colorimetry. They are:

1. Visual colorimetry
2. Photo electric colorimetry

1. **Visual colorimetry**: visual colorimetry is the method of colorimetric analysis in which natural or artificial white light is generally used as light source and eye is used for color comparision.

Instruments used in Visual Colorimetry

Instruments used in visual colorimetry are called as comparators or colorimeter.

Name of the visual method	instrument/ apparatus used
1. Standard series method	1. Modified test tube rack 2 BDH Lovi bond Nessleriser mark 3 3 Lovibond 1000 comparator & Nessler tube.
2. Duplication method	Nessler tubes
3. Dilution method	Nessler tube
4. Balancing method	1 Hehner cylinders 2 Duboscq colorimeter.

Principles and Laws:

- When monochromatic light passes through a solution, some part of the light is absorbed by the solution and intensity of emitted light will be less than intensity of incident light.
- Laws explaining colorimetry are:

a) **Lamberts Law:** It was originally developed by Bouger and extended by Lambert. Hence called Lamberts-Bouger's law. It states that, when monochromatic light passes through a transparent medium, intensity of emitted light decreases exponentially as the thickness of the medium increases arithmetically.

It is expressed by,

$$le/lo = e^{-kt}$$

where, le= intensity of emitted light.

lo= intensity of incident light.

e =exponential.

K =constant

t= thickness of absorbing medium (length of the light path through the medium)

b) **Beer's Law**: Beer's law states that, when monochromatic light passes through a transparent medium, intensity of emitted light decreases exponentially as the concentration of the colored component in the medium increases arithmetically.

It is expressed by

$$\mathbf{le / lo = e^{-kct}}$$

or $\mathbf{\log\ lo / le = Kct}$

where, le = intensity of emitted light,

lo = intensity of incident light,

e = exponential

K = constant

c = concentration and

t = thickness (length of the light path through the absorbing medium)

2. **Photo electric colorimetry:** Photo electric colorimetry is the method of colorimetric analysis in which light of narrow wavelength within visible range is used and eye is replaced by photocell for comparison of color intensities. This is the advantage in photo electric colorimetry where color matching is done by photo cell and not by eye visibility which is more accurate.

Photo electric colorimeter uses light of wave length in the visible range i.e. 380-760 millimicrons only approximately as incident light.

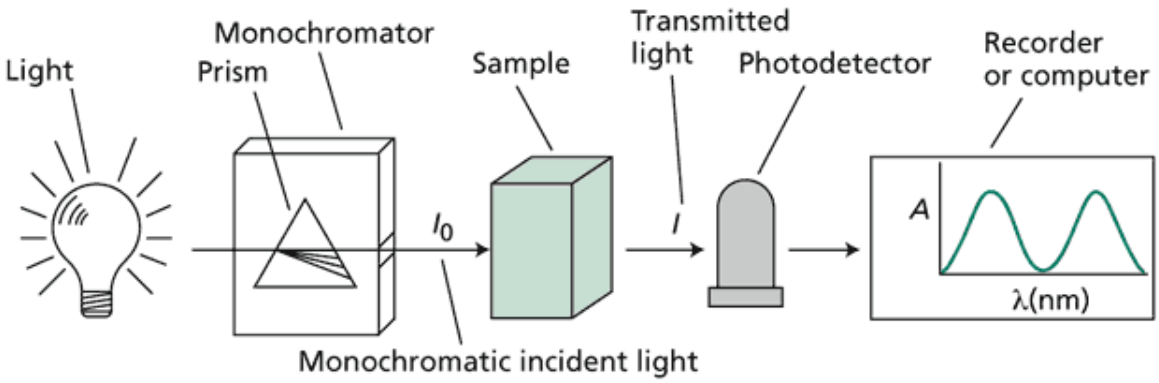


Fig: Components of photo electric colorimeter.

Principle and laws:

- When monochromatic light passes through a solution, some part of light is absorbed by the solution and the intensity of emitted light will be less than the intensity of incident light.
- This is in accordance with Beer-Lambert's law which states that, the intensity of emitted light decreases exponentially as-

a. The thickness of the absorbing medium increases and
b. Concentration of the colored component in the medium increases arithmetically.

Operation (Single cell photometer)

1. Take similarly treated test, standard and blank solutions.
2. Switch on the power supply to the colorimeter and the light source.
3. Set the colorimeter to zero optical density or 100% transmittance using blank at wavelength specified to determination.
4. Determine the optical densities of similarly treated test and standard solutions.
5. Calculate the concentration of unknown by using the formula-

$$\text{Concentration of unknown sample} = \frac{\text{Optical density of test}}{\text{Optical density of standard}} \times \text{concentration of standard.}$$

6. Set the colorimeter to zero optical density with distilled water and switch off the power supply.

Applications:

Photoelectric colorimeter is the most important tool in a biochemical laboratory.

1. It is useful in determination of components of biological fluids/serum/plasma, urine, CSF etc.
   EX: Blood sugar, serum cholesterol, serum uric acid, etc.
2. It is useful in pharmaceutical analysis, research and clinical studies.
3. Photometric titrations have been useful in locating end point in a titration. These titrations are called as photometric titrations, and have been used in several reactions.
   Ex: Amino acids, potassium permanganate, iodine, etc.

Care and maintenance:

1. Cover the photo electric colorimeter with a plastic cover, when not in use.
2. Keep the power supply and light source switched off, when not in use.
3. Check the sensitivity of galvanometer occasionally using a standard dichromate solution.
4. Proper filter and cuvette filled with distilled water is placed in their positions, before starting the colorimeter.
5. Do not keep the instrument near to a vibrating instrument and heating apparatus.

6.2 Spectrophotometry

Spectrophotometry is method of quantitative determination of concentration in ultra violet range (10-400nm), visible range (400-800 nm) and infrared range (0.8-1,000 microns) of electromagnetic spectrum.

Spectrophotometers are the instrumentsused in spectrophotometry. They are more precise than photo electric colorimeter.

Spectrophotometer differs from Colorimeter in thatit uses prism or diffraction gratings as monochromators. These separate the various wave lengths of radiant energy.

Principle and law:

- When monochromatic light passes through the solution, some part of light is absorbed by the solution and the emitted light is measured. Measurement of

absorptivity of the radiation by the components is to be determined in spectrophotometry.

- Beer – Lambert's law is followed in spectrophotometry.

Parts of spectrophotometer:

1. Light source.
2. Monochromators and slits.
3. Associated optics.
4. Cuvettes / sample holders.
5. Photocells.
6. Galvanometer.

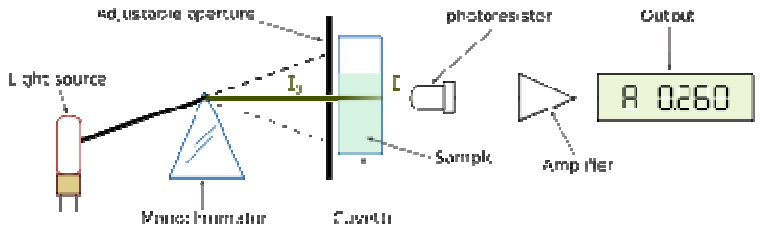


Fig: Components of a Spectrophotometer.

Types of spectrophotometers:

They can be divided into two groups-

1. Manual spectrophotometer.
2. Recording spectrophotometer.

Types of spectrophotometers based on wavelength of light-

1. Ultra violet spectrophotometer.
2. Visible spectrophotometer.
3. Infrared spectrophotometer.

Types of spectrophotometers on the basis of number of optical paths-

1. Single beam spectrophotometers consisting of one optical path.
2. Double beam spectrophotometers consisting of two optical paths.

Fig: Image of a spectrophotometer.

Operation

- Manual spectrophotometers are provided with different controls.
  They are wave length controls, slit width adjustment, dark current control, sensitivity control, readout, etc.
- Recording spectrophotometers also have switch control, scanning speed control, recording control.
- Wave length required for the estimation being done.
- Dark current control is for adjustment of zero transmittance.
- Sensitivity control is for adjustment of 100% transmittance.
- Balancing potentiometer is with a calibration scale forms read out.
- A spectrophotometer is operated using these contols.

Applications:

1. Visible spectrophotometry has wide range of applications. Ex: determination of salicylic acid, urea, glycine, etc.
2. UV spectrophotometry finds application in identification of Hydrocarbons, vitamins, steroids, hetero-cyclins and conjugated aliphatics. UV spectrophotometry is also used for identification of degradation products and for testing the purity in biological and pharmaceutical research.
3. Steroids, enzymes and many other substances can be determined by UV spectrophotometer.
4. Vitamin A can be assayed by measuring the absorbance at 334 millimicrons.

5. UV and visible spectrometry prove useful in elucidating of structures of organic compounds.
6. Spectrophotometry is useful in deciding constitution of compounds.
7. H^+ion concentration can be determined by using spectrophotometry by using suitable indicator.
8. UV spectrophotometry is used in determination of inorganic substances such as Lead in bone ash, Mg, As, Br, Cl_2, etc.

Maintenance and care:

1. Cover the spectrometer with a plastic cover, when not in use.
2. Keep the power supply and light sources switches off when not in use.
3. Put the proper filter and cuvette filled with distilled water in their positions before putting on the spectrophotometer.
4. Check the sensitivity of galvanometer occasionally using a standard dichromatic solution.
5. Maintain the instrument clean.
6. Do not keep the instrument nearer to vibrating instruments and heating apparatus.

Conclusion

Colorimetry is a method of quantitative analysis based on absorption of light transmitted through the solution of a substance in visible range.

Spectrophotometry is used to measure the intensity of light, or the wavelength that the specimen absorbs.

Summary

Colorimetry and spectrophotometry are important methods in instrumental analysis in any biochemical work.

Colorimeters use two types-a) visual colorimeter b) Photo electric colorimeter. Colorimetry Spectrophotometry is based on Beer-Lambert's law. Spectrophotometry is more accurate than colorimetry. It can be used in UV, visible, and infrared range.

Model questions:

Short Answer Type Questions

1. Define colorimetry.
2. Mention the types of colorimeters.
3. What is the difference between visual and photoelectric colorimetry?
4. Give Beer-Lamberts law.
5. Write any two applications of photoelectric colorimeter.
6. Write wavelength range of UV, visible and infrared rays.
7. Mention the difference between colorimeter and spectrophotometer.
8. Mention the types of spectrophotometers based on wavelength.

Long Answer Type Questions

1. Classify colorimetric methods and explain them.
2. Explain Beer-Lamberts law in detail.
3. Give the principle, construction, operation, care, maintenance and application of photoelectric colorimeter.
4. Give the principle, parts, operation, application, care and maintenance of spectrophotometer.

Chapter -6

Water, Chemicals and Related Substances.

Structure

6.1 Water

6.2 Purity of Chemicals

6.3 Corrosives

6.4 Hygroscopic Substances.

Learning Objectives

1. Student should have the knowledge of chemicals, their handling and storage aspects.
2. Properties of chemicals also should be known to the student for personal safety as well as fellow technicians safety.

6.1 Water

Formula: H_2o

Molecular weight: 18.02

Water is the most important constituent of all forms of life. It constitutes 70-90% of living forms. It is transparent bland liquid. Water is highly reactive. It has high melting and boiling point, heat of vaporization, heat of fusion and surface tension. The preparation of most reagents and solutions used in clinical laboratory requires pure water.

Purified water for laboratory use is prepared by any of the following process:

1. **Distillation**: Water purified by distillation is called distilled water. It is usually prepared by employing electrically heated glass distillation unit or stainless steel distillation unit.
2. **Ion-exchange:** Water obtained by percolation through Ion- exchange resins is called De- ionized water. Strong acid resins will remove anions from water. Commercially available units are Permutit, Elgastat, etc.
3. **Reverse osmosis:** In reverse osmosis, an aqueous solution is separated from pure water by semi-permeable membrane and sufficient pressure is applied to solution. This causes water to flow from aqueous solution in to pure water instead of pure

water in to aqueous solution. This principle of reverse osmosis is adopted in Milli-Q3 system of Millipore Corporation.

Fig 6.1 Distilled Water can.

Standards laid down for purified water: Indian pharmacopoeia prescribes limits for different chemicals and water. The pH of water is 4.5 to 7.0.

6.2 Purity of chemicals: Substances used in the analysis for diagnosis must be pure so as to get results with accuracy. Purity means state of not containing any impurities.

Grading of Reagents*:* Reagents supplied by the manufacturers are graded as per purity into-

1. **Analytical reagent quality (AR) or Anala** "R", a pure quality.
2. **Guaranteed reagent quality (GR),** a pure quality.
3. **General purpose reagent quality (GPR),** not that much pure as AR and GR.
4. **Laboratory reagent quality (LR).** This quality is used for preparing qualitative reagents such as Fehiling's solution, Seliwanoff's reagent, etc.
5. **A.C.S** reagents are the meeting the specifications of American chemical society.
6. **Primary standards,** are the analytical reagents of exceptional purity used for volumetric analysis.
7. **Reagents** are commercially available chemicals of highest purity for which American chemical society has not yet specified any requirement.
8. **Organic reagents (O.R),** are the reagents suitable for research application
9. **Purified chemicals**, are good quality chemicals where there are no official standards specified.

10. **U.S.P, are** Chemicals manufactured under current Good manufacturing practices (G.M.P), and are denoted as U.S.P.
11. **B.P,** are chemicals meeting the specifications of British pharmacopoeia denoted as B.P.
12. **I.P**, are chemicals meeting the specifications of Indian pharmacopoeia denoted as I.P.
13. **N.F**, are chemicals meeting the requirements of National Formulary denoted as N.F.
14. **C.P**, are chemically pure chemicals for general application denoted as C.P.

Sources of Impurities: Types and amount of impurities present in the chemicals depends upon several factors. Some of them are –

1. Raw materials employed in the manufacture
2. Method or process used in the manufacture
3. Chemical processes employed in the processes
4. Plant materials employed in the process
5. Container materials used for storage of final products
6. Decomposition during keeping
7. Adulteration.

Effects of impurities:

1. Impurities bring about changes in physical properties of substances like color, odour, taste, etc.
2. They may bring about chemical changes rendering the chemical reagent useless.
3. They act as interfering substances affecting the accuracy of the result.
4. Impurities may affect active strength of the substance influencing the result.

Test of purity: Tests for purity are tests for detecting impurities in the substances. Governing factor for these tests is to determine how much impurity is likely to bring about technical and other difficulties, when the substance is used in analysis.

6.3 Corrosives

Corrosive chemicals are those substances which can cause destruction of living tissues. Different chemicals of corrosive nature have to be handled by laboratory technicians. While handling these substances, they can cause corrosion, if they fall on any part of the body. A

technician should be well aware with measures of preventing accidents, first aid measures, precautions to be followed while handling with acids and alkalies and their storage aspects.

Accidents may happen by corrosives may be due to:

1. Spilling of splashes on the skin during handling.
2. Spilling of splashes in to eyes.
3. Swallowing on to mouth during pipetting.

Fig 6.2 Symbol showing corrosion.

Classification of corrosive substances: Corrosive substances can be classified onto categories on the basis of corrosiveness, they can cause. They are-

1. Substances with high degree of corrosiveness,
   (a) **Stong acids**: Stong acids are the substances which are ionised to the extent of 100%. Examples are Hydrochloric acid, Sulphuric acid, Nitric acid, etc.
   (b) **Strong alkalies:** Strong alkalies are those, which are ionised to the extent of 100%. Examples are Sodium hydroxide, Potassium hydroxide, Barium hydroxide, etc.
2. Substances with lesser degree of corrosiveness,
   (a) **Weak acids**: weak acids are those, which are ionised to the extent of less than 100%. Examples are Glacial acetic acid, Phosphoric acid, Nitrous acid, etc.
   (b) **Weak alkalies:** Weak alkalies are those, which are ionised to the extent of less than 100%. Examples are Ammonium hydroxide, zinc hydroxide, etc.
   (c) **Phenols and Cresols:** Phenols are aromatic hydroxy compounds in which OH group is directly attached to the Benzene ring. Examples are Phenols, Nitro phenol, Picric acid, etc.

Properties of some important chemicals:

1. **Hydro chloric acid**: (HCl):It occurs as colorless fuming liquid with pungent odour. It is miscible with water and alcohol. It is strong acid and attacks metals. It is to be handled carefully because it is corrosive to body parts like eyes, skin, mouth, lips and tongue. It is stored in well closed container.
2. **Sulfuric acid:(H_2SO_4)** Sulfuric acid is a colorless fuming acid. It has oily consistency. It is miscible with water. It is a strong acid and miscible with water. It is safe handled to avoid corrosion to the body parts like skin, eyes, mouth, etc. It is well closed container.
3. **Sodium hydroxide: (NaOH):** It is also called as caustic soda. It is available as pellets, flakes or sticks. It is extremely hygroscopic. It absorbs atmospheric carbon dioxide and partially converted to Sodium carbonate. It is freely soluble in water, alcohol and Glycerine. Heat is produced during dissolution. It is to be stored in air tight container.
4. **Potassium hydroxide: (KOH):** It is available as dry hard, brittle, white flakes, sticks or fused masses. It is highly hygroscopic and partially. Converts to potassium carbonate by absorbing atmospheric carbon dioxide. Since it is concentrated alkali, avoid spilling on skin, into eyes and swallowing in to mouth.
5. **Acetic acid: (CH3COOH):** It is also called glacial acetic acid. It is commercially available in strength of 45N. It is a colorless liquid soluble in water. It has oily consistency. It is stored in well closed container.
6. **Phenols and cresols:**
   (a) **Phenols:** phenols are colorless crystalline solids or liquids with characteristic odour. They darken in air due to oxidation. They are sparingly soluble in water. They dissolve readily in alcohol and ether. Phenols are weak acids. Ex: Hydroxy benzene(carbolic acid). They are hygroscopic. They produce blisters on skin.

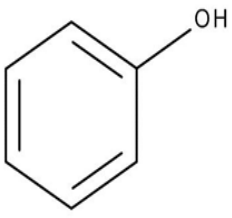
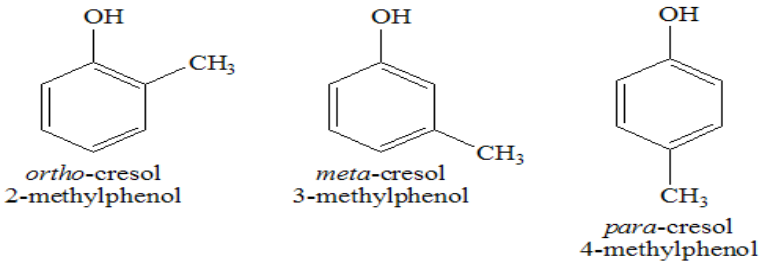


Fig **6.3 Hydroxyl benzene or phenol**

(b) **Cresols:** There are three forms of cresols. They are-Ortho "o", meta "m", and para"p" forms. They have similar properties as phenols. They are less toxic than phenols.

Fig 6.4**Structure of Ortho,meta and para forms of cresol.**



6.4 Hygroscopic Substances: Hygroscopy is the ability of a substance to attract and hold water molecules from the surrounding environment. This is achieved through either absorption or adsorption and the substance becomes physically changed by an increase in volume, stickiness or change of other physical properties. Water molecules gets suspended between the material molecules.

Ex: Aluminium Chloride, Conc. Sulfuric acid, Calcium oxide, Magnesium tri silicate, etc.

Deliquescent substances: These are the substances which absorb moisture from atmosphere and become aqueous solutions. The property of dissolving in water, absorbed from surrounding atmosphere is called deliquescence.

Ex: Sodium hydroxide, Calcium chloride, Potassium hydroxide, etc.

Both hygroscopic substances and deliquescent substances possess one property in common-absorption of water from the atmosphere.

Storage of hygroscopic substances: They are to be stored in sealed containers due to their affinity for atmospheric moisture.

Properties of some hygroscopic substance:

a) **Aluminium Chloride**: It is a hygroscopic salt. It is white in color, but turns to yellow color due to contamination with Iron chloride. It is prepared from Alumina. Alumina is mixed with charcoal and heated in atmosphere of dry chlorine gas to get anhydrous aluminium chloride. It is soluble in organic solvents like alcohol, ether, benzene, etc.

b) **Magnesium Tri Silicate**: It is slightly hygroscopic substance. It is white fine powder used as food additives. It is used in foods to absorb fats. It is odour less, taste less and is insoluble in water and alcohol. It is prepared by running a solution of sodium silicate into equimolar solution of magnesium sulfate or magnesium chloride. It is used as antacid in treatment of peptic ulcers.

Conclusion:

Purified water is used for quantitative determinations. Pure chemicals should be used for accuracy in results.

Summary:

Water is the most important constituents of all forms of life. Water is a good solvent. Purified water is used in laboratories. It is prepared by distillation, ion exchange, reverse osmosis, etc.

Chemical substances used in diagnostics should be pure to get results with accuracy. Reagents are graded into Anala R quality, guaranteed reagent quality GR, General purpose reagent quality GPR, laboratory reagent quality LR, quality according to purity. Qualitative work purest possible reagent i.e. AR. For qualitative work, laboratory reagent quality is enough.

Purity means freedom from impurities. Sources of impurities are raw material employed in the manufacture of reagents, method or process used in manufacture, chemical processes employed, plant materials used for storage, decomposition, adulteration, etc.

Corrosives are the substances which can cause destruction of living tissues. Strong acids, strong alkalies, weak acids, weak alkalies, phenols and cresols can cause corrosion in varying degrees. Hygroscopic substances are those which absorb moisture from atmosphere. Ex: sodium hydroxide, potassium hydroxide etc.

MODEL QUESTIONS.

Short Answer Type Questions

1. What water is used in laboratory?
2. How is purified water prepared?
3. What is ion-exchange?
4. Define reverse osmosis.

5. Define a) strong acids b) Weak acids.
6. Define a) strong alkalies b) weak alkalies.
7. Write the properties of HCl.
8. Write the properties of Acetic acid.
9. What is a hygroscopic substance?
10. Differentiate hygroscopic substance and deliquescent substance.
11. Give some examples of hygroscopic substances.
12. What are corrosives?
13. Write two effects of impurities.
14. What is purity of chemicals?

Long Answer Type Questions

1. Write about preparation and use of water on laboratory.
2. What are the sources of impurities? Discuss.
3. Explain in detail about Corrosives.
4. Give an account of the purity of chemicals.
5. Write in detail about strong acids and strong alkalies.
6. Discuss about hygroscopic substances.

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Chapter-7

Collection of Specimens

Structure

7.1 Blood

7.2 Urine

Learning objectives

1. Student should be able to collect different blood samples.
2. Student should also know about different urine specimens.
3. Student should also be knowing about the preservation methods of the specimen.

7.1 Blood (phlebotomy)

Phlebotomy is the surgical opening of a vein in order to withdraw blood. Specimens of blood are required to be collected for different diagnostic tests like hematological, biochemical, microbiological, serological aspects of diagnosis.

Types of blood Specimens

a) ***Random blood specimen*** is one which is collected at any time without any specific instructions to be followed by the patient before the collection.
b) ***Fasting specimen*** is one which is collected in morning after a night's fasting (12-16hours)
c) ***Post prandial specimen***is specimen collected 2 hours after taking lunch.

Forms of blood used in diagnostic tests

Blood is used for diagnostic testing in the form of-

1. ***Whole blood:*** It is used in blood cell count, differential counts, hemoglobin estimation, pH, lead, etc.
2. ***Serum:***It is used in determination of Amino acids, Free and esterified cholesterol, creatinine, copper, iron, enzymes, etc.
3. ***Plasma***: It is used in determination of Fibrinogen, Ascorbic acid, bicarbonates, chloride, etc.

4. ***Cellular parts:*** It is used in determination of Glucose-6PD, abnormal hemoglobin, etc.

Types of blood collection

Types of blood specimens based on routes of blood collection-

1. **Capillary blood**: Blood collected from capillaries**.**
2. **Venous blood:** Blood collected from veins.
3. **Arterial blood**: blood collected from arteries.

1) Collection of capillary Blood Specimen: Capillary blood is used when small volume of blood is needed.

Uses: capillary blood is used in blood cell counting procedures, hemoglobin estimation, blood grouping, bleeding time, clotting time, etc.

Quantity of blood collected: Volume up to 0.2 ml. can be collected. A skilled technician can obtained up to 1 ml. Amount up to 3 ml. can be collected by repeated expressions.

Sites of selection for capillary blood collection:

1. Tip of finger
2. Thumb
3. Lobe of an ear or great toe
4. Heel for infants.

Requirements:

1. Surgical needle or disposable hypodermic sterile needle or lancet.
2. Cotton
3. Spirit or povidone iodine
4. Thin tourniquet
5. Blood pipettes or slides or small tubes.

Fig 7.1: Blood lancets.

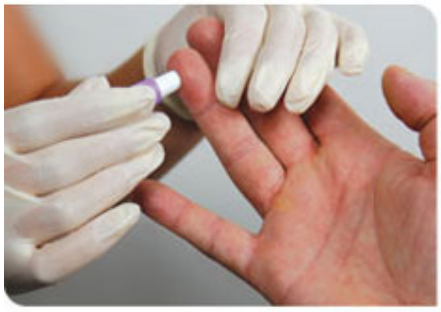


Fig 7.2: capillary blood collection.

Procedure

1. Rub the part with 70% alcohol or spirit.
2. Make a quick and good stab by using a disposable sterile lancet.
3. The cut should deep enough so that blood flows freely without squeezing the stabbed site.
4. Wrap a thin tourniquet at the base of the finger, if felt necessary.
5. By using a cotton the first drop should be wiped away and a separate fresh drop is used for the test.

Precautions

1. A free flow of blood is essential and blood collected by squeezing may lead to errors.
2. Pipettes used should be clean and dry since they ensure filling with ease.

1) Collection of venous blood specimen: When specimen of blood in more volumes than capillary blood is required, venous blood is preferred.

Uses: venous blood is collected for determinations like E.S.R, blood sugar, serum cholesterol, blood urea, etc.

Quantity of blood collected by venous route: Up to 10 ml. of blood is collected by veins. If a number of determinations are to be done, up to 20 ml. of blood can be collected.

Sites of selection for venous blood collection

1. Vein on hand or wrist
2. Antecubital area
3. Vein on the ankle
4. Vein foot.

Requirements

1. Rubber tourniquet or cuff of a sphygmomanometer.
2. Sterile syringe with a disposable needle
3. Spirit or ether
4. Cotton
5. Tubes for transfer the blood.

Procedure

1. Extend the arm of the patient.
2. Apply a tourniquet firmly a few inches above the elbow.
3. Clean the site of collection with cotton soaked in spirit and allow to evaporate.
4. Taking the arm of the patient in to grip with one hand and holding the skin at the site of collection with the thumb of another hand, penetrate the needle into vein by positioning the needle at 30^0 and 40^0 angle.
5. When the needle enters into the vein, withdraw the plunger slightly.
6. Release the tourniquet when blood appears in the barrel. This minimizes congestion.

7. Place a pad of cotton wool soaked on spirit on the site of needle in to the vein after drawing the required amount of blood.
8. Hold firmly for few minutes until blood stops.
9. Remove the needle from the syringe and transfer the collected blood into appropriate container.

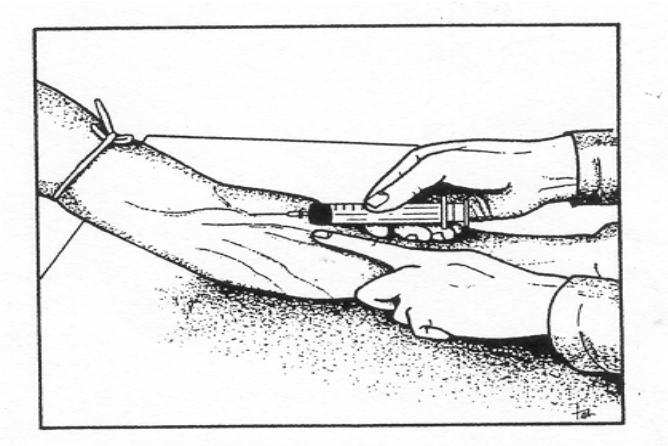


Fig 7.3: collection of venous blood.

Precautions

1. Tourniquet should be loosened once the needle has been inserted into vein.
2. Avoid the areas like healed burn areas and hematoma. Care is taken to prevent hematoma.
3. Follow some useful techniques when patient with difficult veins is to be handled, like making a fist, massaging the arm, choosing the other hand, etc.

2) Collection of arterial blood: venous blood and arterial blood are similar in composition except for a few differences such as O_2/CO_2 tensions. It is rarely examined. Arterial blood collection is painful and requires skill.

Uses: Arterial blood collectionis useful in measuring pH of arterial blood, partial pressure of O_2 and CO_2, oxygen saturation and bicarbonates.

Sites of selection for arterial blood

1. Radial artery
2. Brachial artery
3. Femoral artery.

Requirements

1. Betadine solution as anti-septic lotion.
2. 1% xylocaine as anesthetic drug
3. 20 or 21 gauge sterile needle.
4. 12 ml. heparinized syringe.
5. Cotton.

Procedure

1. Instruct the patient to take sitting position
2. Raise the wrist with a pillow and instruct the patient to extend the fingers downwards.
3. Palpate the artery, and rotate the hand back and forth until a good pulse is felt.
4. Clean the site of collection with antiseptic agent.
5. Anaesthetize the site of collection to reduce pain.
6. Puncture the artery and draw blood in pre heparinized syringe, by drawing the plunger. Care is taken not to pull needle out of artery.
7. After collecting 3-6 ml. of blood withdraw the needle.
8. Keep sufficient absorbent bandage over the site of puncture and apply pressure for about 2 minutes.
9. Expel air bubbles in the sample immediately.
10. Cap the syringe and rotate gently to cause proper mixing of blood with heparin.
11. Preserve by storing in the refrigerator, if it is not tested within 15 - 25minutes.

Precautions

1. Apply enough pressure at the puncture site and watch for bleeding.
2. Collection of arterial blood should be done without trauma.
3. Beware of air bubbles in the syringe as they will cause change of concentration of gases.

Novel blood collection system

Vacutainers: Vacutainers are one of the single use type novel blood collection system working by negative pressure. They are available with or without anticoagulants.They are of single use type.They are used to collect blood by venipuncture or by finger prick method, instead of conventional syringes and needles.

Parts of a vacutainer: The vacutainer system consists of-

1. Sterile single use blood collection needle.
2. A holder, used to secure needle during insertion into tube stopper and venipuncture.
3. A sterile vacutainer primary tube, an evacuated glass tube with rubber stopper containing vacuum.

Procedure

1. When needle is inserted at the site of collection, the rear cannula pushes through the rubber sleeve and punctures the rubber stopper.
2. This allows the vacuum in the tube to draw blood from the vein by negative pressure.
3. When one tube is withdrawn from the back of the needle, to collect blood in another container, the sleeves slides back into position and keeps the blood from flowing out through rear cannula.
4. When last tube has been filled, the entire system is removed from the patient's arm and the needle is disposed.

Precautions

1. While collecting blood, the arm of the patient is held downwards and blood is kept from contact with stopper. This will prevent back flow of blood from blood tubes into veins.
2. Only sterile tubes are used to avoid contamination.

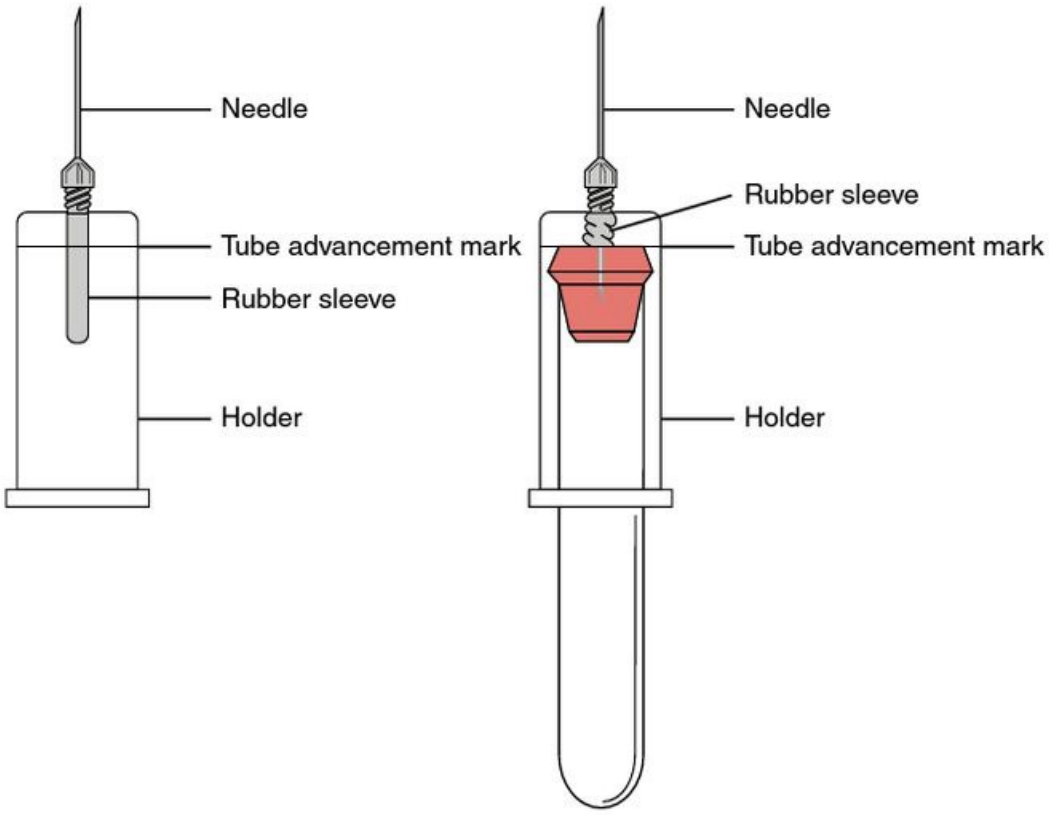


Fig 7.4: Diagram showing parts of a vacutainers.

Advantages of vacutainers

1. It eliminates the preparation of anticoagulant containing tubes
2. There is no processing of containers.
3. It minimizes hemolysis in specimens.
4. There is no possibility of blood spillage.
5. Since syringe is not required, there are fewer disposals. Only the needle is disposed after blood collection.

Preservation of blood

1. Blood is usually preserved at 2-4 ^{0} C in refrigerator.
2. ***Hematoma*** is clotting of blood***. Anticoagulants*** are used to avoid clotting of blood in some tests.

   Ex: a) Double oxalate, which is a mixture of 3 parts of ammonium oxalate and 2 parts of potassium oxalate.

b) Sodium citrate

c) Ethylene diamine tetra acetic acid (EDTA)

d) Sodium fluoride

e) Acid citrate dextrose (ACD)

f) Heparin.

3. ***Hemolysis***means destruction of red blood cells. It causes certain changes in the composition of the specimen. Measure to prevent hemolysis are-

a) Collection tubes must be clean and dry.

b) During collection, minimum amount of constriction should be applied to the arm.

c) Blood should be easily collected into collecting tubes after removing the needle.

d) Speed of centrifugation has to be controlled.

7.2 Urine

Collection of urine specimen is an important aspect in laboratories. It is used in physical, chemical and microbiological examination.

Types of urine samples

1. ***Single specimen:*** Urine specimen collected any time randomly is called as single specimen. It is called as single specimen. It is useful for qualitative analysis.
2. ***Fasting urine specimen:***Urine specimen collected after a fasting of 12-16 hours of overnight fasting is called fasting urine specimen.
3. ***Post prandial urine specimen:*** urine specimen collected 2 hours after lunch is called post prandial urine specimen.
4. ***24hours urine specimen:*** Specimen of urine collected in a period of 24 hours duration is called as 24hrs urine specimen. It is required in quantitative determination.

Method of collection of urine sample:

1. Collect specimen in dry, clean, sterile glass of plastic container which has wide mouth, spill free and has a screw cap top.
2. Label the specimen such as name of patient, type of specimen, ID number, date and time of collection.

Collection of 24-hours urine sample

a) Procedure:

1. Use a 2.5 to 5 liters capacity dark plastic or glass jar which contains 5 grams of sodium carbonate powder. This will adjusts the acidity of urine and helps in preservation.
2. A 24 hour urine collection has to be started at a specific time. Exact 24 hours of time is to be maintained.
3. For example, if 8 A.M is chosen in the morning, the bladder is emptied at that time and that urine is discarded. From that time on, add any urine that is passed into the jug. Recording of each urinated time is not needed. Urine passed during defecation in this 24 hours is also saved, otherwise it is incomplete.
4. During the collection, store the urine jug tightly capped in a refrigerator or an ice chest.
5. Exactly after 24 hours, i.e. at 8 A.M. on the next day morning, urine collection is ended by emptying the bladder into the jug for the last time.

b) Volume:

1. On an average diet and normal fluid intake, the volume of 24 hours urine specimen is between 1.2 to 1.5 liters.

2. In polyuria, the volume of 24 hours urine specimen is more than 2 liters.

3. In oliguria, the volume of 24 hours is reduced below 500 ml.

4. In Anuria, there is total suppression of urine.

c) Risks of 24 hours urine:

1. Forgetting to collect urine within time and going beyond the time resulting in excess collection.

2. Loss of urine from container by spilling.

3. Not keeping urine cold during collection period.

d) Preservation:

1. Freshly passed urine has to be examined. On standing, chemical changes take place. Bacterial growth may also take place. To avoid this it is to be placed in refrigerator at 2-8^{0}C.
2. Different chemicals are used as urinary preservatives.
   Ex: a) Conc HCl
   b) 2N HCl
   c) Acetic acid
   d) Meta phosphoric acid
   e) Formalin
   f) Boric Acid
   g) Thymol
   h) Toluol
   i) Chloroform.

Conclusion

- Specimen of blood and urine should be tested without delay.
- Labelling of the collected specimen is important for identification.
- If any delay is there for examining the urine, it has to be preserved.

Summary

Blood specimens are required for diagnostic tests. Random specimen, Fasting specimen are types of specimens. Different forms of blood used in testing are whole blood, plasma, serum and cellular parts. Different routes of blood collection are capillary, venous and arterial. Novel method is vacutainer method.

Urine specimen is of different types. 24 hours urine, single specimen urine, etc. several preservatives are used to preserve urine.

Model questions

Short Answer Type Questions

1. Mention different blood specimens.
2. What is a fasting specimens?
3. What do you mean by post prandial specimen?
4. Mention different routes of blood collection.
5. Mention some applications of capillary blood.
6. Name the different sites of capillary blood collection?
7. What is the quantity of blood collected by capillary method?
8. What is the quantity of blood collected by venous route?
9. Mention the sites of venous blood collection?
10. What is the angle of insertion of needle into vein?
11. How do you handle a patient with difficult veins?
12. Name the arteries used for blood collection.
13. What is a vacutainer?
14. Define anticoagulant.
15. Give some examples of anticoagulants.
16. Define the term hemolysis?
17. What is single urine specimen?
18. What is 24 hours urine specimen?

Long Answer Type Questions

1. Write about different types of blood specimens. What are different forms of blood used in diagnostic testing? Mention their uses.
2. Write about novel blood collection system.
3. Explain arterial blood collection.
4. Discuss different aspects of 24 hours urine specimen.
5. Explain venous blood collection.

Chapter-8

Solutions

Structure

8.1 Introduction

8.2 Types based on solute and solvent

8.3 Types based on method of expressing concentration and calculation.

Learning objectives

1. Students should prepare different solutions.
2. A student should practice various calculations.

8.1 Introduction

- *A **solution*** is defined as homogenous and monophasic mixture of two or more substance.
  Ex. 0.9% Sodium Chloride solution.
- A ***solute***is a substance present in minor proportion in a solution.
  Ex. Sodium Chloride present in 0.9% Sodium Chloride solution.
- A ***solvent i***s a substance present in major proportions in a solution.
  Ex: Water present in 0.9% sodium chloride.

8.2 Typesof solutions based on solutes and solvents.

Based on states of matter of solutes and solvents, solutions are of different types,

1. **Solid in liquid solutions:** Solid solute dissolved in liquid solvent is called solid in liquid solution.
   Ex: 10% sodium tungstate solution.
2. **Liquid in liquid solution**: Liquid solute dissolved in liquid solvent is called liquid in liquid solution.
   Ex: 2% Acetic acid solution.
3. **Gas in liquid solution**: Gaseous solute dissolved in liquid solvent is called gas in liquid solution.
   Ex:Aerated water.

4. **Solid in solid solution**: Solid solute dissolved In solid solvent is called solid in solid solution.

   Ex: Alloys.
5. **Liquid in solid solution**: Liquid solute dissolved in solid solvent is called liquid in solid solution.

   Ex: Hydrated salts such as $Na_2CO_3.10\ H_2O$
6. **Gas in solid solution**: Gaseous solute dissolved in solid solvent is called gas in solid solution.

   Ex: H_2 in palladium.
7. **Solid in gas solution:** solid solute dissolved in gaseous solvent is called gas in solid solution.

   Ex: Minute particles in inhaled air.
8. **Liquid in gas solution**: Liquid solute dissolved in gaseous solvent is called liquid in gas solution.

   Ex: Moisture in air.
9. **Gas in gas solution:** Gaseous solute dissolved in gaseous solvent is called gas in gas solute

   Ex: Air.

Methods of expressing concentration:

Different methods of expressing concentration are:

1. Percentage
2. Molarity
3. Normality
4. Molality
5. Formality
6. Mole fraction.

8.3 Types of solutions based on method of expressing concentration and calculations

Based on method of expressing concentration, different types of solution are:

1. Percentage solutions
2. Molar solutions
3. Normal solutions

4. Molal solutions

1) **Percentage solutions**: Solutions whose concentrations are expressed in percentage are called as percentage solutions.

There are four kinds of percentage solutions. They are:

a) Weight in volume percentage solutions. (W/V solution)

W/V solutions are solutions of solids in liquids, one part of solid solute by weight dissolved in liquid solvent to produce solution of 100 parts by volume is called as 1% weight in volume solution.

General formula:Solute – 1gand Solvent up to 100ml.give 1% W/V solution.

Calculations:

Example: Prepare 200 ml. of 0.9% W/V sodium chloride.

Answer: For 100 ml. --------0.9 grams of sodium chloride

For 200 ml. ---------?

$$\frac{0.9 \times 200}{100} = 1.8 \text{ grams}$$

1.8 grams of sodium chloride dissolved in enough water and diluted to 200 ml. with water will produce 200 ml. of 0.9% W/V sodium chloride solution (Normal saline)

Method of preparation:

1. Weigh accurately 1.8 grams of sodium chloride.
2. Dissolve in about 150 ml. of the water.
3. Examine for any dust/foreign particles. If necessary filter it using filter paper.
4. Dilute to 200 ml. volume with water in a graduated measuring jar.
5. Transfer into a clean and dry container and label it.

Example2: Prepare 250 ml. of 10% W/V sodium tungstate solution.

Answer: 100 ml. ---------- 10g

250 ml. ----------?

$$\frac{10 \times 250}{100} = 25 \text{ grams}$$

25 grams of sodium tungstate dissolved in enough water and diluted to 250 ml. with water will produce 10% W/V sodium tungstate solution.

Method of preparation:

1. Weigh accurately 25 grams of sodium tungstate.
2. Dissolve in approximately 168 ml. of water.
3. Examine for any dust/foreign particles. If present filter it using a filter paper.
4. Dilute to 250 ml. with distilled water in a graduated measuring jar.
5. Transfer into a clean dry container and label it.

b) Weight in weight solutions. (W/W solution)

W/W solutions are also percentage solutions of solids in liquids. One part of solid solute by weight dissolved in liquid solvent to produce solution of 100 parts by weight is called as 1% weight in solution.

General formula: Solute – 1g and solvent up to 100g will give 1% W/W solution.

c) Volume in weight solutions (V/W solutions)

V/W solutions are percentage solutions of liquids in solids. One part by volume of liquid solute dissolved in solid to produce solution of 100 parts by weight is called as 1% volume in weight solution.

General formula: liquid—1 ml. and solid up to 100g will give 1% V/w solution.

d) Volume in volume percentage solutions. (V/V solution)

V/V solutions are percentage solutions of liquids in liquids. One part by volume of liquid solute diluted to 100 parts by volume with liquid solvent is called as 1% volume in volume solution.

General formula: Liquid—1ml. and solid up to 100 ml. will give 1% V/V solution.

Calculations:

Example1: Prepare 50 ml. of 2% V/V acetic acid.

Answer: 100 ---- 2

50-----?

$$\frac{50 \times 2}{100} = 1 \text{ ml}$$

1 ml. of acetic acid, diluted to 50 ml. of water will produce 50 ml. of 2% V/V acetic acid solution.

Method of preparation

1. Take approximately 45 ml. of water into a measuring jar.
2. Add 1 ml. of acetic acid with stirring.
3. Make the volume to 50 ml.
4. Transfer to a clean and dry container and label it.

Example2: Prepare 150 ml. of 10% V/V formalin in normal saline.

Answer: 100 ml. ---------- 10ml.

150 ml. ----------?

$$\frac{150 \times 10}{100} = 15 \text{ ml.}$$

15 ml. of formalin diluted to 150 ml. with water will produce 150 ml. of 10% V/V formalin.

Method of preparation:

1. Take 15 ml. of formalin in a measuring jar.
2. Dilute to 150 ml. with water.
3. Transfer into a clean, dry container and label it.

2) **Molar solutions:** Molar solution is defined as a solution containing one mole of substances in one liter of solution.

Molarity: Molarity is number of moles of the solute per liter of solution. It is denoted M.

$$M = \frac{\text{Number of moles of solute}}{\text{Volume of solution in liters}}$$

Mole: Mole is defined as one gram molecular weight substance present in one liter solution.

Gram molecular weight: Molecular weight expressed in grams is called as gram molecular weight.

Example: One mole of H_2SO_4 = 98.078 grams of H_2SO_4 in one liter solution.

One mole of HCl = 36.5 grams of HCl in one liter solution.

Calculations:

Example 1:Prepare 75 ml. of 0.1 M HCl

Answer:

Molecular weight of HCl = 36.5

Gram molecular weight of HCl = 36.5 grams.

Weight of HCl required to make 1 liter of 1M HCl = 36.5 g.

Therefore, weight of HCl required to make 0.1M HCl =

1M ---------- 36.5 g

0.1M ----------?

$$\frac{0.1 \times 36.5}{1} = 3.65 \text{ g}$$

Weight of HCl required to make 75 ml. of o.1M solution =

1,000 ml ---------- 3.65 g

75 ml. ----------?

$$\frac{3.65 \times 75}{100} = 0.273 \text{ g}$$

Method of preparation:

1. Take accurately 0.273g HCl.
2. Add about 50 ml. of water gradually while stirring and cool.
3. Dilute to 75 ml. with distilled water.
4. Standardize and adjust molarity if necessary.
5. Mix thoroughly and store in a clean dry container.

Example 2: Prepare 100 ml. of 0.5M HCl with commercially available HCl.

(Specific gravity of commercially available HCl = 1.16. Percentage by weight = 36% W/W.)

Answer:

Molecular weight of HCl = 36.5

Gram molecular weight of HCl = 36.5g

Weight of HCl required to make 1 liter HCl solution = 36.5g.

Therefore weight of HCl required to make 1 liter of 0.5M HCl =

1M ---------- 36.5 g

0.5M ----------?

$$\frac{0.5 \times 36.5}{1} = 18.25$$

Weight of HCl required to make 100 ml. of 0.5M HCl =

1000 ml. ---------- 18.25 g

100 ml. ----------?

100 x 18.25

---------- 1.825 g

1000

Weight of HCl required to make 100 ml. of 0.5M HCl = 1.825g

Quantity of commercially available concentrated HCl required to make 100 ml. of 0.5M HCl =

36 g ---------- 100 g

1.825 g ----------?

1.825 x 100

---------- = 5.07 g

36

Specific gravity of HCl = 1.16

1.16g ---------- 1 ml.

5.07g ----------?

5.07 x 1

---------- = 4.37 ml.

1.16

Therefore, quantity of commercial concentrated HCl required to prepare 100 ml. of 0.5M HCl = 4.37 ml.

Method of preparation:

1. Measure accurately 4.37 ml. of commercially concentrated HCl.
2. Gradually add 75 ml. of distilled water by stirring and cool.
3. Dilute to 100 ml. with distilled water.
4. Standardize and adjust molarity if necessary.

5. Mix thoroughly and store in a clean and dry container.

Example 3: Prepare 50 ml. of 1M NaOH.

Answer:

Molecular weight of NaOH = 40.

Gram molecular weight of NaOH = 40 g

Weight of NaOH required to make 1 liter of 1M NaOH solution = 40 g.

Therefore, weight of NaOH required to make 50 ml. of 1M NaOH =

1000 ml. ---------- 40 g

50 ml. ----------?

$$\frac{50 \times 40}{1000} = 2 \text{ grams.}$$

Weight of NaOH required to make 50 ml. of 1M NaOH = 2 g.

Method of preparation:

1. Weigh accurately 2 g. of NaOH.
2. Dissolve about 40 ml. of distilled water.
3. Dilute to 50 ml. with distilled water.
4. Standardize and adjust molarity if necessary.
5. Mix thoroughly and store in a clean dry container.

3) Normal solution: Normal solution is defined as a solution containing one equivalent of substance in one liter of solution.

Normality: Normality is defined as number of equivalents of substance per liter of solution. It is denoted by N. It is expressed as:

$$N = \frac{\text{Number of equivalents of substance.}}{\text{Number of liters of solution.}}$$

$$N = \frac{\text{Number of milli equivalent of substances}}{\text{Number of milli liters of solution.}}$$

$$\text{Normality} = \text{Molarity} \times \frac{\text{Molecular weight}}{\text{Equivalent}}$$

Examples: Normality of sulfuric acid = 36 N.

Normality of HCl = 12N

Normality of glacial acetic acid = 45N.

Calculations:

Example 1: Prepare 500 ml. of 0.5 N HCl.

Answer:

Molecular weight of HCl = 36.5

Gram molecular weight of HCl = 36.5 g.

Equivalent weight of HCl = 36.5 g.

Weight of HCl required to make 1 liter of 1N HCl = 36.5 g.

Weight of HCl required to make 1 liter of 0.5 N HCl =

1 N ---------- 36.5 g.

0.5 N ----------?

$$\frac{0.5 \times 36.5}{1} = 18.25 \text{ g.}$$

Weight of HCl required to make 500 ml. of N HCl =

$$\frac{500 \times 18.25}{1000} = 9.125 \text{ g.}$$

Therefore weight of HCl required to make 500 ml. of 0.5 N HCl = 9.125 g.

Method of preparation:

1. Take accurately quantity of HCl equivalent to 9.125 g. of HCl.
2. Add gradually to approximately 450 ml. of distilled water with constant stirring and cool.
3. Dilute to 500 ml. with distilled water.
4. Standardize and adjust normality if necessary.

Example 2: Prepare 250 ml. of 0.5 N NaOH.

Answer:

Molecular weight of NaOH = 40

Gram molecular weight of NaOH = 40 g.

Equivalent weight of NaOH = 40 g.

Weight of NaOH required to make 1 liter if 1N NaOH = 40 g.

Weight of NaOH required to make I liter of 0.5 N NaOH =

$$\frac{0.5 \times 40}{1} = 20 \text{ g.}$$

Weight of NaOH required to make 250 ml. of 0.5 N NaOH =

1000 ml. ---------- 20 g.

250 ml. ----------?

$$\frac{250 \times 20}{} = 5 \text{ g.}$$

1000

Weight of NaOH required to make 250 ml. of 0.5 N NaOH = 5 g.

Method of preparation:

1. Weigh accurately 5 g. of NaOH.
2. Dissolve in about 200 ml. of distilled water.
3. Dilute to 250 ml. with distilled water.
4. Standardize and adjust normality if necessary.
5. Mix thoroughly and store in a clean and dry container.

4) Molal solutions: Molal solution is defined as solution containing one mole of solute in one kilogram of solvent.

Molality: Molality is the number of moles of solute per one kilogram of solvent. It is denoted by m.

$$m = \frac{\text{Number of moles of solute}}{\text{Weight of solvent in Kg.}}$$

Example: 1m HCL means 36.5 grams of HCl in Kg water.

5) Formality: It is the number of formula weight in gm. Dissolved per liter solution. When formula weight is equal to the molecular weight, formality is same as Molarity.

6) Mole Fraction: It is the ratio of number of moles of one of the components to the total number of moles of solute and solvent. It is denoted by X.

If n1 is number of moles of solute, n2 is number of moles of solvent, x1 is mole fraction of solute and x2 is mole fraction of solvent,

$$X1 = \frac{n1}{n1 + n2} \quad \text{and} \quad x2 = \frac{n1}{n1 + n2}$$

x1 + x2 = 1.

Conversion of mg % to m eq/ L:

$$\text{Conversion in m eq/L} = \frac{\text{mg. per 100 ml. x 10 x valency}}{\text{Molecular weight.}}$$

Conclusion

Solution are mixtures of more than one component. They are homogenous mixtures and monophasic.

Summary

Solutions are monophasic mixtures of two or more substances in which solute is dissolved in solvent. They are classified on the basis of states of matter of solute and solvent. Concentrations are expressed as percentage, molarity, normality, molality, mole fraction and formality. Percentage solutions are weight in volume (W/V) solutions, weight in weight (W/W) solutions, volume in weight (V/W) solutions and volume in volume (V/V) solutions.

Molarity is number of moles of substance present in one liter of solution. Normality is number of gram equivalents present in one liter of solution. Molality is number of moles present in one Kg. solvent. Mole fraction is ratio of number of moles of one of the components of the solution to the total number of moles of solute and solvent. Formality is number of formula weight in grams, dissolved in liter of solution.

Model Questions

Short Answer Type Questions

1. Define solution.
2. Define solute and solvent.
3. Give examples of a) Solid in liquid solution b) Liquid in liquid solution.
4. What is percentage solution?
5. Define molarity.
6. What is molar solution?
7. Define mole.
8. Name the different types of mole percentages.

9. What is meant by gram molecular weight?
10. Mention the relation between molarity and normality.
11. Define molality.
12. What is Molal solution?
13. Define formality.
14. What is mole fraction?

Long Answer Type Questions

1. Define solutions classify and give examples.
2. Explain the different methods of expressing concentrations.
3. Mention different types of percentage solutions. Write about weight in volume percentage solutions with an example.
4. Write about volume in volume percentage solutions and their preparation with an example.
5. Write about normal solutions giving one example.

Problems

1. Prepare 60 ml. of 0.2 M HCl.
2. Prepare 75 ml. of 0.5 M HCl from commercial concentrated HCl.
   (Specific gravity of commercial concentrated HCl is 1.16, percentage by weight is 36%)
3. How do you prepare 300 ml. of 0.2 M H_2SO_4 from commercially available concentrated H_2SO_4
   (Specific gravity of commercial concentrated H_2SO_4. Is 1.84 and percentage by weight is 95%)
4. Prepare 250 ml. of 0.1 N HCl.
5. Prepare 300 ml. of 0.5 N NaOH.

Chapter – 9

Carbohydrates and Lipids

Structure

9.1 Carbohydrates

9.2 Lipids

Learning Objectives

Student should

1. Get familiar with carbohydrates and quantitative tests of carbohydrates.
2. Know the clinical importance of lipids.

9.1 carbohydrates

Definition: They are defined as polyhydroxy aldehydes or ketones (or) substances which yieldpolyhydroxy aldehydes or ketones on hydrolysis.

Important functions:

- Humans and all animals except carnivores derive the major portion of their food calories from various types of carbohydrates in their diet.
- Most of the energy for metabolic activities of the cell in all organisms is derived from the oxidation of carbohydrates.
- Present In storage organs of plants in the form of starch.
- Present in liver and muscles of animals in the form of glycogen as important food reserves.
- They also serve as an important component of skeletal structures in plants in the form of cellulose.
- Also present peptidoglycan or murein layer in outer structures of bacteria.

Classification:

Carbohydrates may be classified into two major groups-

1. Sugars (sweet substances): They are classified into-
   a) Monosaccharides
   b) Oligosaccharides

2. Non-sugar (which are not sweet.)

1. Sugars:

a) Monosaccharides:

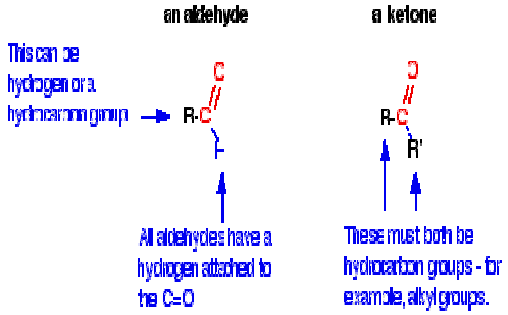
These consists of single polyhydroxy aldehyde or ketone unit which cannot be broken down to simpler substances on hydrolysis.

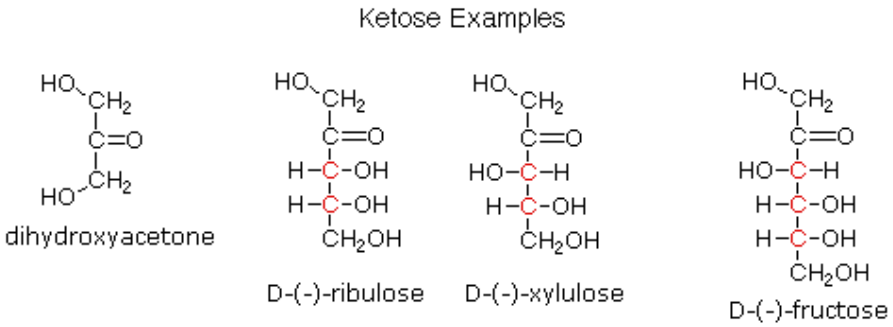
They are classified as:

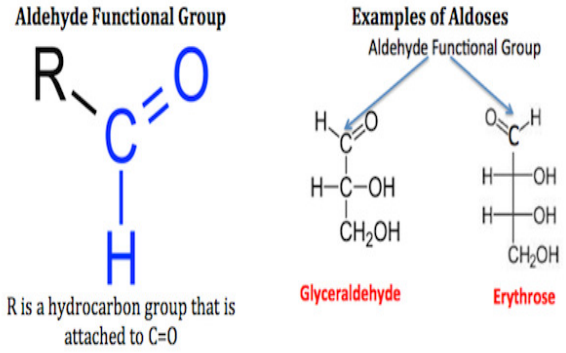
- **<u>Based on functional group:</u>**On the basis of functional group, monosaccharides are classified into:

1) ***Aldoses*** containing aldehyde as functional group.
2) ***Ketoses*** containing ketone as functional group.

Fig 9.1 structures of aldehyde and ketone groups.







- **<u>Based on number of carbons</u>**: On the basis of number of carbon atoms contained, monosaccharides can be classified into-

1) ***Trioses*** which contain 3 carbons atoms.
2) ***Tetroses*** which contain 4 carbons atoms.
3) ***Pentoses*** which contain 5 carbons atoms.
4) ***Hexoses*** which contain 6 carbons atoms.
5) ***Heptoses*** which contain 7 carbon atoms.
6) ***Octoses*** which contain 8 carbon atoms.

Each exists in two series:

Trioses: 1) Aldo trioses Ex: Glyceraldehyde.

2) Keto trioses Ex: Dihydroxy acetone.

Tetroses: 1) Aldo Tetroses EX: D-Erythrose.

2) KetoTetroses Ex: D-Erythrolulose.

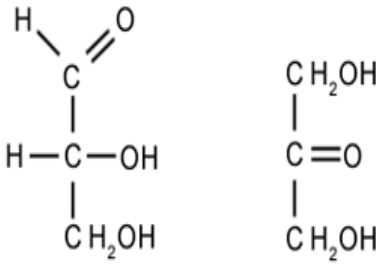
Pentoses: 1) Aldo Pentoses Ex: D-Ribose

2) KetoPentoses Ex: D-Ribulose.

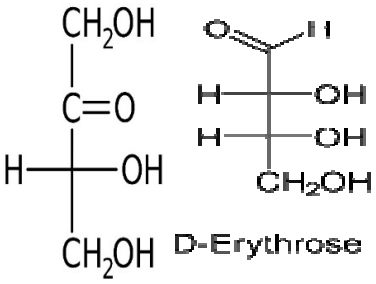
Hexoses: 1) Aldo hexoses Ex: D-Glucose.

2) Keto hexoses Ex: D-Fructose.

Fig 9.2 Structures of carbohydrates:

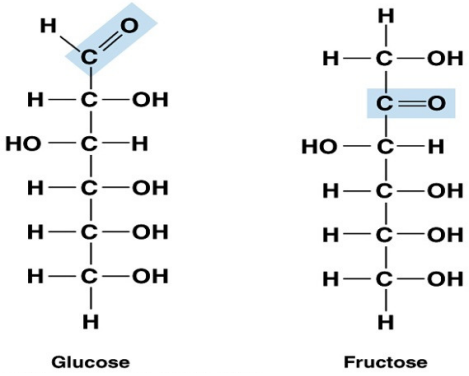


D-Glyceraldehyde Dihydroxyacetone



D-Erythrose

D-Erythrulose



Glucose Fructose

b) **Oligosaccharides:** Oligosaccharides are the carbohydrates which on hydrolysis yield two or more monosaccharides. They are further classified into:

1) Disaccharides.
2) Trisaccharides
3) Tetrasaccharides

1. ***Disaccharides:*** Disaccharides are the Oligosaccharides which on hydrolysis yield two monosaccharides.

Ex: Sucrose ----------> Glucose + Fructose

Maltose ----------> Glucose + Glucose

1. ***Trisaccharides:*** Trisaccharides are Oligosaccharides which yield three monosaccharides on hydrolysis.

Ex: Raffinose ----------> Glucose + Galactose + Fructose

2. ***Tetrasaccharides:*** Tetrasaccharides are Oligosaccharides which yield four monosaccharides on hydrolysis.

Ex: Stachyose ----------> Glucose + Frutose + 2 Galactose.

2. Non – Sugars:

They are usually taste less amorphous solids, insoluble in water and form colloidal suspension in water. On hydrolysis, they yield monosaccharide molecules.

Polysaccharides are further divided into:

a) ***Homo-polysaccharides***: They are polysaccharides made of single type of monosaccharide units.

Ex: Starch, Cellulose, Glycogen, Inulin.

b) ***Hetero-polysaccharides:*** They are polysaccharides made of different types of monosaccharide units.

Ex: Heparin, Hyaluronic acid, Chondroitin sulfate.

Tests to identify carbohydrates:

Qualitative tests: Following are testsused to detect different carbohydrates:

1. **Tollens's Test**: Reducing sugars on reacting with Ag^{+} (Ammonia complex) gives silver mirror.
2. **Benedict 's test:** Reducing sugars on reaction with Cu^{++}(citrate complex) give yellow-red precipitate.
3. **Fehling's Test:** Reducing sugars on reaction with Cu^{++} (tartrate complex) give yellow-red precipitate of Cu_2O.
4. **Salvinoff's Test for Fructose**: Fructose solution on addition of Salvinoff's reagent and boiling onwater bath for 5 minutes produces deep reddish color.

5. **O-Toluidine** Test for Galactose: Addition of O-Toluidine reagent to galactose solution and heating over boiling water bath for 10 minutes produces green color.
6. **Rubner's Test for Lactose:** Add 2.5 grams of Lead acetate solution to solution of Lactose and filter. Boil filtrate in another tube, add 1.5 ml. of NH_4OH and boil again for few seconds. Red color with red precipitate forms on cooling.
7. **Tauber's test for Pentose Sugars:** Addition of Tauber's reagent to Pentose sugar solution, boiling for 30 seconds and cooling develops pink to red color.
8. **Osazone Test for Carbohydrates**: Solution of carbohydrate taken in a dry test and acidified with in a dry test tube and acidified with a few drops of glacial acetic acid on addition of Osazone mixture should be placed in boiling water bath for 40 – 60 minutes. This tube should be cooled overnight and its deposit to be observed under microscope. Glucose forms yellow sheaves of slender needle like glucosazone crystals. Lactose forms close tufts of short fine crystals of lactosazone. Maltose forms clusters of broad bladed crystals of maltosazone.

9.2 Lipids

Definition: Lipids are cell components insoluble in water and soluble in non-polar solvents like acetone, ether, chloroform or benzene.

Biological Importance:

1. Lipids are used as energy storage component of the body. They are used during different metabolisms of the body.
2. Prostaglandins are a family of fatty acid derivatives. This lowers blood pressure and stimulates contraction of muscles.
3. Most membranestructure contain 40% of lipid. Membranes serve not only as barriers but also for binding certain enzymes and also acts as transport systems.
4. Gangliosides are the group of lipids present in gray matter of the brain. They function in the transmission of nerve impulses.
5. Acetic acid is used for synthesis of cholesterol. Cholesterol is precursor of many other steroids in animal tissues including bile acids, androgens- the male sex hormone, Oestrogen -- female sex hormone, Progesterone and Adreno-cortical hormone.
6. Some of the neutral lipids are found on the surface of erythrocytes and give them blood group specificity. They are partly responsible for the necessity of matching donor's and recipient's blood before transmission.

7. Cancer cells have characteristic glycosphingo lipids different from those in the normal cells.

Classification: On the basis of nature of products obtained on hydrolysis, lipids are mainly divided into three types. They are-

1. **Simple lipids**
2. **Conjugated lipids**
3. **Derived lipids**

1. **Simple lipids:** Simple lipids are esters of fatty acids with glycerol or fatty alcohols. Simple lipids are two types:
   - **a)** ***Fats and Oils:*** Fats and oils are esters of fatty acids with glycerol. Difference between fats and oils is, fats are solids at ordinary temperature whereas oils are liquids at ordinary temperature.
     Ex: Coconut oil, Arachis oil, olive oil, etc.
   - **b)** ***Waxes***: Waxes are esters of fatty acids with higher molecular weight monohydric alcohols or with sterols.
     Ex: Bees wax, Lanolin, Spermaceti, etc.
2. **Conjugated lipids:**Conjugated lipids are lipids conjugated with non-fatty prosthetic group. There are different types.
   - a) ***Phospholipids:***Phospholipids are lipids conjugated with phosphoric acid and nitrogenous component in the molecule.
     Ex: Lecithin, Cephalin, sphingomycins, etc.
   - b) ***Glycolipids:*** Glycolipids also called as cerebrosides are lipids containing sugar in glycosidic linkage with glycerol.
     Ex: Phrenoson, cerebron, Narvon, etc.
   - c) ***Sulpholipids***: They are lipids conjugated with sulfuric acid.
     Ex: sulfoquinovosyl diacylglycerol.
   - d) ***Lipoproteins:*** Lipids in the state of conjugation with proteins are called as lipoproteins. There are 4 types of lipoproteins: They are-
     - Chylomicrons
     - Very low density lipoproteins
     - Low density proteins
     - High density lipoproteins.

3. **Derived lipids:**Derived lipids are substances derived by hydrolysis of simple and compound lipids. Saturated and unsaturated fatty acids, sterols, alcohols, Glycerol's, etc. come under derived lipids.
   a) ***Saturated fatty acids:*** They are fatty acids that have no double bonds between the individual carbon atoms of the fatty acid chain. That is, the chain of carbon atoms is fully saturated with hydrogen atoms.

Ex: **Acetic acid – CH_3COOH**

Propionic acid-- C_2H_5COOH

   b) ***Unsaturated fatty acids***: Fatty acids containing one or more double bonds are unsaturated fatty acids. They can be sub-divided into different types based on number of double bonds.
      - **Oleic series**: Oleic series contain one double bond**. Ex**: Oleic acid
      - **Linoleic series**:Linoleic series contain two double bonds**. Ex**: Linoleic acid.
      - **Linolenic series**: Linolenic series contain three double bonds. **Ex:** Linolenic acid.
      - **Arachidonic acid**: It contain four double bonds.

   c) ***Sterols:*** They are derived lipids having cyclic structure obtained from nature. Steroids have cyclopentano per hydro phenanthrene ring. They are classified into different categories.
      - Sterols – cholesterol
      - Bile salts – Glycocholic and Taurocholic acids
      - Sex hormones – Testosterone, estradiol
      - Adreno cortical hormones – Corticosterone, cortisone.
      - Vitamins A, D, E and K
      - Cardiac glycosides – Strophanthin
      - Saponins – Digitonin.

   d) ***Essential fatty acids***: Fatty acids which cannot be synthesized in the body and have to be supplied through food are called as essential fatty acids. Ex: Linoleic acid is a precursor for the biosynthesis of arachidonic acid, which is not found in plants.

Clinical significance of lipids:

- Determination of lipids have diagnostic importance.

- Serum cholesterol is elevated in atherosclerosis, necrosis, and diabetes mellitus. It is decreased in hyperthyroidism, malabsorption and anemia.
- Elevation of plasma triglycerides indicates the risk of atherosclerotic diseases.
- Hyperlipidemias can be inherited trait or they can be secondary to nephrosis, diabetes mellitus, biliary obstruction and metabolic disorders of endocrine glands.

Conclusion

Carbohydrates are polyhydroxy aldehydes or ketones or substances which yield these on hydrolysis. Carbohydrates have significant role on living cells. Lipids are water insoluble components of cells. Both carbohydrates and lipidshave significant role in diagnosis.

Summary

Carbohydrates are polyhydroxy aldehydes or ketones. They have significant role in living cells. They are classified into monosaccharides, disaccharides, trisaccharides and tetra saccharides. Monosaccharides are further divided into trioses, Pentoses, hexoses, heptoses and Octoses. Non- sugars are polysaccharides which are divided into homopolysaccharides and heteropolysaccharides.

Quantitative tests for carbohydrates are Tollens's test, Benedicts test, Fehling's test, Salvinoff's test, O-Toluidine test, Rubner's test and Osazone test.

Lipids are water insoluble components of cell. They are soluble in non-polar solvents like acetone, ether, chloroform, benzene, etc. They are classified into simple lipids, conjugated lipids and derived lipids. Essential fatty acids cannot be synthesized in the body, hence must be supplied through plant sources.

Model Questions

Short Answer Type Questions

1. Define carbohydrates.
2. Mention two important functions of carbohydrates.
3. What are trioses?
4. Name the number of carbon atoms in a) Tetroses b) Hexoses
5. What are monosaccharides?
6. Define Oligosaccharides.

7. Write the products of hydrolysis of sucrose and maltose.
8. What are homopolysaccharides? Give examples.
9. What are heteropolysaccharides? Give examples.
10. What is a lipid?
11. Define simple lipids.
12. Differentiate between fats and oils.
13. Give examples of phospholipids.
14. Give some examples of glycolipids.
15. What are Sulpholipids?
16. What are lipoproteins?
17. Mention different types of lipoproteins.
18. What are derived proteins?
19. Write the definition of saturated fatty acids?
20. What are unsaturated fatty acids?
21. What are essential fatty acids?
22. Name the lipids present in brain and nervous tissue.
23. Which lipids give blood group specificity?
24. Name the fat soluble vitamins.
25. What are sterols?
26. Mention the sterols which are synthesized from cholesterol
27. What are prostaglandins?
28. What is the function of plasma lipoprotein?

Long Answer Type Questions

1. Write about the classification of carbohydrates.
2. What are lipids? Classify them.
3. What are different tests used to identify carbohydrates.
4. Define derived lipids and classify them.
5. Write the important functions of lipids and carbohydrates.

Chapter -------- 10

Amino Acids and Proteins

Structure

10.1 Amino Acids

10.2 Proteins

Learning Objectives

1. Student should know the importance of amino acids and proteins.
2. Student should know the biological importance of amino acids and proteins.

10.1 Amino Acids

Definition: Amino acids are molecules containing an amino group ($-NH_2$), a carboxylic acid group (-COOH), and a side chain (R group) specific to each amino acid.

These organic compounds contain the elements hydrogen, carbon, nitrogen, oxygen and in some cases Sulphur. Hence they are also called as α – amino carboxylic acids (α- amino acids) due to attachment of amino group to carbon atom next to carboxyl group.

Important Functions

1. Amino acids are the building blocks of proteins.The primary functions of amino acids are to build and repair muscles. They are essential in the replacement of daily wear and tear.
2. Amino acids produce chemicals that allow our brains to function at its highest potential. **Glycine** and **Glutamic acid** are involved in the transmission of impulse in the nervous system.
3. **Glutamic acid**is useful in body building and fitness because of its ability to maintain muscle mass while trying to shed fat.
4. Many amino acid derivatives are hormones. **Adrenalin / epinephrine** is the hormone for fight and flight**. Norepinephrine** is an important neurotransmitter released from sympathetic neurons affecting the heart and it also increases the rate of contraction.
5. **Leucine** is an amino acid that provides ingredients for the production of components in the body are utilized for the production of energy.

6. **Arginine** forms intermediate products in urea synthesis. Arginine plays an important role in cell division, healing of wounds, removing ammonia from the body, immune function and release of hormones.
7. **Tyrosine** is a precursor for the formation of melanin, which is a pigment of hair, skin and eyes.

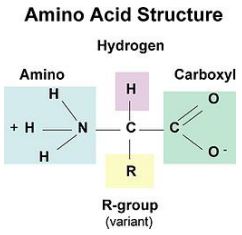


Fig 10.1: Structure of Amino Acid.

Classification

a) On the basis of capability/ non- capability of biosynthesis of amino acids in the body, they can be classified into-

1. Essential Amino Acids
2. Non- Essential Amino Acids

1. **Essential Amino Acids:** These cannot be synthesized in the body and hence must be supplied through diet by eating complete protein food of combination of vegetables. The **nine** essential amino acids include histidine, leucine, lysine, methionine, phenylalanine, tryptophan and valine.
2. **Non –essential amino acids:**Amino acids which can be synthesized in the body are called as non-essential amino acids. The **thirteen** non – essential amino acids are alanine, arginine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, hydroxyproline, proline, serine and tyrosine.

b) Amino acids classified based on number amino group and carboxyl groups:

1. **Mono amino mono carboxylic acids (Neutral amino acids**): They contain one amino acid group and one carboxylic group. Ex: Glycine, Alanine.
2. **Mono amino dicarboxylic acids**: They contain one amino group and two carboxyl groups.

   Ex: Aspartic acid, Glutamic acid.

3. **Di amino mono carboxylic acids:** They contain two amino groups and one carboxyl group.
   Ex: Lysine.

c) Amino acids classified on the basis of side chain:

1. Amino acids containing aliphatic side chain. Ex: Alanine.
2. Amino acids with side chains containing hydroxyl group. Ex: Serine.
3. Amino acids with side chain Sulphur atom. Ex: Cysteine
4. Amino acid containing aromatic rings. Ex: Phenyl alanine.
5. Amino acid with side chain pyrimidine. Ex: Proline.

Qualitative tests to identify amino acids:

1. **Solubility:** Amino acids are essentially soluble in water. The solubility vary from compound to compound. They may be soluble in water, dilute alkali or dilute acids.
2. **Ninhydrin Test:** Amino acids react with Ninhydrin to give violet color. This test is used for qualitative and quantitative identification of amino acids. Ninhydrin reagent is useful as detector in separation of amino acids in a specimen by paper chromatography.
3. **Stability to alkali:** Amino acids, unlike amides and volatile amines, do not evolve NH or alkaline vapors when boiled with alkali.
4. **Specific reactions for individual amino acids:**
   a) Xanthoproteic test
   b) Millan's test
   c) Hopkin's test.

10.2 Proteins

Definition: Proteins are high molecular weight polymers of a group of low molecular weight monomers of amino acids united by peptide bonds.

Important Functions:

1. They serve as structural elements. Collagen and elastin provide a fibrous framework in animal connective tissue. Keratin is the protein of hair, horns, nails, skin.
2. Some proteins act as storage components. Ovalbumin is used as an amino acid source of developing embryo. Casein is an important protein in milk.

3. Enzymes are proteins used in selective acceleration of bio chemical reactions like digestion and other metabolic reactions.
4. Insulin is a protein hormone produced by pancreas which regulates the concentration of sugar in blood of vertebrates.α
5. Some proteins have defensive function. Thrombin and fibrin are responsible for blood clotting and prevent loss of blood.
6. Defensive proteins are antibodies which help in protection against diseases.
7. Transport function is conducted proteins like-
   a) Hemoglobin – transport oxygen and carbon dioxide
   b) Myoglobin – transport oxygen in muscles
   c) Lipo – protein – transport lipids
8. Spiders and silk worms produce thick solution of protein fibroin which quickly solidifies into insoluble thread.

Classification

a) **Proteins are classified into** –

1. **Fibrous proteins:**
   - Fibrous proteins are polypeptide chains arranged in parallel along a single axis to yield fibers or sheets.
   - They are soluble in strong acids and alkalis, but insoluble in common solvents.

   Ex: Collagen present in tendons and bones.
   α-Keratin present in hair, nails, skin, etc.
   Elastin present in elastic connective tissue.
2. **Globular proteins:**
   - Globular proteins consists of polypeptide chains folded into compact spherical shapes.
   - They are soluble in water, dilute acids, dilute alkalis, etc.
   - Almost all enzymes, antibodies and many hormones are globular proteins. These proteins also serve function in transportation.

   Ex: Hemoglobin, Albumin, etc.
3. **Intermediates:**
   - Proteins resembling fibrous proteins in structure and resembling globular proteins in solubility properties fall into this category.

   Ex: Fibrinogen (plasma protein)

Myosin (muscle protein)

b) Another method of classification of proteins are-

1. **Simple proteins**: They are proteins which on hydrolysis yield amino acids only.

   Ex: Albumin or egg white

   Globulin or egg yolk

   Keratin of hair, nails, skin, etc.

   Collagen of bones and tendons

   Elastin of elastic connective tissue.

2. **Conjugated proteins:** They are simple proteins combined with non-prosthetic group. They include-
   a) ***Nucleoproteins:*** Proteins combined with nucleic acid as prosthetic group. Ex: DNA.
   b) ***Glycoproteins:*** Proteins combined with carbohydrates. Ex: Mucin (saliva).
   c) ***Lipoproteins:*** Proteins combined with lipids. Ex: Serum lipoproteins.
   d) ***Phosphoproteins***: Proteins combined with phosphoric acid. Ex: Casein of milk.
   e) ***Chromo proteins:*** Proteins combined with pigments. Ex: Hemoglobin.
   f) ***Metalloproteins:*** Proteins combined with metals. Ex: Ceruloplasmin (copper containing protein of serum.)
3. **Derived proteins:** They are the products resulting from hydrolysis by the action of acids, alkalis and enzymes.

Qualitative Tests to identify proteins

1. **Coagulation tests:** Proteins on heating, on exposure to U.V radiation, on reaction with acids undergo *coagulation i.e. precipitation*. When precipitation is irreversible.
2. **Color reactions:** Proteins give colored reactions with many reagents.
   a) ***Biuret test:*** Proteins on treatment with alkaline copper sulphate solution gives red or violet color. Proteins with 2 or more peptides linkages give this test.
   b) ***Millan's test:*** Addition of Millan's reagent to a protein solution gives white precipitate. This test is given by proteins yielding tyrosine on hydrolysis.
   c) ***Xanthoproteic test***: Protein on warming with concentrated nitric acid gives yellow color. This test is given by proteins containing amino acids with benzene ring.
   d) ***Ninhydrin test***: Protein on boiling with dilute aqueous solution of Ninhydrin produce violet color. This test is given by α-amino acids, proteins and dipeptides.

e) ***Hopkins-Cole test:*** Concentrated sulfuric acid when added down the side of a test tube containing protein and glyoxylic acid, violet ring appears between two layers.

Conclusion

- Amino acids build proteins.
- Proteins on hydrolysis yield amino acids.

Summary

Amino acids are building blocks of proteins in which an amino group is attached to carbon atom next to carboxyl group and hence also called as α- amino carboxylic acids. Essential amino acids are those which are not synthesized in the body and hence must be supplied through diet. Non-essential amino acids are those which can be synthesized in the body. Mono amino carboxylic acids contain one amino group and onecarboxyl group. Mono amino dicarboxylic acids contain one amino group and two carboxyl groups. Di-amino mono carboxylic group contains two amino groups and one carboxyl group Amino acids are classified based on the side chains. Side chains may be hydroxyl group, sulfur group, aromatic ring, etc. Amino acids are identified on the basis of solubility, Ninhydrin test, stability to alkali, etc.

Proteins are chemically polypeptides having variety of amino acids united by peptide bonds. Proteins are classified into 3 types. a) Fibrous proteins, b) globular proteins and c) intermediates. They are also classified into simple proteins, conjugated proteins and derived proteins. Proteins give positive result to coagulation test. They also give color reactions.

Amino acids and proteins have several important functions. They are principally responsible for replacing wear and tear of the body. Enzymes, hormones, hemoglobin, etc. are proteins.

Model Questions

Short Answer Type Questions

1. What are essential amino acids?
2. Write the definition of amino acids.
3. Name the amino acid responsible for transmission of impulses in the nervous system.
4. What are fibrous proteins?
5. What are glycoproteins?

6. Write about Ninhydrin test.
7. Name the amino acid with side chain Sulphur.

Long Answer Type Questions

1. Define amino acids. Give different classifications of amino acids.
2. What are proteins? Classify them.
3. Write the important functions of amino acids and proteins.

Write the qualitative tests for

Chapter – 11

Diagnostic tests

Structure

11.1 Serum Uric Acid

11.2 Serum Creatinine

11.3 Blood urea

11.4 Identification of Calcium in serum.

Learning objectives

1. Student should know about different biochemical estimations
2. Student should practice to calculate the concentrations using O.D values of different determinations in any medical lab.

11.1 Serum Uric Acid

Introduction: Uric acid is the end product of purine (nucleoprotein) metabolism. The serum uric acid level is often raised in gout. This test is used in the differentiating of gout from non-gouty arthritis.

Chemical structure: 2, 6, 8 trihydroxy purine.

Site of synthesis: Liver is the chief site of synthesis.

Normal value: Blood contains 2 - 7 mg. of uric acid for 100 ml.Daily output is 0.75 to 1 g.

Clinical significance: its value in blood is increased in-

1. Gout
2. Leukemia
3. Pneumonia
4. Arteriosclerosis with hypertension
5. Cardiac decompensation.
6. Uremia.

Determination of Serum Uric Acid

Principle: Uric acid reacts with phosphotungstic acid in alkaline medium to form a blue colored complex. Concentration of uric acid is directly proportional to the color intensity. Concentration of uric acid in serum is determined by comparing with similarly treated uric acid standard. Alkaline medium is provided by sodium carbonate.

Name of the method: Henry-Caraway's method.

Requirements:

1. Test tubes
2. Serological tubes
3. Centrifuge tubes
4. Colorimeter

Reagents:

1. **Sodium tungstate 10% w/v**
   a) 1o g/dl. Sodium tungstate : 50 ml.
   b) 2/3N, sulfuric acid : 50 ml.
   c) Orth phosphoric acid : 1 drop
   d) Distilled water : 800 ml.
   - Mix well and store at room temperature in an amber colored bottle.
2. **Sodium carbonate 10% w/v:**
   a) Anhydrous sodium carbonate : 10 mg.
   b) Distilled water : 100 ml.
   - Dissolve 10 mg. of anhydrous sodium carbonate in 75 ml. of distilled water and make to 100 ml. with distilled water. The reagent is stable at room temperature when stored in polythene bottle.
3. **Stock uric acid standard (100 mg %):**
   a) Lithium carbonate : 60 mg.
   b) Uric acid : 100 mg.
   c) Formalin : 2 ml.
   d) 50% Acetic acid : 1 ml.
   e) Distilled water : 100 ml.

- Heat about 80 ml. of distilled water in 250 ml. beaker to 60^0C. Add 60 mg, lithium carbonate and mix well. Add 100 mg. uric acid and mix thoroughly. Add 2 ml. formalin and then, slowly with shaking add 1 ml. acetic acid. Mix well and make the final volume to 100 ml. by adding distilled water. Store in amber colored bottle at 2—8^0C.

4. **Working uric standard (5 mg %):**
   a) Stock uric acid standard solution : 5 ml.
   b) Distilled water : 100 ml.
   - Dilute 5 ml. of stock uric acid solution to 100 ml. with distilled water.
5. **Stock phosphor tungstic acid reagent:**
   a) Sodium tungstate (molybdate free): 50 mg.
   b) Ortho phosphoric acid : 40 ml.
   c) Distilled water : 400 ml.
   - Mix and reflux gently for two hours. Cool and make final volume 500 ml. store at 2 – 8^0C in an amber colored container.
6. **Working phosphotungstic acid solution:**
   a) Stock phosphotungstic acid : 5 ml.
   b) Distilled water : 100 ml.
   - Dilute 5 ml. of stock phosphotungstic acid to 100 ml. volume with distilled water.

Specimen: Serum (or plasma).

Wave length: 660 nm (Red filter)

Procedure:

1. Take three test tubes and label them as T, S and B representing Test, Sample and Blank respectively.
2. Take the reagents as follows:

Reagent	T	S	B
Distilled water	3.5 ml.	3.5 ml.	4 ml.
Serum	0.5 ml.	--- ----	
Uric acid (standard)	----	0.5 ml. ----	

Sodium tungstate 10% w/v ml.	0.5 ml.	0.5 ml.	0.5
Sulfuric acid	0.5 ml.	0.5 ml.	0.5 ml

This step gives protein free serum filtrate and similarly treated standard and blank.

3. Mix well and centrifuge after five minutes.
4. Take the supernatant into test tubes as follows and mix well.

	T	**S**	**B**
Supernatant ml.	3 ml.	3 ml.	3
Sodium Carbonate ml.	1 ml.	1 ml.	1

5. Set the colorimeter to 100% transmission with blank at 660 nm wave length (red filter).
6. Determine the O.D. of the test and standard.
7. Determine the concentration of serum uric acid with formula.

$$\text{Concentration of serum uric acid} = \frac{\text{O.D. of Test}}{\text{O.D. of standard}} \times 5 \text{ mg/ dl}$$

Normal value: 2 to 7 mg / dl.

11.2 Serum Creatinine

Introduction: Creatinine is the waste product of creatinine (nucleoprotein) metabolism.

Creatinine level raises in the body from spontaneous break down of creatinine phosphate.

Clinical Significance: Serum creatinine is increased in renal failure. Increased serum creatinine indicates renal disease. Elevation values are also observed in certain conditions like congestion heart failure, shock and mechanical obstruction of the urinary tract.

Normal values: Serum creatinine ranges from 0.6 to 1.5 mg/ dl. Daily output is 1.5 to 3 gm.

Determination of Serum Creatinine

Principle: Creatinine reacts with picric acid in alkaline medium to form a reddish yellow complex, intensity of which is directly proportional to the concentration of creatinine in the specimen and can be measured at 520 nm (green filter).

Name of the method: Alkaline- picrate method (Jaffe's reaction)

Specimen: Serum (or plasma)

Requirements:

1. Test tubes
2. Serological pipette
3. Volumetric flask.
4. Test tube stand
5. Centrifuge tubes
6. Centrifuge.
7. Colorimeter.

Reagents:

1. **Picric acid reagent:** 0.91 gm/dl (0.04 M)
2. **Sodium hydroxide**: 10 gm/dl
3. **Working creatinine standards**: 1 mg/dl, 5 mg/dl and 10 mg/dl.
    - These standards are prepared in 0.01 N hydrochloric acid by using stock creatinine standard 100 mg/dl. They are stable at 2 -- 8^0C.
4. **Alkaline picrate solution**:
    - It is prepared by mixing 4 parts of picric acid reagent and 1 part of sodium hydroxide. This reagent is stable for one day.

Specimen: Serum (or plasma)

Wave length: 520 nm (green filter)

Procedure:

1. Prepare protein free blood filtrate as follows
   - Distilled water – 3 ml.
   - Serum -- 1 ml.
   - 2/3 N H_2SO_4 ---- 0.5 ml.
   - 10% Sodium tungstate solution --- o.5 ml.

   Mix all the contents, centrifuge and take the supernatant.
2. Pipette it into the tubes labelled as T, S and B, representing test, sample and blank as follows

Reagent	T	S	B
Distilled water	3 ml.	3 ml.	3 ml.
Filtrate	2 ml.	---	-----
Working standard	----	2 ml.	-----
Alkaline picrate	1 ml.	1 ml.	1 ml.

3. Mix all the contents and keep at room temperature for 20 minutes.
4. Set the colorimeter to 100% transmission with blank.
5. Determine the O.D. of test and different standards.
6. Determine the concentration of unknown using the following formula-

$$\text{Concentration of serum creatinine} = \frac{\text{O.D of test}}{\text{O.D of standard}} \times \text{Concentration of standard mg/dl.}$$

Normal values:

Males : 0.9 to 1.5 mg/dl

Women : 0.8 to 1.2 mg/dl.

11.3 Blood Urea

Introduction: Urea is a main excretory product of protein catabolism. It is water soluble. Determination of blood urea is significant in assessment of kidney functioning.

Structure:

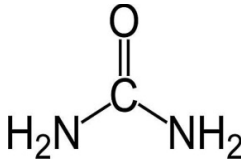


Fig 11.1: Structure of Urea

Site of formation: Formation of urea takes place in liver in the ureotelic organisms by sequence of reactions called as Urea cycle or Krebs – Hanseleitcycle.

Clinical significance: Elevated levels of blood urea are observed in pre-renal, renal and post renal conditions. Diabetes Miletus, dehydration, cardiac failure, etc. are pre – renal conditions. Kidney diseases are renal conditions. Enlargement of prostate, stones in urinary tract, etc. are post-renalconditions. Blood urea is decreased in severe liver diseases, pregnancy, malnutrition, etc.

Normal range: Average of 30 mg. of urea will be present per 100 ml. of blood. Average of 30 gm. Of urea is excreted through urine in 24 hours in an adult.

Determination of Blood Urea

There are different methods of determination of blood urea. They are:

1. Diacetylmonoxime method (DAM).
2. Enzymatic method/ Berthelot reaction method.
3. Titration method

1. **<u>Diacetylmonoxime method (DAM):</u>**

Principle: Urea reacts with Diacetylmonoxime in hot acidic medium and in the presence of thiosemicarbazide and ferric ions to form a pink colored compound which can be measured on a green filter (520 nm)

Requirements:

1. Test tubes

2. 10 ml. pipette
3. 0.1 serological pipette
4. 100 ml. measuring cylinder
5. Water bath
6. Stop watch
7. Colorimeter.

Reagents:

1. DAM – TSC reagent:

a) Diacetylmonoxime : 1 g.
b) Thiosemicarbazide : 0.2 g.
c) Sodium chloride : 9 g.
d) Distilled water : 1 liter

- Dissolve Diacetylmonoxime in 600 ml. of distilled water. To this solution dissolve thiosemicarbazide and sodium chloride. Dilute it to 1 liter with distilled water. This stable at room temperature for one year.

2. Acid reagent:

a) Ortho-phosphoric acid : 10 ml.
b) Sulfuric acid : 60 ml.
c) 10% aqueous ferric chloride solution : 1 ml.
d) Distilled water : 1 liter.

- Add Ortho-phosphoric acid and sulfuric acid of 750 ml. of distilled. Cool and add ferric chloride solution. Make it to 1 liter with distilled water. This reagent is stable at room temperature for one year.

3. Stock Urea Standard Reagent (1% w/v):

a) Dry urea : 1 g.
b) 0.2% w/v benzoic acid aqueous solution : 100 ml.

- Dissolve urea in 75 ml. of benzoic acid solution and dilute to 100 ml. with benzoic acid solution. This is stable at room temperature for one year when refrigerated.

4. Urea working standard (50 mg/dl.):

a) Stock urea standard : 5 ml.
b) 0.2% benzoic acid aqueous solution : 100 ml.

- Dilute stock urea standards to benzoic acid solution. This is to be freshly prepared for each batch of determination.

Sample: Blood

Wave length: 520 nm (green filter)

Procedure:

1) Prepare protein free blood filtrate (T) as follows-
   a) Blood : 0.1 ml.
   b) 2/3 N Sulfuric acid : 0.2 ml.
   c) 10% Sodium tungstate solution : 0.2 ml.
   d) Distilled water : 3.5 ml.
   - Mix all the contents and centrifuge after 5 minutes. Take supernatant in a test tube and label as test (T).
2) Give similar treatment to 0.1 ml. of urea working standard, take the supernatant in a test tube and label as standard (S).
3) Give similar treatment to 0.1 ml. of distilled water, take the supernatant in a test tube and label it as blank (B).
4) Label the tubes as T, S and B and take the reagents as follows:

Reagent	**T**	**S**	**B**
Supernatant	2 ml.	2 ml.	2 ml.
DAM – TSC reagent	3 ml.	3 ml.	3 ml.
Acid reagent	3 ml.	3 ml.	3 ml.

5) Mix the contents and plug the tubes with cotton.
6) Heat in a boiling water bath for 15 minutes and cool.
7) Set the colorimeter to 100% transmission using blank at 520 nm wave length.
8) Determine the O.D. of standard and test.
9) Determine the concentration of blood urea using the formula:

$$\text{Concentration of blood urea, mg/dl.} = \frac{\text{O.D. of test}}{} \times \text{concentration of standard}$$

O.D. of standard

Normal value: 15 – 45 mg/dl.

Blood urea

Blood urea Nitrogen, mg/dl. = ----------

2.15

2) Enzymatic method (Berthelot reaction method):

Principle: The procedure is based on Berthelot reaction. Urease splits into ammonia and carbon dioxide. Ammonia and carbon dioxide reacts with phenol in the presence of hypochlorite to form Indophenol, with which alkali gives a blue colored compound.

Requirements:

1. Test tubes
2. 1.0 ml, 5.0 ml., 0.1 ml. graduated pipette
3. Water bath
4. Colorimeter.

Reagents:

1. **Urease reagent**:
   a) Urease : 1000 units
   b) Phosphate buffer : 100 ml.
   - Dissolve urease in 75 ml. of phosphate buffer and dilute to 100 ml. with phosphate buffer, pH 7.0 (0.05 M). It is stable at 2 – 8^0C for 3 months.
2. **Phenol reagent:**
   a) Phenol : 5 g.
   b) Sodium Nitroprusside : 0.025 g.
   c) Distilled water : 500 ml.
   - Dissolve phenol and Sodium nitroprusside in 450 ml. of distilled water and dilute to 500 ml. with distilled water. It is stable at 2 – 8^0C for 3 months.
3. **Hypochlorite Reagent:**
   a) Sodium hypochlorite : 0.21 g.
   b) Sodium hydroxide : 2.5 g.
   c) Distilled water : 500 ml.

- Dissolve the reagents in 450 ml. of distilled water and then make it to 500 ml. with distilled water. It is stable for 3 months at 2 – 8^0C.

4. Standard urea nitrogen 20 mg/dl.:

a) Urea : 42.8 g.

b) Saturated benzoic acid : 100 ml.

- Dissolve urea in 75 ml. of benzoic acid and make it to 100 ml. using benzoic acid. This is stable for 3 months at 2 – 8^0C.

Sample: Serum or heparinized plasma.

Wave length: 546 nm. (Green filter)

Procedure:

1. Take 3 test tubes and label them as T, S and B representing test, standard and blank respectively.
2. Take reagents in the tubes are taken as follows-

-- **Reagents**	**T**	**S**	B
Urease reagent	0.5 ml.	0.5 ml.	0.5 ml.
Serum/plasma	0.02 ml.	----	---
Standard urea nitrogen	-----	0.2 ml.	-----

3. Mix and keep at 37^0C in a water bath for 10 minutes.
4. Now add the reagents as follows-

Reagent	**T**	**S**	**B**
Phenol reagent	1 ml.	1 ml.	1 ml
Hypochlorite reagent	1 ml.	1 ml.	1 ml.

5. Mix the contents and keep the tubes in water bath again at 37^0C for 10 minutes.

6. Add 5 ml. of distilled water to each tube and mix thoroughly.
7. Set the colorimeter to zero O.D. with the help of blank at 546 nm wave length.
8. Determine the O.D. of test and standard.
9. Determine the values of blood urea nitrogen and blood urea using the formulae—

$$\text{Blood urea nitrogen} = \frac{\text{O.D. of test}}{\text{O.D. of standard}} \times 20 \text{ mg/dl.}$$

$$\text{Blood urea} = \text{Blood urea nitrogen} \times 2.14$$

Normal values:

Blood urea nitrogen = 5 – 21 mg/dl.

Blood urea = 11 – 45 mg/dl.

11.4 Identification of Calcium in Serum

Introduction: The calcium of the blood is confined to plasma. Serum calcium is used to screen a range of conditions related to bones, heart, nerves, kidneys and teeth. The test may be done patients having parathyroid disorder, malabsorption or thyroid problem.

Normal range: Total serum calcium ranges from about 9 to 11 mg/dl.

Clinical significance: Decrease serum calcium values are found in hypoparathyroidism, rickets, etc. A fall in serum calcium can occur in acute pancreatitis and renal diseases. Increased serum calcium values are observed in hyperparathyroidism, more intake of vitamin D and multiple myeloma.

Determination of Serum Calcium:

Principle: Calcium reacts directly with cresolphthalein complexion (CPC) reagent containing dimethyl sulfoxide and 8 – hydroxylquinoline. Since magnesium reacts with CPC, the addition of 8 – hydroxylquinoline eliminates the interference from magnesium.

Requirements:

1. Test tubes
2. 100 ml. graduated cylinder
3. 100 ml. beaker

4. 10 ml. graduated pipette
5. Serological pipette
6. Stop watch
7. Colorimeter.

Reagents:

1. **Calcium reagent 1:**
   a) Cresolphthaleincomplexion: 40 mg.
   b) Concentrated hydrochloric acid : 1.0 ml.
   c) 8 – Hydroxylquinoline : 2.5 g.
   d) Dimethyl sulfoxide : 100 ml.
   e) Glass distilled water : 900 ml.
      - Mix all the contents and finally make it to 1 liter by using glass distilled water. The reagent is stable at room temperature for 3 months.
2. **Calcium reagent 2:**
   a) Potassium cyanide : 500 mg.
   b) Dimethylamine : 40 ml.
   c) Glass distilled water : 960 ml.

   Mix the contents thoroughly and adjust it to 1 liter by using glass distilled water. It is stable at room temperature for 3 months.
3. **Calcium standard 10 mg/dl. (5.0 meq/liter):**
   a) Calcium carbonate : 25 mg.
   b) Hydrochloric acid : 50% (v/v)
      - It is prepared with 25 g. calcium carbonate hydrochloric acid 50% v/v. This reagent is stable at 2 – 8^0C.
4. **EDTA** : 4.0 g/dl.

Specimen: Serum or heparinized plasma.

Wave length: 575 nm (yellow filter)

Procedure:

1. Prepare fresh working reagent by mixing equal volumes of calcium reagents 1 and 2. The color of the reagent should be light purple.

2. Take 3 test tubes and mark them as T, S and B indicating test, standard and blank respectively.
3. Take reagents as follows-

Reagent	**T**	**S**	**B**
Working reagent	6.0 ml.	6.0 ml.	6.0 ml.
Serum/heparinized plasma	0.05 ml.	-----	------
Standard 10 mg/dl.	-----	0.05 ml.	------
Distilled water	-----	------	0.05 ml.

4. Mix the contents thoroughly and keep at room temperature for exactly 10 minutes.
5. Set the colorimeter to zero O.D. at 575 nm wave length (yellow color) using blank.
6. Read the intensities of test and standard.
7. Calculate serum calcium using the formula-

$$\text{Serum calcium} = \frac{\text{O.D. of test}}{\text{O.D. of standard}} \times 10 \text{ mg/dl.}$$

Normal range: 8.5 – 10.5 mg/dl.

Conclusion

Different diagnostic tests are useful for assessing clinical condition of a patient. Different tests help to diagnose different diseases.

Summary

Determination of blood urea is significant in assessment of renal functioning. Different methods of determination of blood urea are Diacetylmonoxime method, enzymatic method and Titration method. Determination of serum uric acid is significant in determination of Gout, Leukemia, etc. It is determined by Henry- caraway method. Determination of serum creatinine is significant in the diagnosis of renal functioning, cardiac functioning, etc. It is

determined by alkaline picrate method. Serum calcium is determined to screen conditions related to bones, heart, teeth, etc.

Model Questions

Short Answer Type Questions

1. What is uric acid?
2. Give the normal values of serum uric acid.
3. Write the clinical significance of determination of serum uric acid.
4. Give the principle of determination of serum uric acid by Henry- Caraway method.
5. Write the composition of stock uric acid reagent (100 mg/dl).
6. How do you prepare 5 mg/dl working serum uric acid solution from stock uric acid standard solution?
7. Write the clinical significance of serum creatinine determination.
8. Write the composition of alkaline picrate reagent.
9. Give the principle of serum creatinine determination by alkaline picrate method.
10. Mention the normal values of serum creatinine for male and female.
11. What is urea? Give its chemical structure.
12. Write the principle of determination of blood urea by Diacetylmonoxime method.
13. Mention the wave lengths selected for determination of blood urea by
    a) Diacetylmonoxime method b) Berthelot reaction method.
14. Write the principle of determination of blood urea by enzymatic method.
15. Name the enzyme reagent used in blood urea determination by Berthelot reaction method and give its composition.
16. What is the normal value of serum calcium?
17. Write the clinical significance of serum calcium determination.

Long Answer Type Questions

1. How do you determine serum uric acid by Henry- Caraway method?
2. Describe the determination of serum creatinine by alkaline picrate method.
3. Explain the method of determination of blood urea by Diacetylmonoxime method.
4. Write the Enzymatic method of determination of blood urea.
5. Explain the method of determination of serum calcium.

Chapter – 12

Vitamins and Minerals

Structure

12.1 Vitamins

12.2 Minerals

Learning Objectives

1. Student should get familiar with types of vitamins, daily requirement and deficiency diseases.
2. Student should get the knowledge of minerals, their sources, daily requirement and related deficiency diseases.
3. Student should conduct a survey of deficiency of different vitamins and minerals in his locality to have a view over the vitamin and mineral deficiency situations.

12.1 Vitamins

Definition: A vitamin is an organic compound required as a nutrient in small amounts by an organism.

Classification: Depending on solubility property, vitamins are classified into two types. They are:

1. **Water soluble vitamins**: There are nine water soluble vitamins. Eight B vitamins and vitamin C.
2. **Fat soluble vitamins**: There are four fat soluble vitamins. Vitamins A, D, E and K.

Water soluble vitamins:

The nine water soluble vitamins are:

1) Thiamin (Vitamin B_1)
2) Riboflavin (Vitamin B_2)
3) Nicotinic acid or Niacin (Vitamin B_3)
4) Pantothenic acid (Vitamin B_5)
5) Pyridoxine (Vitamin B_6)
6) Biotin(Vitamin B_7 or Vitamins H)
7) Folic acid (Vitamin B_9)
8) Cyanocobalamin (vitamin B_{12})

9) Ascorbic acid (Vitamin C)
10) Miscellaneous.

1) **Thiamin (Vitamin B_1):** It is also called aneurin. It occurs in living cells in its active coenzyme form- Thiamin pyrophosphate (TPP). It participates in decarboxylation of α-Keto acids and forms Acetyl coenzyme A.

Dietary Sources: This vitamin is adequately available in many natural foods. It is available in the germ of cereals, brans, egg yolk, yeast extracts, peas, beans and nuts, pork, liver, heart, kidney, etc.

Daily requirement: Adults: 1 – 1.5 mg. per day.
Children: 0.7 – 1.2 mg. per day.

Deficiency diseases: Deficiency of this vitamin leads to loss of appetite, gastrointestinal disturbances, muscular weakness, pain in arms and legs, decrease in blood pressure. Severe deficiency affects entire nervous system leading to Beriberi.

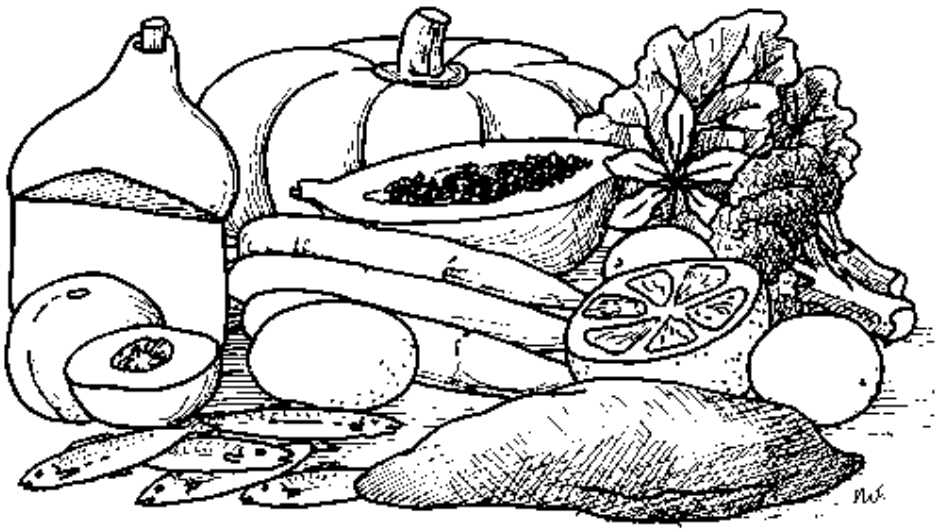


Fig 12.1: Sources of Vitamin B1

2) **Riboflavin (Vitamin B_2):** It is also called as Lacto Flavin. It is heat stable, light sensitive yellow crystalline solid.

Dietary sources: Liver, milk, cheese, almonds, leafy green vegetables, kidneys, yeast, legumes, tomatoes and mushrooms are good sources of vitamin B_2.

Daily requirement: Adults: 1.2 – 1.7 mg. per day

Pregnant and Lactation females: 1.5 – 2.0 mg. per day.

Deficiency diseases: Deficiency of Riboflavin in man causes inflammation of tongue and lining of mouth cheilosis–cracking of lips and corners of mouth, dry and scaling skin. The eyes may also become itchy, watery and sensitive to bright light.

Fig 12.2: Sources of vitamin B2

3) **Nicotinic Acid or Niacin (Vitamin B_3):** It is called as Pellagra preventing factor. It was first prepared by oxidation of Nicotine. After 1913, it was isolated from yeast.

Dietary sources: Niacin is found in variety of foods including liver, chicken, beef, fish, cereal, peanuts, dates, broccoli, carrots, nuts and legumes. It is synthesized from tryptophan, which is found in meat, fish, dairy and eggs.

Daily requirement: Adults: 15 – 20 mg. per day.

Children: 10 – 15 mg. per day.

Deficiency diseases: severe deficiency of niacin in diet causes disease called pellagra, characterized by diarrhea, dermatitis and dementia. Common psychiatric symptoms are anxiety, fatigue, poor concentration, restlessness and depression.

Fig: 12.3 Vitamin B3 foods

4) **Pantothenic acid (Vitamin B_5):**This is structural component of coenzyme A. its function is to serve as carrier of acyl groups in enzymatic reactions.

Dietary sources: The rich sources of pantothenic acid are eggs, cereals, legumes, meat, pork, yeast, milk, etc.

Daily requirement: Children: 2 – 4 mg. per day

Adults, pregnant and lactating women: 5 – 10 mg. per day.

Deficiency diseases: Clinical cases related to deficiency if this vitamin is very rare. Symptoms of deficiency are due to impaired energy levels leading to irritability, fatigue and apathy.

5) **Pyridoxine (vitamin B_6):**Pyridoxine is biologically converted into pyridoxal and pyridoxine. Active coenzyme forms Pyridoxal phosphate and Pyridoxine phosphate which take active part in amino acid metabolism.

Dietary sources: The important food sources of this vitamin are milk, fish, meat, eggs, banana, cabbage, corn, whole grain, etc.

Daily requirements: Adults: 2.2 – 2.2 mg. per day.

Pregnant and lactating women**:** 2.5 mg. per day.

Deficiency diseases: In some infants, its inadequate supply in diets lead to epileptic like seizures. This deficiency of this vitamin leads to hypochromic anemia.

Fig 12.4 Vitamin B6 Rich food

6) **Biotin**:Biotin is made by intestinal bacteria. It is necessary for the production of fatty acids and the metabolism of fats and amino acids. It plays an important role in the citric acid cycle by which energy is released during aerobic respiration.

Dietary sources:The sources of biotin are eggs, liver, kidney, milk, tomatoes, grains, nuts, string beans, spinach and grass.

Daily requirements: Adults: 100 – 300 µg. per day**.** Minimal requirement of this vitamin is not established because quantity of vitamin provided by bacteria cannot be determined.

Deficiency diseases: Biotin deficiency is caused by prolonged feeding of raw egg white. Raw egg white contains a protein called Avidin. It has the property of binding with biotin and preventing its absorption from intestine.

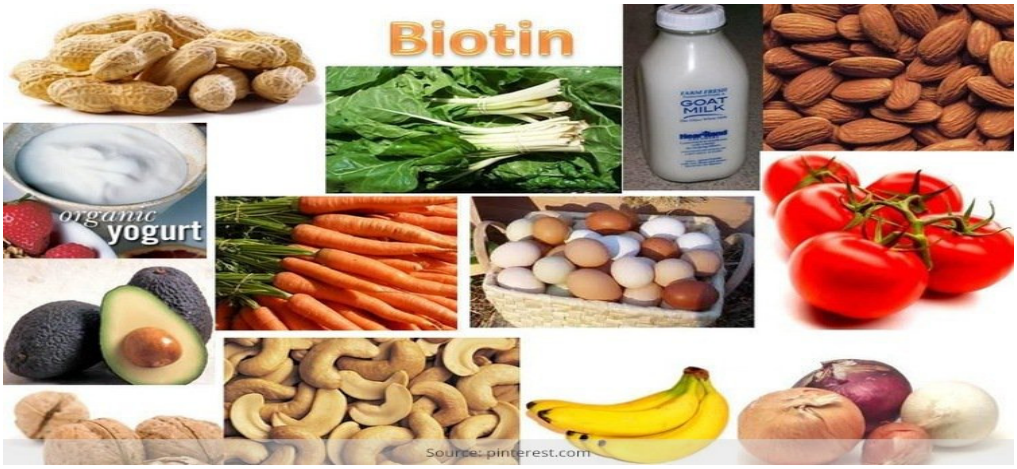


Fig 12.5: Sources of Biotin

7) **Folic acid (Vitamin B_9):** It is a pteridine derivative synthesized by intestinal bacteria. It is rich in leafy vegetables.

Dietary sources: Leaves and foliage of spinach, whey, mushrooms, liver, yeast, bone marrow, soya beans and fish. In moderate amounts, it is also present in fruits like oranges, bananas, etc. It is synthesized by bacteria.

Daily requirement: Adult: 100 µg. per day.

Pregnant women**:** 300 µg. per day.

Lactation women: 150 µg. per day.

Deficiency diseases: Folic acid deficiency leads to impairment in the biosynthesis of purines and pyrimidines. This leads to diarrhea, depression, confusion, and during pregnancy, fetal neural defects and brain defects.

Fig 12.6: Sources of Folic acid.

8) **Cyanocobalamin (Vitamin B_{12}):**It is a vitamin containing cobalt. It is essential for the growth of epithelial cells. It is required in the formation of myelinated nerve fiber, blood, and fatty acid synthesis and energy production.

Dietary sources: the rich sources of this vitamin are milk, fish, liver, kidney, pork, egg, chicken curd, etc.

Daily requirement: Adults: 3µg. per day.

Children: 0.5 – 1.5 µg. per day.

Pregnancy and lactating women**:** 4µg. per day.

Deficiency diseases: deficiency of this vitamin causes pernicious anemia and peripheral neuritis. It is essential for normal maturation and development or erythrocytes. Deficiency of this vitamin leads to neurological disorder rand degeneration of peripheral nervous tissue causing numbness, tingling in fingers and toes.

Fig 12.7: sources of vitamin B $_{12}$

9) **Ascorbic acid (Vitamin C):**It is a vitamin which is found in citrus fruits, involved in the repair of tissues and the enzymatic production of certain neurotransmitters. It is important for proper functioning of immune system. It works as antioxidant.

Dietary sources: Citrus fruits like orange, lemon, etc. tomatoes, potatoes, cabbage, papaya, amla, etc.

Daily requirement: Children: 45 mg. per day

Adults: 80 mg. per day

Pregnant women: 110 mg. per day.

Deficiency diseases: Deficiency of this vitamin results in dry and rough skin. It also results in increased rate of infections, sore joints and bones and scurvy.

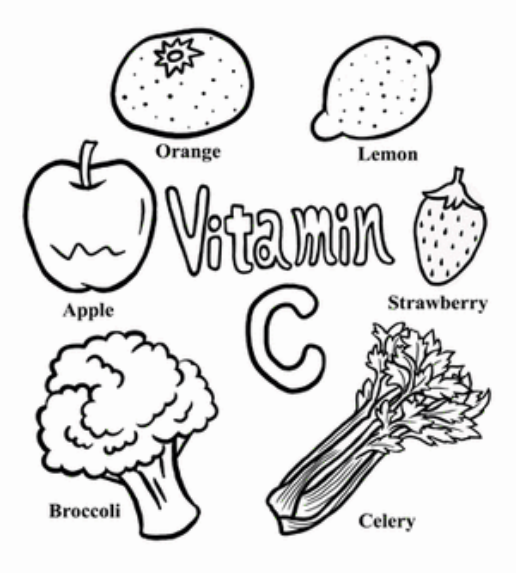


Fig 12.8: Sources of vitamin C

10) **Miscellaneous vitamins:** They are compounds which function like vitamins.

a) **Choline**:

- It is a component of phosphor-lecithin involved in membrane structure and lipid transport.
- Choline is present in liver, chicken, cauliflower, spinach, peanuts and almonds.
- Daily requirement is 425 mg. per day in adults. In pregnant women about 550 mg. is required.
- Choline deficiency include liver diseases and neurological disorders.

b) Lipoic acid:

- It is also called as acetate replacement factor. It is also called as Protogene.

- Lipoic acid is found in all foods like kidneys, heart, liver, broccoli, etc.
- Lipoic acid is involved in dicarboxylic reactions.

c) Inositol:

- It is a carbohydrate, but does not belong to any specific sugar.
- It is required for synthesis of phosphatidylinositol, constitute of cell membrane. It also acts as a lipotropic factor.
- It is found in cereals, high bran content, beans, fruits, etc.

Fat soluble vitamins:

These are soluble in fat. Bile salts facilitate their absorption through GIT. The fat soluble vitamins are:

1. Vitamin A
2. Vitamin D
3. Vitamin E and
4. Vitamin K.

1) **Vitamin A**: It is useful for maintenance of integrity of epithelial tissue. It is necessary for healthy skin and general growth also.

Dietary sources: yellow vegetables and fruits, cod liver oil, Shark liver oil, etc.

Daily requirement: Children: 1500 – 5000 I.U.

Adults: 5000 I.U.

Deficiency diseases: Xerophthalmia, Keratomalacia, etc.

Diseases caused by excess Vitamin A: Sluggishness, head ache, roughening of skin, etc.

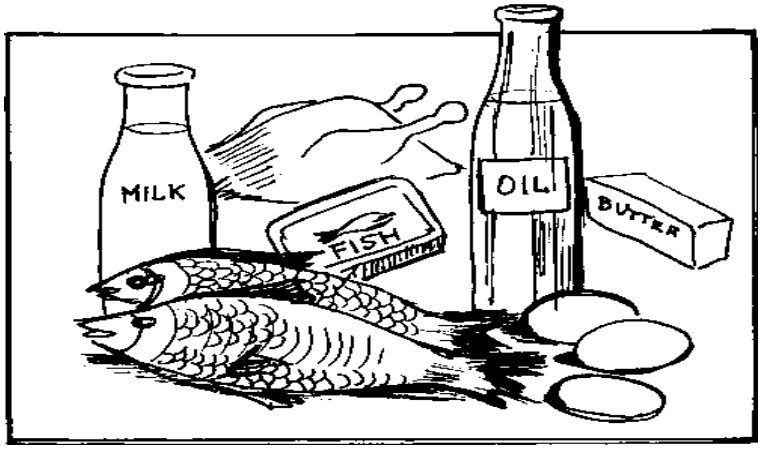


Fig 12.9: Sources of vitamin A

2) **Vitamin D:**They are sterols. It is essential for increasing absorption of calcium, magnesium, etc.

Dietary sources: Liver of fish, eggs, milk, butter etc.

Daily requirement: 200 – 400 I.U.

Deficiency diseases: Rickets in children and osteomalacia in adults.

3) **Vitamin E**: It is also called Tocopherol.

Dietary sources: Eggs, meat, liver, fish, corn oil, cotton seed oil, etc.

Daily requirement: 25 – 30 mg. per day.

Deficiency diseases: Hemolysis, anemia, hepatic necrosis, etc.

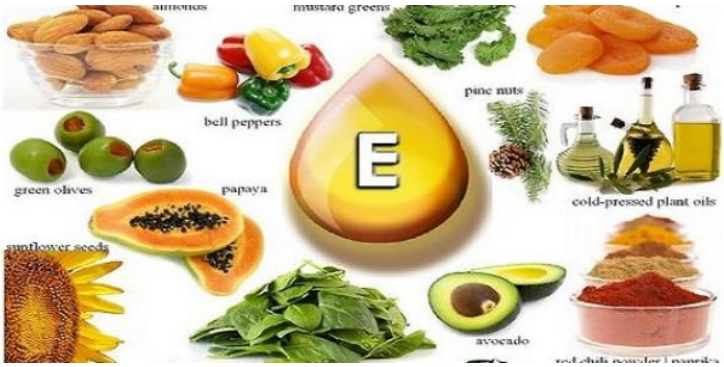


Fig 12.10: sources of vitamin E

4) **Vitamin K:** It is essential for coagulation of blood. It catalyze the synthesis of prothrombin by liver.

Dietary sources: Green leafy vegetables, cauliflower, carrots, milk, etc.

Daily requirement: Average diet contains quantities adequate for adults.

Deficiency diseases: Its deficiency is not reported in healthy individuals. Its deficiency is reported in infants and new born with mother's diet of low quantities of vitamin K. its deficiency causes hemorrhagic conditions.

Fig: sources of vitamin K

Fig 12.11: Vitamin K rich food

12.2 Minerals

Definition: Minerals are substances found in food that are essential for growth and health. They are supplied through diet.

Classification: They are classified into-

1. Macro elements(Principal elements)
2. Trace elements.

1) ***Macro elements (Principal elements)***: They are seven in number. They are Sodium, Potassium, Calcium, Phosphorous, Magnesium, Chlorine and Sulphur.

a) **Sodium:** It is the major cation of extracellular fluid. It is required for maintaining – neuromuscular function, heart beat initiation, cell permeability and normal water balance.
Dietary sources: Bread, cheese, table salt, carrots, cauliflower, etc.
Daily requirement: Adults: 5 – 15 g. per day.
Deficiency diseases:In Hyponatremia, lower concentration of Sodium in blood than normal leads to dizziness, lethargy, malaise. In Hypernatremia, higher values are present which leads dehydration.

b) **Potassium**: Itis the major cation of intracellular fluid. It is useful for maintaining cardio-muscular activity, osmotic pressure, acid - base balance, and water retention and protein biosynthesis in ribosomes.
Dietary sources: Chicken, beef, liver, banana, etc.
Daily requirement: About 4 g. per day.

Deficiency diseases:Deficiency of potassium is called hypokalemia, which causes injury to myocardium and kidneys, etc. Condition of excess of potassium is called hyperkaliemia, which causes renal failure, severe dehydration and Addison's disease.

c) **Calcium**: It is essential along with Phosphorous for the formation of bones and teeth. Ionic calcium is essential for coagulation of blood, nervous excitability and neuro – muscular transmission.

Dietary sources: Milk, cheese, egg, cabbage, cauliflower, etc.

Daily requirement: 1 – 1.2 g. per day.

Deficiency diseases: Tetanus, rickets, osteomalacia, osteoporosis, renal rickets, etc.

d) **Phosphorous**: It is essential along calcium for formation of bones and teeth.

Dietary sources: Milk, cheese, egg yolk, meat, etc.

Daily requirement: 240 – 1200 mg. per day.

Deficiency diseases: Low values leads to rickets, renal rickets, hyper - parathyroidism.

e) **Magnesium**: 70% of body's magnesium content is combined with calcium and phosphorous.

Dietary requirement: Milk, eggs, cabbage, etc.

Daily requirement: 100 – 300 mg. per day.

Deficiency diseases: Deficiency leads to depression, muscular weakness, convulsions, etc.

f) **Chlorine**: It is essential as chloride of sodium for acid base balance. Chloride ion is also essential for water balance, osmotic regulation, production of HCl, activation of amylase.

Dietary sources: Sodium chloride.

Daily requirement: 5 – 20 mg. per day.

Deficiency diseases:Diarrhea, sweating and vomiting.

g) **Sulphur**: It is present in Sulphur containing amino acids, cysteine and methionine.

Dietary sources: Cysteine and methionine.

Deficiency diseases: Increased levels are observed in renal impairment, pyloric and intestine obstruction, leukemia, etc.

2) ***Trace elements:*** They are required in small quantities. On the basis of essentiality, they can be classified into-

a) Essential trace elements: Iron, Iodine, Copper, Cobalt, Fluorine**.**

b) Possibly essential elements: Nickel, Tin, Vanadium, etc.

c) **Non-essential elements:** Aluminium, Boron, Lead, Mercury, etc.

a) Essential trace elements:

1. **Iron:**Total iron present in normal adult body is about 5 g. It is present in hemoglobin, myoglobin and plasma.

Dietary sources: Liver, heart, kidney, fish, egg, etc.

Daily requirement: 10 – 18 mg. per day.

Deficiency diseases: Anemia.

Symptoms due to excessiveness: Siderosis, nutritional Siderosis and hemochromatosis.

2. **Iodine**: It is required for biosynthesis of Thyroxine and Tri iodothyronine.

   Dietary sources: sea foods, vegetables, etc.

   Daily requirement: 100 – 200 µg. per day.

   Deficiency diseases:Goiter in adults, Cretinism in children.

3. **Copper:** It is required for normal functioning of metabolism along with other nutrients.

   Dietary sources: Sea food, whole grains, legumes, lemons, etc.

   Daily requirements: 1 – 3 mg. per day.

   Deficiency diseases: Illnesses that reduce digestion, loss of appetite, anemia, etc.

4. **Cobalt:** Cobalt is an important micronutrient in our body. It is present in liver. It helps in blood formation. It is utilized in the synthesis of vitamin B_{12}.

   Dietary sources: Liver, meat, clams, goat milk, oysters, apricots, etc.

   Daily requirement: 5 – 8 mcg. Per day.

   Deficiency diseases: Its deficiency results in lack of vitamin B_{12} which leads to pernicious anemia and nervous system disorders.

5. **Fluorine:** It is essential for development of teeth and bones.

   Dietary sources: Drinking water.

   Daily requirement: Drinking water containing 1 – 2 ppm. Of fluoride.

   Deficiency diseases: Dental caries in children.

   Symptoms due to excessiveness: Dental fluorosis, hyper calcification of bones, etc.

Minerals	
Amounts Per Serving Size 339 g **(1 Cup)**	
Calcium	20.3 mg
Iron	1.4 mg
Magnesium	6.8 mg
Phosphorus	13.6 mg
Potassium	176 mg
Sodium	13.6 mg
Zinc	0.7 mg
Copper	0.1 mg
Manganese	0.3 mg
Selenium	2.7 mcg
Fluoride	23.7 mcg

Fig 12.12: Table showing minerals acquired from one cup of food.

Conclusion

Vitamins are organic substances essential for life. They are classified into

1. Water soluble vitamins
2. Fat soluble vitamins

Minerals are of two types:

1. Macro elements
2. Trace elements.

Summary

Vitamins are organic substances essential for life. They are classified into –1) Water soluble vitamins and 2) fat soluble vitamins. Water soluble vitamins are Thiamin, Riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, folic acid, cyanocobalamin, lipoic acid, etc. Fat soluble vitamins are vitamins A, D, E and K.

Minerals are also important for the body. They are two types-1) Essential elements and 2) Trace elements.

Macro elements are seven. They are sodium, potassium, calcium, phosphorous, magnesium, chlorine and Sulphur. Trace mineral elements can be classified into essential, possibly essential and non-essential elements.

Model Questions

Short Answer Type Questions

1. Write the sources of vitamin A and D.
2. Mention the daily requirements of A and D vitamins.
3. Write the deficiency diseases of vitamin A.
4. What are the deficiency diseases of vitaminC?
5. Mention the deficiency diseases of vitamin K and E?
6. What are macro elements?
7. Write the functions of Sodium.
8. What is hyperkaliemia?
9. Name the conditions of hyponatremia.
10. How is chlorine essential for our body?
11. Give the symptoms that occur due to excessiveness of Fluorine in water.

Long Answer Type Questions

1. Classify vitamins and minerals.
2. Write about fat soluble vitamins.
3. Discuss various water soluble vitamins.
4. Write a note on macro elements or principal elements.

Chapter - 13

Blood Glucose Levels

Structure

13.1 Blood Glucose

13.2 Determination of Blood Glucose or Blood sugar.

Learning Objectives

1. Student should know about different biochemical estimations.
2. Student should practise to calculate the concentration using O.D. values of different determination in any medical.
3. Student should conduct a survey in the surroundings to get view of types of blood sugar and increasing cases in children and elders.

13.1 Blood Glucose or Blood Sugar

The end products of carbohydrates in food are glucose, galactose, fructose and pentose. The major function of carbohydrate in metabolism is as fuel to be oxidized and provide energy for metabolic activities. The main blood glucose is α-β-D glucose and is added in blood by the following ways:

1) Absorption from the intestine.
2) By glycogenolysis.
3) By gluconeogenesis.

From the blood circulation, glucose is reduced by;

1) Conversion of liver glycogen.
2) Conversion to tissue glycogen.
3) By synthesis of fats.
4) Synthesis of lactose and glycoprotein.

Because of these processes, the blood glucose levels remain constant up to 100 mg/dl.

Diabetes mellitus:

Definition: Diabetes mellitus is a chronic disease due to disorder of carbohydrate metabolism.

Conditions leading to diabetes mellitus:The blood sugar level remains steady between 70 - 100 mg/dl during the 24 hours and following food intake, it rises up to 140 – 150 mg/dl. ***Insulin***is a hormone produced by Beta cells of Islets of Langerhans of Pancreas. It increases utilization of glucose by tissues. When there is absent or deficient secretion of insulin by Beta cells of Islets of Langerhans, glucose cannot be utilized by the cells properly resulting in its increased level in blood which is the characteristic condition of diabetes mellitus.

Hyper glycaemia:When the fasting blood glucose level is more than 120 mg/dl, the condition is called Hype glycaemia, which is the characteristic condition of diabetes mellitus.

Hypo glycaemia: When fasting blood glucose levels fall below 72 mg/dl, the condition is called Hypo glycaemia. It is most common when associated with treatment of diabetes.

Glycohemoglobin:Glycohemoglobin is the type of hemoglobin seen in red cells in patients with chronic diabetes. These are represented as HbA1a, HbA1b and HBA1c which comprise about 1.6%, 0.8% and 4% respectively of the total hemoglobin. These are collectively measured as***HbAabc or HbA1***. HbA1 levels reflect carbohydrate imbalance than GTT or fasting glucose concentration. Normal range of HbA1 is 5 – 8 %.

Types of diabetes mellitus:The new classification system identifies four types of diabetes mellitus. They are –

1. **Type 1 diabetes mellitus:** It is characterized by Beta cell destruction caused by autoimmune process, usually leading to absolute insulin deficiency. Over 95% of persons develop this before the age of 25. This is seen in persons having a family history of type 1 diabetes mellitus.
2. **Type 2 diabetes mellitus**: It is characterized by Insulin resistance in peripheral tissue and an insulin secretory defect of beta cells. This is the most common form of diabetes mellitus and is highly associated with a family history of diabetes, older age,obesityand lack of exercise.

3. **Other specific types of diabetes mellitus**: Types of diabetes mellitus of various known etiologies are grouped together to form the 'other specific types of diabetes mellitus'. This group includes persons with genetic defects of beta cells function.
4. **Gestational diabetes:**Women who develop type 1 diabetes mellitus during pregnancy and women with undiagnosed asymptomatic type 2 diabetes mellitus during pregnancy are classified with gestational diabetes mellitus. This gets resolved in most women after delivery but they have increased risk of developing type 2 diabetes mellitus later in her life.

Detection of diabetes mellitus: Determination of blood sugar is important in diabetes mellitus. Blood sugar is determined by the following methods:

a) **Fasting blood sugar (FBS):**The blood sample is collected after the patient fasts for 12 hours or overnight.
b) **Post-prandial blood sugar (PPBS):** Blood is collected one and half to 2 hours after the intake of rich carbohydrate meal.
c) **Random blood sample (RBS):** Blood is collected at any time, regardless the time of food intake.

13.2 Determination of Blood Glucose.

Blood glucose is determined mainly by two tests. They are:

1. GOD – POD method.
2. GTT method.

1) GOD – POD method: This is the enzymatic method to determine blood glucose.

Principle:

Glucose undergoes oxidation by the action of the enzyme glucose oxidase to give gluconic acid.

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

H_2O_2 formed in this reaction is cleaved into water and oxygen by the action of peroxidase enzymes.

POD

H_2O_2 ----------> $H_2O + ½ O_2$

Nascent oxygen formed in this reaction reacts with 4 amino phenazone to give pink colored compound.

Color intensity of this compound is directly proportional to the concentration of glucose in plasma/serum. Concentration of glucose in plasma/serum can be calculated by comparing with the intensity of color developed with similarly treated standard.

Wave length: 530 nm.

Specimen: Fluoride plasma / serum. (It is to be collected within 30 minutes after collection of blood.)

Reagents:

1. **Buffer – Enzyme reagent:**
   a) Glucose oxidase (GOD) : 650 units
   b) Peroxidase (POD) : 500 units
   C) 4 – Amino phenazone : 20 mg.
   d) Sodium azide : 30 mg.
   e) Phosphate buffer (M/0) : 100 ml.
   - Dissolve all the contents in 100 ml. of M/10 phosphate buffer.
2. **Phenol reagent:**
   a) Phenol : 1 g.
   b) 0.1 N HCl : 1 liter.
   - Dissolve phenol in 750 ml. of 0.1 N HCl and dilute to 1 liter with 0.1 N HCl.

Procedure:

1. Take 3 test tubes and label them as T, S and B representing test, standard and blank respectively.
2. Pipette the reagents into the tubes as follows-

S No.	Reagent	T	S	B
1.	Glucose reagent	3 ml.	3 ml.	3 ml.
2.	plasma/serum	0.02 ml.	---	----
3.	Glucose Standard	---	0.02 ml.	----
4.	Distilled water	---	---	0.02 ml

3. Mix and keep at 37^0C for 15 minutes or at lab temperature for 3 minutes.

4. Determine O.D. at 530 nm. Wave length.

5. Determine plasma glucose concentration using the formula-

$$\text{Plasma / Serum glucose} = \frac{\text{O.D. of the test}}{\text{O.D. of the standard}} \times \text{concentration of standard (100 mg/dl)}$$

Normal values: 70 – 120 mg/dl on fasting.

2) GTT method: In some circumstances, a glucose tolerance test is significant to diagnose the unknown cases of diabetes mellitus. Assessment of glucose tolerance of an individual is called glucose tolerance test (GTT).

Preparation of the patient: Patient should be kept on balanced diet containing 300 g. of carbohydrate per day for three days prior to the test. Patient should be on fasting for 10-14 hours before reporting to the laboratory. Coffee or tea without sugar can be allowed in the morning. Patient is restricted from smoking or chewing tobacco till the analysis is over.

Specimens

1. Fasting blood specimen.

2. Fasting urine specimen.
3. Post glucose blood specimens for fasting urine specimen negative cases in the interval of 30 minutes (4 0r 5 specimens) in adults and children.
4. For pregnant patient, blood samples are obtained every hour for 3 hours. Timing is begun when the patient begins to drink the glucose solution.
5. One post prandial blood specimen for fasting urine specimen positive cases.
6. Post glucose urine specimen for unknown diabetics after each blood specimen (at least 2 specimens)

Procedure

1. Collect fasting blood and urine specimens.
2. Test the fasting urine specimen by Benedict's qualitative method.
3. If the fasting urine specimen is positive for glucose, collection of post prandial blood specimen is enough.
4. If the fasting urine specimen is negative for glucose, adults are then given a drink containing 75 g. of glucose. Children are given ideal 1.75 g/kg body weight up to 75 g. Pregnant patients are given 100 g. of glucose. This quantity of glucose is given by dissolving in about 300 ml. of cold water. Risk of vomiting may be avoided by addition of lemon juice to the glucose solution.
5. Collect 4 or 5 post glucose blood and urine specimens at ½ an hour intervals.
6. Determine glucose content in each blood specimen and test qualitatively each sample of urine for sugar and ketone bodies.

Interpretation

1. **Normal glucose tolerance:**
    - Concentration of glucose in fasting blood specimen will be in the normal range.
    - Maximum blood glucose level is reached in the first or second post glucose specimen.
    - Blood glucose reaches normal within 2 hours after oral glucose administration.
    - Urine sugar is absent all throughout.

2. **Decreased glucose tolerance**:
    - Values in fasting blood specimens are high. Post glucose/ post prandial values are higher. Return of these values to normal is delayed.
    - Urine specimens are positive for Benedict's qualitative test.
3. **Increased glucose tolerance**:
    - Fasting blood glucose may be below limits. Only a small raise may be observed in blood glucose.

Case study of Normal Response:

S. No.	Time	Blood Glucose (mg/dl)	Urine sugar
1.	Zero hour	80	Negative
2.	½ hour	130	Negative
3	1 hour	140	Negative
4	1 & ½ hour	100	Negative
5	2 hours	80	Negative

1. After administration of oral glucose solution, zero hour blood glucose level is normal.
2. Half an hour specimen shows raise in glucose level to 130 mg/dl.
3. One hour blood specimen shows maximum raise to 140 mg/dl.
4. One and half hour blood specimen shows a fall of glucose level to 100 mg/dl.
5. Two hours sample shows further fall to 80 mg/dl.
6. It shows that, there is maximum raise in blood glucose within 1 and ½ hour and has fallen down to normal and it equals zero hour sample at the end, and urine sugar is negative throughout.

Conclusion

Study of disease and its related diagnostic tests are useful for assessing the clinical conditions of a patient.

Summary

Insulin is the hormone secreted by beta cells of islets of Langerhans in Pancreas which maintains blood glucose levels. Raise of blood glucose is termed as diabetes mellitus. Normal blood glucose is 75 – 120 md /dl. Hyper glycaemia is raise in blood glucose and hyper glycaemia is fall of blood glucose levels. There are 4 types in diabetes mellitus. Type 1, type 2, other specific type and gestational diabetes.

Blood glucose determination is an important in diagnosis and assessment of diabetes mellitus. Fasting blood, post prandial blood and random blood is collected from patient to diagnose diabetes mellitus, depending on the requirement. GOD – POD method is important method to determine blood glucose. Glucose tolerance test (GTT) is significant in the diagnosis of unknown cases of diabetes mellitus.

Model Questions

Short Answer Type Questions

1. What is diabetes mellitus?
2. How is Glucose added in to the blood?
3. From blood circulation, how is glucose reduced?
4. Write the normal values of blood sugar?
5. Mention the different types of diabetes mellitus.
6. Define a) Hyper glycaemia b) Hypo glycaemia.
7. Name the hormone which helps to maintain blood glucose levels normal. Where is it secreted?
8. What is Glyco hemoglobin?
9. Write the normal range of HbA1.
10. Mention the different types of blood samples collected to determine blood sugar.
11. What is fasting blood specimen?
12. Give the principle of blood sugar determination by GOD – POD method.
13. What is GTT?
14. Give the list of specimens required for glucose tolerance test.

15. While giving glucose to patient during GTT, how do you avoid vomiting?
16. What is normal glucose tolerance in GTT?
17. How do you interpret decreased glucose tolerance in GTT?

Long Answer Type Questions

1. What is diabetes mellitus? Explain the types of diabetes mellitus and types of blood samples collected to determine blood glucose.
2. Write in detail about GOD – POD method for the determination of blood sugar.
3. In which conditions GTT is preferred? Write in detail about GTT.

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