"शिक्षा मानव को वन्धनों से मुक्त करती है और आज के युग में तो यह लोकतंत्र की भावना का आधार भी है। जन्म तथा अन्य कारणों से उत्पन्न जाति एवं वर्गीकरण विषयों को दूर करते हुए मनुष्य को इन सबसे ऊपर उठाती है।"

— इंदिरा गांधी

“Education is a liberating force, and in our age it is also a democratising force, cutting across the barriers of caste and class, smoothing out inequalities imposed by birth and other circumstances.”

— Indira Gandhi
## PRACTICAL MANUAL — BIOCHEMISTRY

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In BNS-102, Applied Sciences, you have studied 5 blocks namely Biochemistry, Biophysics, Microbiology (2 blocks) and Nutrition and Dietetics. To understand some of the basic concepts and principles explained in these blocks, we need to do practical work in various laboratories.

The practical manuals are prepared under 4 blocks.

Block 1, Biochemistry Practical Manual, gives you a brief introduction to laboratory techniques, verify some of the properties of matter, and an opportunity to do observation-demonstration of biochemistry principles of body fluids.

Block 2, Biophysics Practical Manual, gives you a chance to do experiment on principles of forces, density, light and electricity.

Block 3, Microbiology Practical Manual, gives you practice in the use of microscope and through that study of various microorganisms, live and stained. You will know through practice why aseptic technique is essential when caring for the sick and debilitated.

Block 4, Nutrition and Dietetics Practical Manual, gives you a good practice on planning and evaluating diets for individuals with various characteristics in life. The Practical manual has the guideline for you as a nursing personnel to plan the therapeutic diets in various diseased conditions. You may apply these skills while practicing nursing care in the hospital and community.
Dear Student:

- Fill up the checklist given at the end of this manual.
- Get it signed by the respective nursing personnel.
- Detach the checklist and submit it to your PIC along with the file of self activities.
INTRODUCTION TO THE MANUALS

Applied Sciences Practicals (BNSL-102) is comprised of four Blocks, namely Biochemistry (Block-1), Biophysics (Block-2), Microbiology (Block-3) and Nutrition and Dietetics (Block-4). Each block contains a number of activities, check your progress exercises and Experiments/Demonstrations.

The experiments/demonstrations are expected to be observed/conducted by you during the placement in the laboratory so that you get an opportunity to understand the scientific principles and their relation to nursing.

Evaluation

Applied Science practicals carry 100 marks. Each block is given a weightage of 25 marks. In order to prepare an assessment of your practical work for Block 1, 2, 3, 4 and 5 the list of self activities and supervised activities is provided.

You will be given the demonstration of the listed supervised activities by your academic counsellors in respective course practicals. The marks and the number of hours for activity is allotted.

After completing your self and supervised activities you must record in the practical record book which will be evaluated by your academic counsellor of the respective course. Out of 25 marks 13 marks will be for the supervised activities and 12 marks for the practical examination.

Note: This pattern of evaluation is applicable only to the Applied Sciences Practicals.

Time for planning and conducting practicals and recording observations

Applied Sciences practical is a 4 credit course. This means that the total time you would spend on completing the practicals should not exceed 120 hours i.e. each block of the course is allotted 30 hours. These 30 hours is inclusive of contact hours and self-study hours and for conducting the experiments. Some of practicals may take longer to complete, others may take less time. You will need to adjust the time accordingly. On the whole you may organize your work as follows:

Example: 1 credit = 30 hours.

1. Planning, Observing and Conducting experiments — 28 hrs. (inclusive of self study hours)

2. Recording the observation — 2 hrs.

30 hrs.
The practical course in Biochemistry for Post Basic B.Sc (Nursing) consists of a block of six units, corresponding in content and correlation to the theory course. The course covers experiments and demonstrations in Biochemistry topics.

Unit 1 introduces you to a Chemistry/Biochemistry laboratory and explains its set up briefly. Safety precautions and first aid treatments for the most common mishaps likely to occur in the laboratory, have been discussed in the very beginning and in quite some detail as these are of primary importance to you. After familiarizing you with the common laboratory glassware equipment and chemicals, some basic operations such as volume measurement, filtration, etc., have been described. This is followed by a description of preparation of solutions of different strengths and their dilutions. Necessity of maintaining a proper laboratory record, is also discussed.

Unit 2 Matter: Its classification and properties, describes how to classify matter and spells out on experiments for you to do to distinguish a compound from a mixture. There are also experiments to show various physical and chemical changes. The later part of the Unit discusses concepts such as diffusion, osmosis and dialysis and has included experiments/demonstrations to help you understand these physiologically and clinically important processes.

Proteins, carbohydrates and lipids (especially triglycerides) are the major dietary components. Unit 3 explains some of the chemical and biochemical tests for the detection of these components. Colour reactions, heat and coagulation tests for proteins, Benedict’s test for carbohydrates, solubility and emulsification of fats, are all of diagnostic importance too. Clinical correlations of some of these tests have been stressed at this stage. This will make these tests more meaningful for you while performing them. Some of these tests are applied while analyzing urine and blood samples in routine clinical investigations.

Units 4, 5 and 6, basically concerns experiments of interest in clinical investigation and diagnosis of various diseases or pathological conditions. So, it deals with the analysis of components in the major body fluids namely, blood, urine and cerebrospinal fluid (CSF). Unit 4 describes the composition of urine in normal health and compares it with that in various disease states. The discussion on proper collection and preservation of urine samples, is followed by tests for detecting normal and abnormal constituents. The appendix on preparation of artificial or simulated urine samples for laboratory analysis by you will eliminate the need to collect actual urine samples and thus avoids possible contamination and risk of infection to you from pathological samples.

Clinical analysis of blood forms the text of Unit 5. Methods of collection and preservation of blood have been given due importance. Investigations of major clinical significance such as blood sugar estimation, Glucose Tolerance Test (GTT), and their interpretations; hemoglobin estimation, bilirubin, urea and creatinine estimations, have all been discussed in the necessary detail. As you know, cholesterol estimation is of immense clinical significance, especially in correlating it with the risk for the development of heart diseases. Hence, a brief description of the method of cholesterol estimation in serum and its clinical relevance to the development of atherosclerosis and heart diseases, has also been included in the concluding part of the unit.

The last unit, i.e. Unit 6, highlights the importance of analysis of cerebrospinal fluid for clinical diagnosis. The two major investigations concern quantitation of protein and glucose in CSF and their correlation to various pathological conditions. These two tests suffice for most clinical situations.

We hope that by reading and understanding the chemical and biochemical concepts discussed in the above units, your curiosity and enthusiasm to actually perform the tests/experiments would be kindled. After actually doing the experiments in the laboratory, you as Nurses would be in a position not only to grasp their chemical significance but also appreciate the chemical bases behind the tests. At the end of the block list of self and supervised activities is provided.
UNIT 1  BASIC LABORATORY TOOLS AND TECHNIQUES

1.0 OBJECTIVES

After going through this unit and performing the experiments and activities, you should be able to:

- follow the safety measures while working in the laboratory;
- perform the first aid procedures in case of an accident;
- identify and use common laboratory apparatus and equipment;
- measure the volume of liquids and perform basic operations like heating and filtration; and
- prepare solutions of desired strength from bulk drugs or stock solutions.

1.1 INTRODUCTION

Welcome to this laboratory course in biochemistry. The first unit of this course has been devised to familiarize you with the basic tools and techniques used in a biochemistry laboratory. You will be introduced to the laboratory and the precautions to be followed while working there, so that, you do not have any unpleasant experience during your stay in the laboratory. Some first aid procedures have also been explained in detail to tackle an unfortunate mishap. After introducing you to different apparatus used in the chemistry laboratory, we shall take up basic laboratory operations like, washing glassware, measurement of volume, heating filtration etc. Preparation of solutions and doses, an important procedure for a practicing nurse, is explained with the help of a number of examples. Some simple experiments have been included for you to practice the basic operations. The idea and importance of maintaining laboratory record has also been discussed. The unit ends with a brief summary.
1.2 KNOW YOUR LABORATORY

You are going to work in a laboratory arranged by your study centre for this course. This may be a totally new setup for you. In order to work effectively in such a laboratory, it is advisable to get acquainted with it. In a chemistry laboratory you need to know about the location of the common chemicals, reagents, acids, glassware, gas supply, balances, safety equipment, fire extinguishers, first aid box etc. Your counsellor would take you around the laboratory and show you all the items mentioned above. Listen carefully to his/her instructions. Do clarify if you have any doubts.

When you enter the laboratory you will notice rows of working benches equipped with burners and wash basins along with water taps. The benches may have some shelves with certain chemicals on them. On the side walls of the laboratory you may find side shelves carrying a number of reagents etc, commonly used by all the students. You will also find a small room called as preparation room adjoining the laboratory. This is where the laboratory staff does all the prior preparations related to the experiments.

You will be allocated a seat and a cupboard with some apparatus in it. Check the glassware issued to you carefully for any chips or cracks. Get these replaced; otherwise these may cause harm while in use. Having got familiar with the laboratory and before actually going ahead with the work, it is worthwhile to know about the safety measures to be followed in the laboratory.

1.3 SAFETY MEASURES IN THE LABORATORY

A chemistry laboratory is intrinsically a hazardous place, quite prone to accidents. Accidents are a consequence of carelessness and lack of knowledge about chemicals and the apparatus being used. These can occur anywhere – even in the most well run laboratories. However, their occurrence can be minimized by following certain precautions while working in a laboratory. Some general precautions are given below:

- Always wear a lab coat or an apron while working in the laboratory.
- Never wear loose clothes and tie your hair, if you have long hair.
- Never eat, drink or smoke in the laboratory.
- Know the location of fire extinguishers, and the nearest exit.
- Work only during scheduled laboratory hours.
- No unauthorized experiments should be performed in the laboratory.
- Treat all chemicals as dangerous. Never taste or smell chemicals.
- Use a match stick and not paper to light the burner.
- Do not use broken or cracked glassware. Check glassware before using it.
- Don’t pipette out corrosive liquids with mouth.
- Never put the used reagents back into their container.
- Put paper trash and broken pieces of glass in dust bins.
- Avoid contact of chemicals with skin.
- Never keep flammable liquids near an open flame.
- Don’t touch electrical switches with wet hands.
- Always clean your work area and put away all glassware before leaving.
- Always close the gas and water taps before leaving the laboratory.
1.3.1 First Aid

As said above, we can minimize the accidents by following the precautions but we can't eliminate them. There is always a probability of accidents occurring. It is imperative, therefore, that a certain degree of preparedness should exist to cope with any eventuality. The relevant first aid procedures for the common accidents likely to occur in a chemistry laboratory are given below. These are not the treatments for the accidents but are mere procedures that would minimize the possible damage caused by accident. A timely first aid can, at times, provide more relief than a delayed medical aid.

a) Chemical Burns: These are caused by accidental exposure to acids, bases and other corrosive chemicals. The long-term effect of these burns is scarring and, depending on the site of the burn, can be quite disastrous. Phenols are exceptionally dangerous. These are highly caustic and are very rapidly absorbed through the skin.

First aid

- The first step in such a situation is to remove the offending agent at the earliest.
- Remove contaminated clothes; longer they stay, greater the damage.
- Remove rings, watches and other ornaments in the affected area as these may trap the contaminants.
- Wash the affected portion under running water for about 10-15 minutes to limit the tissue destruction.
- Take care that the washings do not go onto the unaffected portion of the skin. Save yourself also.
- Avoid using neutralizing solutions. These generate heat, which increases damage.

In case the corrosive chemical falls in the eye (i.e. it is a case of an ocular burn):

- Remove the contact lens, if it is there, as the chemical can get in between the lens and cornea and cause damage. Do not let the patient rub his/her eyes.
- Wash the eye with gently running water from the tap or an eyewash bottle for 10 to 15 minutes.
- Open the eyelids and ensure that water drenches the eyeball.
- All eye injuries must be seen by a doctor preferably within an hour.

b) Thermal Burns: These can be contact burns caused by direct contact with open flame or contact with a hot object like a hot flask, the injury being confined to the point of contact or scalds which result from contact with hot liquids.

First aid

- Remove the victim from the source of the burn. Cool the burnt area using large amount of cold water.
- Do not use ice except in small superficial burns. It may cause body heat loss.
- Once the burn has been cooled, cover the burnt area using dry, sterile dressing or the cleanest cloth available.
- Refer patients to critical burns/ a regional burn centre for further management.

c) Cuts and Wounds from broken glass: Cuts and wounds are probably the most likely accidents in a laboratory. The injury may vary from a minor cut to a profusely bleeding wound.

First aid

- In case of a minor cut or abrasion, clean the wound and surrounding skin with soap and water.
• To avoid contamination, wash away from the wound and not towards it.
• Pat the wound dry, with a sterile pad or the cleanest cloth available and, apply a suitable bandage as required.
• For a major wound involving profuse bleeding, apply firm pressure over and around the wound with a sterile pad or the cleanest cloth available, to reduce bleeding as much as possible.
• Make the victim lie down with bleeding-part raised higher than the rest of his/her body.
• Apply a tight bandage and call for the physician, if not done so far.
• Meanwhile keep the victim warm and give him (if he is conscious) a lot of nonalcoholic liquid to drink.

d) Ingestion of Chemicals: Incidents of ingestion of chemicals are far less common than those involving other routes of exposure. However, significant quantities of toxic chemicals can be ingested, e.g. in a mouth-pipetting experiment.

First aid

• If the corrosive material is confined to mouth only then repeated mouthwash is probably the best method.
• However, if the chemical has been ingested, give about 250 ml of water to dilute the contents.
• Do not try to neutralize the chemical as the heat generated may cause additional injury.
• Do not try to induce vomiting. This may cause injury to the delicate tissues of the oesophagus.
• Arrange for the medical help, or better, transfer to the hospital.

e) Inhalation of Gases and Vapours: Inhalation of toxic gases and obnoxious vapours of certain volatile liquids is another common situation in a laboratory that demands prompt first aid. Inhalation of gases and vapours can cause varied effects ranging from irritation of eyes and mucous membrane to drowsiness to unconsciousness.

First aid

• Move the victim immediately to fresh air.
• If the victim is unconscious, place in a face down position and check for the breathing.
• At the first sight of difficulty in breathing or stopped breathing, apply artificial respiration immediately (mouth to mouth method).
• Keep the patient warm and as quiet as possible till the help arrives.
• Call for the medical help. If needed, transfer the victim to the hospital.

f) Splashing of chemicals on the skin and in the eye: Splashing of chemicals on the skin and in the eye is a common occurrence in chemistry laboratory. No chemical can be considered totally hazard free, though some are relatively safer. It is good, therefore, to regard all chemicals as hazardous and proceed for the first aid.

First aid

• Wash the affected portion immediately under running water for about 10-15 minutes. Take care that the washings do not go onto the unaffected skin.
• Remove contaminated clothes, rings, watches and other ornaments in the affected area.
- In such cases the quickness with which the splashed chemical is removed really matters.
- If situation so warrants, a medical advice should be sought.

For the splashing of the chemical into the eye, proceed as instructed under chemical burns.

### 1.4 COMMON LABORATORY GLASSWARE AND EQUIPMENT

In this laboratory course you will be doing some simple experiments. For these you will be using a few items of glassware and equipment. These are shown in Fig. 1.1. An alphabetical list of the same is also given. The method of using some of these have been explained in the next section. Others you will learn as you perform the experiments contained in this course.

![Common laboratory glassware and apparatus](image-url)

**Fig. 1.1: Common laboratory glassware and apparatus**
1. Beakers (different capacities)  
2. Boiling tubes/test tubes  
3. Burette  
4. Burner  
5. Clamp with boss head  
6. Clay triangle  
7. Conical flask  
8. Dropper  
9. Filter paper  
10. Funnel  
11. Glass rod  
12. Graduated Pipette  
13. Measuring cylinder  
14. Pipette  
15. Porcelain dish  
16. Porcelain tile  
17. Spatula  
18. Test tube holder  
19. Test tube stand  
20. Tongs  
21. Tripod stand  
22. Wash bottle  
23. Watch glass  
24. Water bath  
25. Wire gauze

1.5 BASIC LABORATORY OPERATIONS

In any experiment, the procedure may be visualized as a set of operations executed with an overall aim of achieving the objective. Certain operations are very basic in nature and find place in a wide variety of experiments. Some of these operations are explained here. Every student in a chemistry laboratory must be able to perform them correctly. Therefore, some simple activities have been introduced so that you may practice these operations.

1.5.1 Cleaning Laboratory Glassware

It is important to use clean glassware to get satisfactory results in an experiment. The impurity in the unclean apparatus may interfere with the experiment. Unfortunately this important aspect of the practical work is normally neglected.

Generally, one can clean the glassware using water and a laboratory detergent. Scrub the apparatus to be cleaned with a little detergent and wash with plenty of water. You may need to use a brush to reach the inaccessible portions of the apparatus. Sometimes, organic residues do not get removed with detergent. In such cases you may use an organic solvent like acetone to clean these. Wash with a little of acetone should be followed by soap and water wash.

CARE: Do not use acetone near a flame, it is highly inflammable.

At times it may be difficult to clean the apparatus even with acetone. In such cases one can use a solution of chromic acid. You are advised to request the laboratory attendant to exchange your apparatus rather than venturing into this hazardous activity. It is advisable to clean the apparatus as soon as possible after the experiment so that when you have to use it again, it is clean.

1.5.2 Measurement of Volume

Measurement is an essential component of any experimental setup. In chemistry experiments we make different types of measurements like, measurement of mass, volume, temperature, length etc. For the present purpose we intend to focus our attention on the measurement of volume only. A given liquid can be measured by different apparatus depending on the volume and accuracy required. Let us learn about some of these.

**Measuring cylinder:** Measuring cylinder is used for approximate measurement of specified volumes. These are available in different capacities. To measure a given liquid, the cylinder is filled with it up to the marking corresponding to the required volume. The volume of the liquid in the cylinder is read by adjusting the level of the eye to that of the liquid and the lower meniscus of the liquid is read. The meniscus refers to the curved
portion of the liquid surface. It is advisable to put a white card or paper behind the markings (as shown in Fig. 1.2) to facilitate reading the level.

![Diagram of a liquid level with markings labeled Card, Meniscus, Eye level, and Darkened area.]

**Fig. 1.2: Reading the level of the liquid**

**Pipette:** A pipette is an accurately measuring apparatus. It is of two types, a fixed volume or transfer pipette and a graduated or measuring pipette. These pipettes come in different sizes.

**Fixed volume pipette:** As the name suggests, it is used to measure and transfer a fixed volume of liquid. To use a pipette,

- Rinse the pipette with the liquid to be measured. For this, take the liquid in a clean dry beaker and dip the pipette into it with the tip nearly touching the bottom. Suck a little of the liquid with mouth. (In principle one should use a sucking device to avoid any accident due to ingestion of the liquid.)
- Remove the pipette from the mouth and immediately close the upper end of the pipette with your index finger (the one next to the thumb). Tilt the pipette and rotate it so as to rinse the inside of the pipette.
- Remove the finger from the upper end of the pipette and drain the liquid through the lower end (i.e. tip) only.
- Suck the liquid again, a little above the etched mark on the pipette and close the upper end of the pipette with moist index finger.
- Release the solution back into the beaker till the level of the liquid (as ascertained by the lower meniscus) reaches the etched mark.
- Insert the tip of the pipette into the container in which the liquid has to be transferred and remove the finger to discharge the solution. Let the solution flow on its own.
- Do not blow out the last drop in the pipette. Touch the tip of pipette to the inner wall of the container; a part of the solution passes into the container. Neglect the remaining liquid still at the tip.

**Graduated pipette:** A graduated or measuring pipette is used to accurately measure and transfer a variable volume of the liquid. It is used in a manner similar to that of a fixed volume pipette, the only difference being that it is not discharged completely. Instead the flow of the liquid is stopped by pressing the finger tightly when the desired volume of the liquid has been transferred.

**Why don’t you try doing a simple experiment before going further?**

**Experiment 1 (a): Calibration of a measuring cylinder.**

**Objectives**

- Correctly reading the level of a liquid
- Practicing the use of a pipette
- Recording data
Principle

As said earlier, a measuring cylinder is used for an approximate measurement. It has an accuracy of ± 5-10%. The measuring cylinders made of soda glass are even more inaccurate because at times these do not have a uniform diameter. As a consequence, the markings on it do not correspond to the volume contained in it. We may use a pipette to calibrate such a measuring cylinder.

Requirements

100 cm³ measuring cylinder-1
10 cm³ pipette-1
Marker.

Procedure

Follow the instructions given below sequentially:

1) Measure 10 cm³ of water with the help of a pipette and transfer it to a clean, dry measuring cylinder.

2) Mark the level of water in the measuring cylinder with a colored pencil or a glass marker. Label the mark as 10.

3) Read the level of water in measuring cylinder according to the original markings, and record it at serial number 1 in Table 1.1 given below.

4) Add another 10 cm³ of water with the help of a pipette and mark and label the level as 20.

5) Read and record the level as done above in Table 1.1 given below.

6) Repeat the procedure till you add 100 cm³ of water.

Table 1.1: Calibration of Measuring Cylinder

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Volume of water added (in cm³)</th>
<th>Reading in the measuring cylinder</th>
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<tbody>
<tr>
<td>1</td>
<td>10</td>
<td></td>
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<tr>
<td>2</td>
<td>20</td>
<td></td>
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<td>3</td>
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<td>90</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Result/Inference

Compare your markings on the cylinder with the original ones and record your inference regarding the correctness of the original markings, in the space provided below.

Experiment 1 (b): Preparation of a calibrated test tube.

Objectives

- Practising the use of a graduated and a fixed volume pipette
- Correctly reading the level of a liquid
Requirements

Test tube
Glue
Paper
Marker
Graduated pipette

Procedure

Follow the instructions given below sequentially:

1) Take a test tube
2) Cut a thin strip of paper and paste on the test tube as shown.
3) Transfer 1 cm$^3$ of water into the test tube with the help of a graduated pipette
4) Keep the test tube upright in a test tube stand, and carefully mark the level of water in the test tube with a colored pencil or a glass marker on the paper strip.
5) Label the mark as 1.
6) Repeat the same procedure up to 10 cm$^3$ of water and label the markings accordingly.
7) Measure 10 cm$^3$ of water with the help of a pipette and transfer it to the calibrated test tube.
8) Read the level in the test tube and report.

Result

1.5.3 Heating

Heating is a common laboratory operation. A number of heating devices are available in a chemistry laboratory. The choice of heating method depends on the required temperature and the flammability of the substance being heated, besides convenience. We shall discuss about two such devices.

- Bunsen burner
- Water/steam bath

Bunsen burner: It is a simple and convenient heat source. In principle it is something similar to the gas stove we use at home. A typical burner is a metallic barrel mounted on a platform. The lower end of the barrel has an inlet for the fuel supply and a air hole or air vent. The burner mixes the fuel (LPG) with air at the base and allows the mixture to burn at the top.

To use a Bunsen burner, open the gas knob and light the burner with the help of a match stick or a gas lighter. The appearance of the flame depends on the amount of the air being mixed with gas. Adjust the air flow by rotating the movable ring at the air hole, till you get a hot luminous flame which divides into different regions as shown in Fig. 1.3(a). The outermost region is the hottest while the innermost region is relatively cooler.
Fig. 1.3 (a): A Bunsen burner    Fig. 1.3 (b): Correct way of heating a test tube on the burner

The temperature of the Bunsen burner flame is quite high. It can bring a directly heated liquid to its boiling point very soon. To heat a liquid in a test tube fix the test tube in a test tube holder and bring it to the burner [Fig. 1.3(b)]. While heating it make sure that the open end of the test tube points away from you as well as others so that in ease of a spurt of the liquid it does not hurt anybody. Further, never heat the test tube at a particular point, keep swirling it while heating.

CARE: Never heat any flammable liquid directly on the flame in an open container.

Water bath: Sometimes we do not need to heat a liquid to a very high temperature, a direct heating on the Bunsen burner is not advisable as we can’t control the temperature of the flame. In such cases one uses a water bath. Water bath is basically a kind of saucepan of water and provides a gentle, even heating. Commercially different types of water baths are available, but a simple improvised water bath can be made simply by taking water in a medium sized beaker and heating it over Bunsen burner in a set up as shown below.
1.5.4 Filtration

Separation of a liquid or a solution from a solid is called as filtration. In simple filtration method, also called as gravity filtration method, we take a circular filter paper (or you may cut a circular filter paper from the filter paper sheet available in the laboratory) and fold it as shown in Fig. 1.5(a). We get the filter paper in a cone shape with four leaves. Open the cone in such a way that there are three leaves on one side and one on the other. Take a moist funnel and put the filter paper into it, pressing it evenly against the sides of the funnel, ensuring that no air gap is left between the filter paper and the funnel. Set up the filtration apparatus as shown in Fig. 1.5(b).

Fig. 1.5 (a): Folding of filter paper

Fig. 1.5(b): Correct way of pouring mixture for filtration

To filter the given mixture pour it over the funnel along a glass rod, (as shown above) till the funnel is about three forths full. Allow the liquid to filter, keep refilling the funnel till all the mixture has been filtered. Remove the filter paper, dry and collect the solid.

Experiment 2: Separation of a mixture of sand and water by filtration.

Objectives

- Practicing the use of a graduated and a fixed volume pipette
- Correctly reading the level of a liquid

Principle

The filtration operation is based on the principle of separation on the basis of particle size. The filter paper contains pores of a certain size and does not allow particles bigger than it to pass through.

Requirements

Test tube
Glue
Paper
Marker
Beaker
Funnel

Procedure

Follow the instructions given below sequentially:

1) Fold a piece of filter paper and set up the filtration assembly as explained in subsection 1.5.4.

2) Take about 20 cm³ of sand-water mixture in a small beaker, mix it well, and pour it over the funnel using a glass rod.

3) Collect the filtrate (water) in a clean container and dry the filter paper to recover the sand.

4) Check the quality of water in the filtrate. If turbid, discuss with your counsellor.

5) Record the results below.

Result


1.6 PREPARATION OF SOLUTIONS AND DOSES

As a practicing nurse you would have come across solutions in situations like, medicinal baths, irrigations, stomach lavage etc. You may be expected to prepare a given amount of solution of desired strength from pure or full strength drug or from a stock solution. It is therefore, essential that you develop the ability to make solutions when needed and be able to compute dosage and recognize the strength of a solution irrespective of how it is represented.

You have learnt about solutions in block 1 of your course BNS-102. Let us recall a few important aspects of solutions, before taking up their preparation. A solution is defined as a homogenous mixture of two or more substances and has no tendency to settle upon standing. In a solution containing two components the one in larger amount is called as solvent while the one in lesser amount is referred to as the solute, e.g. in a solution of sugar in water, the sugar is solute and water is the solvent. In principle, both solute and the solvent can be in any physical state viz., solid, liquid and gas. A nurse normally has to deal with solutions of liquids or solids in liquids.

A solution is dilute if it contains a small amount of solute in a given volume of solvent. Addition of more solute to a solution makes it concentrated. A solution containing a spoonful of sugar in a glass of water is dilute as compared to a solution containing two spoons of sugar in the same amount of water, which is relatively concentrated.

The concentration of a solution can be expressed in a number of ways. The most common way of expressing the concentration is per cent. You would have read in your school mathematics that the term percent, represented by the symbol, %, is basically a fractional means that we divide the total into 100 parts (given as denominator) and refer to some of hundred parts. Or say, in a nursing class of 100 students, if there are six boys, the

As expressed above, both numerator as well as the denominator are in the same units and
we represent percent by a number and the % sign. However, in a context of solutions, the amounts of solute and the solution may be measured in the same or different units. Therefore, we have different ways of expressing percentage concentration of solutions. Let us understand it.

**Weight/volume per cent (w/v %):** This is the most common concentration expression. It is used for the solutions where a solid solute is dissolved in a liquid solvent to give the solution. It refers to the grams of the solute dissolved to give 100 cm³ of the solution. A 0.9% (w/v) solution of NaCl contains 0.9 g of NaCl in 100 cm³ of the solution.

**Weight/weight per cent (w/w %):** Weight/weight per cent of a solution is equal to the number of grams of solute present in 100 g of the solution, i.e., 100 g of 5% (w/w) solution of glucose would contain 5 g of glucose.

**Volume/volume per cent (v/v %):** Such an expression of percent is used when both the solute as well as the solvent are gases or liquids. It refers to the number of volumes of solute present in 100 volumes of the solution. For example, if you look at the label of a bottle of cough syrup, under composition you may find an entry like, alcohol – 8% (v/v), this means that 100 cm³ of the cough syrup contains 8 cm³ of alcohol.

### 1.6.1 Preparation of Solutions from Full Strength Drugs

Having learnt about solutions and various methods of representing percent concentration, let us take up the preparation of a solution. In any situation of solution preparation, one has to consider four parameters. These are:

- Desired strength (D)
- Strength available (H)
- Amount of solute (A)
- Total volume of the solution (Q)

These parameters are related by the following formula

\[
\text{Desired strength} \times \frac{\text{Total volume of the solution}}{\text{Strength available}} = \text{Amount of solute} \\
\Rightarrow D \times \frac{Q}{H} = A
\]

In any problem, three of these parameters would be given, and you will have to find the fourth to solve the problem. Let us take two examples.

**Example 1:** How would you prepare 100 cm³ of 7% (v/v) alcohol solution for gastric analysis?

Analysing the problem, we see that

- Desired strength = 7%
- Strength available = 100 % (since we have pure alcohol)
- Amount of solute = to be determined
- Total volume of the solution = 100 cm³

Using the formula, we have

\[
\text{Amount of solute} = \frac{\text{Desired strength} \times \text{Solution volume}}{\text{Strength available}} \\
A = \frac{7\% \times 100 \text{ cm}^3}{100\%} = 7 \text{ cm}^3
\]

This means we should take 7 cm³ of pure alcohol and add enough water to it to make 100 cm³ of solution to get the desired 7% (v/v) alcohol solution.
Example 2: How much of 1 in 20 solution can be prepared from 7.5 g of glucose?

We see that here the desired strength has been expressed differently. 1 in 20 means 1 part in 20 parts and we are familiar with percent which is certain parts in 100 parts. To convert the given strength to percent we may use common sense or basic mathematics. Let us see how.

We write 1 in 20 as $\frac{1}{20}$; if we multiply the denominator by 5, we get 100.

So, we multiply both, the numerator and the denominator, by 5

$$1 \times 5 / 20 \times 5 = \frac{5}{100} \quad \text{or} \quad 5\%$$

So the method of conversion is to find a suitable factor which converts the denominator to 100, then apply the same factor to the numerator also.

Coming back to the problem we see that

Desired strength = 1 in 20 = 5 %

Strength available = 100 % (since pure substance)

Amount of solute = 7.5 g

Total volume of the solution = to be determined

We had the formula, $D \times Q / H = A$

Rearranging it, we have $Q = A \times H / D$

Total volume of the solution, $Q = \frac{7.5 \times 100}{5} = 150 \text{ cm}^3$

So, we can prepare 150 cm$^3$ of 1 in 20 solution from 7.5 g of glucose.

Why don't you perform the two computational activities given below to assess whether you have understood the concept or not?

Activity 1

How much of water must be added to 1 g of mercurochrome to make 1 in 200 solution of mercurochrome.

Activity 2

How many tablets of 0.5 g each are required to prepare 1 dm$^3$ of 1: 500 solution of tyrothricin for irrigation?
1.6.2 Preparation of Solutions from Stock Solutions (Dilutions)

Many a times we need solutions of concentrations lower than the stock solutions available, which are relatively strong solutions. The method of preparation is similar to the one given above, but we have to keep in mind that in stock solutions the strength is less than 100%. We use a formula similar to that used above. Let us take a few examples.

Example 3: How would you prepare 500 cm$^3$ of physiological saline. The available sterile saline solution is of 10% strength.

Analysing the problem we see that

Desired strength (D) = 0.9%
Strength available (H) = 10%
Volume of stock solution (A) = to be determined
Total volume of the solution (Q) = 500 cm$^3$

We had the formula, $D \times Q / H = A$

Substituting the values, we get, $A = 0.9\% \times 500\, cm^3 / 10\% = 45\, cm^3$

So, we should take 45 cm$^3$ of sterile saline solution of 10% strength and add enough sterile water to it to make 500 cm$^3$ of solution to get the desired physiological saline solution.

Example 4: How much of the diluent (sterile water) will need to be added to 10 cm$^3$ of 1:50 atropine solution to prepare a 1:200 solution? (Hint: 1:50 is same as 1 in 50)

Analysing the problem we see that,

Desired strength (D) = 1:200 = 1/200 = 0.5 / 100 = 0.5%
Strength available (H) = 1:50 = 1/50 = 2/100 = 2.0%
Volume of stock solution (A) = 10 cm$^3$
Total volume of the solution (Q) = to be determined

We had the formula, $D \times Q / H = A$

Rearranging it, we have $Q = A \times H / D$

Total volume of the solution, $Q = 10\, cm^3 \times 2\% / 0.5\% = 40\, cm^3$

We had 10 cm$^3$ of stock, and the total volume comes to 40 cm$^3$.

So, we need to add $40 - 10 = 30\, cm^3$ of the diluent.

Let us actually do an experiment on preparation of solution by dilution.

Experiment 3: Prepare the following solutions of glucose from a stock of 5% (w/v) glucose solution.

- 40 cm$^3$ of 0.25%
- 60 cm$^3$ of 0.75%
- 50 cm$^3$ of 1.50%
- 20 cm$^3$ of 2.50%

Objectives

After performing this experiment you should be able to:

- calculate the volume of stock solution required for a given dilution
- measure the required volume of the liquid
perform the said dilution
- correctly read the level of a liquid

Requirements
Measuring cylinder
Graduated pipette
Wash bottle
5% glucose solution

Procedure
Follow the instructions given below sequentially:

1) Calculate the volume of stock solution required for the given dilutions using the formula $D \times \frac{Q}{H} = A$

2) Record the calculated values in the observation table given below.

3) For first solution, measure the calculated amount of the solution with the help of a graduated pipette into a calibrated measuring cylinder. If the required volume is large you may use the measuring cylinder itself for the purpose.

4) Slowly add sufficient amount of water to obtain the required volume of the solution. Read the level carefully.

5) Repeat the above steps for the rest of the solutions.

6) Save these solutions, suitably labelled for a later experiment.

<table>
<thead>
<tr>
<th>SL.No.</th>
<th>Desired solution</th>
<th>Volume of the stock solution required</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40 cm$^3$ of 0.25 %</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>60 cm$^3$ of 0.75 %</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>50 cm$^3$ of 1.50 %</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>20 cm$^3$ of 2.50 %</td>
<td></td>
</tr>
</tbody>
</table>

Result

1.7 LABORATORY RECORD BOOK

Recording observations and maintaining a record of the activities performed in the laboratory, is an essential component of any experiment. As you are aware that in this course 70% of your marks are based on continuous evaluation, the maintenance of proper laboratory record by you makes an important component of this evaluation. You are, therefore, advised to purchase a practical notebook for this purpose. You are expected to faithfully record the date, title of the experiment, principle involved, observations and the calculations, etc.

The observations and calculations normally are recorded on the left hand page while other details are given on the right hand page. However, it is not mandatory. You may discuss with your counsellor about how exactly the experiments are to be recorded. What is more
Important is that you complete your records daily and submit the notebook daily to your counsellor for evaluation.

1.8 LET US SUM UP

In this introductory unit we have discussed the basics of a biochemistry laboratory. We began with an introduction to the biochemistry laboratory. It was followed by the precautions to be observed while working in a biochemistry laboratory and also an introduction to general first aid procedures for an untoward incident. The next section familiarised you with the common laboratory glassware and equipment. Thereafter we have discussed about basic laboratory operations like, cleaning laboratory apparatus, measurement of volume, heating and filtration. Two simple experiments make you actually perform some of these operations. Preparation of solutions and doses, an important aspect of a practicing nurse has been dealt in detail with the help of a number of practical examples, followed by an experiment. Finally the idea and importance of maintaining laboratory record has been discussed.
UNIT 2 MATTER: CLASSIFICATION AND PROPERTIES

Structure

2.0 Objectives
2.1 Introduction
2.2 Classification of Matter
   2.2.1 Demonstration 1
2.3 Physical and Chemical Change
2.4 Diffusion, Osmosis and Dialysis
2.5 Let Us Sum Up
2.6 Answers to Check Your Progress

2.0 OBJECTIVES

After going through this unit and performing the experiments contained herein you will be able to:

- classify matter on the basis of physical state and chemical nature;
- differentiate between physical and chemical change with the help of suitable examples;
- state and demonstrate the difference between element, compound and a mixture;
- explain and differentiate between the phenomena of diffusion, osmosis, and dialysis; and
- demonstrate the phenomenon of osmosis experimentally.

2.1 INTRODUCTION

In the previous unit you have learnt about the basic tools and techniques used in a chemistry laboratory, along with the safety aspects of laboratory working. In this unit we shall take up simple experiments related to the concepts covered in Units 1 and 2 of the theory course BNS-102 (Biochemistry). These experiments are designed to provide hands-on experience of basic aspects of chemistry and also to reinforce your knowledge. You will be performing the experiments on:

- Performance and classification of some Physical/Chemical Changes.
- Demonstration of the phenomenon of osmosis using fresh potatoes/RBC’s.

2.2 CLASSIFICATION OF MATTER

You have learnt about matter and its classification in your theory course BNS-102 (Biochemistry). Let us recapitulate some of the basic concepts relevant to this unit. You have read that “Any thing visible/invisible that occupies space and has mass is called as matter.” Matter is concrete; we can feel it with our sense organs of sight, smell, taste and touch etc. There are innumerable examples of matter—table, chair, water, pen, book, oxygen ... the list is endless.

You have also learnt that, at a given temperature, matter can exist in three different forms viz. solid, liquid and gas. These states differ from each other in the way its constituents are packed. In solids the constituent particles of matter are packed quite closely/tightly and do not move around much, thereby giving the solids a defined shape and a fixed volume.
Liquids on the other hand have a fixed volume but do not have a defined shape. A soft drink in the bottle has the shape of a bottle, when transferred to a glass acquires the shape of the glass. It is due to a relatively loose packing of its constituents, which are somewhat free to move around. In gases the constituent particles are far apart from each other and move about quite freely; in fact, they move about so freely that the gases neither have a definite shape nor a definite volume. Further, a given substance can exist in any of these different states. Water is a liquid at normal temperature but it can be solidified by cooling in the freezer or converted into steam by heating. Thus, we can classify matter on the basis of the physical state it occupies at a given temperature.

Activity 1

Look around and make a list of twenty different substances/materials and classify them on the basis of physical state they are in.

All matter is made up of tiny units called as atoms. There are more than 100 different types of atoms known today. These atoms combine in different ways to make all the matter. When atoms of a given type combine amongst themselves in large numbers they constitute what is called as an element. A small piece of iron can be visualized as made up of billions or trillions of atoms of iron, while a similar collection of atoms of copper would give a piece of copper. Sometimes two or three atoms of a given type combine together to form a small group called a molecule. A large collection of such molecules also constitute an element e.g. two atoms of oxygen combine to form a molecule of oxygen (O₂). Billions of these oxygen molecules along with other gases enter our body every time we respire. So an element can be defined as matter that is made up of identical atoms as individuals or as a group.

A combination of two or more atoms of different types in a fixed ratio also gives molecules. These molecules when present in large numbers constitute what is called as a compound. For example the atoms of carbon, hydrogen and oxygen can combine in fixed ratio of 6, 12 and 6 to give a molecule of glucose. This molecule is represented as C₆H₁₂O₆ giving the ratios of atoms of carbon(C), hydrogen (H) and oxygen (O) in it. A large collection of these molecules makes the compound called Glucose—a common nutritional component. A compound can also be visualized as a product of chemical reaction between two or more elements in a fixed ratio.

The water we drink is a chemical compound made up of two elements hydrogen and oxygen combined in a ratio of 2 and 1. (H₂O).

All the substances are not pure substances like elements or compounds. In fact most of the matter around us exists in the form of mixtures. For example the air we breathe in is also a mixture of different gases or the lemon squash you drink is a mixture of water, sugar and lemon juice. A mixture is a substance formed by mixing two or more substances in any proportion.

You may be wondering that why don’t we call a compound a mixture as it is also made by combination of two or more elements. Let us look at a simple experimental demonstration to understand the difference between an element, compound and a mixture.

Demonstration 1: To demonstrate the difference between a compound and a mixture.

(Your academic counsellor will demonstrate the following experiment to you. Watch the demonstration carefully and try to understand the difference between a compound and a mixture. If you have any doubt or confusion, discuss and clarify it with your counsellor.)
Step 1 Spread five small pieces of paper on the working bench and label them from 1 to 5. Put some iron fillings on the first paper and sulphur powder on the second. Take 1, 2 and 3 spatula full of sulphur on papers numbered 3, 4 and 5 respectively. Add a spatula full of iron fillings to each of these and mix them well.

Step 2 Roughly weigh 4 g of iron fillings and 7 g of sulphur powder and put them into a china dish. Keep the mixture for heating on a burner. Occasionally mix the contents with the help of a glass rod. You would observe that the mixture starts boiling and a blackish mass is obtained. Cool it.

Step 3 Bring a magnet near the contents of papers numbered 1 and 2. You would observe that iron fillings cling on to the magnet while sulphur does not.

Step 4 Clean the magnet and bring it to papers number 3, 4 and 5 one at a time. You would observe that iron fillings in the mixtures also cling on to the magnet while sulphur still does not. Put the magnet into one of the mixtures and gently mix it. The whole of iron fillings get attached to the magnet and sulphur stays in the paper. You have been able to separate the mixture of iron and sulphur with a simple device.

Step 5 Bring the magnet close to the black mass obtained in the china dish. It does not get attracted to the magnet. And you can’t even spot sulphur in it. What has happened here is that iron and sulphur have reacted in a certain ratio (4:7) to give a compound called as iron sulphide. This compound does not show the properties of iron. Also we can’t even separate iron and sulphur from the compound iron sulphide.

What do we conclude from this experimental demonstration?

- A mixture can be prepared by mixing two or more components in any ratio.
- The constituents retain their properties in the mixture also.
- A mixture can be separated into its components by simple means.
- Two or more elements can react in a fixed ratio under certain conditions (e.g. heating) to give a compound.
- The constituent elements of a compound lose their properties in a compound.
- It is not easy to separate the components of a compound.

Check Your Progress 1

1) Write 3-4 differences between a compound and a mixture.

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>Compound</th>
<th>Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2) Sulphur powder dissolves in a solvent called carbon tetrachloride while iron fillings do not. Suggest a method of separating a mixture of iron fillings and sulphur powder.

(Hint: You need to use a basic operation learnt in the previous unit.)
2.3 PHYSICAL AND CHEMICAL CHANGE

Chemistry has been defined as the study of matter in terms of its composition and the changes it undergoes. The matter in turn can undergo changes in its state, condition and composition. A physical change refers to a change in the condition or the state of the matter while its composition does not change. The transformation of water into steam or ice on heating or cooling is mere change of state. In all the three states the composition of water is same viz., H₂O. Ice is frozen water while steam is simply the vapour of water. Suppose we take a big piece of ice and crush it into pieces, it is still a physical change, as the composition has not changed.

Activity 2

Take some water in a beaker or a glass and put some sugar in it. Stir the mixture with a glass rod or a spoon. What do you get? Is this a physical change?

A chemical change is the one involving change in the composition of the matter. Here the initial substance/s combine or break to give one or more new substances. For example, if we keep an iron nail in a moist place for a long time it gets rusted. Rust is a new chemical compound formed combining iron with oxygen. Here the composition has changed. Further, chemical changes are permanent i.e. these cannot be reversed by simple means. The rust, formed as a reaction between iron and oxygen cannot be converted back into iron or oxygen. If we burn a piece of paper it changes into ashes, carbon dioxide and water. We cannot create the paper back from ashes.

Activity 3

List five examples each of a chemical and a physical change. Discuss your responses with your counsellor.

Experiment 1: To perform and classify some physical/chemical changes.

Requirements

- Beaker
- Test tubes-3
- Small pebbles
- Sugar
- Glass rod
Procedure

Perform the following actions, observe carefully and classify them as physical/chemical change:

a) Pour 10 cm$^3$ of water each in two test tubes with the help of a pipette.

b) Add a few pieces of small pebbles to the first test tube and shake.

c) To the second test tube add a few crystals of common salt and mix with a glass rod.

d) Put some sugar crystals in another test tube and heat it over the flame till you get a brownish mass.

Check Your Progress 2

Classify each of the following as physical change or chemical change.

1) Fixing a nail in the wall

2) Making a chair from wood

3) Burning wood for heat

4) Kneading dough from flour

5) Making chapatis from dough

2.4 DIFFUSION, OSMOSIS AND DIALYSIS

Having learnt about the matter and its classification, let us now learn about some important physical properties of solutions. Solutions, as you know are homogenous mixtures of two or more substances. There are three properties associated with solutions i.e. diffusion, osmosis and dialysis, which are closely related. As a student of nursing you must understand these properties as well as the distinction between them clearly. Let us learn these one by one.

Diffusion is the process by which a solute distributes itself in the solvent to make a solution. The particles of solute move spontaneously, i.e. on their own, from a region of higher concentration to the one of lower concentration. This process continues till the concentration of the solute is same throughout the solution. You can perform a simple activity and visualize the diffusion phenomenon.

Activity 4

Take a small beaker and place one or two crystals of potassium permanganate or of copper sulphate in it. Now tilt the beaker and carefully pour water into it along the sides of the beaker. Ensure that you do not shake the beaker. Keep the beaker on a flat surface like that of a table and leave it undisturbed. Watch the beaker closely for a few minutes and observe the spreading of colour in the beaker—you are watching the phenomenon of diffusion in action. Write your observations in the space provided below.

Osmosis is a special kind of diffusion in which there is a net movement of solvent (water in case of living systems) molecules across a semi-permeable membrane. A semi-permeable membrane is one, which is permeable to the solvent i.e. allows the solvent molecules to pass through but does not let the solute particles pass through it. Suppose we have an aqueous solution separated from its solvent (water) by a semi-permeable membrane. In such a situation there will be a flow of water molecules in both the directions, i.e. from the solution side to the solvent side and in the opposite direction.
Since the concentration of water is more on the solvent side than on the solution side, more water molecules will pass through the membrane from solvent to the solution than in the reverse direction. As a consequence, there will be a net movement of water from solvent side to the solution side and this exactly is osmosis.

This may also be visualized as diffusion of water from a region of higher concentration (of water) to that of lower concentration (of water). A similar effect will be observed if we separate two solutions of different concentrations from each other by a semi permeable membrane. Here we will observe a net movement of water from the dilute solution to the concentrated solution.

Due to the movement of water from the solvent side to the solution side, the volume of the solution will increase. This increased volume exerts a pressure, on the membrane, which opposes the net movement from the solvent side. This pressure gradually increases with more and more water movement and comes to a stage where it completely stops further net movement of solvent. This pressure is called as osmotic pressure.

Why don’t you do an experiment to see the phenomenon of osmosis in operation?

(We are giving two of the possible experiments to demonstrate the phenomenon of osmosis. These are given as experiments 2A and 2B respectively. Your Academic counsellor will select one of these depending on the availability of the materials. You may, however, read both of these experiments so as to appreciate that one can use different ways of demonstrating the phenomenon of osmosis)

**Experiment 2A: To demonstrate the phenomenon of osmosis using fresh potatoes.**

**Principle**

The wall of the potato cells can act as a semi permeable membrane. We make use of this fact to demonstrate the phenomenon of osmosis. Thin slices of fresh potatoes are kept in solutions of different salt concentrations. This is expected to change the volume of the cells and thereby the size of the potato slices. The change in the size can be correlated with the movement of water into and out of the cells.

**Requirements**

Medium to large sized fresh potato-1

Beakers-3

20 % and 0.9 % NaCl solutions-about 100 cm³ each

Knife-1.

**Procedure**

1) Take three beakers and label them as water, 0.9 % NaCl solution and 20% NaCl solution respectively and fill with these liquids respectively.

2) Peel off the potato and cut into lengthwise slices in such a way that each piece has two flat sides somewhat like the ones cut for French fries. These slices should be of about 7-8 mm thickness. Cut six pieces each of 70 cm length.

3) Measure their lengths and put two pieces each in the three beakers. Record the lengths of the added potato pieces in the second column of the Table 2.1 given below.

4) Soak the potato pieces for about two and a half to three hours, take them out and keep them separately in three watch glasses or some other clean container.

5) Measure the length of the potato slices taken out and record them in Table 2.1 given below.
### Table 2.1: Observation of Phenomenon of Osmosis Using Fresh Potatoes

<table>
<thead>
<tr>
<th>Liquid in the beaker</th>
<th>Length of the potato slices added ($l_1 + l_2$) (In mm)</th>
<th>Average Length of the potato slices added ($l_1 + l_2$) *1/2 (In mm)</th>
<th>Lengths of the potato slices after soaking ($l_1, l_2$)</th>
<th>Average Length of the potato slices after soaking ($l_1 + l_2$) *1/2 (In mm)</th>
<th>Change in the average length (In mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9% NaCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20% NaCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Result**


**Alternatively, the phenomenon of osmosis can be demonstrated using RBCs.**

**Experiment 2 B: To demonstrate the phenomenon of osmosis using red blood cells.**

**Principle**

The membrane of the red blood cells acts as a semi-permeable membrane. We make use of this fact to demonstrate the phenomenon of osmosis. A drop of sheep’s blood is added to each of the solutions of different salt concentrations. Due to osmosis, it is expected that the water will move into the cell or out of it depending on the difference in concentration of water inside and out of the cell. This movement of water into and out of the cells may lead to breakdown or shrinkage of the cells. These changes can be observed under a microscope.

**Requirements**

Sheep’s blood (citrated)
Test tubes – 3
Glass slides – 3,
Microscope – 1

Sodium Chloride solutions (0.2%, 0.9% and 2%)—about 10 cm³ each.

**Procedure**

Follow the instructions given below sequentially:

1) Take three test tubes and label them as 0.2% NaCl, 0.9% NaCl solution and 2% NaCl solution and fill with 10 cm³ of these liquids respectively.

2) Put a drop of Sheep’s blood into each of the three test tubes. Observe the changes in the appearance of the solutions.

3) Put a drop of the solution from the test tube marked 0.9% NaCl on a glass slide and observe under the microscope. Make a rough sketch of it in the space provided below.

4) Repeat the same with solutions from the other two test tubes.
Observations
a) The appearance of the solutions is given below (write your responses in the space provided)
   - 0.2% NaCl
   - 0.9% NaCl
   - 2.0% NaCl
b) Draw roughly the shape of RBC’s observed under the microscope in the following boxes.

| 0.2% NaCl | 0.9% NaCl | 2% NaCl |

Result

Dialysis: This is a very important phenomenon in living systems. A living cell needs to take in nutrients and dispose off the waste materials. It cannot survive with the membranes permeable only to water and also it cannot have membranes, which are permeable to everything—in such a case nothing will stay in the cell. The membranes permeable to solvent as well as small solute particles are called as dialysing membranes. In our body the most significant example of dialysis is the maintenance of blood’s solute and electrolyte balance through kidney functions.

Activity 5
Learn about “How kidneys perform the function of maintenance of blood’s solute and electrolyte balance”. Also find out “What is hemodialysis and its significance in kidney damage”.

Check Your Progress 3
What is the difference between
1) Diffusion and osmosis
### 2.5 LET US SUM UP

In this unit we have tried to briefly recapitulate the basic concepts of matter and its classification on the basis of physical state as well as the chemical nature. A demonstrative experiment has been used to differentiate between element, compound and a mixture. The concepts of physical change and chemical change have been clarified with examples and an experiment has been introduced to perform and classify some physical/chemical changes. The closely related concepts of diffusion, osmosis and dialysis have also been explained and an experiment has been included to demonstrate the phenomenon of osmosis using fresh potatoes/RBC’s. In addition to these experiments, some simple activities which can be done at home also have been included to give a better grasp of the concepts discussed.

### 2.6 ANSWERS TO CHECK YOUR PROGRESS

**Check Your Progress 1**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Compound</th>
<th>Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>Two or more elements react in a fixed ratio to give a compound.</td>
<td>A mixture can be prepared by mixing two or more components in any ratio.</td>
</tr>
<tr>
<td>2)</td>
<td>The constituent elements of a compound lose their properties in a compound.</td>
<td>The constituents retain their properties in the mixture also.</td>
</tr>
<tr>
<td>3)</td>
<td>It is not easy to separate the components of a compound easily.</td>
<td>A mixture can be separated into its components by simple means.</td>
</tr>
</tbody>
</table>

2) We need to use the basic operation of filtration over here. To separate the mixture, dissolve it in the solvent, carbon tetrachloride. As given, sulphur will dissolve in it while iron will not. Filter the solution, the filtrate contains sulphur in carbon tetrachloride and the undissolved iron stays on the filter paper. Sulphur can be obtained by evaporating the filtrate.

**Check Your Progress 2**

1) Physical change
2) Physical change
3) Chemical change
4) Physical change
5) Chemical change

**Check Your Progress 3**

1) Diffusion refers to the process of net movement of solute particles from a region of higher concentration to that of a lower concentration. Osmosis on the other hand refers to the movement of solvent particles through a semi permeable membrane. When a solution is separated from its solvent by such a membrane, there is a net movement of the solvent from the solvent side to the solution side.

2) In osmosis the membrane used is permeable only to the solvent molecules whereas in case of dialysis the membrane allows the passage of solvent as well as small solute particles.
UNIT 3 QUALITATIVE TESTS FOR PROTEINS, CARBOHYDRATES AND TRYGLYCERIDES

Structure

3.0 Objectives
3.1 Introduction
3.2 Tests for Proteins
  3.2.1 Colour Reactions of Proteins
  3.2.2 Heat Test for Proteins
  3.2.3 Coagulation and Precipitation of Proteins
3.3 Tests for Carbohydrates
  3.3.1 Reducing and Non-reducing Carbohydrates
  3.3.2 Tests for Polysaccharides
3.4 Tests for Triglycerides
3.5 Let Us Sum Up
3.6 Answers to Check Your Progress

3.0 OBJECTIVES

After going through this unit and performing the experiments and activities contained herein you will be able to:

- state the principle behind the qualitative tests for proteins, carbohydrates and triglycerides;
- establish the presence of protein in a sample, with the help of colour reactions and heat test;
- enumerate different agents causing the coagulation of the proteins;
- confirm the presence of carbohydrate in a sample through Molisch test;
- differentiate between a reducing and a non reducing sugar using Benedict test;
- describe and experimentally demonstrate different physical and chemical properties of lipids; and
- identify the presence of unsaturated fats in a sample.

3.1 INTRODUCTION

Our body is a highly sophisticated system, consisting of billions of cells in which thousands of biochemical reactions occur continuously to keep them functioning properly. The input of energy and different molecules in the form of food is utilized in these biochemical reactions. The breakdown products of the food constituents are used to generate ATP i.e., the energy currency of the cells and synthesis of a wide variety of biomolecules like, proteins, hormones, nucleic acids, lipids, vitamins, etc. so as to maintain the normal functioning of the body. As a consequence of this a healthy working cell acquires an optimum level of its constituents.

A disease may be visualized as a malfunctioning of some aspects of cellular functioning, which may disturb the cellular equilibrium leading to subtle chemical and microscopic changes in blood or other body fluids. Thus, the samples of blood and other body fluids can provide vital information about the state of health of a person. Laboratory tests on key
constituents of blood, urine and body fluids are used in diagnosis and in the selection of proper treatment for the disease. These tests are also used to follow the effectiveness of the treatment.

In this unit the principles and procedures involved in the qualitative tests for proteins, carbohydrates and triglycerides are described. A few simple experiments based on these principles have also been included. Some of these experiments will be used in the next unit, where we take up analysis of urine samples.

### 3.2 TESTS FOR PROTEINS

You have learnt in your theory course (BNS-101) that proteins are polymeric molecules, which perform a number of functions in the cell.

The presence of proteins can be detected by performing heat test, and with the help of colour reactions. These tests are based on the denaturation, i.e. breaking down of the native structure, or the reaction of different chemical reagents with peptide bonds or the amino acid side chains. Further, coagulation and precipitation of proteins by the action of precipitating agents like, acids and bases, salts of heavy metals and solvents like alcohol are also important. Though the development of colour can be used for the quantitative determination of the proteins, we are going to discuss only the qualitative aspect of these experiments.

#### 3.2.1 Colour Reactions of Proteins

The presence of a protein in a sample can be detected with the help of a number of colour reactions or tests. Certain reagents react with some specific amino acids (tyrosine, tryptophan etc.) or groups (e.g. peptide bond) etc. in the protein molecule to give colored products. Three such tests are included in the experiment given below.

**Experiment 1: Detection of proteins using colour reactions.**

We are going to use three tests here, these are:

**Biuret test**

**Principle**

The biuret test is in fact a test for the presence of a peptide bond (-CONH-). In this test biuret reagent (alkaline solution of copper sulphate) reacts with a molecule containing at least two peptide bonds to give violet/purple coloured complex. Since proteins contain a large number of peptide bonds, these give a positive biuret test.

**Xanthoproteic test**

This test is due to the presence of aromatic amino acids like tyrosine or tryptophan in the protein molecule. In this test addition of concentrated nitric acid (HNO₃) to the protein gives a yellow coloration, which turns orange on adding sodium or ammonium hydroxide. This reaction is responsible for the yellow coloration of the skin when some nitric acid falls on it.

**Millon’s Test**

This test is due to the presence of the amino acid, tyrosine, in the protein molecule. Addition of Millon’s reagent (mercuric sulphate in sulphuric acid) to the protein solution and heating followed by addition of sodium nitrite gives a red coloration.

**Requirements**

- Test tubes
- Biuret reagent
Nitrile acid
40% solution of sodium hydroxide

Millon’s reagent
1% solution of sodium nitrite

Procedure
Perform the tests as described in Table 3.1 given below and record your observations in the relevant columns.

Table 3.1: Colour Reactions of Proteins

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Experiment</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Biuret test: Take about 2 cm³ of the protein solution in a test tube, add about same amount of biuret reagent to it and observe the colour change.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Xanthoproteic test: Take about 2 cm³ of the protein solution in a test tube, add roughly 1 cm³ of nitrile acid to it and observe the colour change. Add about 2 cm³ of 40% solution of sodium hydroxide to the above and observe the change in colouration.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Millon’s Test: Take about 2 cm³ of the protein solution in a test tube, add a few drops of Millon’s reagent to it and heat for about 10 minutes. Cool the solution and add a few drops of 1% solution of sodium nitrite.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Result

3.2.2 Heat Test for Proteins

Experiment 2: To perform heat denaturation test for detecting the presence of protein.

Principle
As discussed above, action of heat causes the denaturation of the protein. The denatured protein becomes less soluble and causes the solution to become turbid. There may be an interference due to the presence of phosphate as it also can make the solution turbid. Such a turbidity can be removed by adding dilute acetic acid solution.

Requirements
Test tube
Test tube holder
Protein test solution (5% egg albumin or bovine serum albumin)
Dilute acetic acid.
Procedure

Fill roughly three fourth of a test tube with the test solution containing protein and gently heat the upper part of the test tube (take care not to boil). Compare the heated portion with the rest of the solution. If turbidity appears, add 2-3 drops of dilute acetic acid solution. If the turbidity still persists it indicates the presence of a protein. Record your observations and inferences in the space provided below.

Observations and inferences

3.2.3 Coagulation and Precipitation of Proteins

Experiment 3: To study coagulation of proteins by chemical means

Principle

Coagulation of proteins occurs due to the neutralization of the charge on them. This can be achieved in a number of ways. The details of mechanisms are not needed in the text.

Requirements

Protein sample solution (~ 5% egg white or bovine serum albumin)
2% acetic acid solution
2% NaOH solution
pH paper,
Mercuric chloride solution
Lead acetate solution
95% alcohol

Table 3.2: Precipitation Reactions of Proteins

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Experiment</th>
<th>Observations</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Take about 2 cm³ each of the protein solution in a test tube, add 2% acetic acid solution drop wise and observe. If you get the precipitate of the protein, take out a drop of the solution with the help of a glass rod and place on a piece of pH paper and record the pH of the solution. Repeat the same experiment by using 2% NaOH solution instead of acetic acid.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Take about 2 cm³ of the protein solution in a test tube, add 1-2 drops of NaOH solution to it and then add mercuric chloride solution drop wise till excess? Repeat the same experiment by using lead acetate solution instead of mercuric chloride.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Take about 5 cm³ of 95% alcohol in a test tube and add a few drops of the protein solution to it.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.3 TESTS FOR CARBOHYDRATES

The presence of a carbohydrate can be ascertained by Molisch test while Tollen’s test and Benedict’s test establish the reducing/ non-reducing nature of the carbohydrate.

Let us perform some experiments.

Experiment 4: To perform Molisch test to detect the presence of carbohydrate in the given sample.

Principle

As mentioned above, carbohydrates are polyhydroxy aldehydes and ketones or compounds that yield these on hydrolysis. On treating with concentrated sulphuric acid, carbohydrates get dehydrated by losing a molecule of water from two adjacent hydroxyl groups. The resulting compound called furfural (or its derivatives) combines with Molisch reagent (alcoholic solution of α-naphthol) to give a purple colored complex.

Requirements

Test tubes
Starch solution
5% aqueous solutions of glucose, fructose, lactose, maltose and sucrose
(Subject to availability any number of carbohydrate samples may be used)
Concentrated sulphuric acid
Molisch Reagent.

Procedure

To perform Molisch test, take 2-3 cm³ of the given test sample and add 2-3 drops of Molisch reagent to it. Mix the solution well and carefully add about 2 cm³ of concentrated sulfuric acid to it along the sides of the test tube (your counsellor would demonstrate it for you).
Take care not to shake the test tube. Sulfuric acid being heavier settles down making the lower layer. Observe the region where the two layers meet. Formation of a purple ring at the junction of the two layers indicates the presence of carbohydrate in the given sample.

Perform the Molisch test with all the given samples and record your observations in Table 3.3 given below.
<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Result: P.39

Sample number ................................and..........................contain carbohydrate.

3.3.1 Reducing and Non-reducing Carbohydrates

Let us do some simple experiments using Benedict test.

Experiment 5:  

A) To perform Benedict’s test to establish reducing/non reducing nature of the carbohydrate

B) To perform Benedict’s test for a rough estimate of the concentration of reducing sugar (glucose)

Principle

In Benedict’s test a solution of the carbohydrate is heated with Benedict’s reagent (alkaline solution of CuSO₄). In this reaction the cupric ions are reduced to cuprous ions and a red colored precipitate of cuprous oxide is obtained. This red precipitate is obtained only if the concentration of the reducing sugar (normally glucose) is about 2% or above. However, if the amount of the reducing sugar is less, it gives greenish, yellowish or orange colored precipitate depending on the amount.

Requirements

Test tubes
Test tube stand
Water bath
Starch solution
5% aqueous solutions of glucose, fructose, lactose, maltose and sucrose
(Subject to availability any number of carbohydrate samples may be used)
Benedict’s solution.

Procedure

A) To perform Benedict’s test, take 2.5 cm³ of Benedict’s solution in a test tube and add 4 drops of given carbohydrate solution with the help of a dropper. Mix well and keep the test tube in a water bath for about 5 minutes, cool and observe the change in color or formation of precipitate, if any.

Perform the test with all the given samples and record your observations in Table 3.4 given below.
Table 3.4: Benedict's Test for Carbohydrates

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Result

Sample number ........................................... contain reducing sugars.

For part B of the experiment, proceed as follows:

1) Take 5 clean test tubes and label them from 1 to 5.
2) Make solutions of different concentrations of glucose in these test tubes by mixing stock solution (5%) of glucose and water as per the table given below. (You may use a measuring pipette or a syringe for this purpose)
3) Take 2.5 cm$^3$ of Benedict's solution in a test tube and add 4 drops of carbohydrate solution from test tube number 1 with the help of a dropper and keep the test tube in water bath for about 5 minutes.
4) Record your observations in the table given below,
5) Repeat step 3 and 4 for the rest of the test tubes.

Table 3.5: Benedict's Test for a Rough Estimate of Concentration

<table>
<thead>
<tr>
<th>Test tube Number</th>
<th>Volume of 5% glucose (In cm$^3$)</th>
<th>Volume of Water (In cm$^3$)</th>
<th>Percentage of Glucose</th>
<th>Colour of the solution Or precipitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>5.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>4.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>4.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>3.5</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>3.0</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

Result


3.3.2 Tests for Polysaccharides

Polysaccharides like starch can be easily hydrolysed i.e. broken down into smaller units with the help of dilute solutions of strong acids. With continued breakdown, the polymeric
molecule of starch eventually breaks down into glucose units. This breakdown of starch into monosaccharides can be easily demonstrated experimentally.

Why don’t you do an experiment to check it for yourself?

**Experiment 6:** To demonstrate the breakdown of starch to monosaccharides with hydrochloric acid.

**Principle**

As discussed above starch is a polysaccharide containing a large number of alpha type of linkage joining the monosaccharides units. A strong acid like hydrochloric acid can break these linkages. Since starch gives a characteristic blue color with iodine in acidic medium, the breakdown of starch can be followed with the help of iodine test, a negative test indicates the absence of starch. Further, in Experiment No. 5 you would have noticed that starch does not give Benedict’s test while the monosaccharides do. This fact can be used to establish the formation of monosaccharides in the hydrolysis reaction.

**Requirements**

Starch solution
Dilute hydrochloric acid
Concentrated hydrochloric acid
Iodine solution
Benedict solution
Water bath
Test tubes

**Procedure**

Perform the experiments as outlined in the table given below. Record your observations and inferences in the appropriate columns of Table 3.6.

### Table 3.6: Hydrolysis of Starch

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Experiment</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Iodine test: Take about 1 cm$^3$ of starch solution in a test tube and add 2-3 drops of dilute HCl followed by 1-2 drops of iodine solution.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Benedict’s test: Take 2.5 cm$^3$ of Benedict’s solution in a test tube, add 4 drops of starch solution with the help of a dropper and keep the test tube in water bath for about 5 minutes.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Hydrolysis: Take about 3 cm$^3$ of starch solution in a test tube, add 10-12 drops of concentrated hydrochloric acid and boil the solution gently for 5-7 minutes. Take out 2-3 drops of the above solution on a porcelain tile and mix with a drop of iodine solution. If you get a positive iodine test, boil the above solution a little more and check again. Continue till you get a negative test.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Cool the hydrolysed solution obtained in step 3 and add NaOH solution drop-wise till the solution is slightly alkaline. (Use a litmus paper or a pH paper to check it) Take 2.5 cm$^3$ of Benedict’s solution in a test tube, add 4 drops of the above solution with the help of a dropper and keep the test tube in water bath for about 5 minutes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Check Your Progress 2

You are given three carbohydrates namely, glucose, sucrose and starch. Complete the table given below by marking a ✓ mark for an expected positive test and a × mark for an expected negative test.

<table>
<thead>
<tr>
<th>Carbohydrate Test</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molisch test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benedict test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodine test</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.4 TESTS FOR TRIGLYCERIDES

Lipids or triglycerides have got typical properties like, solubility in non-polar solvents, emulsification, saponification etc. A simple experiment based on these properties is described here.

Experiment 7: To perform simple tests of triglycerides based on their physical and chemical properties.

Principle

As mentioned above, fatty acids present in triglycerides contain long chains of carbon atoms. Since these non-polar chains make the major bulk of the triglycerides the triglycerides are non polar in nature. As per the basic rule of solubility, "Like dissolves like" the triglycerides are soluble in non-polar solvents like alcohol or ether and are insoluble in polar solvents like water. In this experiment you would check the solubility of a triglyceride (an oil) in different solvents.

It is a common experience that if we mix oil with water and shake, we get an emulsion in which tiny droplets of oil are dispersed in water. These droplets recombine and the emulsion breaks to give two layers. The emulsion however can be made to stay by adding a suitable agent (like soap solution) called as emulsifying agent. The emulsification of oils and fats is an important part of the digestion process. Bile salts and pigments play the role of emulsifying agents in the body.

The nature of fatty acids (saturated/unsaturated) present in triglycerides determines whether it is going to be an oil or fat. Triglycerides containing saturated fatty acids are generally solid at room temperature while the unsaturated fatty acids make it a liquid-oil. The presence of unsaturation in oil can be established with the help of bromine water test. A solution of bromine in water decolorizes on shaking with a solution of oil in an organic solvent.

Triglycerides can be hydrolysed to glycerol and salts of fatty acids with the help of concentrated alkali solution. The salts of fatty acids so obtained act as soaps. This process of getting soaps from hydrolysis of oils is called saponification.

Requirements

Vegetable oil
95% Alcohol
Chloroform
Carbon tetrachloride
Bromine water (freshly prepared)
Soap solution
40% solution of NaOH

**Procedure**

Perform the experiments as outlined in the table given below. Record your observations and inferences in the appropriate columns of Table 3.7.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Experiment</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><strong>Solubility test</strong>: Take about 2cm³ each of water,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>alcohol, chloroform and carbon tetrachloride in four</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>different test tubes and label them. Add 4 drops of</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>oil in each of them and shake well.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><strong>Emulsification</strong>: Take about 5cm³ of water and a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>drop of oil each in two test tubes. Add a few drops</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>of soap solution in one of them. Shake both the test</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>tubes well and observe carefully.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td><strong>Bromine water test</strong>: Take about 2-3cm³ of</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>chloroform and 4 drops of oil in a test tube and</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mix well to dissolve. Add 2 drops of bromine water</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and shake.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td><strong>Saponification</strong>: Take 10 drops of oil in a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>porcelain dish and add about 10 cm³ of alcohol and</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-15 drops of 40% NaOH solution. Stir the</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>contents with a glass rod and heat it over a small</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>flame till all the alcohol evaporates. Cool and</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>touch the contents.</td>
<td></td>
<td><strong>Caution</strong>: Do not heat the dish over strong flame;</td>
</tr>
<tr>
<td></td>
<td>the reaction mixture may catch fire. If it so</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>happens, cover the dish with a wire gauge and put</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>off the flame.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Result**

-----------------------------------------------------------------------------------------------------

3.5 **LET US SUM UP**

In this unit different qualitative tests for proteins, carbohydrates and lipids have been described. A total of seven experiments have been included. The detection of proteins with the help of colour reactions (e.g., biuret test) based on the presence of certain amino acids or groups in the protein molecules and heat denaturation test have been discussed along with precipitation reactions of proteins.

The tests of carbohydrates include their detection with the help of Molisch test and establishing their reducing/non-reducing nature with Benedict's test. The variation of the results in Benedict test as a function of the concentration of glucose has also been included along with the iodine test and hydrolysis test for the polysaccharides.

The section on lipids discusses simple physical and chemical properties like, solubility, emulsification and saponification etc.
3.6 ANSWERS TO CHECK YOUR PROGRESS

Check Your Progress 1

If the pH of intra venous fluid is not comparable to that of the blood then it may lead to coagulation of the blood proteins causing severe damage.

Check Your Progress 2

<table>
<thead>
<tr>
<th>Carbohydrate Test</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molisch test</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Benedict test</td>
<td>✓</td>
<td>✗</td>
<td>✗</td>
</tr>
<tr>
<td>Iodine test</td>
<td>✗</td>
<td>✗</td>
<td>✓</td>
</tr>
</tbody>
</table>
UNIT 4  COLLECTION AND ANALYSIS OF URINE

Structure

4.0  Objectives
4.1  Introduction
4.2  Composition of Urine in Health and Disease
   4.2.1  Normal Constituents of Urine
   4.2.2  Abnormal Constituents of Urine
4.3  Collection and Preservation of Urine Samples
   4.3.1  Collection of Urine Samples
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4.4  Analysis of Urine
   4.4.1  Physical Examination of Urine
   4.4.2  Chemical Examination of Urine
4.5  Let Us Sum Up
4.6  Answers to Check Your Progress

4.0  OBJECTIVES

After going through this unit and performing the experiments and activities contained herein you will be able to:

- describe the normal composition of urine;
- discuss the composition of urine under pathological conditions;
- state different methods of collection and preservation of urine;
- perform physical examination of the given urine sample;
- test the given urine sample for abnormal constituents; and
- correlate the results of urine analysis with pathological conditions.

4.0  INTRODUCTION

You have learnt in Unit 5 of your theory course, BNS-102 that urine is one of the major body fluids, which serves as a route for eliminating waste materials from the body. A normal sample of urine has a certain composition reflecting the normal metabolic state of the person. However, under pathological conditions, the volume and composition of urine can fluctuate a great deal from the normal values. Analysis of urine serves as a diagnostic tool and can reveal diseases like diabetes mellitus, various forms of glomerulonephritis, and chronic urinary tract infections, etc; that normally go unnoticed because of absence of striking signs or symptoms. Further, analysis of urine plays an important role in monitoring the response of the patient to the treatment.

In this unit you are going to learn about urine analysis. This includes the normal and abnormal constituents of urine, collection and preservation of urine samples, followed by the physical and the chemical examination of the collected samples. In the chemical analysis you will observe an application of the principles and procedures involved in the qualitative tests for proteins, carbohydrates and triglycerides that you learnt in Unit 3. You will perform experiments on physical examination of urine as well as the chemical tests for abnormal constituents of urine. You would also learn about the clinical significance of the possible outcomes of these examinations.
4.2 COMPOSITION OF URINE IN HEALTH AND DISEASE

You may be aware that kidneys maintain the electrolyte and buffer level of the blood by a process called Diuresis meaning, formation of urine. Nephron, the functional unit of the kidneys, extensively filter water and non-colloidal constituents of the blood and then reabsorb, part of water and the substances that are not to be discarded, back into the blood. Most of the wastes are left in the urine under formation. Let us look at the normal and abnormal constituents of urine.

4.2.1 Normal Constituents of Urine

In its normal constitution roughly 96% of urine is water and rest 4% consists of dissolved organic and inorganic substances. Urea (~ 30 g/day), creatinine (1-2 g/day) and uric acid (0.7 g/day) are the three main nitrogenous organic constituents of urine. A number of pigments (responsible for the colour of urine) and certain enzymes like trypsin and amylase are also present in the urine. Chlorides, phosphates and sulphates of sodium, potassium, calcium, magnesium and ammonium are the chief inorganic constituents.

4.2.2 Abnormal Constituents of Urine

In addition to the above-mentioned constituents certain other substances are also present in urine, though in very low amounts, at times undetectable. Under pathological conditions the amount of these constituents, however, increase and their detection gives useful clues about the disease. The abnormal constituents include, proteins, reducing sugar, ketone bodies, blood, bile salts and bile pigments. The normal and pathological values of the abnormal constituents of urine along with the possible pathological conditions are summarized in Table 4.1. These will be useful in interpreting the results obtained in urine analysis. The chemical tests for the abnormal constituents are given in the next section.

<table>
<thead>
<tr>
<th>Abnormal constituent</th>
<th>Safe value</th>
<th>Abnormal value</th>
<th>Likely pathological reasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing sugar (mainly glucose)</td>
<td>&lt;100 mg/day</td>
<td>More than normal</td>
<td>Pregnancy glycosuria</td>
</tr>
<tr>
<td></td>
<td>Glucose, 65-72 mg/day</td>
<td>Could be as high as 5 g/day or more</td>
<td>Excessive stress, Diabetic mellitus, Renal glycosuria, Brain damage</td>
</tr>
<tr>
<td>Protein</td>
<td>20-750 mg/day</td>
<td>More than normal</td>
<td>Diabetic mellitus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Could be as high as 3.5 g/day or more</td>
<td>Pregnancy, Nephrotic syndrome</td>
</tr>
<tr>
<td>Ketone bodies</td>
<td>&lt; 50 mg/day</td>
<td>More than normal</td>
<td>Severe vomiting, Starvation,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High fat diet, Metabolic abnormalities,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diabetic mellitus</td>
</tr>
<tr>
<td>Blood</td>
<td>Undetectable</td>
<td>Detectable</td>
<td>Renal stones, Acute-glomerulonephritis,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Malignancies of kidney or urinary tract</td>
</tr>
<tr>
<td>Bile salts</td>
<td>Undetectable</td>
<td>Detectable</td>
<td>Obstructive jaundice</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post hepatic jaundice</td>
</tr>
<tr>
<td>Bile pigments</td>
<td>Undetectable</td>
<td>Detectable</td>
<td>Obstructive jaundice</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hepatic jaundice</td>
</tr>
</tbody>
</table>
Check Your Progress 1
Give the normal and abnormal constituents of urine.

4.3 COLLECTION AND PRESERVATION OF URINE SAMPLES

The analysis of urine sample can provide a great deal of information regarding the metabolic state of the person. However, the result of such an analysis depends on the way the sample has been collected and also the time gap between the collection and analysis of the samples. Further, different types of analysis require the samples to be collected differently. Let us learn about different methods of urine collection.

4.3.1 Collection of Urine Samples

In random collection method the sample is collected at any time of day with no precautions regarding contamination. Such sample may be hypotonic, isotonic, or hypertonic and may contain white cells and bacteria as contaminants. In females, the specimen may contain vaginal contaminants such as trichomonads, yeast, and red blood cells during menstrus.

In early morning collection, the sample is collected before ingestion of any fluid in the morning. Such a sample reflects the ability of the kidney to concentrate urine during dehydration that occurs overnight. For such a collection the patient is advised to avoid fluid ingestion from about 6 p.m. the previous day.

In midstream urine collection, the first half of the bladder urine is discarded and the collection vessel is introduced into the urinary stream to catch the last half. Such a specimen is collected after cleansing the external urethral meatus with a cotton sponge soaked in benzalkonium hydrochloride or some other non-irritating agent.

As mentioned above time gap between the collection of the sample and its analysis in the laboratory is an important factor. Several changes can occur with time, these include:

- decreased clarity due to crystallization of solutes
- increase in pH
- loss of glucose, ketone bodies and bilirubin
- destruction of cells and casts
- growth of contaminating microorganisms

Therefore, urine analysis may not reflect the status of fresh urine if the sample is kept for more than about an hour. Urine samples should be analysed as quickly as possible. If, however, such an analysis is not possible it becomes necessary to preserve such samples. A number of methods are available for this purpose.

4.3.2 Preservation of Urine Samples

Any of the following methods can be used for preserving the urine samples:

a) Refrigeration: It is a simple method of preservation that delays the deterioration of the sample. The sample may be refrigerated with or without the preservatives mentioned below.
b) **Concentrated hydrochloric acid**: Concentrated hydrochloric acid (10 cm³) is used as a preservative in a 24 hr. sample collection.

c) **Toluene**: In this method about 2 cm³ of toluene is added for every 100 cm³ of the urine sample. Toluene acts by forming a thin layer on the surface of the sample. It is a good preservative for the chemical constituents, but does not prevent the growth of the microorganisms.

d) **Formalin (40%)**: Formalin is 40% aqueous solution of formaldehyde. 7-8 drops of Formalin are added to every 100 cm³ of the urine sample. Addition of Formalin may precipitate protein and may also give a false test for sugar.

e) **Thymol**: 100 mg of thymol is added to every 100 cm³ of the urine sample. Like Formalin this may also give a false test for sugar. However it is a good preservative for inorganic constituents.

**Check Your Progress 2**

When is it important to preserve a urine sample?

---

**4.4 ANALYSIS OF URINE**

Having learnt about the normal and abnormal constituents of urine let us learn about the analysis of urine samples. Let us begin with physical examination of urine and see how we perform physical examination and what do the results of such an examination indicate.

**4.4.1 Physical Examination of Urine**

The first step of urine analysis is its physical examination in terms of its color, appearance, odour (smell), amount, specific gravity (thickness) and reaction to litmus. The physical examination can also provide important lead into the diagnosis. Let us take up different physical examinations one by one.

**Appearance**: A normal fresh sample of urine is clear. A turbidity or cloudiness in the sample may be due to the,

- presence of cellular material, like RBC, WBC or bacteria
- presence of protein
- precipitation of salts upon standing at room temperature or in the refrigerator
- presence of fat globules

The presence of cellular material, like RBC, WBC or bacteria is established by microscopic examination while heat test (given later) is used to confirm the presence of protein. If, however, the turbidity disappears on adding a few drops of acid then turbidity is likely to be due to the precipitation of salts.

**Colour**: Normal, fresh urine is pale yellow or amber in colour due to the presence of the pigment *urochrome*. The colour may be pale or dark depending on the volume of the urine excreted. However, an abnormal sample may have distinctly different colours.

A greenish yellow sample may indicate the presence of excessive amount of bile pigments, possibly due to jaundice.

A red or red-brown colour could be from a food dye, eating fresh beets, a drug, or the presence of either hemoglobin or myoglobin, suggesting hemoglobinuria or
myoglobinuria. If the sample contains many red blood cells, it would be cloudy as well as red.

A brownish black colour is due to alkaptonuria, methemoglobinuria or may be due to poisoning with lead, mercury or phenol etc.

The presence of pus or fat globules impart a milky appearance to urine.

Specific Gravity: Specific gravity (sp.gr.) is a measure of urine density, which is directly proportional to the concentration of dissolved solutes. It is normally measured for a 24-hour sample and its value reflects the ability of the kidney to concentrate or dilute the urine over that of plasma. A value of specific gravity between 1.015 and 1.025 is considered normal. A value below this range indicates hydration while above it indicates relative dehydration.

The sp.gr. of urine may go down to 1.002 on ingesting a large amount of water while could increase on fluid restriction. If, however, sp. gr. is not >1.022 after a 12 hour period without food or water, renal concentrating ability is impaired and the patient either has generalized renal impairment or nephrogenic diabetes insipidus. Any urine sample having a specific gravity over 1.035 is either contaminated or contains very high levels of glucose. The specific gravity is measured with the help of a floating urinometer.

Odour: A fresh sample of urine has an aromatic odour; however, in the case of a disease urine may have abnormal smells. An ammonical smell is indicative of a urinary tract infection while a fruity smell of acetone is observed in ketosis. The presence of pus or decomposing tissues in the urine makes it smell putrid.

Volume: An adult normally excretes between 800-2000 cm$^3$ of urine per day depending on the fluid intake and loss of fluid through skin or otherwise. The volume of urine may increase or decrease under pathological conditions.

A urine volume of more than 3000 cm$^3$ per day is referred to as polyuria and is indicative of diabetes, recovery from acute renal failure or a high protein diet. While oliguria, a condition wherein the urine volume goes below 400 cm$^3$ per day could be due to acute renal failure, vomiting, fever, burns or edema. A condition when the urine output decreases to below 100 cm$^3$ per day is called anuria and may be indicative of poisoning with heavy metal salts or acute nephritis.

Reaction to litmus: A normal sample of urine has a pH of about 6 and gives a slightly acidic response to litmus. A strongly acidic urine points to metabolic or respiratory acidosis, methanol poisoning, or metabolic disorders like phenylketonuria, while a strongly alkaline urine is indicative metabolic and respiratory alkalosis and urinary tract infection which should be confirmed by chemical and other means. In severe acidosis the pH of urine may go as low as 4 while in alkalosis the pH may go beyond 8.

After having read the theory of the physical examination of urine and its significance, why don’t you do an experiment now?

Experiment 1: To perform physical examination of the given sample of urine.

**Principle**

This experiment is based on the details given above.

**Requirements**

- Measuring cylinder
- Boiling tube
- Floating urinometer
- pH paper

**Procedure**

Perform the experiments given in second column of Table 4.1 and record your observations and the inferences in the appropriate columns.
### Table 4.2: Physical Examination of the Urine Sample

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Experiment</th>
<th>Observation</th>
<th>Inferences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Study the appearance of the sample in terms of its clarity/turbidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Note the colour of the Urine sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Observe the smell of the given sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Measure the volume of the given sample with the help of a measuring cylinder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Measure the specific gravity of the given sample. For this fill three fourths of the urinometer cylinder or a measuring cylinder with the urine sample and float a urinometer into it. Ensure that the urinometer is floating freely and does not touch the sides of the cylinder. Carefully record the level of the urine surface on the scale marked on the urinometer.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Determine the pH of the sample by putting a drop of urine on a small strip of pH paper and comparing its colour with the comparison chart given.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 4.4.2 Chemical Examination of Urine

Having performed the physical examination of the urine sample let us understand about the chemical examination of urine. In chemical examination we test for the presence of abnormal constituents like, reducing sugar, protein, ketone bodies, blood, bile pigments and bile salts in urine. Let us discuss these one by one.

**Test for reducing sugars:** The reducing sugar, if present, in urine is normally glucose; however, other reducing sugars like lactose, galactose, fructose, or some pentoses may also be present. High levels of glucose are due to diabetes mellitus, pregnancy, excessive stress, renal tubular damage and brain damage. As you have already learnt in section 3.3.1 that the reducing sugars give a positive test with Benedict’s solution, we make use of the same test to check for the presence of reducing sugar in urine samples.

To perform **Benedict’s test**, take about 2.5 cm³ of Benedict’s solution in a test tube and add 4 drops of the given urine sample to it with the help of a dropper. After mixing, the test tube is kept in a water bath for about 5 minutes. A change in colour or formation of precipitate, if any, indicates the presence of reducing sugar. You would recall (experiment 5B of Unit 3) that the colour of precipitate depends on the concentration of the reducing sugar.

The presence of thymol, formaldehyde (used as preservatives) or ascorbic acid and lactic acid etc. in urine may give a false positive test. It is, therefore, advisable that the results obtained should be correlated with the clinical symptoms and other tests.

**Test for proteins:** In renal diseases and diseases affecting renal function, there may be glomerular damage leading to the presence of protein (normally albumin) in urine. Such a condition is called proteinuria (chiefly albuminuria). In the absence of glomerular damage, elevated urine protein may result from excessive exercise, exposure to cold and acute
abdominal diseases. The presence of protein in urine is tested by means of its precipitation/denaturation in heat test. You have learnt and performed this test in the previous unit.

To perform heat test, fill roughly three fourths of a test tube with the urine sample and gently heat the upper part of the test tube (take care not to boil). Compare the heated portion with the rest of the solution. If turbidity appears, add 2-3 drops of dilute acetic acid solution. If the turbidity still persists it indicates the presence of a protein.

Test for ketone bodies: Ketones of various types, which are normal liver metabolites, are normally not found in detectable amounts in the urine. In diabetes mellitus and under starvation conditions there is an increased breakdown of fatty acids leading to over production of ketone bodies (acetone, acetoacetic acid and β-hydroxybutyric acid), which may lead to ketonuria, a condition characterized by the presence of ketone bodies in urine. Ketone bodies may appear in the urine of healthy people on exposure to cold, fasting or due to dietary imbalance, like having a diet rich in fatty acids and poor in carbohydrates. The presence of ketone bodies in urine is identified by Rothera's test.

To perform Rothera's test, take 2-3 cm$^3$ of urine sample in a test tube, add a few crystals of ammonium sulphate to it and shake. Add more crystals if earlier lot dissolves, continue till you have some crystals undissolved i.e. you saturate urine sample with ammonium sulphate. Add 4-5 drops of freshly prepared solution (1%) of sodium nitroprusside and mix well. Carefully add some liquor ammonia to it along the sides of the test tube. Observe the region where the urine and ammonia layers meet. Formation of a blue violet ring at the junction of the two layers indicates the presence of ketone bodies in the given sample.

Test for blood: The presence of blood in urine is called as haematuria, and may be recognized by the coloration of the urine. The presence of blood in urine can be tested chemically by benzidine test.

To test for the presence of blood in urine, take about 5cm$^3$ of urine sample in a test tube, add 2 drops of saturated benzidine solution (prepared in glacial acetic acid) to it and shake well. Add 3 cm$^3$ of hydrogen peroxide to this solution and observe color change, if any. Development of a blue or green colour that changes to brown in a few minutes indicates the presence of blood in urine.

Test for bile salts: Bile salts are the sodium or potassium salts of bile acids. In a normal sample of urine these are present in undetectable amounts. However, in case of obstructive or post hepatic jaundice their amount become significant. The bile salts are emulsifying agents and reduce the surface tension. Hay's test makes use of this property of bile salts for detecting the presence of bile salts in urine.

To perform Hay's test for bile salts, fill two thirds of a test tube with urine sample and take about same amount of water (control) in a second test tube. Gently sprinkle sulphur powder on both the liquids. If the particles of sulphur sink in the urine sample and not in water, it indicates the presence of bile salts in urine sample.

Test for bile pigments: The greenish-yellow or brown colour of urine and its yellow foam indicates the presence of bile pigments, bilirubin and biliverdin, in urine. These are present in undetectable amounts in the urine of a healthy person. However, in case of hepatic jaundice the presence of bile pigments is increased. Fouchet's test is used to detect the presence of bile pigments in urine.

To perform Fouchet's test, take about 10-15 cm$^3$ of urine sample in a boiling tube and add a few crystals of magnesium sulphate (or 2-3 drops of its saturated solution) and about 5 cm$^3$ of 10% solution of barium chloride. A white precipitate is obtained, allow it to settle down and filter. Open the filter paper and dry the precipitate in the folds of filter papers. Place a drop of Fouchet's reagent on the dry precipitate. Development of a green or bluish-green colour indicates the presence of bile pigments.

Based on the information given above, perform the following experiment to check for the presence of abnormal constituents, if any, in the sample provided.
Experiment 2: To perform chemical tests on the given sample of urine to check for the presence of abnormal constituents.

**Principle**

This experiment is based on the details of the chemical tests, for abnormal constituents in urine, given above.

**Requirements**

<table>
<thead>
<tr>
<th>Measuring cylinder</th>
<th>1% solution of sodium nitroprusside</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling tube</td>
<td>10% solution of barium chloride</td>
</tr>
<tr>
<td>Test tubes</td>
<td>Liquor ammonia</td>
</tr>
<tr>
<td>Funnel</td>
<td>Saturated benzidine solution</td>
</tr>
<tr>
<td>Dropper</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>Benedict's solution</td>
<td>Sulphur powder</td>
</tr>
<tr>
<td>Dilute acetic acid</td>
<td>Magnesium sulphate</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>Fouchet's reagent</td>
</tr>
</tbody>
</table>

**Procedure**

Perform the experiments given in second column of Table 4.3 and record your observations and the inferences in the appropriate columns. You are required to give a clinical interpretation of the results obtained.

### Table 4.3: Chemical Tests for Abnormal Constituents in Urine

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Experiment</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><strong>Benedict's Test for reducing sugars:</strong> Take about 2.5 cm³ of Benedict's solution in a test tube and add 4 drops of given urine sample to it with the help of a dropper. Mix and keep the test tube in a water bath for about 5 minutes.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><strong>Heat test for proteins:</strong> Fill roughly three fourth of a test tube with the urine sample and gently heat the upper part of the test tube (take care not to boil). Add 2-3 drops of dilute acetic acid solution.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td><strong>Rothera's test for ketone bodies:</strong> Take 2-3 cm³ of urine sample in a test tube, saturate it with ammonium sulphate. Add 4-5 drops of freshly prepared solution (1%) of sodium nitroprusside, mix and add some liquor ammonia to it along the sides of the test tube. Observe the region where the urine and ammonia layers meet.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td><strong>Benzidine Test for Blood:</strong> Take about 5 cm³ of urine sample in a test tube and add 2 drops of saturated benzidine solution, mix and add 3 cm³ of hydrogen peroxide to it and look for a color change.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td><strong>Hay's test for bile salts:</strong> Fill two thirds of a test tube with urine sample and take about same amount of water (control) in a second test tube. Gently sprinkle sulphur powder on both the liquids and observe.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td><strong>Fouchet's test for bile pigments:</strong> Take about 10-15 cm³ of urine sample in a boiling tube and add a few crystals of magnesium sulphate and about 5 cm³ of 10% solution of barium chloride. Allow the white precipitate to settle down and filter. Dry the precipitate and put a drop of Fouchet's reagent on it.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Check Your Progress 3

1) A sample of urine on analysis gave the following results:
   - Acid response to litmus
   - Negative Benedict's test
   - Negative heat test
   - Positive Rothert's test
   - Negative Benzidine test
   - Negative Fouchelet's test

   What are the possible reasons for such a response?

2) A sample of urine on analysis gave the following results:
   - 2800 cm$^3$ of urine/24 hours
   - Positive Benedict's test
   - Negative heat test

   What are the possible reasons for such a response?

4.5 LET US SUM UP

Urine analysis is an important diagnostic tool. Both physical and chemical examination are useful in diagnosis as well as in following the response of the patient to the treatment. In this unit the composition of urine in health and its change under pathological conditions has been discussed to highlight the importance of urine analysis in diagnostics.
For the results of urine analysis to be meaningful, the sample must represent the true condition of the patient. For this different methods of collection and preservation of urine have been described.

The physical examination of urine refers to its analysis in terms of its color, appearance, odour (smell), amount, specific gravity (thickness) and reaction to litmus. While in chemical analysis the presence of abnormal constituents like reducing sugar, protein, ketone bodies, blood, bile pigments and bile salts in urine is tested with the help of chemical tests, the reducing sugars respond positively to the Benedict’s test, ketone bodies are checked by Rothera’s test while the heat denaturation test establishes the presence of protein in urine. The presence of blood in urine is tested by Benzidine test while bile salts and bile pigments are tested by Hay’s and Fouchet’s tests respectively.

4.6 ANSWERS TO CHECK YOUR PROGRESS

Check Your Progress 1

Urea, creatinine, uric acid, a number of pigments and certain enzymes are the main organic constituents, while chlorides, phosphates and sulphate of sodium, potassium, calcium, magnesium and ammonium are the chief inorganic constituents of urine.

Check Your Progress 2

If a sample of urine is not analysed immediately, several changes like, crystallization of solutes, decomposition of some abnormal constituents and growth of contaminating microorganisms can occur with time. The analysis of such a sample may not reflect the status of fresh urine and the diagnostic advantage of the analysis is lost. Therefore, if immediate analysis is not possible, it becomes necessary to preserve such samples.

Check Your Progress 3

1) Since the urine is acidic and tests positive in Rothera’s test, it appears to be a case of keto-acidosis. As the Benedict’s test is negative, this condition may be because of starvation.

2) It is a case of glycosuria probably due to diabetes mellitus.
Preparation of artificial urine: To perform the experiments on the physical and chemical examination of urine it may become difficult to arrange for samples. One option is to collect samples from the group and exchange them amongst the students. However in such a situation most of the samples happen to be normal and the students do not get to analyse the variety of possible abnormal urine samples. It is, therefore, advisable to fabricate such samples.

Preparation of Normal sample of urine:
Requirements per dm³

Urea: 18.2 g
Sodium chloride: 7.5 g
Potassium chloride: 4.5 g
Sodium phosphate: 4.8 g
Creatinine: 2.0 g
Albumin: (egg or bovine) 50 mg

Distilled water
To about 700 cm³ of distilled water add 18.2 g of urea and mix well till all the crystals are dissolved. Add 7.5 g of sodium chloride, 4.5 g of potassium chloride and 9.6 g of monobasic sodium phosphate to it and mix till the solution is clear. Check the pH with indicator paper, for normal urine it should be between 5 to 7; if the solution is out of this pH range the pH may be adjusted with 1N hydrochloric acid or with 1N sodium hydroxide as required.

Then, place a urine hydrometer into the solution and dilute with water until the solution has specific gravity between 1.015 and 1.025. This solution of “normal urine solution” can be kept refrigerated for several weeks or frozen in plastic containers for months. Before use, the stock solution should be brought to room temperature. To this solution add 2.0 g of creatinine and 50 mg of egg or bovine albumin and mix slowly. If needed add some distilled water to make the volume to 1 dm³.

Preparation of abnormal samples of urine: The artificial normal human urine prepared above may be modified by adding suitable additives to represent different diseases. More than one abnormality could be included in a given sample of urine by mixing the appropriate amounts of the respective additives. For example, to create a urine sample of a patient with diabetes mellitus one may add additives for glycosuria and ketonuria. The details of the additives to be added to create urine samples representing different pathological conditions are given below.

Glycosuria: Add a minimum of 60 mg of glucose (dextrose) to 100 cm³ of “normal urine solution” to obtain a minimally detectable level of glycosuria. A moderated to high level of glycosuria can be achieved by adding 250 to 500 mg of glucose to 100 cm³ of the solution.

Proteinuria: Add at least 30 mg of albumin per 100 cm³ of normal urine solution to get a positive result. Severe renal damage may be exemplified by adding 100 mg or more of albumin to 100 cm³ of the urine solution.

Ketonuria: Add a minimum of 10 mg of acetoacetic acid or at least 0.1 cm³ of acetone to 100 cm³ of normal urine solution.

Haematuria: Add a little of heparinized or defibrinated sheep blood normally used in microbiological and cell culture work.

Specific gravity: Sp.Gr. of the normal urine solution may be lowered by adding distilled water.

Reaction to litmus: Acidic urine can be obtained by adjusting the pH of the normal urine solution to a pH of 4.0 to 4.5 with 1N HCl. While for alkaline response 1N NaOH can be used for adjusting the pH to 8 to 9.
UNIT 5  CLINICAL ANALYSIS OF BLOOD

Structure
5.0  Objectives
5.1  Introduction
5.2  Collection and Preservation of Blood
5.3  Haemoglobin Estimation
5.4  Blood Sugar Estimation
  5.4.1  Collection of Samples
  5.4.2  Interpretation of Blood Sugar Test
  5.4.3  Methods of Estimation of Blood Sugar
  5.4.4  Glucose Tolerance Test (GTT)
5.5  Serum Bilirubin Estimation
5.6  Serum Urea and Creatinine Estimation
5.7  Serum Cholesterol Estimation
5.8  Let Us Sum Up
5.9  Answers to Check Your Progress

5.1 OBJECTIVES

After going through this unit and performing the observation-demonstration experiments, you should be able to:
- collect and preserve the blood samples needed for various investigations;
- describe the methods of separation of serum and plasma from the whole blood;
- explain the methods of preparation of slides for certain investigations;
- interpret the results of blood sugar test;
- perform oral glucose tolerance test;
- diagnose the patients for diabetes mellitus, based on blood sugar test and glucose tolerance test;
- perform the Sahli’s method of haemoglobin estimation from the sample provided;
- observe and understand the application of colorimetric method for estimation of haemoglobin;
- observe and explain the method used for estimation of serum bilirubin; and
- interpret the results of blood urea and creatinine in kidney function.

5.0 INTRODUCTION

Blood can be considered to be a circulating tissue that helps in the transport of nutrients, waste products, gases and hormones. It maintains body pH, fluid balance and body temperature. It also has a defence mechanism against infection. Prevention of hemorrhage is a mechanism of self-preservation, as blood gets clotted at the site of an injury and, thus prevents the loss of blood from the body. The blood constitutes 6.8% of total body mass in adults. The blood volume is about 80 ml per kg body weight in men and 65 ml per kg body weight in women. A 70 kg man thus has about 5.5 litres of blood.

In this practical session, we shall study methods of collection and preservation of blood. We also aim to explain the methods of estimating haemoglobin, blood sugar, serum bilirubin and blood urea and creatinine by using observation-demonstration practical sessions.
5.2 COLLECTION AND PRESERVATION OF BLOOD

In this section, first, you will study some methods of collection of blood.

Collection of Blood

Capillary or venous blood is taken for almost all kinds of investigations. Sometimes, but rarely, arterial blood is also taken. Here we will study only the collection of capillary and venous blood.

Capillary Blood

Capillary blood is similar in composition to arterial blood. It is obtained from a finger or thumb. Some use the lobe of an ear. In the case of infants, the heel is used. Generally the tip of middle or the third finger of the left hand is chosen and massaged a little to obtain one or two drops of blood needed:

- to prepare the slides for total leucocyte count (TLC) and differential leucocyte count (DLC),
- for the detection of the malarial parasite, or
- for the estimation of haemoglobin.

The upper portion of the middle or third finger is cleaned with spirit thoroughly. It is allowed to dry completely (with a little pressure by the thumb of the technician who is going to take the blood). A prick with a sterilized needle or disposable needle or with a lancet is given. When a drop of blood is formed, it is taken at one side of the slide and, a thin film of blood is spread with the help of another slide. In case of estimation of haemoglobin, the blood is directly sucked by a micropipette and put in the tube of haemoglobinometer.

Meanwhile, a cotton plug soaked in spirit is kept on the finger till bleeding is stopped.

Venous Blood

Most frequently collected blood is the venous blood, which may be taken from any prominent vein. A vein on the front of the elbow or forearm is generally chosen. The arm should be kept warm as it improves the circulation and distends the vein. To the extended arm, a tourniquet is firmly tied a few inches above the elbow. However, this should not obliterate the arterial pulse at the wrist. The skin over the vein is cleared with spirit. A well sharpened sterile hypodermic needle or disposable needle fixed to a syringe of appropriate capacity (2 ml, 5 ml or 10 ml), depending upon the number of investigations to be carried out, is inserted into the vein. If blood appears, the tourniquet is released, and a small pad of cotton soaked with spirit is placed on the arm where the needle was inserted, and then, the needle is withdrawn. The cotton is held on firmly for a few minutes until the bleeding stops. The needle is removed from the syringe and the blood is transferred to an appropriate vial. The needle and syringe are immediately washed with water to remove the sticking blood and then sterilized for the next patient.

Precautions

1) To prevent infection, the needle should be well sterilized or it is better to use disposable needles.
2) The syringe should also be very well sterilized.
3) The syringe, needle and the tube should be moisture free.
4) Excessive venous stasis by prolonged application of tourniquet should be avoided.
5) Blood should be withdrawn by needle of gauge less than 21.
6) Clotted blood should not be disturbed for 20-30 minutes.
Preservation of Blood for Different Investigations

The blood is collected in different vials for different investigations. Some investigations are done with serum, or plasma and some are done with whole blood. As time is required between the collection of blood and the actual conduct of the investigation, the whole blood is preserved by reacting them with some anticoagulant which is already kept in the vial. The blood from the vein is poured in such vials. Thus blood is prevented from clotting.

The following chart shows the type of vial which should be used for specific investigations.

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Anticoagulant</th>
<th>Mass of the anticoagulant per ml of blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Blood sugar test and glucose tolerance test</td>
<td>Potassium oxalate and sodium fluoride</td>
<td>3mg</td>
</tr>
<tr>
<td>2. Blood urea</td>
<td>Potassium oxalate</td>
<td>1 mg</td>
</tr>
<tr>
<td>3. Blood counts - ESR (erythrocyte sedimentation rate) and PVC (packed cell volume)</td>
<td>Salts of ethylene diamine tetra acetic acid (EDTA)</td>
<td>2.3 mg</td>
</tr>
<tr>
<td>4. ESR</td>
<td>Potassium oxalate and ammonium oxalate in 2:3 ratio</td>
<td>a pinch</td>
</tr>
<tr>
<td>5. Some special investigations</td>
<td>Heparin (powder or liquid)</td>
<td>—</td>
</tr>
<tr>
<td>6. For blood banking</td>
<td>Acid citrate-dextrose (ACD)</td>
<td>—</td>
</tr>
</tbody>
</table>

Whenever possible, preparation of blood films and the necessary tests should be done immediately.

For serum, the whole blood is kept undisturbed in a plain vial for about 20-30 minutes. Then it is centrifuged. The clear pale yellow supernatant (serum) is withdrawn by a pasteur pipette into a small test tube. As the contents of serum and plasma are almost same except for fibrinogen, generally serum is chosen for the investigations. Serum is analysed for the estimation of calcium, cholesterol, bilirubin, uric acid, etc.

For plasma, the whole blood collected with anticoagulants is centrifuged immediately without waiting to get it coagulated. The supernatant obtained is called plasma.

In the next section, we shall discuss the method of estimation of blood sugar.

Check Your Progress 1

Name the anticoagulant used for blood sugar test and glucose tolerance test.

5.3 HAEMOGLOBIN ESTIMATION

There are many methods of estimating the concentration of haemoglobin in blood. They vary widely in terms of performance, complexity of apparatus and accuracy. In general, the
less complex the apparatus, the less accurate is the method. Most methods involve a comparison of an unknown solution and a coloured solution. Each method uses its own standard. Results should be expressed as grams haemoglobin per 100 ml of blood rather than percentage.

**Sahli Method**

The apparatus consists of a pipette that can measure 20 cmm of blood, a graduated diluting tube and a coloured standard.

Fill the dilution tube up to the mark 20 with 0.1 N-HCl. To this add 20 cmm blood observing due precautions as in the experiment in cell counts. Mix and allow to stand for five minutes. The end point is indicated by the development of brown colour, which is due to the formation of acid haematin.

Add distilled water till the colour of the haematin solution matches that of the standard. Take the reading indicating haemoglobin concentration. Alternatively, a reading may be taken when the colour of the solution is just a shade darker than that of the standard, and another when it is just a shade lighter. The average of the two readings gives the haemoglobin concentration.

The haemoglobinometer tube is graduated both in gm per 100 ml and percentage. The former readings are preferable because what constitutes 100% haemoglobin is arbitrary, and therefore varies from instrument to instrument.

**Photo-colorimetric Method**

This involves the use of photoelectric colorimeter that measures the amount of light absorbed by a solution. The light used in these instruments is of the wave length that corresponds with the part of the spectrum that is maximally absorbed by the solution being tested. The one that is commonly used is the cyanmethaemoglobin method.

**Principle**

Blood is diluted with Drabkin’s reagent.

NaHCO₃ 1 gm. Potassium cyanide: 20 mg,
Potassium Ferricyanide 50 mg, distilled water 100 ml.

Haemoglobin, methaemoglobin and carboxyhaemoglobin (but not sulphahemoglobin) are converted into cyanmethaemoglobin. The absorbance of the solution is measured in a photoelectric colorimeter provided with yellow-green filters because this compound has absorption spectrum with a peak at 540 nm. The optical density is compared with that of the standard.

The standard solution of cyanmethaemoglobin is available as such from ‘BDH’ as clinical reagent, containing 57.2 mg/100 ml.

**Procedure**

Add 0.02 ml of blood and 5 ml of Drabkin’s reagent. Mix well and wait for 10 min (the reaction time). The photo-electric colorimeter is set to zero with the reagent blank (Drabkin solution). Next the optical density of the standard (which should have been brought to room temperature) is measured, followed by the optical density of the sample.

**Calculation**

\[
Hb (g/100 ml) = \frac{O.D. \times \text{Conc. of Std.} \times \text{Dilution factor}}{\text{O.D. of sample}}
\]

**Recording and Analysis of Data**

Haemoglobin by Sahli’s method = gm/100 ml

Haemoglobin by Photo-colorimetric Method

*O.D. — Optical Density
5.4 BLOOD SUGAR ESTIMATION

Glucose is the principal sugar in blood. In this section, let us see the procedure for the collection of blood for carrying out blood sugar test. We shall then discuss the method of estimation of blood sugar.

5.4.1 Collection of Samples

Blood specimen in each case discussed below is collected from vein. Further, a mixture of 1 mg of sodium fluoride and 3 mg of potassium oxalate is used as the anticoagulant per ml of blood.

Specimen for Testing the Fasting Blood Sugar

For this, the specimen of blood should be taken after 10-12 hours of the last meal. Generally, it is taken in the morning before taking breakfast after the overnight fasting (say at 8 a.m., if the dinner was at 8 p.m.) The normal fasting blood sugar is around 80-110 mg per 100 ml of blood. It is the minimum level of blood sugar in an individual.

Specimen for Testing Post Parandial (PP) Blood Sugar

After taking the fasting blood specimen, the patient is given a good breakfast containing 100 g of carbohydrate or 100 g of glucose. The blood specimen is taken 2 hours after taking the breakfast in the same way as stated above. The PP blood sugar shows the maximum level of blood sugar which can be reached after a meal. The normal PP blood sugar level is between 120-160 mg/100 ml of blood.

Specimen for Testing Random Blood Sugar

Random blood sugar test is carried out using the blood taken from the vein at any time.
Before explaining the details of blood sugar test, let us see how to interpret the blood sugar test.

### 5.4.2 Interpretation of Blood Sugar Test

The following points may be kept in mind while interpreting the blood test results:

1) The normal fasting blood sugar is around 80-110 mg/100 ml of blood. It is the minimum level of blood sugar in an individual. The normal level of post-parandial blood glucose is 120 to 160 mg/100 ml.

2) If the fasting blood sugar value exceeds 110 mg per 100 ml, it is indicative of hyperglycaemia. One of the main causes of hyperglycaemia is diabetes mellitus, although there are other reasons also.

If the fasting blood glucose level falls below 45 mg./100 ml, it is indicative of hypoglycaemia. Hypoglycaemia could be due to:

1) starvation
2) excessive exercise
3) drugs, alcohol etc.
4) endocrine diseases

We shall now discuss the methods of estimation of blood sugar.

### 5.4.3 Methods of Estimation of Blood Sugar

This is an observation-demonstration practical.

#### Principle

In this method, as a first step, the proteins are to be removed and we have to obtain a colourless filtrate; otherwise the final colour development for colorimetric estimation will be interfered with.

#### Requirements

1) Sodium sulphate-copper sulphate solution (isotonic solution)
2) Sodium tungstate solution
3) Alkaline tartrate reagent
4) Arsenomolybdic acid (colour reagent)
5) Working standard glucose solution of concentration 1.25 mg/100 ml
6) Blood sample.

#### Procedure

**Step 1**

Place 0.1 ml of blood in a centrifuge tube. Add 3.8 ml of the isotonic solution and 0.1 ml of sodium tungstate solution to it. Gently mix the solution. Centrifuge the tube at 2000-2500 rpm. Collect the supernatant clear liquid. This is the test sample for further analysis.

**Step 2**

Label three test tubes as T (test), S (standard) and B (blank). Proceed with the addition of various solutions in these tubes as follows:
<table>
<thead>
<tr>
<th>Solution</th>
<th>T</th>
<th>S</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test sample</td>
<td>1.0 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Supernatant from step-1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard glucose solution</td>
<td></td>
<td>1.0 ml</td>
<td></td>
</tr>
<tr>
<td>Isotonic solution</td>
<td></td>
<td></td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Alkaline tartarate reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

Cover the tubes with cotton plugs. Keep them in a boiling water bath for 10 minutes.

**Step 3**

Cool the test tubes to room temperature. Add 3 ml of the colour reagent to each of the above three tubes. Allow the tubes to stand for 5 minutes at room temperature.

**Step 4**

Using a green filter (540 nm wavelength), measure % T of the test and standard solutions after adjusting for 100% T with the bank solution. Obtain the O.D. values using Table 6.2 of Practical 6.

\[
O.D_s = \frac{O.D_T}{O.D_s} \\
\]

**Calculation**

Calculate the amount of glucose present in the blood sample as follows:

\[
\text{Glucose concentration in blood} = \frac{O.D_T}{O.D_s} \times \text{Dilution factor} \times \text{Concentration of standard glucose solution} \\
\]

\[
= \frac{O.D_T}{O.D_s} \times 40 \times 1.25 \text{ mg/100 ml of blood} \\
= \frac{O.D_T}{O.D_s} \times 50 \text{ mg/100 ml of blood} \\
\]

(Note that 0.1 ml blood is diluted to 4 ml; it indicates a dilution factor of 40.)

**Report:** The concentration of glucose in blood sample is .................

**Interpretation**

........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................

**Check Your Progress 3**

Why do we remove protein from blood before the colorimetric estimation of glucose?

........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
5.4.4 Glucose Tolerance Test (GTT)

Glucose tolerance is defined as the capacity of the body to tolerate an extra load of glucose. Glucose tolerance test (GTT) is a definite diagnostic test for diabetes mellitus. As mentioned earlier, the fasting blood sugar concentration exceeding 120 mg per 100 ml is indicative of diabetes mellitus. If this value is between 110-120 mg per 100 ml, GTT should be performed to diagnose diabetes mellitus.

Glucose Tolerance Test

This is an observation-demonstration practical.

Principle

GTT is performed through the following stages:

- After the overnight fast lasting for 10-16 hours, the venous blood is taken from the patient and analysed for blood sugar as per details given in blood sugar estimation test.
- The patient is then given orally 75 g of glucose dissolved in 250-300 ml of water. The patient should be allowed 15 minutes to drink this. In children, the dose of glucose should be 1.75 g/kg body weight up to a maximum of 75 g.
- Again venous blood sample is collected 2 hours after the administration of glucose. This sample is also analysed for blood sugar as explained in sub-section 5.4.3.

In this experiment, you will not be doing glucose administration, collection of fasting and 2 hour samples of venous blood, and protein removal from the samples (see Step I in sub-sec. 5.4.3).

You will assume that all these steps have been done by your counsellor.

Let us now see the interpretation part of GTT

- The 2 hour venous blood glucose value in the range 120-160 mg per 100 ml occurs in a normal individual.
- The 2 hour venous blood glucose value in the range 160-180 mg per 100 ml is classified as impaired glucose tolerance. Patient with impaired glucose tolerance is to be followed for diabetes mellitus at a later date.

In severe diabetes, urine may show the presence of ketone bodies, Hence it is worth analysing urine also.

In general, glucose is detectable in urine only if blood glucose level is equal to or greater than 180 mg per 100 ml of blood. The blood glucose value of 180 mg per 100 ml of blood is called renal threshold and it may vary from person to person. Upto the renal threshold value, glucose does not appear in urine due to tubular reabsorption. If the blood glucose level is greater than the renal threshold, glucose appears in urine and, this is termed renal glycosuria. Glycosuria may be considered to be present when urine contains more glucose than 140 mg/dl per 100 ml. This occurs in diabetic cases and also under some other physiological conditions.

In untreated or inadequately treated diabetes mellitus, blood glucose levels rise and ketosis may develop; this could lead to diabetic hyperglycaemic coma. The blood sugar may increase up to 550 mg per 100 ml of blood.

If blood glucose falls below normal level, hypoglycaemia is said to be present. Hypoglycaemia could give rise to weakness, cold sweat, anxiety or even coma. In hypoglycaemia, blood sugar levels decrease upto 30 mg per 100 ml.

Some of the drugs which are to be withdrawn before GTT are oral contraceptives (1 month earlier), salicylates, thiamide, insulin, sulphonamides and oral hypoglycaemic agents.
If the 2 hour venous blood glucose value exceeds 180 mg/100 ml, the patient is diabetic. The patient is said to have decreased glucose tolerance.

If the 2 hour venous blood glucose value is less than 120 mg per 100 ml, the patient is said to have increased glucose tolerance. The patient is then hypoglycemic.

![Glucose tolerance curves](image)

**Fig. 5.1: Glucose tolerance curves**

**Precautions**

The following precautions must be observed while doing GTT:

i) Only venous blood must be taken for estimation.

ii) Patient must be taking a diet of 300 g of carbohydrate per day for at least the last 3 days.

iii) Patient should be in post-absorptive stage. Fasting should not be less than 10 hours and more than 18 hours.

iv) Patient should be seated and smoking prohibited during the test.

v) Patient should not be taking drugs which affect carbohydrate metabolism.

vi) The physical activity of the patient should be normal during the test. No exercise is allowed.

**Requirements**

1) Isotonic solution
2) Sodium tungstate solution
3) Alkaline tartarate reagent
4) Arsenomolybdic acid (colour reagent)
5) Working standard glucose solution of concentration 1.25 mg/100 ml
6) Samples A and B of protein-free blood.

**Procedure**

**Step 1**

In two test-tubes, labelled A and B, you are given two samples of protein-free blood. We have given below the contents of samples A and B. You proceed to Step 2 as such with these samples.
Sample A: The supernatant liquid obtained by centrifuging 0.1 ml of fasting blood, 3.8 ml of the isotonic solution and 0.1 ml of sodium tungstate solution.

Sample B: The supernatant liquid obtained by centrifuging 0.1 ml of blood taken 2 hours after glucose administration, 3.8 ml of the isotonic solution and 0.1 ml of sodium tungstate solution.

Step 2

Label four test tubes as T1, T2, S and B. Then proceed with the addition of various solutions as follows.

<table>
<thead>
<tr>
<th>Solution</th>
<th>T1</th>
<th>T2</th>
<th>S</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>1.0 ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample B</td>
<td></td>
<td>1.0 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard glucose</td>
<td></td>
<td></td>
<td>1.0 ml</td>
<td></td>
</tr>
<tr>
<td>Isotonic solution</td>
<td></td>
<td></td>
<td></td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Alkaline tartrate</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

From the above table, you can understand that
- the test tube T1 contains centrifugate derived from fasting blood (sample A),
- the test tube T2 contains centrifugate derived from the blood sample taken 2 hours after glucose administration (sample B),
- the test tube S contains standard glucose solution, and
- the test tube B (which is the blank) contains isotonic solution.

Cover the tubes with cotton plugs. Keep them in a boiling water bath for 10 minutes.

Step 3

Cool the test tubes to room temperature. To each of the test tubes T1, T2, S and B, add 3 ml of the colour reagent. Allow these test tubes to stand for 5 minutes at room temperature.

Step 4

Using green filter (540 nm) adjust for 100% T with the blank solution (test tube B). Measure the %T values of the test samples (in T1, T2) and the standard sample (in test tube S).

Obtain O.D. values using Table 6.2.

\[
\begin{align*}
O.D_S &= \\
O.D_{T1} &= \\
O.D_{T2} &= 
\end{align*}
\]

Calculation

Calculate the amount of glucose present in the blood sample as follows:

\[
\text{Concentration of glucose in fasting blood sample} = \frac{O.D_{T1}}{O.D_S} \times 40 \times 1.25 \text{ mg/100 ml of blood}
\]

\[
= \frac{O.D_{T1}}{O.D_S} \times 50 \text{ mg/100 ml of blood}
\]
(Multiplication by 40 is done to account for the dilution of 0.1 ml of blood to 4 ml i.e., by 40 times. The strength of standard glucose solution is 1.25 mg per 100 ml blood.)

Report

The concentration of glucose in the fasting blood (T1) is

The concentration of glucose in the blood sample (T2) taken 2 hours after glucose administration.

Interpretation

Check Your Progress 4

Define the following terms:

1) Impaired glucose tolerance

2) Decreased glucose tolerance

3) Increased glucose tolerance

5.5 SERUM BILIRUBIN ESTIMATIONS

Serum bilirubin estimation is one of the important investigations to diagnose any deviation/abnormality in the liver function. Some of the other tests for identifying any abnormality in liver function include estimating enzymes such as serum glutamate oxaloacetate transaminase (SGOT or Serum AST), serum glutamate pyruvate transaminase (SGPT or serum AST), serum alkaline phosphate and serum proteins etc, which we are not going to discuss here. We shall only discuss about serum bilirubin.
Bilirubin as you know is formed by the reticuloendothelial cells of the liver from ferroprophyrin ring obtained mainly from haemolysed erythrocytes and also from myoglobin and iron containing enzymes.

The normal level of bilirubin in blood is 0.3-1 mg/dl. In case of jaundice the level of bilirubin is usually more than 2-2.5 mg/dl in serum.

Bilirubin exists in blood in two forms—conjugated and unconjugated. Conjugation of bilirubin with glucuronic acid is accomplished in the liver. Conjugated bilirubin can pass through in the urine while unconjugated one cannot.

Bilirubin is excreted in the bile mainly in the form of bilirubin diglucuronide. In the intestine, it is converted to urobilinogen by the action of bacterial flora. Some of the urobilinogen is reabsorbed and excreted in the urine as urobilinogen. The rest is excreted in the faeces as stercobilinogen. Urobilinogen gets oxidised to urobilin by atmospheric oxygen after excretion in the urine and so also is stercobilinogen oxidised to stercobilin.

**Determination of serum bilirubin by Van-den Bergh Reaction**

Bilirubin is determined qualitatively and quantitatively by means of diazo reagent. Diazotised sulphanilic acid (sulphanilic acid treated with a mixture of sodium nitrite and hydrochloric acid) in the presence of bilirubin forms a red compound, azobilirubin, which is measured colorimetrically.

Two types of reaction can occur:

a) Direct and
b) Indirect

When the reaction is carried out in aqueous medium, the water soluble conjugated bilirubin reacts to give so called direct van den Bergh reaction. When the reaction is carried out in methanol, the intramolecular hydrogen bonds are broken; thus both conjugated and unconjugated pigments react giving a measure of total bilirubin.

**5.6 SERUM UREA AND CREATININE ESTIMATION**

Blood urea or serum urea is the most commonly performed investigation for assessing kidney function. The normal value of blood urea is up to 40 mg/100 ml, but it may fall during pregnancy. It usually increases in any condition which adversely affect normal kidney function. Renal function is affected by damage to the kidneys due to decreased blood flow or obstruction to the flow of urine.

Serum creatinine is more a specific diagnostic test than blood urea. It is normally below 1.6 mg/100 ml. However, the serum urea and creatinine are not good markers for diagnosis of early renal damage because their level in blood rises only after a significant kidney damage.

**5.7 SERUM CHOLESTEROL ESTIMATION**

You are already aware of the main functions of cholesterol in the body (section 3.3.2 of theory) and also its clinical relevance in relation to its elevated levels in blood. In this unit you would read about elevated levels in blood. A brief description of the measurement of cholesterol levels in serum and its clinical significance is also described.

Recollect that cholesterol like other types of lipid, is transported in blood as their protein complexes called lipoproteins (See Section 6.4.1 of theory) lipoproteins are micelles of high molecular weight that transport non-polar lipids (triacylglycerols and cholesterylesters) via plasma. Each lipoprotein particle consists of an outer monolayer of protein (called apoprotein and polaripids).
Phospholipid and unesterified cholesterol (and an inner core of neutral lipids (triaclylglycerols and cholesteryl esters). The core neutral lipids are primarily inactive passengers, whereas, the apolipoproteins are mainly responsible for further metabolism of the particle by binding to specific enzymes or transport proteins on cell membranes.

**Clinical Significance**

The hyperlipoproteinemias are disturbances of lipid transport that result from abnormalities in the synthesis or degradation of plasma lipoproteins. The elevated lipoproteins can cause two life-threatening diseases: atherosclerosis and pancreatitis.

Lipoproteins are quantitated by estimating the cholesterol content in them. Specimens of blood should be taken after the patient is kept for a week on normal diet and in the morning after fasting for 12 hours. Freshly separated plasma or serum is used. Heparin or Ethylene Diamine Tetra Acetic aid (EDTA) is used as the anticoagulant.

**Colorimetric Estimation of Serum Cholesterol**

**Step 1:** Extract total cholesterol from 0.1 ml serum by mixing thoroughly with a glass rod for 15 minutes with 9.9 ml of Ferric chloride. Acetic Acid solution (Fecl3-Acetic Acid) and centrifuge at 2000 rpm for 10 minutes.

**Step 2:** In three tubes, prepare the Test (T), Standard (S) and Bank(B) reaction mixture with Fecl3 – Acetic Acid (4.9 ml in T, 4.9 ml in S and 5.0 ml in B), 0.1 ml Cholesterol standard only in S. Add 3.0 ml concentrated H2SO4 to all the three tubes. Mix well and keep in a water bath at 50-60°C for 10 minutes.

**Step 3:** Cool the tubes to room temperature. Now measure the absorbance (or % transmittance) of each in a Colorimeter. Calculate the cholesterol content of the Test (serum/sample in mg/100 ml of serum (mg/deciliter or mg/dl)

**Interpretation:**

1) Normal level of total serum cholesterol: 150-250 mg/dl
2) Hypercholesterolemia – serum cholesterol > 250mg/dl
3) Hypocholesterolemia – serum cholesterol < 100 mg/dl
4) Normal LDL Cholesterol: < 150mg/dl
5) Normal HDL Cholesterol: > 40mg/dl

HDL-Cholesterol values show a negative risk factor for coronary heart disease. That is, higher the HDL-Cholesterol in blood, less is the risk for developing coronary heart diseases (CHD). Whereas, in case of LDL-cholesterol, higher levels actually increase the risk of CHD.
5.8 LET US SUM UP

In this practical, we have explained the methods of collection and preservation of blood. We have discussed the procedures for estimation of haemoglobin, blood sugar test and glucose tolerance test. We have given some hints which will be useful in interpreting the results of blood sugar test and glucose tolerance test. We have also explained about the estimation of serum bilirubin. We have discussed about the estimation of blood urea and serum creatinine. This will help you to follow the observation demonstration you will have in the laboratory. In the end cholesterol estimation and its clinical significance has also been given.

5.9 ANSWERS TO CHECK YOUR PROGRESS

Check Your Progress 1

A mixture of 1 mg sodium fluoride and 3 mg of potassium oxalate is used as the anticoagulant per ml of blood, for Blood Sugar Test and Glucose Tolerance Test.

Check Your Progress 2

1) Add distilled water till the colour of the haematin solution matches that of the standard.

2) Sahli method can be done anywhere — universally, easy to perform and gives not very accurate result.

Photocolorimetric method can be done in well established laboratory. It is a cumbersome procedure and gives more accurate results.

Check Your Progress 3

Proteins are removed to obtain a colourless and clear filtrate. Otherwise the final colour development for colorimetric estimation will be interfered with.

Check Your Progress 4

1) The 2 hour venous blood glucose value in the range 160-180 mg per 100 ml is classified as impaired glucose tolerance.

2) If the 2 hour venous blood glucose value is more than 180 mg/100 ml, the patient is said to have decreased glucose tolerance.

3) If the 2 hour venous blood glucose value is less than 120 mg/100 ml, the patient is said to have increased glucose tolerance.
UNIT 6 CLINICAL ANALYSIS OF CEREBROSPINAL FLUID

Structure

6.0 Objectives

6.1 Introduction

6.2 Cerebrospinal Fluid (CSF)
  6.2.1 Functions of CSF
  6.2.2 Characteristic Features of CSF
  6.2.3 Collection and Preservation of CSF

6.3 Analysis of Cerebrospinal Fluid
  6.3.1 Estimation of Proteins in CSF
  6.3.2 Estimation of Glucose in CSF

6.4 Let Us Sum Up

6.5 Answers to Check Your Progress

6.0 OBJECTIVES

After reading this practical and performing the tests mentioned in it, you should be able to:

- explain the methods of collection and preservation of CSF;
- state the normal constituents of CSF; and
- estimate the amount of proteins and glucose in CSF and state the probable reasons for any abnormal values.

6.1 INTRODUCTION

You have learnt in Practical 3 that urine and cerebrospinal fluid (CSF) are the two major body fluids. In Practical 4 you learnt that urine chiefly acts as a route for the elimination of waste products, while CSF has other functions, apart from that of excretion. The presence of various chemical compounds, in specified amounts, in these fluids, reflects the normal metabolic state of the organism. However, in the event of any alteration in the metabolic functions, caused by damage to any organ or tissue, the quantity of the compounds in the fluids, shows an increase or decrease. Thus, the analysis of the body fluids for certain specific biochemical components, becomes necessary to gauge the deviation from the normal metabolic level. Such an analysis, then assumes considerable significance as an important diagnostic tool.

In Practical 3 we discussed some qualitative tests for proteins, carbohydrates and triglycerides. In Practical 4 we discussed the methods of testing urine.

In this practical you will have a chance to study cerebrospinal fluid (CSF) through observation-demonstration. In order to make useful inferences from these tests, we shall discuss the constituents of CSF in normal and pathological conditions. You will observe the performance of two experiments that deal with the estimation of proteins and glucose in CSF. We shall make use of a colorimeter for these two experiments. Thus in this unit, you will come across both the qualitative and quantitative techniques.
6.2 CEREBROSPINAL FLUID (CSF)

The analysis of cerebrospinal fluid is required in the clinical investigation of the central nervous system which consists of the brain, spinal cord and peripheral nerves. Cerebrospinal fluid is a clear and colourless fluid which fills the ventricles, cisternae and subarachnoid spaces of brain and spinal cord.

6.2.1 Functions of CSF

The main functions of CSF are:

1) To support and protect the delicate structures of the brain and spinal cord;
2) To keep the brain and spinal cord moist;
3) To act as a cushion and shock absorber against injuries;
4) To act as a reservoir of fluid to regulate the contents of the cranium (when the volume of brain or blood increases, the CSF is drained away; more CSF is retained, when the brain shrinks).

6.2.2 Characteristic Features of CSF

The constituents of CSF under normal and abnormal conditions are explained hereunder.

Normal conditions: Some of the characteristic features of CSF under normal health conditions are given below in Table 6.1.

<table>
<thead>
<tr>
<th>Table 6.1: Characteristic Features of Normal CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volume</td>
</tr>
<tr>
<td>Specific gravity</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Colour</td>
</tr>
<tr>
<td>Transparency</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Proteins</td>
</tr>
<tr>
<td>Bilirubin</td>
</tr>
</tbody>
</table>

Abnormal conditions: The abnormal conditions with respect to glucose and proteins are discussed below:

- The protein content is increased in case of haemorrhage into the CSF (for example cerebral haemorrhage). Protein content is also increased in inflammatory diseases like meningitis, encephalitis, tumors of spinal cord, etc.
- The CSF glucose is normal or slightly elevated in encephalitis and poliomyelitis. In meningitis, glucose level is markedly decreased and may reach up to zero level.

Keeping in view the clinical importance of glucose and proteins in CSF, we are going to estimate these two. Such a quantitative study could help in the diagnosis of the pathology involved regarding brain and other areas of central nervous system.

The methods employed for the determination of glucose and proteins in CSF are different from those used in the analysis of urine. More sensitive methods are needed for CSF analysis because:
1) CSF sample is obtained in small amounts;

2) Concentration of glucose and proteins in CSF is much less.

6.2.3 Collection and Preservation of CSF

Collection of CSF

CSF is collected by lumbar puncture in which, a large bore needle is passed between the 3rd and 4th lumbar vertebrae, into the subarachnoid space, with the patient lying in a lateral position the fluid is allowed to flow automatically.

The first few drops of the fluid are discarded and the rest of the fluid is collected in sterile containers. The entire procedure is done under strict asepsis.

Preservation

It is advisable to carry out the investigations on CSF as soon as possible after collection. However, if there is an expected delay, the CSF sample should be properly preserved by:

- refrigeration at temperatures between 2°C and 10°C, and
- adding a small amount of sodium fluoride (NaF) to prevent glycolysis (that is, oxidation of glucose).

6.3 ANALYSIS OF CEREBROSPINAL FLUID

The estimations of proteins and glucose in CSF are to be performed using light intensity measurement. A colorimeter is used for this purpose.

6.3.1 Estimation of Proteins in CSF

Observation-Demonstration

Principle

For the estimation of proteins in CSF, turbidimetry is employed. Turbidimetry involves the measurement of turbidity of solutions. Turbidimetry is based on the measurement of the amount of light transmitted by a turbid (or opalescent) solution. In a turbid solution, an insoluble product of a reaction may be suspended in a solution in a finely divided form. When light is passed through such a suspension, part of it is scattered in different directions and the rest of it is transmitted. Thus the intensity of the light transmitted by a solution is decreased (due to turbidity). Turbidimetry actually measures this decrease in transmitted light. A colorimeter can be used to measure the turbidity of solution. The principles of colorimetry and turbidimetry have been explained in Practical 1 of this section. A colorimeter is generally used to measure the intensity of light transmitted by a coloured solution.

The turbidity is measured in terms of percentage transmittance (% T). The logarithm of percentage transmittance is called absorbance (A) or optical density.

\[ \text{O.D.} = \log \frac{100}{T}, \text{ where } T \text{ is } \% \text{ transmittance.} \]

Although modern colorimeters give O.D. values directly, a chart is given in Table 6.2 which is useful in calculating O.D. from % transmittance.
Table 6.2: Relationship Between Per Cent Transmittance (% T) and Optical Density (O.D.)

<table>
<thead>
<tr>
<th>% T</th>
<th>O.D.</th>
<th>% T</th>
<th>O.D.</th>
<th>% T</th>
<th>O.D.</th>
<th>% T</th>
<th>O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.000</td>
<td>26</td>
<td>0.585</td>
<td>51</td>
<td>0.292</td>
<td>76</td>
<td>0.119</td>
</tr>
<tr>
<td>2</td>
<td>1.699</td>
<td>27</td>
<td>0.569</td>
<td>52</td>
<td>0.284</td>
<td>77</td>
<td>0.114</td>
</tr>
<tr>
<td>3</td>
<td>1.523</td>
<td>28</td>
<td>0.553</td>
<td>53</td>
<td>0.276</td>
<td>78</td>
<td>0.108</td>
</tr>
<tr>
<td>4</td>
<td>1.398</td>
<td>29</td>
<td>0.538</td>
<td>54</td>
<td>0.268</td>
<td>79</td>
<td>0.102</td>
</tr>
<tr>
<td>5</td>
<td>1.301</td>
<td>30</td>
<td>0.523</td>
<td>55</td>
<td>0.260</td>
<td>80</td>
<td>0.097</td>
</tr>
<tr>
<td>6</td>
<td>1.222</td>
<td>31</td>
<td>0.509</td>
<td>56</td>
<td>0.252</td>
<td>81</td>
<td>0.092</td>
</tr>
<tr>
<td>7</td>
<td>1.155</td>
<td>32</td>
<td>0.495</td>
<td>57</td>
<td>0.244</td>
<td>82</td>
<td>0.086</td>
</tr>
<tr>
<td>8</td>
<td>1.097</td>
<td>33</td>
<td>0.481</td>
<td>58</td>
<td>0.237</td>
<td>83</td>
<td>0.081</td>
</tr>
<tr>
<td>9</td>
<td>1.046</td>
<td>34</td>
<td>0.469</td>
<td>59</td>
<td>0.229</td>
<td>84</td>
<td>0.076</td>
</tr>
<tr>
<td>10</td>
<td>1.000</td>
<td>35</td>
<td>0.456</td>
<td>60</td>
<td>0.222</td>
<td>85</td>
<td>0.071</td>
</tr>
<tr>
<td>11</td>
<td>0.959</td>
<td>36</td>
<td>0.444</td>
<td>61</td>
<td>0.215</td>
<td>86</td>
<td>0.066</td>
</tr>
<tr>
<td>12</td>
<td>0.921</td>
<td>37</td>
<td>0.432</td>
<td>62</td>
<td>0.208</td>
<td>87</td>
<td>0.060</td>
</tr>
<tr>
<td>13</td>
<td>0.886</td>
<td>38</td>
<td>0.420</td>
<td>63</td>
<td>0.201</td>
<td>88</td>
<td>0.056</td>
</tr>
<tr>
<td>14</td>
<td>0.854</td>
<td>39</td>
<td>0.409</td>
<td>64</td>
<td>0.194</td>
<td>89</td>
<td>0.051</td>
</tr>
<tr>
<td>15</td>
<td>0.824</td>
<td>40</td>
<td>0.398</td>
<td>65</td>
<td>0.187</td>
<td>90</td>
<td>0.046</td>
</tr>
<tr>
<td>16</td>
<td>0.796</td>
<td>41</td>
<td>0.387</td>
<td>66</td>
<td>0.180</td>
<td>91</td>
<td>0.041</td>
</tr>
<tr>
<td>17</td>
<td>0.770</td>
<td>42</td>
<td>0.377</td>
<td>67</td>
<td>0.174</td>
<td>92</td>
<td>0.036</td>
</tr>
<tr>
<td>18</td>
<td>0.745</td>
<td>43</td>
<td>0.367</td>
<td>68</td>
<td>0.167</td>
<td>93</td>
<td>0.032</td>
</tr>
<tr>
<td>19</td>
<td>0.721</td>
<td>44</td>
<td>0.357</td>
<td>69</td>
<td>0.161</td>
<td>94</td>
<td>0.027</td>
</tr>
<tr>
<td>20</td>
<td>0.699</td>
<td>45</td>
<td>0.347</td>
<td>70</td>
<td>0.155</td>
<td>95</td>
<td>0.022</td>
</tr>
<tr>
<td>21</td>
<td>0.678</td>
<td>46</td>
<td>0.337</td>
<td>71</td>
<td>0.149</td>
<td>96</td>
<td>0.018</td>
</tr>
<tr>
<td>22</td>
<td>0.658</td>
<td>47</td>
<td>0.328</td>
<td>72</td>
<td>0.143</td>
<td>97</td>
<td>0.013</td>
</tr>
<tr>
<td>23</td>
<td>0.638</td>
<td>48</td>
<td>0.319</td>
<td>73</td>
<td>0.137</td>
<td>98</td>
<td>0.009</td>
</tr>
<tr>
<td>24</td>
<td>0.620</td>
<td>49</td>
<td>0.310</td>
<td>74</td>
<td>0.131</td>
<td>99</td>
<td>0.004</td>
</tr>
<tr>
<td>25</td>
<td>0.602</td>
<td>50</td>
<td>0.301</td>
<td>75</td>
<td>0.125</td>
<td>100</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Proteins in CSF are precipitated as a fine white suspension on treating with an acid like trichloroacetic acid. The measurement of O.D. of this suspension helps us in estimating the quantity of protein in the specimen.

The normal level of proteins in CSF is 20-40 mg per 100 ml. In case of the following abnormal conditions, increase in CSF proteins is generally observed:

- haemorrhages,
- inflammation, and
- encephalitis, polio etc.

Requirements

- Working standard protein solution – 50 mg/100 ml solution of bovine serum albumin (BSA).

3% Trichloroacetic acid (TCA) solution

Test sample

Procedure

Take three test tubes and label them separately as B (blank), S (standard), and T (test). Proceed with the additions of solutions as follows (T is the test sample or unknown sample).
Mix the contents of each tube well and allow them to stand for 5 minutes.

Measure the transmittance (%T) of B, S and T solutions in a colorimeter using a blue filter (to obtain light of wavelength 450 nm).

Using Table 6.2 the values of % T are converted into O.D. values.

\[
\begin{align*}
O.D_B &= \_ \\
O.D_S &= \_ \\
O.D_T &= \_ \\
\end{align*}
\]

**Calculation**

\[
\text{Concentration of test sample (expressed as mg/100 ml)} = \frac{O.D_T - O.D_B}{O.D_S - O.D_B} \times \text{Concentration of standard solution}
\]

\[
= \frac{O.D_T - O.D_B}{O.D_S - O.D_B} \times 50 \text{ mg/100 ml}
\]

(The concentration of standard proteins used is 50 mg/100 ml)

**Report of Observation**

The concentration of proteins in CSF is:

The following abnormalities may be present:

Note that absorbance of the blank (O.D_B) is subtracted from the absorbance values of the test and standard samples to eliminate absorbance due to the medium.
6.3.2 Estimation of Glucose in CSF

The normal glucose level in CSF is about 50-80 mg in 100 ml. This is about 65-70% glucose found in plasma or blood. Further, following a meal, CSF glucose increases, like blood glucose, though the extent of increase is slightly less. After a gap of 3 or 4 hours following a meal, blood and CSF glucose levels equilibrate. Thus, while estimating glucose in CSF, determination of blood glucose also should be done simultaneously.

Decrease in the glucose levels of CSF is of great significance. Low levels of CSF glucose are seen in the following conditions:

- pyogenic meningitis (glucose level is 10-20 mg/100 ml).
- tuberculous meningitis (glucose level is 30-50 mg/100 ml).

For CSF glucose determination, a fasting CSF specimen is used. We make use of alkaline copper reduction for CSF glucose determination.

Principle

Glucose and other sugars possessing a free aldehyde or keto group, are converted into their corresponding alcohols in an alkaline medium. These are termed enediols, which are strong reducing agents. The enediols reduce the cupric ions (in cupric sulphate) to cuprous ions (as cuprous oxide). Under carefully controlled conditions, the cuprous oxide formed is directly proportional to the quantity of glucose present. The cuprous oxide so formed, then reduces the molybdenum in arsenomolybdic acid to give a final product that has a blue colour. The intensity of blue colour is directly proportional to the glucose content of the sample. Using a colorimeter, the O.D. is measured which is used in calculating the glucose content.

The method described below is the same one that is normally used for the determination of glucose in blood, which we have seen in Practical 5.

Requirements

1) Sodium sulphate-copper sulphate solution known as isotonic solution
2) Sodium tungstate solution
3) Alkaline tartarate reagent (alkaline reagent)
4) Arsenomolybdic acid reagent (colour reagent)
5) Working standards glucose solution of concentration 1.25 mg/100 ml
6) Test sample

Procedure

Step 1

Place 0.1 ml of the test sample in a centrifuge tube, add 3.8 ml of the isotonic solution and 0.1 ml of sodium tungstate solution to it. Gently mix the solution and allow the proteins in the sample to precipitate as a white suspension, (sodium tungstate is responsible for precipitating proteins). Place the centrifuge tube in rotor of a centrifuge and balance it with another centrifuge tube containing the same amount (about 4.0 ml) of water. Centrifuge the tubes at 2000-2500 rpm (revolutions per minute) for 5-10 minutes. Collect the clear liquid at the top; proteins settle down at the bottom of the tube. The clear liquid at the top is to be used in Step 2 as test sample.
Step 2

Label three test tubes as B (blank), Standard (S) and T (test). Then proceed with the addition of various solutions as follows:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Test Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test sample (T)</td>
<td>T 1.0 ml</td>
</tr>
<tr>
<td>(supernatant from Step 1)</td>
<td></td>
</tr>
<tr>
<td>Standard glucose (S)</td>
<td></td>
</tr>
<tr>
<td>Isotonic solution (B)</td>
<td>-</td>
</tr>
<tr>
<td>Alkaline tartarate reagent</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

Cover the tubes with cotton plugs and keep them in a boiling water bath for 10 minutes.

Step 3

Cool the test tubes to room temperature. Add 3 ml of the colour reagent to each of the three tubes. Allow the tubes to stand for 5 minutes (at room temperature) for the complete development of colour (green in this case).

Step 4

Using a green filter (wavelength - 540 nm) measure the % transmittance (% T) of the test and standard solution, after adjusting the instrument (that is colorimeter*) to 100% T with the blank solution. Convert the %T values into optical density (O.D.) values using the chart given in Table 3.2.

\[
O.D. = \frac{O.D.}{O.D.}
\]

Calculation

Calculate the amount of glucose present in the CSF sample as follows:

\[
\text{Concentration of glucose in CSF sample} = \left( \frac{O.D._T}{O.D.} \right) \times \frac{\text{Concentration of standard solution}}{\text{Dilution factor}}
\]

\[
= \frac{O.D._T}{O.D.} \times 1.25 \times 40 \text{ mg glucose/100 ml of CSF}
\]

\[
= \frac{O.D._T}{O.D.} \times 50 \text{ mg glucose/100 ml of CSF}
\]

(Note that 0.1 ml of CSF is diluted to 4 ml, which means a dilution factor of 40.)

Report

The concentration of glucose in CSF sample is ..................
The following abnormalities may be present:

Based on the study of this section, answer the following Questions.

Check Your Progress

1) Why is that Benedict's test is not employed to estimate glucose in CSF?

2) What is the basis behind the use of sodium fluoride as a preservative for CSF samples?

6.4 LET US SUM UP

This unit portrays the pattern of biochemical components of one of the important body fluid called Cerebrospinal Fluid (CSF) under normal and pathological situations. An analysis of CSF helps us to diagnose the diseases affecting the central nervous system. In the CSF analysis, we have discussed the methods of estimation of proteins and glucose in CSF.

6.5 ANSWERS TO CHECK YOUR PROGRESS

Check Your Progress

1) Glucose content in CSF is much low, hence, a more sensitive test is employed rather than Benedict's test.

2) Sodium fluoride inhibits the action of certain enzymes involved in the oxidation of glucose, i.e., it prevents glycolysis. If sodium fluoride is not added, glycolysis will take place and glucose will be degraded. Such a sample cannot give a proper quantitative idea of glucose in it.
# LIST OF ACTIVITIES

## Self Activity

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Unit No.</th>
<th>Title of the Activity</th>
<th>Area</th>
<th>Hours</th>
<th>Marks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td>Physical Set up of Laboratory</td>
<td>Lab</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

## Supervised Activities

<table>
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<th>Unit No.</th>
<th>Title of the Activity</th>
<th>Area</th>
<th>Hours</th>
<th>Marks of Lab.</th>
</tr>
</thead>
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<tr>
<td>1.</td>
<td>Unit 1, Section 1.1-1.5</td>
<td>Introduction to the apparatus and their uses</td>
<td>Lab</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>Unit 1, Section 1.6</td>
<td>Preparation of solution/dilution</td>
<td>-do-</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>Unit 2, Section 2.2</td>
<td>Demonstration of:</td>
<td>-do-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Difference between compound and mixed element</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Mixture separation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Miscibility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Unit 2, Section 2.4</td>
<td>Phenomenon of Osmosis using red blood cell</td>
<td>-do-</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>5.</td>
<td>Unit 3, Section 3.2</td>
<td>Tests for Proteins</td>
<td>-do-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>6.</td>
<td>Unit 3, Section 3.3</td>
<td>Test for Carbohydrates:</td>
<td>-do-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Molisch test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Benedict’s test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Iodine test for Starch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Unit 3, Section 3.4</td>
<td>Tests for Triglycerides</td>
<td>-do-</td>
<td>2</td>
<td>1</td>
</tr>
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<td>8.</td>
<td>Unit 4, Section 4.4</td>
<td>Physical Examination of Urine</td>
<td>-do-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9.</td>
<td>Unit 4, Section 4.4</td>
<td>Chemical Examination of Urine</td>
<td>-do-</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>10.</td>
<td>Unit 5, Section 5.6, 5.7</td>
<td>Interpretation of CSF and Blood Reports including Cholesterol</td>
<td>-do-</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>
# CHECKLIST

**Indira Gandhi National Open University (IGNOU)**  
**School of Health Sciences**  
**Post Basic B. Sc. Nursing**  
**BNSL 102 Applied Sciences (Nutrition & Dietetics)**

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Signature of Nurse Incharge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Have you been able to conduct a survey among the following category of individual belonging to:</td>
<td></td>
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<tr>
<td></td>
<td>• Low income group (LIG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Middle income group (MIG)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>• High income group (HIG)</td>
<td></td>
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<tr>
<td>2.</td>
<td>Have you been able to prepare a sample guide on the selection of food items by individuals in each of the income groups mentioned in item</td>
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<td>3.</td>
<td>Have you been able to conduct a survey in your neighbourhood and find out which foods are avoided by older adults and why?</td>
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<tr>
<td>4.</td>
<td>Have you been able to present your survey findings in a tribular form</td>
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<tr>
<td>5.</td>
<td>Have you been able to conduct a survey among 10 lactating mother in your neighbourhood</td>
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<td>6.</td>
<td>Have you been able to find out what special food items/snacks are prepared and eaten by them during lactation period</td>
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<tr>
<td>7.</td>
<td>Have you been able to find out why there mother eat these selected food items</td>
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<tr>
<td>8.</td>
<td>Have you been able to visit any urban slum/resettlement colony or any other disadvantage group living close to your residence and/or place of work</td>
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<tr>
<td>9.</td>
<td>Have you been able to identify how soon after birth, the infants are breastfed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
10. Have you been able to observe how soon mother feed colostrums to the infant

11. Have you been able to find out what is the first food given to the newborn child

12. Have you been able to find at what age complementary foods are introduced

13. Have you been able to find out how long the infant is breastfed

14. Have you been able to find out what are the complementary foods and at what age these foods are given

15. Have you been able to identify/select five patients (each) suffering from the following diseases:
   - Renal failure
   - Hypertension
   - Hepatitis

16. Have you been able to interview them and find out about the foods included and avoided by them

17. Have you been able to compile the data and list the food included/avoided by them in a tabular form

Signature of Student

Signature of Nursing Officer