



NATIONAL OPEN UNIVERSITY OF NIGERIA

SCHOOL OF SCIENCE AND TECHNOLOGY

COURSE CODE: BIO 204

COURSE TITLE: BIOLOGICAL TECHNIQUES

MODULE 1

Unit 1: Types of microscopes and their uses.

Unit 2: Preparation of microscope slides / Examination

Unit 3: Dissection Guides

UNIT 1: TYPES OF MICROSCOPES AND THEIR USES CONTENT

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1.0 INTRODUCTION

In this unit 1 of module 1, those of you that are users of laboratory microscopes, as well as those seeing the tool for the first time will learn about modern microscopes, a tool used in various health professions, research institutes, and Universities to magnify small objects that are difficult to see with the naked eye. The intention is to let you have a better understanding of the microscopes and their uses.

2.0 OBJECTIVES

At the end of this unit, you will be able to:

- appreciate the use of microscopes
- describe the structure and functions of microscopes
- develop the skills, for focusing objects, under a microscope.
- handle and take care of a microscope

3.0 MAIN CONTENT

3.1 CLASS OF MICROSCOPE

There two kinds of microscopes that you can identify in any laboratory. They are the simple and compound microscopes magnify an object directly, and on the other hand, compound microscopes magnify an object, and show the objects in their reverse position. You can observe that what is left in the image is right in the object when moving the slide in a certain direction.

3.2 TYPES OF MICROSCOPES

There are many types of microscopes that you can find in the market today. All you need to do is to determine what it is used for. The different types of microscopes and their basic functions are as listed below.

- *Complex Compound Microscope*: Light or optical types of *complex compound microscopes* combines objective, eyepiece lenses, and light condenser lens and magnify the image of small objects (Fig1.1)

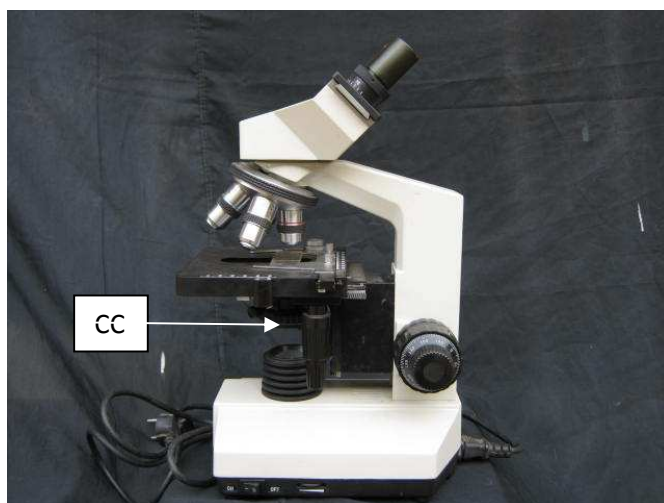


Fig. 1.1 :Complex compound (CC) microscope with Condenser Chamber. (Photo from Bup Oyesiku Bryolab, 2011)

- *Simple Compound Microscope.* A simple compound microscope is similar to complex compound microscope in function. However, the difference is that a simple compound microscope, is most used to magnifying objects generally. It has no light condenser attached beneath the stage as compared to complex compound microscope. (Fig 1.2)



Fig.1.2 Simple compound microscope without condenser lens beneath the stage. (Photo from Bup Oyesiku Bryolab, 2011)

- *Simple Magnifying Lens:* The simplest light microscope is the magnifying lens (Fig 1.3). It is usually hand held. It magnifies object and usually useful for field work.



Fig1.3: Simple Magnifying Lens (Photo from Bup Oyesiku Bryolab, 2011)

Stereo / Dissecting Microscope: A stereo or dissecting microscope, combines two objectives lenses, and two eyepieces to view an object. When you use this microscope, you will see three-dimensional images of the object on the stage. (Fig 1.4)

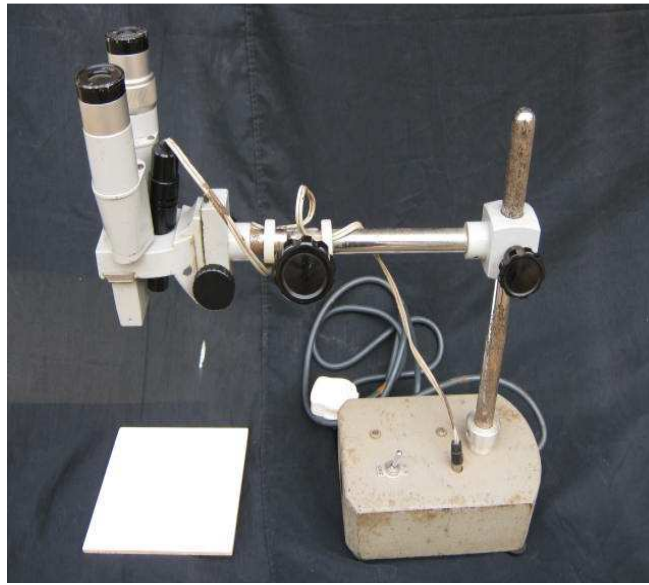


Fig 1.4 Stereo microscope (Photo Bup Oyesiku Bryolab, 2011)

- *Fluorescence Microscopes:* Fluorescence microscope is a special microscope that uses fluorescence and phosphorescence lights to view samples and determine their properties.
- *Electron Microscope:* An electron microscope is one of the most sophisticated types of microscopes with highest magnification (10,000X – 2,000,000X). Electrons used to illuminate the smallest particles, which passed through magnetic field onto a photographic film. When the film is developed magnified images of the particles appears. We have two types, the Transmission Electron Microscope (TEM), and Scanning Electron Microscope (SEM). Fig 1.5. They are useful for bacteria and studies on Viruses etc.



Fig 1.5: Transmission Electron Microscope (TEM)(Photo Bup Oyesiku Bryolab, 2011)

- *Digital Microscope:* A digital microscope you will see in Fig 1.6 has a digital camera attached to it and connected to a computer screen to view the object directly. It has the advantages of taking the picture of the object as well.



Fig. 1.6: Digital Microscope with USB connector (Photo from Bup Oyesiku Bryolab, 2011)

- *Digital Imager Microscope:* This imager microscope (Fig 1.7) is a type of digital video capturing microscope you mount on compound microscope, and connect with USB or AV cable to record the activities of mobile specimens



Fig 1.7; Digital Imager Microscope (Photo from Bup Oyesiku)

3.3 LIGHT MICROSCOPY AND FUNCTIONS

You have learnt above, the types of microscopes that require visible light to detect small objects as a well-used research tool in biology. Yet, many of you are unaware of the important features available in these microscopes. For you to use light microscope efficiently, you need to understand the basic microscopy: bright field, dark field, in addition, phase contrast, and oil immersion. When you use any of these fields, you should consider the following; Contrast, Focal Plane, Resolution, and Recognition (CFRR) of the sample when you see it. You should also note that the oil immersion objective (1000x) called “wet” objective, and other lower objectives (400x, 100, and 50x) called “dry” objectives.

- **Bright field microscopy:** In bright field microscope, light source is from below the stage. Light travels through the specimen, through the objective lens to your eye through the eyepiece. The microscope controls over the intensity and shape of the light to give an image you see. Bright field microscope gives you best images of stained specimens, naturally pigmented, or living photosynthetic organisms. You should know here that bright field microscope is best used for stained specimens.
- **Phase contrast microscopy:** Most of the detail of the transparent living cells is detectable in phase contrast microscopy. However, insufficient contrast between structures with similar transparency may occur. You should know that each transparent structure has a tendency to bend light, providing an opportunity to distinguish them.

This translates to mean the reduction in appearance of a structure depends on the refractive index. Highly refractive structure, bend light at much greater angle than do structure with low refractive index.

Phase contrast is better than bright field microscopy when the specimen is transparent and high magnifications (400x, 1000x) are required. The health professions and in some university programmes use phase contrast microscopy in teaching.

- **Dark field microscopy:** Dark field microscopy is a cheaper alternative to phase contrast microscopy. The resolution and

contrast obtained with the dark field is superior to what you will get from phase contrast, it is important for you to know that in dark field, reflected light from particles on the slide passes through combined lenses (i. e. objectives and eyepieces) to your eye. While phase contrast transmits **refracted** light through specimen on your slide to your eye. To get a dark field effect you need to place an opaque disc underneath the condenser lens of a bright field microscope; so only external light source (from side of stage or above it) that is scattered by the object on the slide reaches the eye. Any time you want to view specimens in liquid sample, dark field (100X) is best.

- **Oil immersion microscopy:** In microbiology lab, you use oil immersion microscopy to observe stained smears of mixed bacteria. You use immersion oil designed especially for oil immersion microscopy. Oil immersion lens is essential for viewing individual bacteria or detail of fixed specimens. To use an immersion lens, you first focus the area of specimen to be observed with the high and dry (400x) lens. Next, you place a drop of immersion oil on the cover slip. Click in the immersion lens, and bring the lens down the stage nearly touching the cover slip, while looking from the side. Then you focus by moving the lens up away from the slide until you hit the focal plane to see clear image.

3.4 PARTS AND FUNCTIONS OF A MODERN MICROSCOPE

Before you buy or use a microscope, it is important to know the parts and the functions of each one. The parts of a typical

modern complex compound microscope are described below (Fig 1.8)

Eyepiece (EP): The eyepiece lens is attached to the top of the tube you are looking at in Fig 1.8. The eyepieces standard lens powers are 5X or 10X or 15X or 16X.

Revolving nosepiece (RN): This moveable part house two or more objectives lenses.

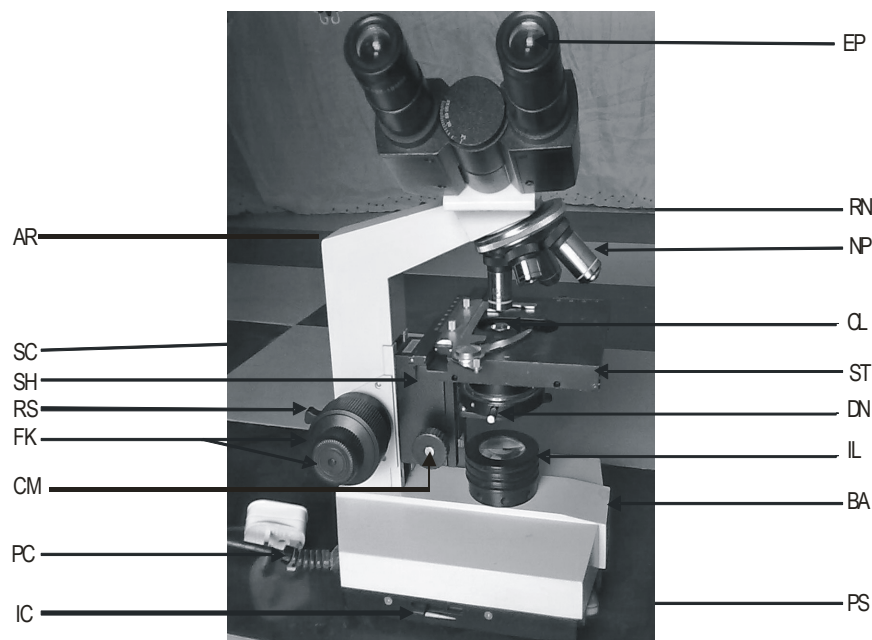


Fig 1.8: Modern Complex Compound Microscope with condenser lens beneath the stage (Photo from Bup Oyesiku Bryolab, 2011)

Nosepiece (NP): You often find three or four objective lenses on a nosepiece with 4X, 5X, 10X, 40X, 100X powers. The low powers (4X, 5X, 10X, 40X) are called “dry” scan objectives. Power 100X forms the oil immersion. You should note that the shortest lens has the lowest power; the longest one has the highest power.

Condenser Lens (CL): You may use CL to focus light onto the specimen on your microscope stage. You need to use it most at a

higher powers (40X and 100X) to give you a sharper image than those microscopes without *CL*. Your *CL* adjusting knob is located on the other side of your microscope to move the *CL* up and down to focus a good quality images. The *CL* rated at 0.65 – 1.25 will give you maximum benefit if you operate your microscope at 40X and 100X objective powers. You should set very close to the slide at 100X and further down away at the lower powers.

Stage (ST): The flat plate where you place your slide for viewing.

Diaphragm Knob (DN): This knob controls the disc directly above the *CL*. You may use the *DN* to vary the amount and size of cone light reaching the slide from below.

No rule of thumb as to setting you should use for a particular power. The setting is a function of transparency of specimen, contrast, and objective lens in use.

Illuminator (IL): A lighting source used in place of a mirror

Base (BA): The bottom of the microscope use for support and house the power source, fuse and illuminator.

Power Switch (PS): This switch controls light supply to the microscope

Illumination Control: This bar control the amount of light passing through the illuminator condenser.

Power Cable (PC): This wire connects to the power source to provide light to the microscope through the illuminator (*IL*)

Condenser Mover (CM): A knob you use to move the condenser up or down, to attain a good quality of light.

Focus Knobs (FN): Knobs that make rough and fine adjustments to be focused. You have two knobs on a microscope, large knob is for coarse focus, and the small one is for fine focus.

The latter is used at a higher power objective. *FN* moves the stage up and down when you turn the knobs.

Rack Stop (RS): You may use this knob to lock the stage level after adjusting how close the objective lens can get to the slide. You do this to avoid making the high power objective lens down into the slide and break it! It is often set at the factory.

Stage Holder (SH): Seat on which the stage is resting, connect it with the *FK* knob, which in turn moves the stage up or down when you turn the *FK* knob.

Stage Clip (SC): The stage clip holds the slide in place.

Arm (AR): It attaches the eyepieces and the objectives to the base.

Stage and Slide Mover: Stage mover knob (big upper knob) is used to move the stage forward or backward to position the best quality specimen. While, the small lower knob moves the slide to either left or right to position the best specimen for view (Look at the other side of your microscope for the combined knobs).

3.5 OPERATING THE MICROSCOPE

Now that you are conversant with the microscopes and their parts let us now consider the operation of the microscope. The proper way to operate your microscope is to start to focus with the lowest power objective lens (*OB*) while you look through the side and move the lens down as close to the slide without touching it. Next, you look through the eyepiece lens and focus upward only until the image is

sharp. If you cannot get it in focus, repeat the process over. When you have a sharp image at low power lens, you turn the revolving nosepiece (*RN*) to click the next higher power lens and do a minor adjustments with the fine focus knob. Continue with subsequent objective lenses and fine focusing each time.

3.6 HANDLING AND CARE OF THE MICROSCOPE

It is important at this stage to know that a good quality microscope is unbelievably expensive! Therefore, you must observe the general procedures and precautions when handling your microscope.

1. Always carry your microscope with one hand on the Arm (*AR*) and one hand on the Base (*BA*), and close to your body.
2. Plug the microscope in and place excess wire on the table.
3. Always start and end focusing with low power Objectives lens (*OB*)
4. Always make sure the stage and lenses are clean before you put away the microscope.
5. Always use good quality lens tissue to clean before you put away the microscope.
6. Always cover your microscope with a dust jacket when not in use.

4.0 CONCLUSION

In this unit, you have learnt about the types of microscopes in the laboratory, their component parts and uses. You have also been provided with sufficient information on the skills needed for the manipulation, handling and care of the microscope.

5.0 SUMMARY

Microscopes are special lenses designed to magnify small objects that are difficult to see with the naked eyes. There are many types of microscope; simple, compound, stereo dissecting, fluorescence digital and electron. The type you will choose to use at any time will depend on the type of study you want to carry out and the size of object you want to examine. Since microscopes are fragile and expensive equipment, care must be taken in their handling and protection.

6.0 TUTOR-MARKED ASSIGNMENTS (TMA)

1. List the kinds of microscopes you have learnt in this unit.
2. Distinguish between a compound and a simple microscope in relation to object they magnify.

7.0 REFERENCES / FURTHER READINGS

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UNIT 2: PREPARATION OF MICROSCOPE SLIDES**CONTENT**

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Preparation of Slides
 - Temporary Preparation
 - Permanent Preparation
 - Use of Stains
 - 3.2 Labelling of slides
 - 3.3 Storage of Prepared slides
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References / Further Reading

1.0 INTRODUCTION

In this unit you will be introduced to the terminology used in slide making, and you will also learn about a variety of techniques which will enable you to make slides. Similar techniques are widely and routinely used in biological laboratories in schools, colleges, research institutions as well as in hospital pathology labs to examine tissues and micro-organisms and to look at chromosomes.

This unit has been designed to introduce you to the care and preparation of microscope slides.

2.0 OBJECTIVES

After completing the study of this unit you should be able to:

1. describe some simple techniques for slide preparation.
2. distinguish between temporary and permanent slide preparations.
3. describe the use of stains in slide preparations
4. describe how slides are labeled and stored

3.0 MAIN CONTENT

3.1 PREPARATION OF SLIDES

Slides making is an important part of many areas of biological, medical, veterinary and forensic sciences and you will often be required to prepare different kinds of slides in-house. Specimens may be smears of fluids, thin sections or whole mounts of all or part of an organ or organisms. In all cases the material is mounted on a glass slide prior to its examination.

Two main types of preparation are used:

1. Temporary and
2. Permanent

Temporary preparations are examined and then discarded, usually on the same day; whereas permanent preparations may remain in good condition for years. The techniques involved in preparation are described here.

a. Temporary Preparations

These preparations may be needed for matter of minutes or hours only. They are mounted in water or dilute 1, 2, 3-propanetriol

(glycerol) or other fluid of low volatility. After examination they are discarded.

The procedure is as shown in Fig. 2.1

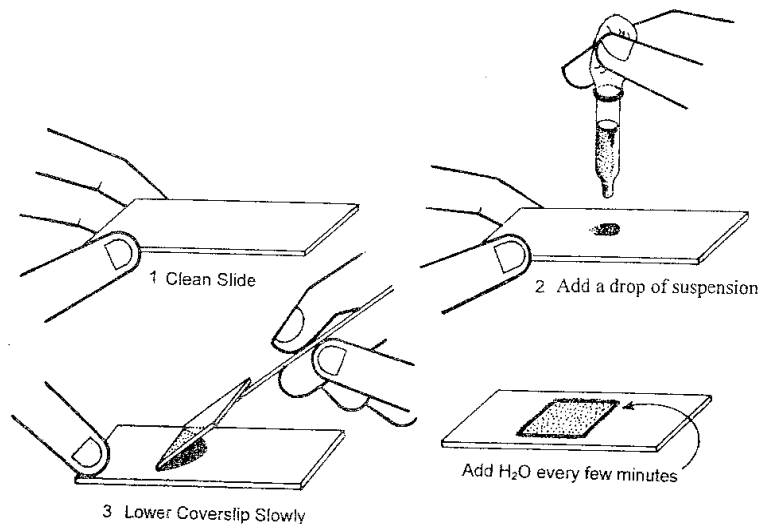


Fig 2.1: The technique for lowering the cover slip over the slide.

The material under examination can be fixed and stained or even examined in a living state, for example protozoa such as *amoeba*. In this case a harmless aqueous stain such as 1% methylene blue can be used – it would then be known as a “vital stain”.

If it is necessary to keep the slide for a matter of hours it is possible to reduce evaporative losses from the edges of the cover-glass by painting a ring of gum or molten wax or nail polish around its edge.

It is always desirable to use a cover-glass over your temporary preparation. If it is omitted, the curvature of the drop of liquid in which the objects is mounted causes optical distortions. An additional problem is the danger of contaminating your microscope’s objectives lenses.

The preparation of stained specimens for microscopical study either temporary or permanent generally involves the following three processes (additional processes are used in making permanent preparations).

(1) Fixation

For fresh tissues, the main aim of fixation is to kill tissues rapidly by precipitating proteins. This minimizes post-mortem changes. The reagent used is called a fixative; the most commonly used being 70% alcohol. Other common fixatives are Bouin fluid and formalin. Different fixative are used depending on the nature of the tissue whether soft or hard. Tissues should be washed well after fixation, using the same solvent as the stain, in order to remove all traces of the fixative. If this is not done, tissues may not stain properly and some types of fixative may crystallize out (fixation is not necessary if the material is already preserved).

(2) Staining

The object of staining is to accentuate the distinction between the different components of a tissue or organ.

(3) Mounting

Mounting media employed for temporary preparations include water and 1, 2, 3-propanetriol (glycerol) 30%-50% aqueous solution.

b. Permanent Preparation

If the slide is to be kept for long-term reference, for days or even years, it must be made as a permanent preparation. This is achieved by:

1. Dehydrating the specimen after staining (usually with a graded series of alcohols);
2. Clearing it – treating it with a solvent that is mutual soluble between alcohol and the mounting medium [traditionally clove oil or 1-2 dimethylbenzene (xylene); and
3. Mounting it under a coverglass in a preservative which dries hard and has a refractive index similar to glass, e.g. Canada balsam or Euparal or DPX mountant which gets dries up easily as opposed to Canada balsam which takes longer time.

The making of a permanent stained preparation mounted in Canada balsam involves five process:

- (i) Fixation (ii) Staining (iii) Dehydration,
- (iv) Clearing, and (v) Mounting

Fixation and staining have already been mentioned in the earlier sub-section and the purpose of dehydration, clearing and permanent mounting is outlined below.

1. Dehydration

The purpose of dehydration, i.e. the removal of water, is to allow complete infiltration of tissues with Canada balsam. Unless all traces of water are removed, infiltration is incomplete, the tissues appear opaque and bacterial decay ultimately sets in.

If carried out too rapidly, dehydration causes distortion and shrinkage, especially of delicate tissues, by setting up violent diffusion currents. It should therefore be done gradually and sufficient time allowed for the complete extraction of water. Dehydration is commonly effected by the passage of the stained

specimen or slide through successively stronger solutions of ethanol (ethyl alcohol) ending with immersion in absolute alcohol 100% ethanol (2 changes).

CAUTION: *Alcohol is a highly flammable material. Adequate ventilation and no naked flames are essential safety precautions.*

2. Clearing

Where the dehydrating agent is immiscible with the mounting medium, it is necessary to introduce an intermediate fluid that is miscible with both. Such a fluid is known as a clearing agent. The purpose of clearing is to remove all traces of alcohol, thus allowing the tissues to be infiltrated with the Canada balsam or other mountant. Examples of clearing agents are 1, 2-dimethylbenzene (xylene) for small soft tissues; clove oil and cedarwood oil for thick tissues. Incomplete dehydration is indicated by cloudiness in the clearing agent and the slide should be returned to absolute alcohol. Toluene is another good clearing agent but it is a bit costly.

3. Mounting

Permanent preparations are obtained by enclosing tissues in solid, resiniferous media such as Canada balsam/DPX. After the tissues have been cleared, they are mounted in a semi-fluid 1, 2-dimethylbenzene (xylene) balsam mixture. The 1,2-dimethylbenzene (xylene) subsequently evaporates and the balsam hardens. However, there are some disadvantages of using this mountant. Xylene is inflammable and toxic; the drying process is prolonged and this mountant discolours with time. It is advantageous to use DPX because it dries up easily. To hasten the

process of drying up, slides may be kept in oven at lower temperature for sometime.

Use of Stains

A great variety of stains are in common use. In all cases the aim is to give different parts of the specimen different colours and colour density and hence increase optical contrast between them. Two different staining methods are in common usage.

1. *Progressive staining*: This involves leaving a tissues in the stain until nuclei, for example, are deeply stained and cytoplasm is only faintly stained. It is good plan to dilute the stain beforehand.
2. *Retrogressive (regressive) staining*: This entails deliberate, over-staining followed by destaining which results in differentiation. Best results are obtained when overstaining is prolonged. This method depends on differential rates of stain extraction. For example, acid alcohol removes haematoxylin (haemathoxylin is a nuclear stem that stains only the nucleus) more rapidly from the cytoplasm than the nuclei.

Other important aspects of staining procedures are as follows.

1. Stains may be used singly or in combination i.e. *counterstaining*
2. The use of two stains together is known as *double staining*
3. Stains used in combination should be complementary in colour, e.g. light green and safranin (green and red) used in plant tissue staining.

Stains are either general or specific. A general stain, e. g. borax carmine, stains all parts of a tissue usually in different densities or shades of the same colour. A specific stain acts on one or more

components of a tissue or organ in a selective manner, e.g. safranin stains lignified plant cell walls red.

3.2 LABELLING OF SLIDES

Slides act as a permanent record of tissues, organs and specimens. They may be of pathological origin, e.g. hospital patients, purchased material, prepared “in house”, etc. In all cases it is essential that the slide is properly identified by adequate labelling.

Labels should therefore carry the following information:

1. The name of the organism – if the whole organism is mounted then the slide can be marked **WM=whole mount** or **E=entire**
2. The part of the organism used, e.g. **liver, root**.
3. The type of preparation, e.g. **smear; squash; TS = transverse section; VS = vertical section; LS = longitudinal section**.

The following information is desirable but not essential;

4. Stains(s) used, e.g. **H.E.** = haematoxylin/eosin.

If the slide is prepared “in house” then it should be:

5. Dated
6. Signed.

It is common to use two labels, one on each end. Self-adhesive or gummed slide labels pre-printed with lines are available from lab suppliers – alternatively use ordinary self-adhesive or gummed labels. You will find it easier to write the label before you stick it on the slide. If the label is gummed (rather than self-adhesive) you must not lick it but use a wet sponge.

3.3 STORAGE OF PREPARED SLIDES

Prepared permanent slides are valuable. Even mass-produced slides are expensive these days at least from lab suppliers. They are widely used by educational establishments in the teaching of histology (the study of tissues of plants and animals) and cytology (the study of cells). Slides which are individually prepared 'in house' may represent the investment of considerable time and care of the part of the person who made them. In some cases the preparation may be uniquely valuable. It could for example be an important reference slide or a slide of the diseased tissues of a hospital patient.

So the message is clear – take great care of prepared slides. The following is a short list of some important pointers towards good practice.

1. Freshly prepared permanent slides must be stored flat until the mountant has hardened.
2. Special thermostatically controlled warming plates are available, but alternatively an incubator may be used.
3. Once the mountant is hard the slide may be stored flat in a card tray or upright in a slotted box or cabinet drawer.

4.0 CONCLUSION

Slide preparation is an essential component of biological and medical research. It proceeds the observation of the specimens under the microscope.

Slides could be prepared either for temporary or permanent use.

Prepared slides must be properly labeled for identification and stored.

5.0 SUMMARY

The important points to note in this unit are:

- Slides of biological / medical specimens can be prepared in the laboratory.
- Slides are either prepared for temporary or permanent usage.
- Appropriate stains are used for specimens to reveal their structures in details,
- Slides are viewed under the microscope to reveal their details and
- Prepared slides must be properly labeled and stored.

6.0 TUTOR-MARKED ASSIGNMENT

1. Describe briefly how slides are prepared.
2. Differentiate between progressive and regressive staining.

7.0 REFERENCES / FURTHER READING

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Unit 3: DISSECTION GUIDE**CONTENT**

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Preparation for Dissection
 - 3.2 Procuring Animals
 - 3.3 Anaesthetizing the Animals
 - 3.4 Theoretical Knowledge
 - 3.5 Setting up Dissection Trays
 - 3.6 Pithing
 - 3.7 Flag labeling
 - 3.8 Safe Disposal of Dissected Animals
 - 3.9 General Instructions for Dissection
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1.0 INTRODUCTION

Dissection is a major component of the biology practical. The meaning of dissection is to cut open the animal in order to ascertain the structure of its parts, so as to define their boundaries and display clearly their mutual relations. Dissection consists mainly in removing the connective tissue which binds the several parts together. Dissection requires lots of preparations before hand.

In this unity you are also provided with some dissecting guide to help you through some dissections.

2.0 OBJECTIVES

After doing this experiment you should be able to:

- prepare dissection trays/petriplates
- use the dissection guides provided
- anaesthetize the animals correctly,
- dispose dissected animals safely.

3.0 MAIN CONTENT

3.1 Preparation for Dissection

Materials Required

Dissection trays

Petridishes

Dissection kit

Chloroform

Ethane

Formalin

Urethane

Microscope

Table lamp

Animals

Procedure

In schools, colleges and universities, dissection of chordates/non-chordates is one of the major practical. A lab technician has to perform several tasks before and after dissection.

3.2 Procuring Animals

Orders for animals should be placed with an animal supplier according to the number of students in a class. This exercise should be done two-three days before dissection is to be performed.

1.1 Anaesthetizing the Animals

Before dissection, animals are given anaesthesia. Chloroform and ether are used as anaesthetizing agents. Rats, frogs and pigeons are generally freshly chloroformed for the dissection, though for some of the dissections e. g. cranial nerves in rat and internal ear in frog, preserved specimens are required. Similarly scoliodon is also preserved after anaesthetizing them. Preservatives commonly used are formalin (5% or 8% or 10%) and 70% alcohol.

3.4 Theoretical Knowledge

Keep a well-labelled diagram of the dissection to be done in the classroom. You should have some theoretical knowledge of the dissection to be performed e.g. if students have to dissect male or female reproductive organs of rat, frog or other animals, then you should be able to differentiate them morphologically, just by looking at them externally.

3.5 Setting up of dissection Trays

Large animals like frog, rat, fish, pila, leech and other animals are dissected in dissection trays whereas small animals such as cockroach and small insects can be dissected in petriplates.

It should be seen that wax is spread over trays uniformly and water does not drip from the trays. The trays should be half filled with water so that the animal can be fully immersed in it. The trays should not be completely filled with water.

One set of dissection kit dissection tray, a microscope and a table lamp should be arranged for the teacher/instructor. Similar sets should be arranged for students. It should be seen that dissection instruments are clean and sharp.

Water should be kept clean in the dissection tray during dissection by changing it whenever it is stained with blood etc.; so that visibility remains the same. If any animal is bleeding profusely, alcohol dipped cotton should be applied to the affected organ blood vessel. See that every waste of dissection is put in the petridish. It should not be thrown carelessly on the top of table or lab floor.

3.6 Pithing

Some of the experiments like muscle twitch and heart perfusion in frog do not require chloroform-anaesthetized animals. Before dissection is started, you have to anesthetise the frog by injecting 2.5 ml of 20% urethane intramuscularly. This would quieten the frog. Alternatively you could immobilize the frog by pithing. You have learnt how to pith a frog in your BIO 191. Check this out if you have forgotten the method.

3.7 Flag Labeling

Frequently, the students are asked to dissect some organs, blood vessels or nerves etc. and flag label them. For flag labeling, small

pieces of paper (2.5 x 0.7 cm) are prepared and a needle is passed through each close to one end.

The names of organs, blood vessels or nerves are written on these flags and each flag is inserted in the dissecting trays close to the organ, blood vessel or nerve bearing the name on the flag.

Some of the dissections require black-papering. You have to cut black paper into small and thin strips which can be inserted underneath blood vessels and nerves.

3.8 Safe Disposal of Dissected Animals

You have already studied in Unit 4 and Exercise 13 of LT – 1 course that the dissected animals should be buried deep in the soil. Simply placing the material in a plastic bag and putting it in the dustbin is not good enough.

3.9 General Rule of Dissection.

As you have learnt earlier in this unit, you need to give some anaesthesia to animals before you dissect them. The common anaesthesia used in the biology laboratory and chloroform and ether. For rats, frogs and pigeons, it is better to dissect them immediately after anaesthesia.

However, certain studies for example, of the nervous system are better done with preserved animals. Formalin (5%, 8% or 10%) and 70% alcohol are the common laboratory preservatives. It is usually better to do dissection after you have done the theoretical studies.

Opening Up

Invertebrates are better opened up from their dorsal side while vertebrates from their ventral side. In both cases, you pick the skin

up with a pair of forceps (if the animal is big), if it is small, you will have to hold the animal with your left hand while with a pair of scissors you make your incision with the right hand on the mid-line of the animal with the scissors pointing upwards to avoid damage to lower internal structures. This incision should go along the line of the animal as much as possible. Then make side slits in the same manner, with the scissors pointing upwards to avoid damage to underlying tissues.

With the scapel, gently scrape the inside layer of the skin to separate skin from the underlying tissues by cutting through subcutaneous tissue. Once the skin has been removed the animal must be laid down (even if it is small) and pinned securely to the dissecting board. It is important to pin specimen securely to the dissecting board. It is important to pin specimen securely down so that both hands will be free for use. Seekers could then be used to probe parts, when other walls have to be opened up to reach internal organs, the same cautions as we took for opening up the skill will apply.

You have to be very gently in dissecting so as to avoid damage to internal organs. If the dissection involves cutting through tissues (especially blood vessels) then you must take steps to ensure that the blood flow or the contents of the cut tissues or organs do not interfere with your studies. Usually, you may have to wash, and soak away blood with cotton wool or blotting paper.

4.0 CONCLUSION

In this unit, you have learnt about the materials required for dissection; the anaesthetizing agents; dissection proper, the precautions you must take and safe disposal of used animals.

5.0 SUMAMRY

- Dissection generally is a complex exercise
- It requires patience
- It also requires a sound theoretical knowledge of the animal to be dissected for easy location of organs/structures.
- Dissection is done in dissection trays using kits
- Dissection animals must be buried properly

6.0 TUTOR MARKED ASSIGNMENT

1. Describe the process of setting up a dissection tray in the laboratory.
2. What are the rules one should observe in the process of carrying out dissection?

7.0 REFERENCES / FURTHER READING

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Soper, R. (Ed) Biology Sciences Cambridge U. K., Cambridge Press

Stone, R. N. Cozens, A. B. Emia, F. I. (1972), New Biology for Tropical Schools, Longman Group Ltd

MODULE 2

Unit 1: MICROTOMY AND HAND SECTIONING**CONTENT**

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Microtomes
 - 3.1.1 Rocking
 - Rotary
 - Sledge
 - Freezing
 - 3.1.2 Microtome Knives
 - 3.1.3 Hand Cut sections (hand sectioning)
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References / Further Reading

1.0 INTRODUCTION

Biology owes much to the allied sciences of chemistry and physics for the development of new tools and techniques. In this unity you will study about the equipment which is necessary for a biology lab. You should keep in mind that mastery of simple apparatus is a prerequisite for work with more advanced equipment. Some equipment, which are mostly used in chemistry and physics, has merely been referred to and you will study them in detail in respective courses.

2.0 OBJECTIVES

After studying this unit you will be able to:

- describe various types of microtomes, their working and use .
- list the microtome knives and describe its working.
- describe hand cut sections

3.0 MAIN CONTENT

3.1 MICROTOMES

You will come across microtomes in a biology lab. The mechanical cutting of plant/animal materials are done by the aid of microtomes and the process is commonly known as microtomy.

Microtomes can be divided into four basic groups:

- 1) Rocking
- 2) Rotary
- 3) Sledge (sliding)
- 4) Freezing

All microtomes, however, have a few things in common; all have a means of holding a block, the chuck; all have a knife past which the chuck can be moved in order to cut the block, and all have a mechanism by which the chuck can be advanced micron by micron, or in some cases by sub-micron steps, towards the blade. The blade in all types is angled with respect to the chuck, and that angle may be varied.

1. Rocking Microtomes

The commonest rocking microtome is called a Cambridge Rocker. (Fig 2.1) is a basic diagram. The mechanism of the microtome is mounted on a heavy cast iron base which helps to reduce

vibrations by providing an inertial 'sink' and thus keeping all the fixed points truly fixed.

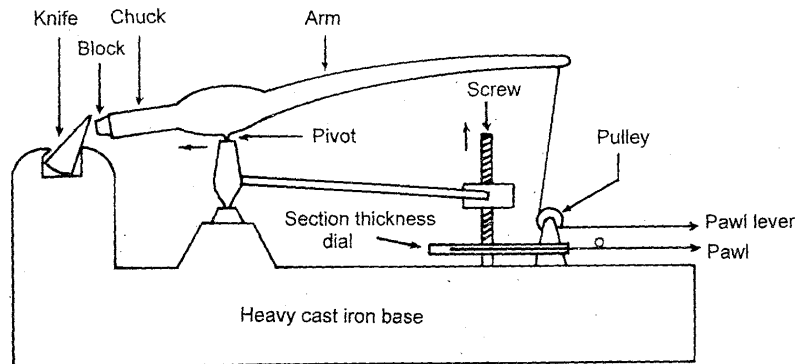


Fig. 2.1: The Cambridge rocking microtome.

The block for sectioning is fixed to the chuck which is rocked up and down about the *pivot* by the action of the *pawl lever* on the arm. With each rocking movement the chuck passes the *knife* which is only a little heavier than a cut throat razor, and which has its cutting edge upward and a slide from the block on the downward stroke. On the upward stroke the *pawl* itself is engaged and moves the chuck forward by an amount which can be varied between two and twenty microns in two micron steps, by means of the *screw*. The downward stroke is neutral.

There are other rocking microtomes, but all such instruments suffer from the same disadvantage, namely that the section is necessarily curved since the chuck moves in an arc. For many purposes this is unimportant but it would not do for an embryological specimen, for example, where the juxtaposition of groups of developing cells might be critical, or for making serial sections of animals to ascertain the position and extent of the internal organs. A rocking microtome is generally best for cutting sections thicker than six microns.

2. Rotary Microtomes

Rotary Microtomes are larger and heavier instruments even more heavily damped to prevent vibrations and are surely capable of making thinner sections than the rocking variety. They are much more expensive. The pass, made past the much heavier knife by the chuck, is straight and relatively fast and is succeeded by a return upward stroke during which the chuck is advanced. A basic diagram of the machine can be seen in (Fig 2.2) which shows the flywheel used to keep sections flowing from the knife by smoothing out the action. Rotary microtomes enable sections of 5 microns thickness to be cut.

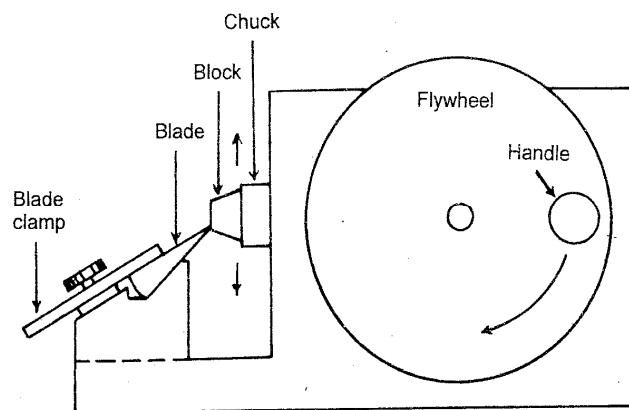


Fig. 2.2: The Rotary Microtome.

3. Sledge Microtomes

The sledge microtome is a device which may weigh as much as fifty kilograms, and consists of a heavy base into which are machined flat tracks on which the chuck sledge slides. The chuck holds the block with the surface to be cut uppermost. The chuck slides past a very heavy blade which is held horizontally on mountings which are bolted to, or drawn from, the base. (Fig 2.3) shows the basic diagram but can convey little of the weight of the machine. Each section is cut by a movement of the sledge

past the blade and the return stroke advances the chuck. Various devices may be attached to sledge microtomes among which are chuck stages for the sectioning of small entire animals. Sledge microtomes are damped extremely well and may be used to cut sections down to two or three microns.

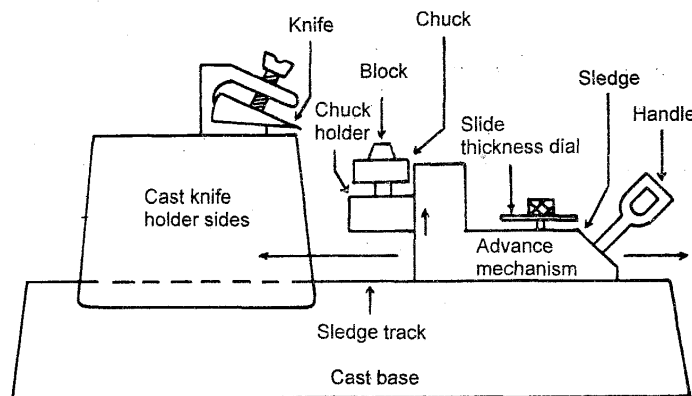


Fig 2.3: A sledge microtome.

4. The Freezing Microtome

The only other type of microtome of interest here is the freezing microtome. This device usually has a wedge blade and the instrument is unusual as the blade is moved past the fixed chuck rather than the other way around. Fresh tissue to be sectioned is placed on the hollow chuck, and carbon dioxide gas passed rapidly through the chuck under pressure from a cylinder. The expanding CO₂ is sprayed on the tissue from above, and on the blade to cool it.

The cold blade is passed through the frozen tissue and the single sections removed with a cooled section lifter or forceps to be floated in water. Such microtomes are sometimes mounted in chilled cabinets to make working with them easier. It is now possible to cut frozen sections using freezer aerosols instead of

CO₂ and freezing chucks are made which are electrically cooled by means of Peltier cells.

Microtomes of all kinds should be cleaned of wax after use, possibly by using a little xylene or less harmful solvent, and any parts liable to rust should be wiped with a little thin oil. The action should be examined and lubricated where necessary and any soiled paintwork cleaned. The cover should always be on if the machine is not in use, but should be removed regularly for examination during periods of prolonged idleness.

3.1.2 MICROTOME KNIVES

There are three basic cross sectional shapes of knives, wedge, plano-concave and double concave, all of which are shown in (Fig 2.4).

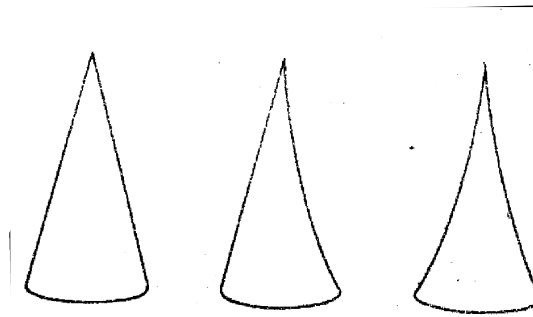


Fig 2.4: Microtome Knives

The plane wedge knife is generally, though not exclusively used for cutting frozen sections, and plano-concave knives are often used for sectioning soft materials such as celloidin embedded tissues. Botanical sectioning razors are generally plano-convex.

The best knives for the cutting of paraffin blocks are the double concave type, which should be of a heavy pattern cross-section to prevent vibration, i.e. the taper should be short and the base wide.

Blades are designed to undertake particular jobs, but microtomists are essentially experimentalists at heart and often use them for other purposes than those for which they were intended. Thus anything that we say about the uses of blades may be contradicted by someone else's experience. Nevertheless, there are certain things about microtome blades which are unalterable: for instance, a blunt one won't cut! Blades should be treated not only as dangerous but as delicate. The weighty hunk of stainless steel which is a microtome blade has been made with the precision of a Swiss watch and for section cutting its edge should look perfect when viewed under a microscope at about fifty times. This is how they come from the manufacturer! The job of a microtomist is to keep it that way.

3.1.3 HAND CUT SECTIONS (HAND SECTIONINGS)

Some tissue like plant stems, roots, cartilage in animals are firm enough to be held in the hand directly or supported in a matrix while cutting a section. The traditional supporting matrix for young stems and roots and also leaves that are soft is pith. A recent substitute (which you can easily obtain) is expanded polystyrene such as that used in packaging or insulating materials. In use, the plant specimen is sandwiched between two rods of polystyrene (in which grooves may be cut to receive it) or pith which could be strips of pumpkin or banana stem or potato. The support is sectioned along with the specimen and they are then separated by flotation in water or preservative. The aim is to obtain thin slices of the specimen, *preferably only one cell thick*. Very skilled workers may be able to cut the complete section to the correct thickness. It is usual,

however, to cut wedge-shaped slices so that at least some part of the section is of correct thickness (see Fig 2.5)

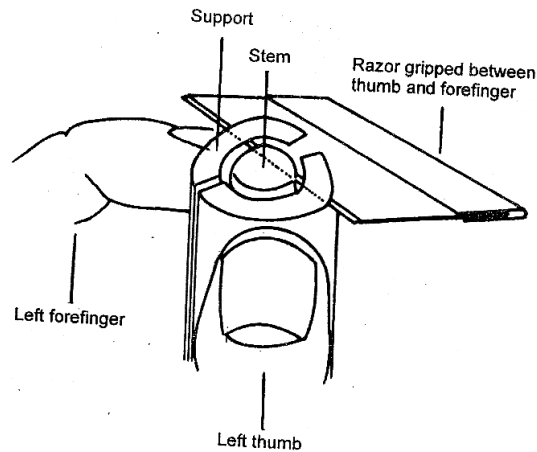


Fig 2.5: Sectioned wedges.

The single hollowground botanical razor is the ideal instrument for cutting sections. However, it is difficult to sharpen the blade adequately. A satisfactory substitute is a single-edged disposable razor blade. The length and size of the botanical razor makes it easier to use than the small disposable blades.

Such sections are then floated on water if the specimen is fresh or in 70% alcohol if the material is preserved. The thinnest sections are selected and stained in aniline sulphate or aniline chloride and mounted in dilute glycerol. If the slide has to be preserved for future use then the sections are stained, dehydrated, cleared and mounted in Canada balsam or any other suitable mountant.

4.0 CONCLUSION

Microtomes as instruments used for cutting plants or animals in the laboratory. There are four basic types of microtomes and the one you were to use will depend on the specimen / material you want to cut.

5.0 SUMMARY

The important points to note in this unit are:

- Microtomy is the mechanical cutting of plant / animal materials in the laboratory.
- The Rocking, Rotary, Sledge / Sliding and the Freezing are the four basic groups of microtomes.
- The actual cutting is done with microtome knives or sharp razor blades.
- The shape of knives are wedge, plano-concave and double concave.
- The purpose of sectioning in the lab is to get a thin section of the object preferably only one cut thick for microscopic viewing.

6.0 TUTOR-MARKED ASSIGNMENT

Fill in the blank.

1. Forsections cutting from rocking microtomes is not advisable
2. A rocking microtome generally cuts section thicker than
3. A rotatory microtome can cut the sections ofthickness.
4. In freezing microtomegas is sprayed on the tissues.
5. All the microtomes should be cleaned with or with some less harmful

7.0 REFERENCES

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Laboratory Course 3.

UNIT 2: PHOTOMETRY

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Photometry and the Eye
 - 3.2 Photometric quantities
 - 3.3 Photometric versus Radiometric Quantities
 - 3.4 Watts versus Lumens
 - 3.5 Photometric Measurement Techniques
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

Photometry is the science of the measurement of light, in terms of its *perceived* brightness to the human eye. It is distinct from radiometry, which is the science of measurement of radiant energy (including light) in terms of absolute power; rather, in photometry, the radiant power at each wavelength is weighted by a luminosity function (a.k.a. visual sensitivity function) that models human brightness sensitivity.

Typically, this weighting function is the photopic sensitivity function, although the scotopic function – and others – may also be applied in the same way.

2.0 OBJECTIVES

At the end of this unit you will be able to

- define Photometry

- explain Photometry with its units of measurement
- compare Photometric and Radiometric quantities

3.0 MAIN CONTENT

3.1 PHOTOMETRY AND THE EYE

The human eye is not equally sensitive to all wavelengths of visible light. Photometry attempts to account for this by weighting the measured power at each wavelength with a factor that represents how sensitive the eye is at that wavelength. The standardized model of the eye's response to light as a function of wavelength is given by the luminosity function. Note that the eye has different responses as a function of wavelength when it is adapted to light conditions (photopic vision) and dark conditions (scotopic vision). Photometry is typically based on the eye's photopic response, and so photometric measurements may not accurately indicate the perceived brightness of sources in dim lighting conditions where colours are not discernible, such as under just moonlight or starlight.

3.2 PHOTOMETRIC QUANTITIES

Many different units of measure are used for photometric measurements. People sometimes ask why there is need to be so many different units, or ask for conversions between units that can't be converted (lumens and candelas, for example). We are familiar with the idea that the adjective "heavy" can refer to weight or density, which are fundamentally different things. Similarly, the adjective "bright" can refer to a light source which delivers a high luminous flux (measured in lumens), or to a light source which concentrates the luminous flux it has into a very narrow beam

(candelas), or to a light source that is seen against a dark background. Because of the ways in which light propagates through three-dimensional space – spreading out, becoming concentrated, reflecting off shiny or matter surfaces – and because light consists of many different wavelengths, the number of fundamentally different kinds of light measurement that can be made is large, and so are the numbers of quantities and units that represent them.

Table 2.1: SI Photometry Units

Quantity	Symbol	SI unit	Abbr.	Notes
Luminous energy	Q_v	lumen second	lm's	Units are sometimes called talbots
Luminous flux	F	lumen(=cd'sr)	Lm	Also called <i>luminous power</i>
Luminous intensity	I_v	candela(=lm/sr)	cd	An SI base unit
Luminance	L_v	candela per square metre	Cd/m ²	Units are sometimes called “nits”
Illuminance	E_v	lux (=lm/m ²)	lx	Used for light incident on a surface
Luminous emittance	M_v	lux (=lm/m ²)	lx	Used for light emitted from a surface
Luminous efficacy		lumen per watt	lm/W	Ratio of luminous flux to radiant flux
SI . Photometry				

3.3 PHOTOMETRIC VERSUS RADIOMETRIC QUANTITIES

There are two parallel systems of quantities known as photometric and radiometric quantities. Every quantity in one system has an analogous

quantity in the other system. Some examples of parallel quantities include:

- Luminance (photometric) and radiance (radiometric)
- Luminous flux (photometric) and radiant flux (radiometric)
- Luminous intensity (photometric) and radiant intensity (radiometric)

In photometric quantities every wavelength is weighted according to how sensitive the human eye is to it, while radiometric quantities use unweighted absolute power. For example, the eye responds much more strongly to green light than to red, so a green source will have greater luminous flux than a red source with the same radiant flux would. Radiant energy outside the visible spectrum does not contribute to photometric quantities at all, so for example a 1000 watt space heater may put out a great deal of radiant flux (1000 watts, in fact), but as a light source it puts out very few lumens (because most of the energy is in the infrared, leaving only a dim red glow in the visible).

SI radiometry units

<i>Quantity</i>	<i>Symbol</i>	<i>SI unit</i>	<i>Abbr.</i>	<i>Notes</i>
Radiant energy	Q	joule	J	energy
Radiant flux	Φ	watt	W	radiant energy per unit time, also called <i>radiant power</i>
Radiant intensity	I	watt per steradian	$\text{W}_{\text{sr}}^{-1}$	power per unit solid angle
Radiance	L	watt per steradian per square metre	$\text{W}_{\text{sr}}^{-1} \cdot \text{m}^{-2}$	power per unit solid angle per unit <i>projected</i> source area. called <i>intensity</i> in some other fields of study.
Irradiance	E, I	watt per square	$\text{W} \cdot \text{m}^{-2}$	power incident on a surface.

		metre		sometimes confusingly called “intensity”.
Radiant exitance /Radiant emittance	M	watt per square metre	$\text{W}\cdot\text{m}^{-2}$	power emitted from a surface
Radiosity	J or J_λ	watt per square metre	$\text{W}\cdot\text{m}^{-2}$	Emitted plus reflected power leaving a surface
Spectral radiance	L_λ or L_ν	watt per steradian per metre ³ or watt per steradian per square metre per hertz	$\text{W}\cdot\text{sr}^{-1}\cdot\text{m}^{-3}$ or $\text{W}\cdot\text{sr}^{-1}\cdot\text{m}^{-2}\cdot\text{Hz}^{-1}$	Commonly measured in $\text{W}\cdot\text{sr}^{-1}\cdot\text{m}^{-2}\cdot\text{nm}^{-1}$
Spectral irradiance	E_λ or E_ν	watt per metre ³ or watt per square metre per hertz	$\text{W}\cdot\text{m}^{-3}$ Or $\text{W}\cdot\text{m}^{-2}\cdot\text{Hz}^{-1}$	Commonly measured in $\text{W}\cdot\text{m}^{-2}\cdot\text{nm}^{-1}$

3.4 WATTS VERSUS LUMENS

Watts are units of radiant flux while lumens are units of luminous flux. A comparison of the watt and the lumen illustrates the distinction between radiometric and photometric units.

The watt is a unit of power. We are accustomed to thinking of light bulbs in terms of power in watts. This power is not a measure of the amount of light output, but rather indicates how much energy the bulb will use. Because incandescent bulbs sold for “general service” all have fairly similar characteristics (same spectral power distribution), power consumption provides a rough guide to the light output of incandescent bulbs.

Watts can also be a direct measure of output. In a radiometric sense, an incandescent light bulb is about 80% efficient: 20% of the energy is lost (e.g. by conduction through the lamp base). The remainder is emitted as radiation, mostly in the infrared. Thus, a 60 watt light bulb emits a total radiant flux of about 45 watts. Incandescent bulbs are, in fact, sometimes used as heat sources (as in a chick incubator), but usually they are used for the purpose of providing light. As such, they are very inefficient, because most of the radiant energy they emit is invisible infrared. A compact fluorescent lamp can provide light comparable to a 60 watt incandescent while consuming as little as 15 watts of electricity.

The lumen is the photometric unit of light output. Although most consumers still think of light in terms of power consumed by the bulb, in the U. S. it has been a trade requirement for several decades that light bulb packaging give the output in lumens. The package of a 60 watt incandescent bulb indicates that it provides about 900 lumens, as does the 15 watt compact fluorescent.

The lumen is defined as amount of light given into one steradian by a point source of one candela strength; while the candela, a base SI unit, is defined as the luminous intensity of a source of monochromatic radiation, of frequency 540 terahertz, and a radiant intensity of $1/683$ watts per steradian. (540 THz corresponds to about 555 nanometres, the wavelength, in the green, to which the human eye is most sensitive. The number $1/683$ was chosen to make the candela about equal to the standard candle, the unit which it superseded).

Combining these definitions, we see that $1/683$ watt of 555 nanometre green light provides one lumen.

The relation between watts and lumens is not just a simple scaling factor. We know this already, because the 60 watt incandescent bulb and the 15 watt compact fluorescent can both provide 900 lumens.

3.5 PHOTOMETRIC MEASUREMENT TECHNIQUES

Photometric measurement is based on photodetectors, devices (of several types) that produce an electric signal when exposed to light. Simple applications of this technology include switching luminaires on and off based on ambient light conditions, and light meters, used to measure the total amount of light incident on a point.

More complex forms of photometric measurement are used frequently within the lighting industry. Spherical photometers can be used to measure the directional luminous flux produced by lamps, and consist of a large-diameter globe with a lamp mounted at its center. A photocell rotates about the lamp in three axes, measuring the output of the lamp from all sides.

Luminaires (known to laypersons simply as light fixtures) are tested using goniophotometers and rotating mirror photometers, which keep the photocell stationary at a sufficient distance that the luminaire can be considered a point source. Rotating mirror photometers use a motorized system of mirrors to reflect light emanating from the luminaire in all directions to the distant photocell; goniophotometers use a rotating 2-axis table to change the orientation of the luminaire with respect to the photocell. In either case, luminous intensity is tabulated from this data and used in lighting design.

4.0 CONCLUSION

Photometry is the measurement of light, in terms of its perceived brightness to the human eye. Many units of measure are used for photometric measurements.

5.0 SUMMARY

The important points to note in this unit are:

- Photometry is the measurement of light as its perceived brightness to the human eye.
- The human eye is not equally sensitive to all wavelengths of visible light
- Photometric and radiometric quantities are the two parallel systems of calorimetry.
- Watts are units of radiant flux while lumens are units of luminous flux.
- The watt is a unit of power

6.0 TUTOR MARKED ASSIGNMENT

- 1a. What is Photometry?
- b. State two units of measure of light.

7.0 REFERENCES/FURTHER READING

Nelken and Parker (1999) Advanced level Physics
Oxford University Press.

<http://en.wikipedia.org/wiki/photometry>

UNIT 3: CALORIMETRY

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Heat and Temperature
 - 3.2 Specific Heat Capacity and Latent Heats
 - 3.3 Experimental Methods of Calorimetry
 - 3.4 Applications
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

Calorimetry deals, mainly, with measurement of heat energy. The term *calorimetry*, as it were, is made up of two key words, *calorie* which, by definition, is the amount of heat required to raise the temperature of 1g of water by 1°C and ‘*metry*’ that is, measurement (from the word *meter* which is a device used for making measurements). You are familiar with the metallic container usually used for measuring the heat content of substances in the laboratory called *calorimeter*. You are also aware that, although the SI unit of heat is called *joule J*, some scientists, such as biologists and nutritionists, still measure heat energy in *calories cal*.

Heat is a form of energy that flows from a part of a system or body to another as a result of the temperature gradient between them.

Naturally, heat flows from high to low temperature parts of a system (body).

2.0 OBJECTIVES

At the end of this unit you will be able to

- differentiate between specific Heat and Latent heat
- carry out simple experiments on Calorimetry
- discuss simple application of Calorimetry

3.0 MAIN CONTENT

3.1 HEAT AND TEMPERATURE

The concepts of *heat* and *temperature* are so intricately related that there is the need to show the distinction between them.

Heat is a form of energy that flows from high to low temperature parts of a system.

As a form of energy, the SI unit of heat is joule (J). However, there are other units of heat in common use. One of them is the calorie(call). One calorie was originally defined as the heat required in raising the temperature of one gram of water by one degree Celsius.

$$1 \text{ cal} = 4.184 \text{ J} \quad \text{exactly}$$

Note that the “calorie” used by the nutritionists is actually the “kilocalorie” and is denoted Calorie (call)

You already know that *temperature* is the degree or measure of hotness or coolness of a body. You are also familiar with the Celsius temperature scale. The standard SI unit of temperature is the *Kelvin* (K). Other older scales such the Fahrenheit (F) are also

available. Inter-conversions among these scales are easily worked out.

Temperature is a property of a body that determines the direction of heat flow in it. Heat naturally flows hot to cold parts of a body.

3.2 SPECIFIC HEAT CAPACITY AND LATENT HEATS.

One of the effects of heat on a body is that when heat flows into a body, its temperature rises and vice versa. One of the measurements we will consider in calorimetry is the ability or capacity of a material to retain or store heat flows into it. Experimental observation reveals that the capacity of a material to store heat depends on its *mass* as well as the arrangement of the atoms or molecules and the bonding forces that hold them together. You know that a bucketful of water, which is more massive, will stores more heat than a cupful of water when both are heated to the same temperature. Also, a metal block will store less heat than wooden block of equal dimensions if both are heated to the same temperature. Different substances require different amounts of heat to raise temperatures by 1°C. The amount of heat required to raise the temperature of a substance by 1°C is proportional to its mass and the change in temperature.

We define specific capacity as the amount of heat required to raise the temperature of one kilogramme of a substance by one degree celsius.

We can measure it as follows:

$$\text{Specific heat capacity} = \frac{\text{amount of heat absorbed or given out by a body}}{\text{mass of substances} \times \text{change in temperature}} \text{ in } J/kg^{\circ}C$$

Note that for a substance of any mass, we can define the *heat* or *thermal capacity* as *thermal capacity = mass of substance \times specific heat capacity in J/°C*

Table of the values of specific heat capacity of some substances are obtaining from standard texts of physics.

As biologists, you will no doubt be interested in the determination of calorific values of food and other substance using the calorimetric techniques.

We have looked at the specific hat capacity of substances. We must note that for a given mass of a substance, all we need to measure in order to determine its specific heat capacity is the change in temperature, which we may achieve through calorimetry. We will now describe another measure of heat which does not involve change in temperature.

Another effect of heat on a substance is the change of phase or state. During a change of phase, the heat that flows into or out of a substance goes to break or make the bonds and the atoms or molecules are rearranged to give rise to a different state of matter. Until this process is completed, the temperature of substance does not change. In other words, the heat that flows into or out of the body is latent (hidden) since it does not manifest as change in temperature of the substance. However, the amount of heat that the body takes in or gives out is measurable in terms of the amount of the substance converted into a different phase. This also depends on the nature of the material, that is, on the type and strength of bonds between its atoms or molecules. The amount of heat required to change the phase of a substance at constant temperature is

proportional to its mass. We define the specific latent heat as the amount of heat required to change/transform one kilogramme of a substance from one phase to another at constant temperature. We can measure this as follows:

$$\text{Specific latent heat} = \frac{\text{amount of heat absorbed or given out}}{\text{mass of substances}} \text{ in J/kg}$$

For solid to liquid transition, we have the specific latent heat of fusion. Similarly, for liquid to vapour transition, we have the specific latent heat of vaporizations.

Table of the values of specific latent heats of some substances may also be obtained from standard texts. We will now proceed to discuss experimental methods of calorimetry.

3.3 EXPERIMENTAL METHODS OF CALORIMETRY

1. Method of Mixtures and Heat Exchanges

The methods depends on the fact that when a hot object and cold object are in thermal contact, heat flows from the hot to the cold object until thermal equilibrium is established, that is, the objects attain the same temperature. In mathematical notation this principle can summarized as follows:

Heat lost by the hot object= Heat gained by the cold object

The method assumes that there is no heat exchange between the mixture and the surrounding. In practice, the mixture will always be held in a container. Thus, part of the heat lost by the hot object is used to heat up the container is thermally insulted or lagged from the surrounding by a poor conductor such as felt or wool. The figure 2.1 below is the diagram of a calorimeter (a metal cup) used for the heat exchange experiments.

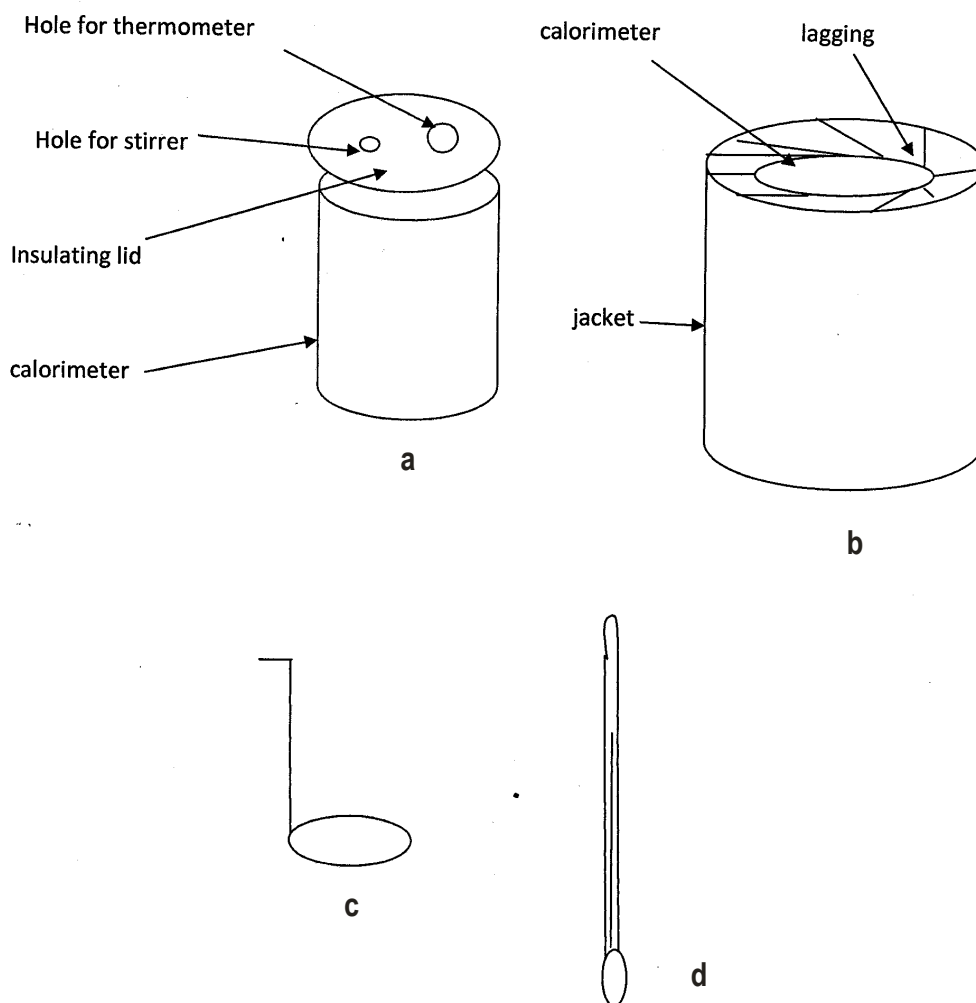


Fig 3.1:a, a calorimeter; b, a lagged calorimeter; c, a stirrer and d, a thermometer

The calorimeter is usually made up of copper, a good conductor so that it reaches the same temperature as its content as soon as possible. It is also usually polished inside and outside so as to reduce loss of heat by radiation. As illustrated in figure 1b, the calorimeter is placed inside a jacket (a larger container) with the space between it the jacket filled with layers of lagging material such as felt or cotton, which is an insulator. This helps to reduce heat loss by conduction and convection. An insulating lid is used to cover the calorimeter to prevent evaporation which leads to cooling and loss of part of the liquid weighed into the calorimeter. The

stirrer is used to maintain uniform temperature and the thermometer is used as a sensor to measure the temperature change of the mixture.

Experiment to Determine the Specific Heat Capacity of a Solid by the Method of Mixtures

We determine the mass m_x and temperature T_x of a solid substance X. T_x may be determined by placing it in boiling water long enough for it to attain the same temperature as the boiling water. X is then quickly dropped into a lagged calorimeter which contains water of mass m_w at temperature T_0 which is less than T_x and gently stirred. We denote the mass and specific heat capacity of the calorimeter by m_{cu} and c_{cu} respectively. The final steady temperature T obtained is recorded. The value of C_x , the specific heat capacity of X is then calculated using the definition we have given before.

We note that

Heat lost by solid X = Heat gained by water and calorimeter

The values of T , T_x , T_0 , m_w , m_{cu} , and m_x are easily measured while c_{cu} can be found from tables of specific heat.

Experiment to Determine the Specific Heat Capacity of a Liquid by the Method of Mixtures

To determine the specific heat capacity of an unknown liquid, we need to make the following modification to the experiment to determine the specific heat capacity of an unknown solid outlined above:

- i. We use a solid of known specific heat capacity
- ii. Use the liquid whose specific heat capacity is required instead of water.

The same procedure and calculations as before can then be carried out.

2. Electrical Methods

The source of heat in this case is the electric heater. We recall that *power* is the rate of which *energy* is generated or expended. Thus, the power rating of the heater and the heat produced by it in a given time interval is defined as

Heat absorbed or given out = power rating of the heater x time in watts (W)

Now, electrical power is defined as the product of the voltage and the current. Thus, for a given voltage, the quantity of heat generated in a given time by an electric heater when current flows in it in a certain time interval is

Heat absorbed or given out = voltage x current x time in joules (J)

So, the measurement of the specific heat capacity of a substance heated electrically can then be obtained from definition as before.

3.4 APPLICATIONS

The method of calorimetry can be used to determine the temperature of a furnace by placing a metal of known specific heat capacity in the furnace and later removing it into a calorimeter to determine change in temperature by the method of mixtures.

Another application of the method of calorimetry is the determination of energy values of food substances mentioned earlier.

4.0 CONCLUSION

Calorimetry is a measure of heat energy. Heat is a form of energy that flows from a part of a system to another through a temperature gradient.

5.0 SUMMARY

The important points to note in this unit are:

- Heat is a form of energy
- Temperature is the degree of hotness or coolness of a body
- Specific capacity is amount of heat required to raise the temperature of one kg of a substance by one degree celcius.
- Specific latent heat is the amount of heat required from one phase to another at constant temperature.

6.0 TUTOR MARKED ASSIGNMENT

Describe one application of calorimetry methods

7.0 REFERENCES/FURTHER READING

Nelken and Parker (1999) Advanced level Physics
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<http://en.wikipedia.org/wiki/photometry>

UNIT 4: CHROMATOGRAPHY AND CONDUCTOMETRY

CONTENTS

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Chromatography
 - 3.2 Types of Chromatography
 - 3.3 Applications of Chromatography
 - 3.4 Conductometry
 - 3.5 Applications of Conductometry
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Readings

1.0 INTRODUCTION

Chromatography and Conductometry are separation and analytical techniques widely used in chemistry and the biological sciences. Most things that occur in nature are a mixture of substances which can only be separated or analysed using any of the techniques known. In this unit (3) you will learn about some of these techniques

2.0 OBJECTIVES

At the end of this unit you should be able to:

- Understand the principle on which chromatography as a separation technique is based.
- Describe the different methods of chromatography available
- Mention some applications of chromatography

- Understand the concept of Conductometry
- Describe the measuring units involved in Conductometry
- List some applications of Conductometry

3.0 MAIN CONTENT

3.1 CHROMATOGRAPHY

Chromatography, firstly introduced by the Russian botanist Micharl Iswett is a method for separating the components of a mixture by differential distribution of the components between a stationary phase and mobile (moving) phase. Initially used for the separation of coloured substances from the plants (Greek, *Chromos* meaning coloured) is now the most extensive technique of separation and purification of coloured/colourless organic compounds.

Separation of two sample components in chromatography is based on their different distribution between two non-miscible phases. The one, the stationary phase, a liquid or solid, is fixed in the system. The other, the mobile phase, a fluid, is streaming through the chromatographic system. In gas chromatography the mobile phase is a gas, in liquid chromatography it is a liquid.

The molecules of the analytes (mixture to be separated) are distributed between the mobile and the stationary phase. When present in the stationary phase, they are retained, and are not moving through the system. In contrast, they migrate with the velocity, v , of the mobile phase when being there. Due to the different distribution of the particular analytes the mean residence time in the stationary phase differs, too, resulting in a different net migration velocity. **This is the principle of chromatographic separation. Separation of two sample components in chroma-**

tography is based on their different distribution between two non-miscible phases.

3.2 TYPES OF CHROMATOGRAPHY

3.2.1 Paper Chromatography is one of the most common types of chromatography in which filter paper serves as a support for immobile liquid phase. Removing liquid flows between the fibres of the cellulose of the filter paper but these are not the stationary phase. The true stationary phase is the very thin film of liquid usually water adhering to the surface of the fibers. (Water is adsorbed on the fibers/cellulose by strong hydrogen bonds with –OH of the cellulose). The substrate to be separated is distributed between the two liquids, stationary liquid that is held on the fibers of the paper and moving liquid in developing solvent. It uses a strip of paper and capillary action is used pull the solvents up through the paper to separate the solutes. A small concentrated spot of solution that contains the sample is applied to a strip of chromatography paper about 2 cm away from the base of the plate, usually using a capillary tube for maximum precision. This sample is absorbed onto the paper and may form interactions with it. Any substance that reacts or bonds with the paper cannot be measured using this technique. The paper is then dipped in to a suitable solvent, such as ethanol or water, taking care that the spot is above the surface of the solvent, and placed in a sealed container. The solvent moves up the paper by capillary action, which occur as a result of the attraction of the solvent molecules to the paper, also this can be explained as differential absorption of the solute components into the solvent. As the solvent rises through the paper it meets and dissolves the sample mixture, which will then travel up

the paper with the solvent solute sample. Different compounds in the sample mixture travel at different rates due to differences in solubility in the solvent, and due to differences in their attraction to the fibers in the paper. The components of the mixture move up the paper with the solvent at different rates, R_f , due to their differing interactions with the stationary and mobile phases.

$$R_f = \frac{\text{Distance the solute moves}}{\text{Distance the solvent front moves}}$$

This method has been largely replaced by thin layer chromatography

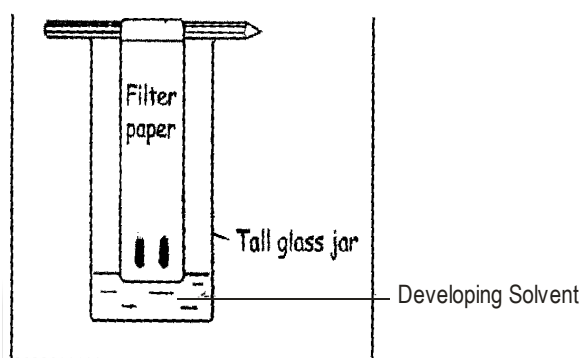


Fig. 4.1: Set up of a typical paper chromatography tank.

3.2.2 Thin-layer Chromatography

The surface of the plate consists of a very thin layer of silica gel on a plastic or Aluminium backing. Silica gel is a form of silicon dioxide (silica). Thin layer chromatography is similar to paper chromatography in that it involves spotting the mixture on the plate and the solvent (mobile phase) rises up the plate in the chromatography tank. It has an advantage over paper chromatography in that its separations are very efficient because of the much smaller size of the particles in the stationary phase. Gas chromatography and high performance liquid chromatography are more sophisticated chromatographic techniques.

3.2.3 Column Chromatography

Column chromatography is frequently used by organic chemists to purify liquids (and solids). An impure sample is loaded onto a column of adsorbent, such as silica gel or alumina. An organic solvent or a mixture of solvents (the eluent) flows down through the column. Components of the sample separate from each other by partitioning between the stationary packing material (silica or alumina) and the mobile elutant. In column chromatography, the stationary phase is packed into a glass tube to form a cylinder or **column** of granules. Solvent or buffer can flow freely between the granules. Stationary phase may be silica gel or ion exchange resin or a variety of other substances that may have particular affinity for amino acid molecules. The sample is applied with care as a layer on top of the stationary phase. Then solvent is added and flows through the column. Samples molecules move while they enter the flowing solvent. The stationary phase in polar compounds are attracted to the polar column packing by hydrogen bonding or dipole-dipole attractions. The more polar component interacts more strongly with the stationary phase. Polar compounds are moved slowly. Non-polar compounds are going to come off the column first, while the polar compounds are going to come off the column last. Usually, one starts with a less polar solvent to remove the less polar compounds, and then slowly increase the polarity of the solvent to remove the more polar compounds. Molecules with different polarity partition to different extents, and therefore move through the column at different rates. The eluent is collected in fractions.

3.2.4 Gas Chromatography (GC)

A gas is the mobile phase and the stationary phase can be either a solid or a non-volatile liquid. There are five basic GC components:

- 1) **Pneumatic system** –gas supply (flow control and measurement).

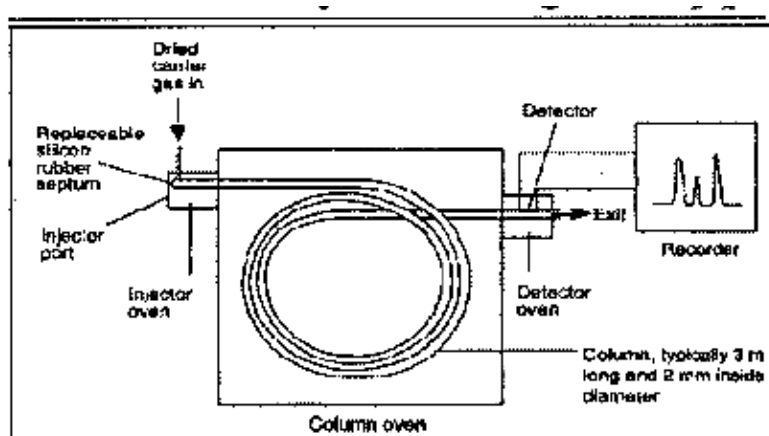


Fig. 4.2: set up of a typical Gas Chromatography.

- 2) **Injection system** – heated injector port, where the sample is vaporized if necessary
- 3) **Column** – where the separation occurs
- 4) **Oven** – the coiled column is wholly contained in a thermostatically controlled oven.
- 5) **Detector** – integral detector or link to a mass spectrometer

3.2.4.1 How does Gas Chromatography Work?

- 1) A carrier gas, examples of which are Helium and Neon flows through the system. A valve controls the flow rate.
- 2) A sample of the volatile mixture is injected into the carrier gas. The sample is vaporized in the heated injector port.
- 3) The carrier gas carries the vaporized sample into the column. The columns are stainless steel or glass tubes. They can be up

to 25 m in length and are of narrow bore (2-10mm). Therefore the column is often wound into a coil. The packed columns contain porous support material. The sample mixture undergoes a series of interactions between the stationary and mobile phases as it is carried through the system by the carrier gas. Due to the wide choice of materials available for the stationary and mobile phases, it is possible to separate molecules that differ only slightly in their physical and chemical properties.

- 4) The coiled column is contained in the thermostatically controlled oven.
- 5) Separated components emerge in the order of increasing interactions with the stationary phase. The least retarded component comes through first. Separation is obtained when one compound is sufficiently retarded to prevent overlap with another component of the sample, as it emerges from the column.
- 6) Two types of detector can be used: (1) thermal conductivity detectors which respond to changes in the thermal conductivity of the gas leaving the column and (2) flame ionization detection (FID), which is more commonly used. In thermal conductivity, as the carrier gas leaves the column, it cools the detector. When a solute emerges with the carrier gas, it does not cool the detector to the same extent. Alternatively, samples can be passed from the oven directly into a mass spectrometer, where they are analysed.

Retention time is defined as the time taken for a component to go from injection to detection. This varies depending on

- a. The nature of and the interactions between the solute and the stationary and mobile phases.
- b. The flow rate of the carrier gas,
- c. The temperature of the column (shorter retention times are obtained at higher temperatures),
- d. The length and diameter of the column,

Once GC has separated a mixture, the components can be indentified using known retention times. For unknown compounds the solutes are collected individually and anlysed using another method, e.g. mass spectrometry.

For each compound in a mixture one peak is observed on the chromatogram. In the particular set of operating conditions relating to the column, the retention time will increase with the size and polarity of the compound. To find the concentration of a particular compound, the peak height should be measured.

3.2.5 High Performance Liquid Chromatography

Basic Components:

1. **Solvent Reservoir.**
2. **The Pump System** controls the flow and measures the volume of solvent (the mobile phase). The flow rates of HPLC columns are slow – often in the range of $0.5 - 5 \text{ cm}^3 \text{ min}^{-1}$
3. **The Injector System:** The sample to be separated is injected into the liquid phase at this point.
4. **The Column** is made of steel and packed usually with porous silica particles (the stationary phase). Different materials can be used

depending on the nature of the liquid. A long column is not needed because separation in HPLC is very efficient. Columns are usually 10-30 cm long, with an internal diameter of 4 mm.

Different components of the sample are carried forward at different rates by the moving liquid phase, due to their differing interactions with the stationary and mobile phases.

- 5. The Detector:** When the components reach the end of the column they are analysed by a detector. The amounts passing through the column are small, so solutes are analysed as they leave the column. Therefore, HPLC is usually linked to a spectrometer (e.g. ultra violet or mass spectrometry).

The length of time it takes for a compound to reach the detector allows the component to be identified. Like the GC, once the retention time of a solute has been established for a column using a particular set of operating conditions, the solute can be identified in a mixture. A chromatogram is obtained for the sample.

3.3 APPLICATIONS OF CHROMATOGRAPHY

Thin layer chromatography is particularly useful in forensic work, for example in the separation of dyes from fibres.

Gas Chromatography is used to analyse blood samples for the presence of alcohol. It is also used to analyse samples taken from athletes to check for the presence of drugs. In each case, it separates the components of the mixture and indicates the concentrations of the components. Water companies test samples of water for pollutants using Gas Chromatography to separate the pollutants, and mass spectrometry to identify them.

HPLC has many uses such as drug testing, testing for vitamins in food and growth promoters in meat. In each case components of the mixture are separated and detected.

1.2 CONDUCTOMETRY

One of the most important characteristics of electrolyte solution is their capability to carry electric current. The electrolyte conductance is possible through movement of positive and negative ions which originate through dissociation of electrolyte.

Conductometry deals with the conductivity of electrolytes. The resistance of the solution is measured by applying an alternating voltage to the measuring cell (if direct current was applied, electrolytic processes occurring within the solution could alter the resistance). Experience has shown that, due to polarization effects, more precise results are obtained if the **measuring frequency** is adapted to the measuring range, e.g. 300Hz at low and 2.4 Hz at higher conductivities (7/12 Conductometer). The conductivity of a solution depends on;

- the **number of ions**. The more ions a solution contain, the higher its conductivity.
- the **ionic mobility** in a general way. The mobility in turn depends on:
 - ✓ the **type of ion**: the smaller an ion, the more mobile it is and the better it conducts electrical current. Ions of very high conductivity are H_2O^+ , OH^- , K^+ and Cl^- . If an ion is surrounded by water molecules (hydratization) and therefore becomes larger, its conductivity decreases.
 - ✓ the **solvent**: the more polar a solvent, the more completely ionized are the compounds dissolved in it. Water is an ideal

solvent for ionic compounds. In alcohols the ionization decreases with increasing chain length (methanol>ethanol >propanol). In non-polar organic solvents, e.g. chlorinated and non-chlorinated hydrocarbons, there is practically no ionization.

- ✓ the **temperature**: in contrast to what is found with solids, the conductivity of solutions increases with increasing temperature at a rate that ranges from 1 to 9% per Kelvin, depending on the ion.
- ✓ the **viscosity**: the ionic mobility decreases with increasing viscosity, which means that the conductivity also decreases.

1.2.1 Measuring setup

The minimal measuring setup consists of a **conductometer** and the **conductivity cell** connected to it. Generally the following items are also used: temperature sensor closed measuring/titrating vessel that can be thermostatted, and a magnetic stirrer.

A conductometer is an instrument for measuring complex resistances using alternating voltages (in contrast to the measurement of the purely ohmic resistances of metallic conductors, liquids, together with the measuring cell, constitute a network of resistances and capacities). The alternating voltages applied are available at minimum two frequencies. Quasi-ohmic conditions can be obtained, however, by the appropriate selection of the measuring frequency, cell constant and electrode material. Under these conditions the electrical conductivity can be determined from the measured resistance.

A conductivity measuring cell consist of two electrodes that face each other and are as inert as possible. Platinum is generally used

as the electrode material. Smooth (shiny) electrodes should only be used for conductivities $<20 \text{ } \square\text{S/cm}$. For higher conductivities, platinized electrodes are used (coated with platinum black). To avoid measuring errors due to changes in the electrical field, the measurement is carried out in a strictly defined volume. This is why immersion measuring cells are usually surrounded by glass or a plastic material. As stated above, the same cell cannot be used for the whole conductivity range. Accordingly, different cells are used that are optimized for the conductivity range to be covered. The measuring cells are characterized by their **cell constant c**. For low conductivities, cells with a small cell constant are used while cells with a high cell constant serve to measure high conductivities.

1.3 APPLICATIONS OF CONDUCTOMETRY

- The experimental determinations of the conducting properties of electrolytic solutions are very important as they can be used to study quantitative behavior of ions in solutions.
- They can also be used to determine the many physical quantities such as degree of dissociation and dissociation constants of weak acids and bases.
- They can be used to determine the ionic product of water, solubility and solubility products of sparingly soluble salts.
- They form the basis for conductometric titration methods.

4.0 CONCLUSION

Chromatography and Conductometry are two types of analytical and separation techniques used in the laboratory by chemists, biologists and other natural scientists to determine the quality and quantity of particular substances in different mixtures.

5.0 SUMMARY

In this unit, you learnt the following:

- in chromatography, the separation of two sample components is based on their different distribution between two non-miscible phases.
- chromatography, High Performance Liquid Chromatography are all type of Chromatography with various applications in the chemical industry.
- conductometry measures the strength of the current between two electrodes in a solution containing ions. This strength depends on the electric potential between the electrodes and the concentration of ions in the solution.
- conductometry is thus used to measure the ion concentration in a solution among other uses.

6.0 TUTOR-MARKED ASSIGNMENT

1. What do you understand by the term Conductometry?
2. Write short notes on the following;
 - a. Paper Chromatography
 - b. Gas Chromatography

7.0 REFERENCES/FURTHER READINGS

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MODULE 3

UNIT 1: SCIENTIFIC METHODS OF INVESTIGATION

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 The Scientific Methods
 - 3.1.1 Observing and Communicating
 - 3.1.2 Observation
 - 3.1.3 Interpretation
 - 3.1.4 Prediction
 - 3.1.5 Repetition
 - 3.1.6 Consensus
 - 3.1.7 Experiment
 - 3.1.8 Keeping record of your investigation
 - 3.2 Writing a report of Scientific Investigation
 - 3.2.1 Introduction
 - 3.2.2 Materials
 - 3.2.3 Methods
 - 3.2.4 Results
 - 3.2.5 Discussion
 - 3.2.6 Conclusion
 - 3.3 Communicating Scientific findings

3.3.1 People

3.3.2 Journal

3.3.3 Reports

3.3.4 Patent

3.3.5 Trade Literature

4.0 Conclusion

5.0 Summary

6.0 Tutor-Marked Assignment

7.0 References / Further Reading

1.0 INTRODUCTION

The methods employed in the various sciences are determined both by the general nature of the objective in mind and by the nature of the subject matter. A prerequisite to nearly every science is a suitable method of description of its subject matter. The language of such description must be capable of reproducing the subject matter with precision and uniqueness. If description is of an object, there should be only one corresponding object, which it should be possible to reproduce or reconstruct from the description. Specialized vocabulary are usually involved, often coined or derived from Greek or Latin words or roots. The specialized languages of the various sciences also usually permit statements to be made much more concisely than is possible with conventional language.

2.0 OBJECTIVES

As you go through this unit, you should be able to:

- know the qualities / attributes of a good Scientist
- appreciate the use of instruments for research
- observe experiments, predict and interpret scientific result
- conduct experiment and keep record.
- report your investigations as done universally and
- communicate your scientific findings.

3.0 MAIN CONTENT

3.1 The Scientific Methods

3.1.1 Observing and Communicating

One of the most important scientific methodologies is the possession of a very keen sense of observation and the skill to vividly describe or express such observation correctly to someone not present at the scene. We should be curious about things we can touch, taste, hear, smell and see. We also use instruments so that we can make observation that would not otherwise be possible. For example, with a magnifying lens or microscope, we can see details not visible to the unaided eye. Such details as the structure of the Amoeba sp. and the hypha or vegetative structure of a fungus. Here are some observations about flowering plants that you can repeat without special equipment.

3.1.2 Observation

- a) Seeds are produced in fruits
- b) If seeds taken from a fruit are sown they will grow into plants if conditions are right.

- c) These plants will be similar to those from which the fruits were taken and will produce similar fruits.

3.1.3 Interpretation

After making such observations about seeds from different kinds of plants you might come to certain conclusions:

- a) Seeds are produced only in fruits
- b) There is something in a seed that can develop into a plant
- c) All plants grown from seed are similar to those from which the fruit was taken and they produced similar seeds.

3.1.4 Prediction

On the basis of these conclusions, a scientist might predict that seeds from the fruit of a kind of plant he has not yet studied would produce more plants of that kind if they were sown in suitable soil.

Thus, observation, interpretation and prediction are things people do well. These are part of the common sense way of working that scientists call the scientific method.

Quite often, many scientific investigations should include many or all of the following:

- a) Identification and classification of the problem your set out to solve or shed some light. Is it on plant, animal, soil or their components?
- b) Working safely.
- c) Observing carefully
- d) Measuring precisely
- e) Recording observations accurately
- f) Analyzing data (recorded observations) carefully

- g) Interpreting the results of your analysis of data in the light of your own and other people's work.
- h) Preparing a written record of your work with appropriate reference to the work of others.

Possible sources of error in any investigation include insufficient thought in planning the investigation, use of impure chemicals or faulty equipment, and insufficient care in reading instructions, observing, measuring, recording and analyzing data, interpreting results or in preparing a written account of the work. Our ability to observe is also affected by the limitations of our senses and by the fact that we interpret observations in terms of our previous experience. This may result in their misinterpretation. The influence of previous experience is also indicated by the fact that different scientists may interpret the same evidence and come to different conclusions.

3.1.5 Repetition

One of the **most potent methods of checking for correctness or truth is repetition**. It is a matter of experience that there are objects and situations which repeat. It is part of the task of scientific investigations to formulate the conditions under which a situation repeats. If scientists can establish the conditions necessary for repetition, they can verify a previous description or observation by finding whether they now get the same result as before. Thus, they guard against possible previous mistakes on their part and at the same time increase the presumptive probability that they have correctly stated the conditions necessary for repetition.

3.1.6 Consensus

Another method of checking or confirming the correctness of an observation is agreement between different observers. Here the multiplicity of observations by different persons corresponds to the multiplicity of the repeated occurrence. The matter of the consensus among different observers is regarded by many people as so important that it is often incorporated into the definition of science, which is sometimes partially defined as the consensus of qualified persons.

3.1.7 Experiment

One of the potent tools of many of the Sciences, both for the discovery of new facts and for more adequate understanding of existing facts, is experimentation. Experiments artificially vary the conditions under which phenomena occur. In this way, they may create conditions never observed in nature. Used in this way, experiment becomes an enormously potent instrument in acquiring understanding and for understanding a situation or phenomenon.

3.1.8 Keeping Record of your Investigations

Scientists keep records as part of their work, to help them think, plan, observe, describe and remember. Usually a record of all practical work is kept in a special science note-book. You should note first the date and give each investigation a title. Then keep a concise note of exactly what you do. Write in carefully constructed sentences and prepared drawings or diagrams to ensure that your records cannot be misunderstood or misrepresented later.

Entering your observation in a table, as you make them, will help you to concentrate, to prepare complete and accurate records, to think about your work, and to arrange your thoughts. Any observations

recorded during an investigation are called DATA. These are facts of any kind recorded as words or numbers (Numerical data) as you count and measure. Counting and measuring enable scientists to be précise.

The records you have made so far during your investigation are the kind a scientist would make in a laboratory note-book or filed note book. Scientists also prepare accounts of their investigations for their own further references and for publication so that other scientists can be informed of any thing new. Communication is an essential part of the scientific method.

Start each account, or report, with a suitable title, your name and the date. Then use these headings to sign post the parts of your account.

3.2 Writing a report of Scientific Investigation

3.2.1 Introduction: What was the purpose of your investigation?

3.2.2 Materials: List the materials you used

3.2.3 Methods: What did you do and how did you do them?

3.2.4 Results: State concisely your findings. These may be presented mainly in tables, graphs or other diagrams.

3.2.5 Discussion: How did you interpret your results?

3.2.6 Conclusion: List any conclusions

The use of these headings makes report writing easier and helps readers find the information they need. If you could suggest possible explanations for your findings, each such possible explanation would be your hypothesis. For example, you might propose the hypothesis

that plants give off oxygen after photosynthesis (process of making food) in the presence of sunlight.

To test this hypothesis, you could repeat your investigation with the same plant recording your observation, use natural bright sunlight, dull sunlight, light with a bulb, light with a fluorescent tube and of course in darkness. Record your observation. In science an experiment is an investigation in which a hypothesis is tested. If oxygen is given off using those light sources and amount and if none is given off, this will be validated by your observations hence your results.

3.3 Communicating Scientific Findings

Scientists publish accounts of their investigations so that other scientists, any anyone else, can read about their work and make use of their findings.

Some of the avenues for publishing their work includes:

- 3.3.1 People:** The first obvious but very important way of spreading information is by word of mouth. People talk to friends and colleagues, passing on news about their own work and often including pieces of information they have read elsewhere. Information is also spread in this way in a more organized fashion by people talking at seminars, conferences and so on.
- 3.3.2 Journals:** New research which does not have to be kept secret for commercial or defence reasons is nearly always published in the form of articles or papers in journals. There area large number of journals publishing research in many scientific fields.

3.3.3 Reports: A significant proportion of applied research and development is first written up in the form of reports. Some reports are made openly available to anyone interested except it is of commercial or military values which are restricted.

3.3.4 Patent: Industrial research and development which has some commercial value is normally published as patent. These are documents granted by governments to the owners of the invention allowing them a monopoly for a limited period of time in order to exploit the invention.

3.3.5 Trade Literature: Trade literature-advertisements, catalogues and company magazines – is a well – used sources of information, particularly in engineering and construction.

Other media of publication are Books, Reviews, Abstracts, Indexes, References and Databases.

4.0 CONCLUSION

The scientific methods of investigation involves experimentation, observation an interpretation of your findings. Your experiments should be capable of repetition by another person anywhere in the world and capable of production of a similar result.

The scientific investigations are also reported and communicated by many conventional methods.

5.0 SUMMARY

The important points to note in this unit are:

- possession of a very keen sense of observation is one of the most important scientific methodologies,

- science involves vivid description or expression of observations made,
- an analytical interpretational method is required of a Scientist
- a good study in science involve experimentation and repetition of such study.
- the report of a scientific investigation should include a title introduction; materials and methods; results, the discussion of the result and perhaps a conclusion.
- accounts of scientists are published in journals, books or patented.

6.0 TUTOR-MARKED ASSIGNMENT

- 1a What is scientific investigation?
- b How do you report your scientific investigation?

7.0 REFERENCES / FURTHER READING

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Unit 2: EVALUATING ORIGINAL RESEARCH PAPERS

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Quality
 - 3.2 Originality
 - 3.3 Focus
 - 3.4 Methodologies
 - 3.5 Contribution(s) to Knowledge
 - 3.6 Application
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

You will be introduced to the parameters one needs to look for when evaluating an original research paper in this unit. You will recall that in unit 1 of this module, we discussed the research methods and techniques and reporting scientific investigations. When evaluating an original research paper, many of but not necessary all the points listed in this unit must be considered.

2.0 OBJECTIVES

After completing the study of this unit you should be able to;

- know an original research paper
- list the criteria for evaluating an original research paper
- evaluate an original research paper

- state if any, the contributions of a research paper to knowledge

3.0 MAIN CONTENT

3.1 Quality:- Quality refers to the standard of something when it is compared to other things like it. When you have a research paper to evaluate, and you compare it with others you have read before or had a knowledge of, will it be able to compare favourably with them? How good or bad is the paper? Does it have all the necessary parameters of a very good research paper? Is it well written following the laid down standards?

Does the paper contain substances or information that will be useful to other researchers nationally and internationally? Will research students learn something from the paper that will facilitate their studies and research?

If most of the answers to these questions are positive, it goes to confirm that the research paper is of good quality.

3.2 Originality:- Ancillary to 3.1 is the originality of the research reported in the paper being evaluated. How genuine are the content of the paper? Do the content contain well thought out and well executed research? Is the work a continuation of somebody's idea or is the idea new? These are a few of the questions to be raised when one thinks about originality of a research paper.

3.3 Focus:- Does the research to be published give attention to one particular problem or does it attend to many problems in a single study? If it is a single study, is it an indepth on superficial study? Is the study directed at a goal? This is all what a focused research paper tends to address.

3.4 Methodologies:- What was done in the study and how this was done are the pertinent questions to be asked in methodology. Does

the method follow the standard techniques and procedure. Were standard equipment and procedure for such study rigidly followed.? Were the steps “diluted” or watered down. Are there improvisation and why? Will this not affect the outcome of such study?

Which ever way, the methodology must follow the laid down standard and procedure for a paper to be rated high.

3.5 Contribution to Knowledge:- What is the outcome of the research findings stated in the research paper? Is the finding of note? Has it added to knowledge on the subject matter? Is there any new information for the scientific community? In all its ramifications methodology, results and findings, has any contribution of note been made to knowledge? These are important questions to be answered to be able to ascertain if the paper had any added value to knowledge.

3.3 Application:- Is the research finding of any immediate or future socio-economic or medical application? Can private sector key in into the findings and “Power” it? Is it of significant for military application? What are the other benefits derivable from the study? Those are some of the questions to be answered regarding application of a research finding.

4.0 CONCLUSION:- In evaluating an original research paper, care must be taken to assess its quality, focus and contribution to knowledge.

5.0 SUMMARY- The important points to note in this unit are:

- The quality of a research paper will determine its acceptability.
- The originality and focus of such paper are of paramount importance.

- The method for the study must be standard and the study should be able to contribute useful information to knowledge now or latter.
- It will also be good if the paper can be of useful application now or thereafter.

6.0 TUTOR-MARKED ASSIGNMENT

- 1a. What is research?
- b. List four major points you need to consider when evaluating an original research paper.

7.0 REFERENCES/FURTHER READING

Adebanjo Afolabi. (1995). Science; History Philosophy and Importance to mankind. Akol publishers, Ibadan.

Giere, R.N. (1979). Understanding Scientific reasoning. Macmillan Press, New York