COURSE GUIDE

BIO 204
BIOLOGICAL TECHNIQUES

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INTRODUCTION

Biological Techniques is a two-unit course, available to students offering biology related courses. The course introduces students to the types of microscopes and their uses; preparation of microscope slides / Examination as well as dissection guides.

At the end of the lectures in this course, students should be able to:

a. Describe the different types of microscopes.
b. Draw and label properly the parts of microscopes.
c. Explain the steps in slides preparation
d. Describe the steps of dissection

COURSE COMPETENCIES

The course will provide general overview of the course synopsis; this course material shall be divided into appropriate sections to help the learners understand and assimilate the contents of the course. The course guide will help students to understand how to go about Tutor-Marked-Assignment which will form part of the overall assessment at the end of the course.

Similarly, structured on-line facilitation classes in this course shall increase the comprehension of the course thus students are encouraged to activity participate. This course introduces students to the knowledge of the types of microscopes and their uses; preparation of microscope slides / Examination as well as dissection guides that will be helpful during in advance studies.

COURSE OBJECTIVES

This course is aimed at providing students the knowledge of the types of microscopes and their uses; preparation of microscope slides / Examination as well as dissection guides
Thus, the course objectives are to;

a. List the different parts of a light microscope and state their functions,
b. State and explain the stages involved in preparation of slides,
c. Describe the basic principles of spectrophotometry, colorimetry, photometry, polarimetry, chromatography, refractometry, melting points and colligative properties.
d. Describe the basic collection and preservation processes of plant and animal materials and their preservation in Herbarium and Museum respectively.

e. Explain the need for experimental design, basis of report writing and presentations

WORKING THROUGH THIS COURSE

The successful completion of this course entails the studying of the course guide and the reference textbooks/materials as well as other materials provided by the National Open University of Nigeria. The course guide is divided into sections, each section has self-assessment exercise. The practice of the assessment will positively influence your academic performance in the course. The course is expected to cover a minimal period of 8 weeks to complete.

Study Units

The Modules of this course shall be in accordance with the course objectives thus;

Module 1

Unit 1: Introduction to Microscope
Unit 2: Biological Drawing Techniques
Unit 3: Dissection guide
Unit 4: Microscope slides preparation technique
Unit 5: Microtomy and Hand Sectioning

Module 2:

Unit 1: Calorimetry
Unit 2: Photometry
Unit 3: Chromatography and Conductometry
Unit 4: Evaluating Original Research Paper & Internet Technology
Unit 5: Scientific Methods of Investigation

REFERENCES AND FURTHER READINGS

In every section or Module, Reference materials shall be provided for further reading.

PRESENTATION SCHEDULE

Assignment	Marks
TMA 1-4	Four T M A s , best three marks of the four count at 10% each - 30% of
End of course examination	70% of overall course marks
Total	100% of course materials

ASSESSMENT

In every section or Module, self-assessment questions shall be provided for further practice.

How to get the Most from the Course
kThe course guide is designed in a simplified form to assist self comprehension. In addition, further references with web links are provided in each sectio/module or unit. Similarly, the course has facilitation session that will provide information on any grey areas.

ONLINE FACILITATION

Eight weeks is scheduled for online facilitation. This facilitation is divided into two session (synchronous and asynchronmous). The synchronous session is a live session that is provided by a facilitator through University approved source (Zoom) for 1 hour. While the asynchronous session is an alternative interaction session that may not be live. In the facilitator dashboard, students have access to the course materials, recorded online facilitation, weblinks, virtual library and host of others that would improve the course comprehension.

MODULE 1

UNIT 1 INTRODUCTION TO MICROSCOPE

Unit Structure

1.1 Introduction

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 intension is to let you have a better understanding of the microscopes and their uses.

1.2 Intended Learning Outcomes (ILOs)

At the end of this topic, students should be able to;

a. define microscope?
b. State the various classes of microscope.
c. Provide proper label of the microscope.
d. State the methods of caring for microscope.

1.3: Main Content

The word microscope is derived from the Greek “mikros”, meaning small and, “skopein” meaning to see. A microscope is an instrument used for looking at objects that cannot be seen with the naked eye and microscopy is the science of using a microscope. In simple terms, it is looking at small things and making them appear bigger so that we can study them. This simple idea has led to a huge number of techniques and methods of observing small things.

The microscope keeps evolving, improving, and expanding its utility. Understanding the fundamentals of microscopy constitutes a significant asset in the workplace, as many scientists work with microscopes even if they have not had significant academic training in the subject. At its core, microscopy is a tool for the exploration of the interaction of electromagnetic energy (here, primarily visible light) with matter. This definition applies equally well across the electromagnetic spectrum. One way to think of microscopy is as visible light spectroscopy in which your eye or a camera function as the detector.

The History of Microscopy

The origins of microscopy can be traced to around 1000 AD when a glass sphere was used to magnify text. In 1021 Iqbal al Haytham wrote a book on "Optics" which increased the understanding of how light behaved but it wasn't until 1590 that Hans and Zacharia Janssen placed lenses in a tube to create the forerunner of modern microscopes. In 1609, Galileo famously developed the compound microscope which was not named until 1625 by Giovanni Faber. In 1874, Ernst Abbe developed a formula that allowed the maximum resolution of a microscope to be calculated. In 1931, Ruske and Knoll built the first Transmission Electron Microscope using an idea from Sziland. Throughout the 20 and early 21 Century, there have been continued innovations in all branches of microscopy. The Nobel Prize has been awarded to microscopy work twice; In 1986 it was awarded jointly to Ruske for work on the electron microscope and Binig and Rohrer for work on scanning and tunneling microscopy. In 2014, the prize was awarded to Betzig, Hell, and Moernerfor the development of super fluorescent microscopy which allows for resolution down to two micrometers.

Optical Microscopy

There are several variations of optical microscopy, one of which is the

a. Compound Microscope. This is the most well-known type of microscope and consists of a tube containing an eyepiece lens at one end, and one or more objective lenses at the other end with different strengths that can be interchanged dependent on what is being studied. There will also be a focusing mechanism and stage to mount the sample and a light source below the sample shining through it. Optical microscopy is usually limited to about 1000 times magnification. Several variations on the optical microscope exist, with some variations having single or binocular eyepieces and different light sources, either light emitting or reflection. It is also possible to have camera attachments or even a digital

microscope that displays the image on a computer screen. The traditional method is to shine a light source through the sample, meaning that sample preparation is critical.

b. Inverted Microscopy is where the light source is above the sample and the lenses are below it. This type of microscopy is particularly useful in biological research Stereo microscopy involves a microscope that has two matched microscopes side by side so that each eye has an individual view of the sample. They are used for dissection, moving microscopic tools, and examining electronic components.

c. Polarizing Microscopy incorporates a polarizing filter into the microscopes so that only a single wavelength of light is transmitted. This type of microscopy finds use in studying crystals and detecting asbestos fibers

d. Metallographic microscopy is used in forensics and diagnostic microscopy where a light that shines onto an opaque surface and is reflected back into the microscope for examination.

e. Reflecting Microscopes use convex and concave mirrors to magnify visible, infrared, and ultraviolet images. This is not an exhaustive list of types of optical microscopy, there are interference techniques that measure the interference to the light as it passes through a sample along with other types. In addition to different types of microscopes, different treatments can be applied to samples such as dye and fluorescent substances to enhance the image or to highlight certain components that need to be studied.

Non-Optical Microscopy

There are several non-optical types of microscopy such as:

a. Scanning Electron Microscopy where high-energy electrons are scanned across a sample and various emissions are emitted and recorded. This type of microscopy can magnify five to 500,000 times Transmission electron microscopy where electrons pass through a thin sample and are recorded. This type of system can magnify up to 50,000,000 times. They can produce detailed three-dimensional images of a sample which allow the topography, structure, and composition of a sample to be examined. This type of microscopy has biological uses as well as industrial uses where they can be used to detect fractures or impurities in minute products such as microelectronics.

b. Transmission Electron Microscope equipment is similar to an optical microscope but uses high-energy electrons instead of light. They are used for study in life sciences, medicine, forensics, and metallurgy amongst many applications.

c. Scanning Probe Microscopy (SPM) can analyze from a nanoscale down to individual atoms. The instrument consists of a sharp tip (as small as one atom) on a cantilever which is moved across a surface to measure deflections. These deflections are recorded and used to produce an image by deflecting a laser off the top of the cantilever. Deflection can be caused by a mechanical, electrostatic, magnetic, chemical bond, Vander Waals, or capillary forces. The probe can be in continuous contact with the surface or, if it is very soft, sometimes the probe will rapidly tap the surface continually. Atomic Form SPM measures electrostatic forces and Magnetic Form Microscopes using Magnetic forces and Scanning Tunnelling Microscopes which measure the current between the probe and the cantilever.

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.

a. *Complex Compound Microscope*: Light or optical types of *complex compound microscopes* combines objective, eyepiece lenses, and light condemner lens and magnify the image of small objects (Fig 1.1)

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

b. *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)

c. *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.

d.

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

e. *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).

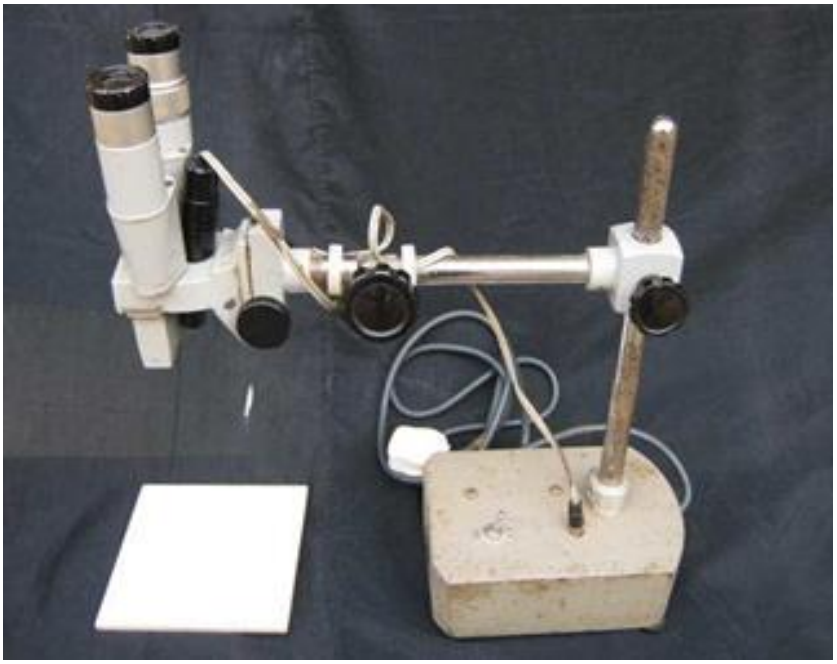

Fig 1.4 Stereo microscope (Photo Bup Oyesiku Bryolab, 2011)

f. *Fluorescence Microscopes:* Fluorescence microscope is a special microscope that uses fluorescence and phosphorescence lights to view samples and determine their properties.

g. *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)

h. *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)

i. *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 Bryolab, 2011)

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.

a. *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.

b. *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.

c. *Dark field microscopy:* Dark filed 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.

d. *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.

Parts And Functions Of A Modern Microscope

Before you buy or use a microscope, it is important to know theparts and the functions of each one. The parts of a typical modern complex compound microscope are described below (Fig 1.8)

a. *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 of 15X or 16X.

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

c. *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.

d. *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 power (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 image. 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.

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

f. *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.

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

h. *Base (BA):* The bottom of the microscope uses for support andhouse the power source, fuse and illuminator.

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

j. *Illumination Control:* This bar controls the amount of light passingthrough the illuminator condenser.

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

l. illuminator (*IL*) *Condenser Mover (CM):* A knob you use to move the condenser upor down, to attain a good quality of light.

m. *Focus Knobs (FN):* Knobs that make rough and fine adjustments to be focused. You have two knobs on a m microscope, largeknob 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.

n. *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.

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

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

q. *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

r. 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).

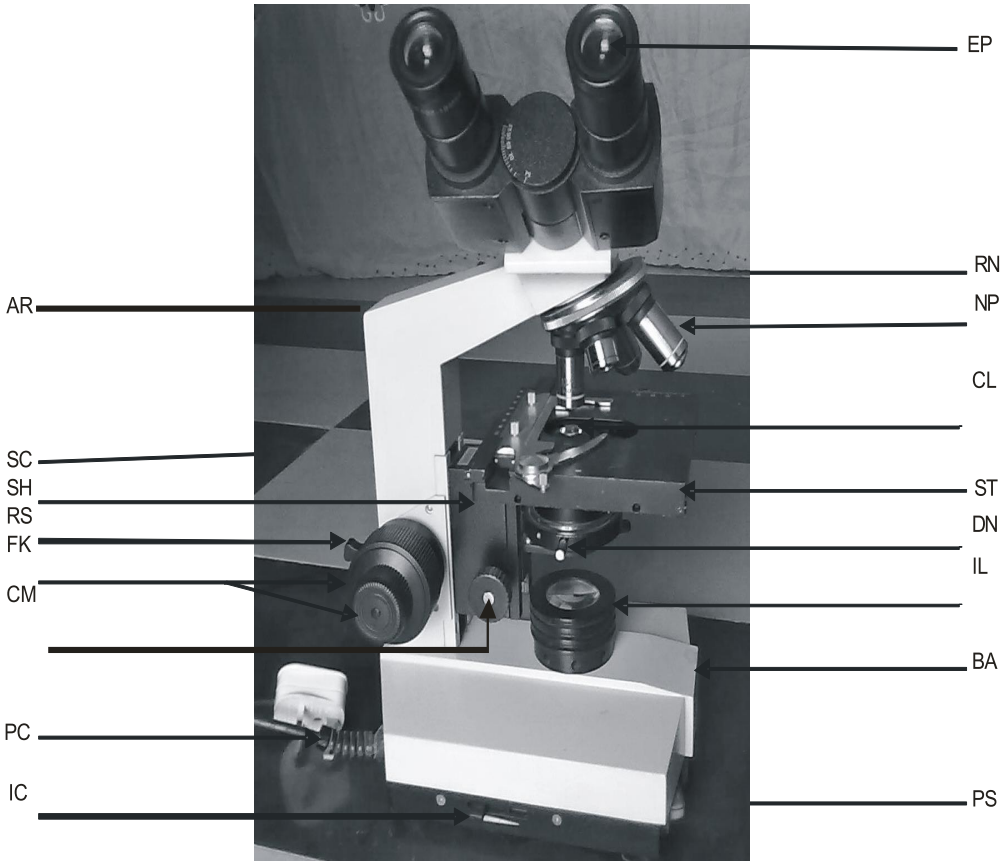

Fig 1.8: Modern Complex Compound Microscope with condenser lens beneath the stage (Photo from Bup Oyesiku Bryolab, 2011)

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 t 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 adjustment with the fine focus knob. Continue with subsequent objective lenses and fine focusing each time.

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. Always carry your microscope with one hand on the Arm (*AR)* and one hand on the Base *(BA)*, and close to your body.

Plug the microscope in and place excess wire on the table. Always start and end focusing with low power Objectives lens (*OB*). Always make sure the stage and lenses are clean before you put away the microscope. Always use good quality lens tissue to clean before you put awaythe microscope.

Always cover your microscope with a dust jacket when not inuse.

What is microscope?

What is microscopy?

Self-Assessment Exercises

1. List the several variations of optical microscopy?
2. What is Bright field microscopy?

1.4 Summary

Microscopes are special lenses designed to magnify small objects that are difficult to see with the naked eyes. There are many types of microscopes; simple, component, stereo dissecting, fluorescence digital and electron. The type you will chose 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.

Microscopy is a varied and valuable science that has many different forms and uses several different techniques. Whatever the object to be studied, there will be a type of microscopy that is suitable. Some techniques are very expensive, so the type of microscopy and sample preparation chosen for a particular project will always depend on the objectives and the budget available. Microscopy is continually developing as science and modern manufacturing techniques allow for better and better equipment to be produced and higher image quality and greater magnification.

1.5 References/Further Reading/Web Sources

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1.6 Possible Answers to Self-Assessment Exercises

1. a. Compound Microscope.
   b. Inverted Microscopy
   c. Polarizing Microscopy
   d. Metallographic microscopy
   e. Reflecting Microscopes

2. 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.

UNIT 2 BIOLOGICAL DRAWING TECHNIQUES

Unit Structure

2.1 Introduction

Knowing the appropriate ways of drawing a biological diagram can surely improve your overall performance in BIOLOGY and also enhance your understanding about the subject. During my teaching, I observed that, many students performing well in the descriptive components of BIOLOGY, but as soon as biological diagrams crop up in their examinations or quality of homework. They develop a tendency to struggle for making diagrams. If you want to score more in biology, you must read the following advised and principles or can say the technically-feasible rules of drawing a biological diagram.

2.2 Intended Learning Outcomes (ILOs)

At the end of this topic, students should be able to;

- State the rules of biological diagram.
- Apply the rules of biological diagram in drawing a biological specimen.
- Calculate magnification.

2.3 Main Content

Rules of Biological Diagram

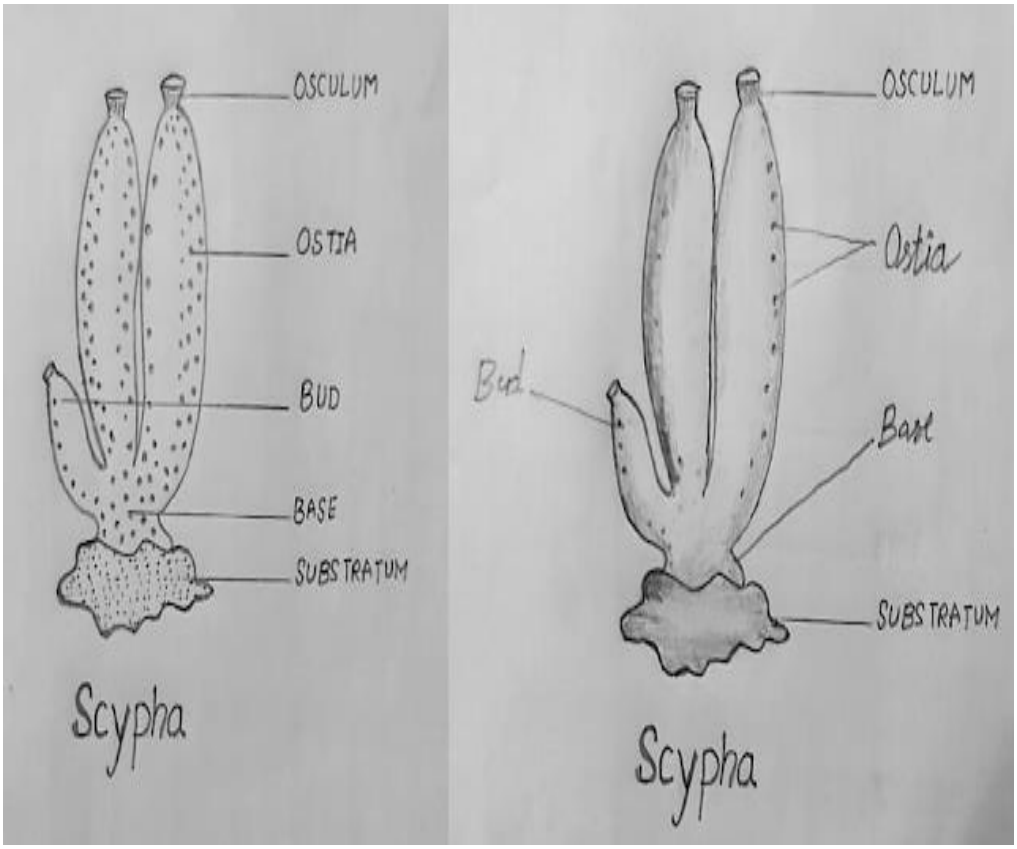
a. No need for Improvisation- You cannot use your creative brain to draw biological diagrams on your answer sheet. You should draw what you see in the textbook, NOT what you WANT to see. So please do not imagine anything.

b. Use just right Pencil- Make sure you use a good quality pencil for your diagrams. Extra bold or 2H lead pencils are more advisable. And remember *NEVER draw your diagrams with a pen.*

c. Simplicity is what's preferable- Try to make your diagrams as simple as possible, but must be Accurate in its general proportions, and should be precise in its overall.

d. Keep away from shading- Shading specific areas of a diagram might look aesthetic to the eyes and you, but this train just not acceptable in biology. Hence, the advice is to AVOID at all costs. Represent darker areas of an object with stippling or dots. Do not shade any areas of the diagram. colouring or graying of the areas should be strictly prohibited.

e.



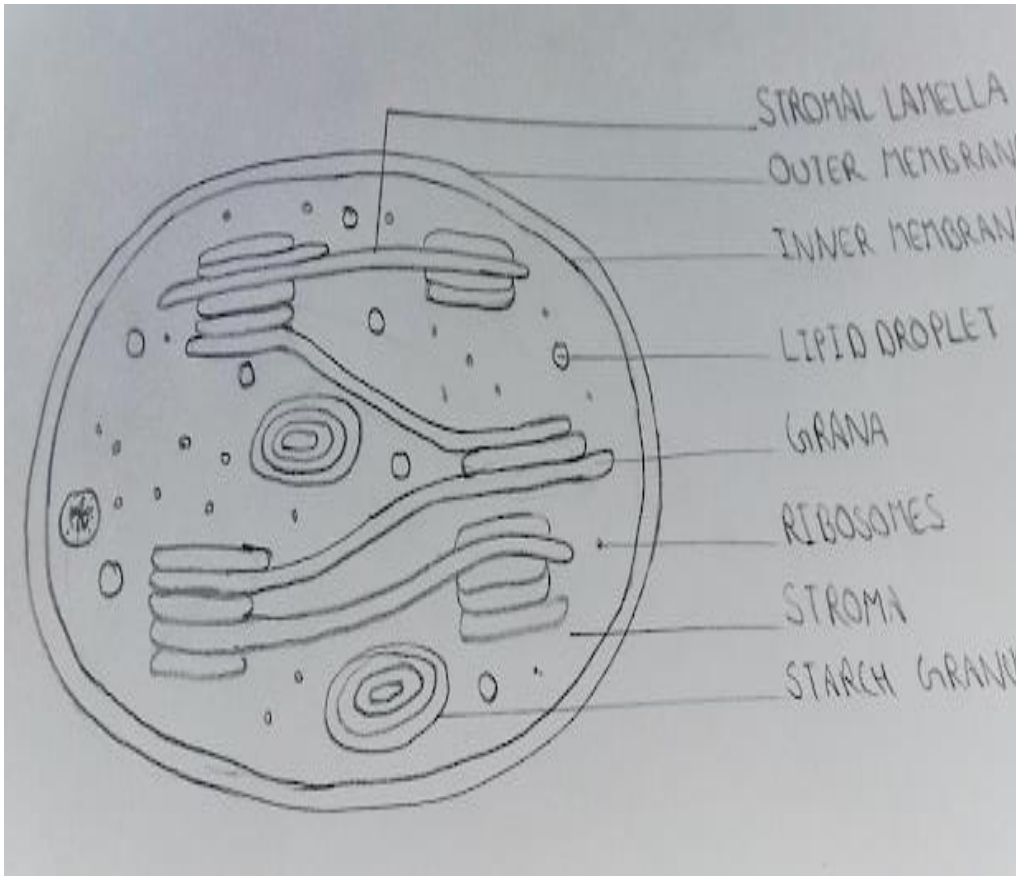
f. Drawings must be executed on an unlined sheet- Biological drawings must be done on unlined sheets or plane sheets, for more authenticity. This principle should be followed particularly when you are drawing a diagram for your biology lab copy or record. *But don't worry about your board exam you get lined copy and you have to draw on that lined sheet.*

g. Biological drawings should be conspicuous for easier comprehensibility- Avoid crowding components of the diagram. A biological drawing must bear all relevant parts that are conspicuous enough to the human eye. It should also be large

enough to current the whole complicated background details of the diagram to the observer.

h. Labeling and positioning: As far as labeling is concerned, follow the following rules as closely as possible for more efficiency: Labeling should be done in a column at the right-hand side of your page. Try to maintain alignment to the best of your abilities. Always use a scale for drawing the lines used for labeling purposes, and it's more advisable to keep those lines parallel to one another. The lettering used for labeling should be kept in a horizontal alignment. Try to avoid vertical lettering unless you are specifically instructed or constrained to do so. Keep your lettering neat and intelligible such that the observer can understand it in a jiffy. For easier comprehensibility, may use block letters for lettering purposes. Do not use the plural form when identifying a single part or object.

i.



j. All biological drawings should be titled. Write the title of the diagram in capital letters and center it

k. Try to draw the diagram on a single stroke of the pencil. Multiple strokes need to be avoided at all possible costs.

l. Use sharp single lines to represent an object. Do not use soft lines, characteristic of sketches

Calculating Magnification

These are the formulae.

$$Magnification = \times \frac{length\ of\ drawing}{length\ of\ specimen}$$

Show your working when calculating the magnification. Do not forget to check the units, make sure they are the same (*cm* or *mm*) before you do the division. *The degree of accuracy for your calculated value should not be more accurate than your measured values.* For example, if the length of drawing measured is 10.0cm and the length of the specimen is 6.0cm, the magnification should be written as x1.7 (to 1dp), not x1.67.

$$Magnification\ (when\ using\ microscope) = \times (magnification\ of\ eyepiece \times magnification\ of\ objective\ lens)$$

Use this when using the microscope for your drawing. For example, the magnification of the eyepiece is x10, multiply that with the magnification of the objective lens, say x40, the combined magnification would be x400.

In-Text Question (ITQ)

Question: State the formular for magnification.

Ans:

$$Magnification = \times \frac{length\ of\ drawing}{length\ of\ specimen}$$

Self-Assessment Exercises

1. Explain labelling and positioning rule of biological diagram.

2.4 Summary

Here are the general principles when drawing biology drawing manually. Use good quality and sharp pencils to draw clean lines and avoid using pencil colors. Make your diagram simple without any sketching, and make it big enough to at least cover 10 lines on the paper to illustrate the complete structure.

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2.6: Possible Answers to Self-Assessment Exercises

- Labeling should be done in a column at the right-hand side of your page.
- Try to maintain alignment to the best of your abilities.
- Always use a scale for drawing the lines used for labeling purposes, and it’s more advisable to keep those lines parallel to one another.
- The lettering used for labeling should be kept in a horizontal alignment.
- Try to avoid vertical lettering unless you are specifically instructed or constrained to do so.
- Keep your lettering neat and intelligible such that the observer can understand it in a jiffy.

- For easier comprehensibility, may use block letters for lettering purposes.
- Do not use the plural form when identifying a single part or object.

UNIT 3 DISSECTION GUIDE

Unit Structure

3.1 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 beforehand.

3.2 Intended Learning Outcomes (ILOs)

At the end of this topic, students should be able to;

- Explain the steps in preparing dissection trays/petri plates.
- Explain the steps in anaesthetizing animals correctly.

3.3 Main Content

Preparation for Dissection

Materials Required

a. Dissection trays
b. Petri dishes
c. Dissection kit
d. Chloroform/Ethane/Formalin
e. Microscope
f. Table lamp
g. Animals

Procedure

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.

a. Anaesthetizing the Animals: Before dissection, animals are given anesthesia. 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, scolion is also preserved after anaesthetizing them. Preservatives commonly used are formalin (5% or 8% or 10%) and 70% alcohol.
b. 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.
c. 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 petri plates.
d. It should be seen that wax is spread over trays uniformly and water does not drip form 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.
e. 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.
f. 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.

Pithing

Some of the experiments like muscle twitch and heart profusion in frog do not require chloroform-anaesthetized animals. Before dissection is started, you have to anesthetize 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. Check this out ifyou have forgotten the method.

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 x0.7 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.

General Rule of Dissection.

As you have learnt earlier in this unit, you need to give some anesthesia to animals before you dissect them. The common anesthesia used in the biology laboratory and chloroform and ether. For rats, frogs and pigeons, it is better to dissect them immediately after anesthesia. 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, andsoak away blood with cotton wool or blotting paper.
Itemize materials required for animal dissection.

Self-Assessment Exercises

1. Explain Pithing

3.4: Summary

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

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3.6: Possible Answers to Self-Assessment Exercises

Pithing is the immobilization of animal (frog) before dissection

UNIT 4 MICROSCOPE SLIDES PREPARATION TECHNIQUE

Unit Structure

4.1 Introduction

Microscope allow us to peek into the tiniest worlds. With a microscope you can open up a whole new world that exists at the cellular level: amazing, unpredictable and full of surprises. One of the great things about prepared microscope slides is that they can be stored for a long time and you can observe and study the process of cell decay over time. But if you can't wait to observe some new interesting samples or if you are planning on major microscopic research, you will need to learn how to prepare microscope slides yourself.

4.2 Intended Learning Outcomes (ILOs)

At the end of this topic, students should be able to;

- Distinguish the different types of slide preparation.
- Explain the steps in preparing slides.
- State the precautions in slide preparation.

4.3: Main Content

Preparing dry mount microscope slides

Dry mount microscope slides are used for studying samples that do not require contact with water in order to survive. The first thing you need to have is a clean blank slide. Very carefully place as thin as possible slice of a sample in the center of the blank slide and cover it with a cover glass. If you wear rubber gloves, you can gently pin down the cover glass to align the sample.

Preparing wet mount microscope slides

Wet mount microscope slides are used for studying samples, which cannot survive without water. Usually those are unicellular organisms and tiny animals. Take a clean blank slide. Place one or two drops of distilled water in the center of the slide using a dropper. Put the sample in the water and cover it with a cover glass. You can pin down the cover glass a little if you are wearing rubber gloves. Do not touch the glass if you are not wearing gloves as fingerprints will certainly appear on the glass, which will significantly impede studying the sample.

A wet mount microscope slide holds the cover glass in place by itself and may be stored for some time. If observed microorganisms are too mobile to be properly studied, you can “slow them down” by adding a binder in the water, for example ProtoSlo (methyl cellulose in a 1.5% solution).

Staining samples

Some organisms are difficult to observe under a microscope without additional staining. One of the best ways to stain microscope a slide is to add a drop of Lugol's iodine (a water solution of iodine and potassium iodide) in the water before placing the sample in it. You can also use solutions of methylene blue or Gram crystal violet.

Preparing Microscope Slides

When preparing microscope slides for observation, it is important first to have all necessary materials on hand. This includes slides, cover slips, droppers or pipets and any chemicals or stains you plan to use. There are two different types of microscope slides in general use. The common flat glass slide, and the depression or well slide. Both are rectangular and measure approximately 1 x 3 inches (25 x 75mm). Depression slides have an indentation in the center to hold a drop of liquid, cost considerably more than the flat variety, and can be used without a cover slip.

Standard slides are made of glass or plastic. For most purposes, glass slides of 1 to 1.2mm thick are used. When working with high power objectives and condensers, the slide thickness should be reduced to 0.8 to 1mm. When ordering slides, always order more than you expect to use. They usually are packaged in increments of 72 (which, incidentally, is 1/2 gross!).

A cover slip or cover glass is a very thin square piece of glass (or plastic) that is placed over the water drop. Because of surface tension, the water drop alone tends to sit in a thick dome. With a cover slip in place, the drop is flattened out allowing the investigator to focus with high power very close to the specimen. The cover glass also confines the specimen to a single plane and thereby reduces the amount of focusing necessary. Finally, the cover glass protects the objective lens from immersion into the water drop.

Glass cover slips should be handled carefully as they are very fragile and break easily. Cover slips measure 18 or 20mm square and the glass variety is available in two thicknesses, Number 1 and Number 2. Number 1 cover glasses are 0.13 - 0.17mm thick and are recommended for oil immersion or high-resolution work. Number 2 slips are 0.17 - 0.25mm thick and are used for general applications. They are sold by the ounce and there are about 120 cover slips per ounce (20 x 20mm, Number 2). With extreme care, glass cover slips can be rinsed and reused many times.

For most general applications, a "pipet" is nothing more than a medicine dropper. They are inexpensive and can easily be cleaned or sterilized (after removing the rubber bulb). Longer pipets are available (for sampling the bottom of a deep jar) and you can make your own micro pipets with glass tubing.

When preparing a well slide, simply transfer one to four drops from the sample container to the depression slide. Focus carefully and do not use the higher power objective lenses as they will likely get wet when focusing too close to the drop. The most common slide preparation is called the "wet mount" slide and utilizes a flat slide and a cover slip. To make one, place a drop of the sample in the middle of a clean slide and lower a cover slip gently over the drop at an angle, with one edge touching the slide first (See Figure 1, below). Allow the liquid to spread out between the two pieces of glass without applying pressure. It takes some practice to determine just how much liquid to use. If too much is placed on the slide, the cover slip will "float", creating a water layer that is too thick (allowing protozoans to swim up and down, in and out of focus). If too little liquid is used, the organisms may be crushed by the cover glass and evaporation will dry up the specimens quickly. A well-prepared slide will last for 15-30 minutes before it dries up.

To extend the life of a wet mount slide, scrape petroleum jelly onto each of the four edges of the cover slip (Figure 2). Place the cover slip over the drop of water ("jelly side down" - Figure 3), and press lightly to seal it to the slide. This sealed slide may last for several days.

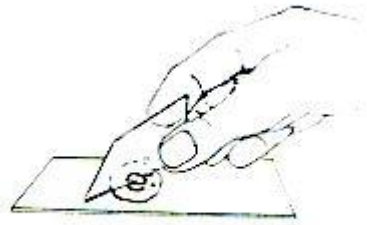


Figure 1

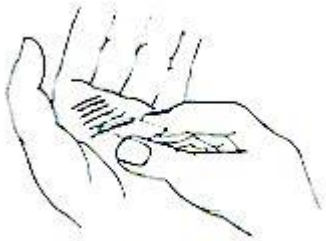


Figure 2

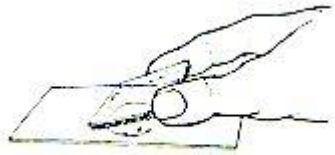


Figure 3

To use a cover slip with larger specimens, add a few broken bits of cover glass to the water drop. When the cover slip is placed over it, the solid particles will add considerably to the thickness of the channel (this is called a "raised cover slip").

To observe Daphnia, use a well slide and carefully hold a tiny piece of twisted paper towel to one edge of the drop. Soak up water with the towel until the Daphnia becomes "grounded" and is unable to move. Use a low power microscope if possible when doing this to avoid soaking up Daphnia in the towel.

Many protozoans move too quickly for accurate observations. Larger ones (i.e., *Paramecium*) can be "corralled" by adding a few strands of cotton fiber from a cotton ball or swab to the drop of the sample before lowering the cover slip. Commercial chemicals called "proto-slow" or "quieting solution" are available from supply companies and work very well, particularly on ciliates. Usually, one drop of quieting solution is added with one drop of sample and the protozoans slow to a stop within ten minutes. When working with these solutions be careful not to contaminate the sample container with the chemical dropper!

Staining techniques can be employed to aid in the observation of cell parts. "Non-vital staining" is the staining of dead cells or tissues. "Vital staining" is the staining of live cells. Staining is a very complex subject, but there are many simple staining techniques that can easily be

performed. Both pre-stained live samples and pre-stained permanent slides (dead organisms) can be inexpensively acquired from science supply companies.

When ordering permanent slides, look for the following abbreviations: "st" is a stained preparation; "wm" means whole mount (the complete organism); "cs" is a cross section, such as a thin wafer of a worm; "ls" is a longitudinal section, a section cut lengthwise; "sq" is a squash preparation; and, "sm" is a smear, such as a blood smear.

How to Prepare Microscope Slides

Purpose of Microscope Slides
To examine single-cell organisms, small plants, and other types of organisms, you'll need a microscope. However, to hold the samples in place and enable light to pass through the samples, they need to be held in place on a microscope slide.

There are two specific types of prepared slides used for this purpose, dry mounts, and wet mounts. Each type of slide is designed for mounting different types of cells. Sometimes when you're wet mounting a specimen that you cannot see particularly clearly, such as a pale or translucent specimen, it may be necessary to stain the specimen; otherwise, you wouldn't be able to see it visible underneath the microscope.

How to Prepare a Dry Mount
C
hoose a Clean Microscope Slide

a. Select a slide and, holding it by the edges, hold it up to the light; you're making sure there are no fingerprints or other dirt and dust on the slide. Typical slides are rectangular and flat on the top and bottom.
b. Slides need to be completely clear to allow the microscope's light to pass through the slide and illuminate the specimen you are studying.
c. Any dirt or smudges or fingerprints will ruin your opportunity of getting a clear view of your specimen. You must wash the slide with soap and water to remove any contamination on the slide if you find any; dry the slide only with a clean cotton cloth.

Specimen Inspection

a. Specimens must be transparent or at least semi-transparent to allow light to pass through them. If, for any reason, light is

blocked and does not pass directly through the specimen and into the microscope's eyepiece, you will not see the specimen.

b. Depending on what you're attempting to view will determine if you need to slice the specimen; some specimens are thin and transparent, for example, an insect's wing, and do not require slicing.

Slice The Specimen

a. Using a dry mount is the simplest and easiest slide to prepare because you do not need to use liquid between the specimen and the slide. Use a dry mount for specimens that will not dry out such as, flower petals, insect legs or wings, feathers, hair, or fur.

b. As we mentioned, transparent or semi-transparent slices of your specimen are necessary to obtain a clear view of the specimen. Using a razor blade for this, you will be able to slice thin enough specimens for light to pass through them.

Position the Specimen onto The Slide

a. Because the specimen is so thin, you'll need to take great care, use forceps to pick up your sliced specimen, and gently position it onto the slide (it doesn't matter which side), unless you have a specimen that you fear might roll off the slide, then use a concave slide to ensure this doesn't happen.

b. If your specimen is not going to roll off the slide, then use a flat-sided slide.

Use A Cover Slip

a. A coverslip is an ultra-thin piece of glass (nowadays, it's more common for coverslips to be plastic), approximately 3/4-inch square.

b. The job of a coverslip is two-fold. One, it prevents the specimen from slipping off the slide. Two, the coverslip prevents any harm from coming to the sample if a user lowers the microscope's lens too much and inadvertently damages the specimen.

c. You now have a fully prepared dry mount slide ready for inspection under your microscope.

How to Prepare a Wet Mount

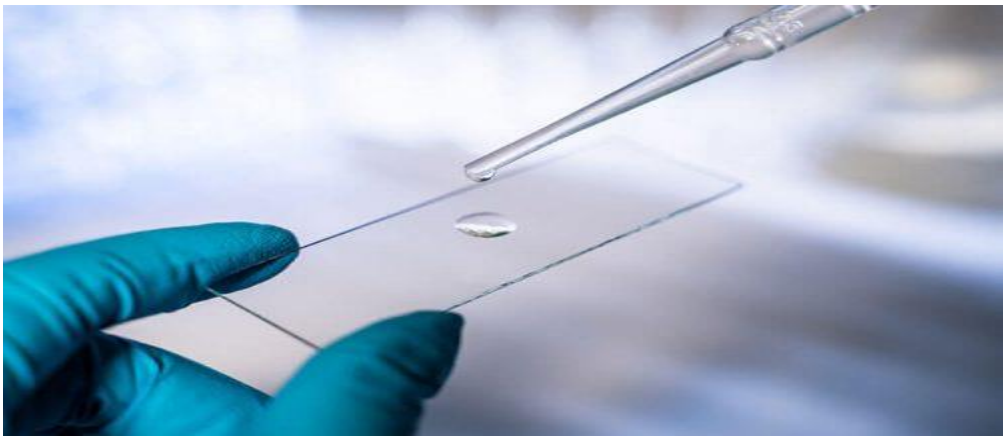
Place A Drop of Water on The Slide

a. Inspect your slide and ensure there is no dirt or fingerprints on the slide. You can use a concave or flat slide; it's up to you.

Position a drop of water in the center of the slide using an eyedropper. The mount takes its name because you're using water on the slide.

b. You need the water to ensure the organic sample your viewing doesn't dry out under the microscope lights. The water will preserve any living organisms you're viewing, for example, single-cell organisms.

c. If you want to create a permanent slide of dead organic material, you can substitute the water for clear nail varnish.



Prepare A Wet Sample Specimen

a. Wet mounts typically feature living or organic materials. You can use a toothpick or razor to cut or scrape to generate a small piece of your wet specimen. There are some common materials used for creating wet mount slides, such as:

b. Human cheek cells or plaque from your teeth.

c. Take a plant stem and cut a thin cross-section with your razor blade.

d. Single-celled organisms such as amoebas or paramecium will require a different method of collection. Easily the quickest way is to find water where you know algae or single-celled organisms swim and use a clean-eyedropper to suck up one or two drops.

Position Specimen onto The Slide

a. How you place the specimen onto the slide depends on what the sample is. You can use anything from tweezers to a toothpick. If you're studying single-cell organisms, use the eyedropper to drop a couple of drops into the water already on the slide.

b. Whatever tool you use to transfer the sample, ensure you place the specimen directly into the center of the water on the slide.

Place A Coverslip onto The Slide

a. Once you place your sample into the water on the slide, place a coverslip over the top. Now position one edge of the coverslip onto the slide next to the water. You should gently lower the other edge of the coverslip until it rests on top of the sample. The pressure from the coverslip will spread the drop of water outwards until it reaches the edges of the coverslip.
b. Do not exert any pressure on top of the coverslip, or you will damage the specimen.

Permanent Slide Preparation

There is a series of processes by which a living organism or its tissue is made fit for microscopic examination in a permanent state. The utility of permanent preparations is that the animal cell or tissue remains as such without undergoing major changes. The method of permanent preparation includes—

a. Killing and narcotization: The first step in permanent preparation is killing instantaneously in order to prevent the change in form of the object as it had in living condition and immediately fixing the object Sometimes killing is preceded by narcotization. The narcotics used are chloroform, menthol, ether, alcohol, acetone and chloroform, etc. The purpose of narcotization and killing is important so as to have the same form and chemically constructed tissue or organism as it had during its lifetime. In certain cases, in smaller animals, killing is done by heating. In the case of the whole mounts and micro-organisms, certain chemicals like corrosive sublimate, bring about killing and fixation simultaneously.
b. Fixing: Fixing is done with various fixative agents for histological elements. Fixing is essential in every type of microscopic preparations either for sections or for whole mounts and also in larger specimens. The function of fixation is manifold.

- The tissues become hard and the hardening resists further post-mortem changes.
- Fixative agent coagulates and renders insoluble the elements of tissues which may not be dissolved in further processing.
- The fixative agent renders insoluble the various constituent elements of cells, alters their refractive indices and thus makes them optically differentiated under the microscope. Various fixative agents generally used are absolute alcohol, 90 per cent alcohol plus glycerine, picric acid, corrosive sublimate, form©!, osmium tetroxide and nitric acid with or without water.

c. Washing: Washing is essential as by this process the uncombined and excess of fixative agent is removed. The presence of fixative agent in tissues or cells will inhibit good staining. The washing agent depends upon the type of fixative agent used. As picric acid in water is removed by water, picric acid in alcohol is removed by 70 per cent alcohol. Fronie and corrosive sublimate are washed with water distillate. Sublimate is washed in alcohol.

d. Staining: The tissues or cell components are stained in various dyes. The dye makes the tissues distinct in its histological sphere. The various dyes are Orange G., Bordeaux red, sudans, congo red* Alizarine oxyqainolne, methylene blue, neutral red, borax carmine, haematoxylin, picro-Indigo carmine, eosin and Gower's carmine. Mainly two kinds of stains are used:

- Nuclear stains which stain nuclear parts of the cell, such as Delafield's or Erhlich's haematoxylin.
- Cytoplasmic stains such as borax carmine, picro-indigo carmine, Gower's carmine and eosin, etc., which stain cytoplasm.
-

For general and whole mount staining borax carmine is used. Aqueous stains are prepared in water, whereas alcoholic stains are prepared in alcohol. When a single stain is used the process is called as simple or single staining. In some cases, two stains, i.e., nuclear and cytoplasmic, are used and this process is called as double staining

e. De-staining or removal of excess of stain: The removal of excess of stain is called as destaining or differentiation. De-staining agents are acid alcohol or acid water. The acid alcohol is used with alcoholic stains, while acid water is used with aqueous stains.

f. Dehydration or removal of water: This process is meant for removal of water from the tissues. The dehydration prevents putrefaction of decaying and maintains the same shape and size of tissues or cells. The moisture or water in tissues absorbs various germs of destructive-nature so that the tissue may be destroyed, hence the necessity of dehydration. Dehydration is done by passing the mounting material dough various grades of alcohol such as 30,50,70,90 and 100 per cent alcohols. The tissue is soaked in gradually increasing strengths of alcohol. The lower grades of alcohol, such as 30, 50 and 70 per cent alcohols, are prepared either from 90 per cent or absolute alcohol. The dehydration is carried out in corked or glass-stoppered tubes.

g. Clearing or de-alcoholization: After dehydration, transparency in tissues is obtained by treating with a clearing agent which removes alcohol and makes the tissue clear and transparent The clearing agents are cedar wood oil, clove oil, xylol and benzol, etc. Xylol is most commonly employed and it makes the tissues

hard and brittle. Clove oil is a superior clearing agent specially in the whole mounts. It also possesses a higher index of refraction than balsam mounting media

h. Mounting on slide: Mounting marks the end of permanent preparation. The choice of mounting media is not much but they should have the same refractive index as that of the cleared tissue. The refractive index of such a stained, dehydrated and cleared cell is N = 1.54. Canada balsam has almost the same refractive index. Mounting is an easy process. The tissue is kept over glass slide in a drop of balsam and cover-slip is lowered slightly. After mounting, the slide may be kept for drying in a hot chamber. The excess of balsam on the slide, as generally happens with beginners, should be removed with cotton soaked with xylol or 90 per cent alcohol. This should be done when the balsam has dried. For much better finishing the edge of the cover glass may be ringed with a cement such as gold size or a varnish. The air bubbles present in balsam under cover glass should be removed by gentle heating. During all the chemical bathing of tissues, two changes of each regent are necessary. The time of keeping tissue in various reagents may vary from 5 to 15 minutes

i. Labelling: After mounting the slides are labelled. Preferably use printed labels or make the labels from ordinary paper. Write over the label the name of the animal or mount, date, time and your name.

Precautions and instructions

- The articles, such as slides, cover-slips and instruments should be perfectly cleaned.
- The working place should be kept in order.
- During dehydration the tissues should be kept in tightly- closed cork or glass-stoppered tubes. The opened tube will spoil material by absorbing moisture from atmosphere. Even breathing closely with dehydrating tube is undesirable.
- The change of solution should be done very quickly, reducing time of exposure to atmosphere to minimum. (5) The chemicals used once should not be reutilized.
- The Canada balsam used should be clean, dust-free and not viscous.
- Embedding, depending on melting point of wax, adjust the oven at 58 °C or 60°C. Take wax with ceresin instead of plain wax. Keep flakes of wax in a beaker of 100 ce. in oven 4-5 hours before embedding. In another beaker keep some wax plus xylene. Now take the tissue from xylene and first keep it in the beaker containing xylene + wax for 30 minutes. Then transfer the tissue in pure melted wax for embedding for 1-2 hours. Normally

double time is given for embedding than the time required by tissue to sink at bottom in xylene.

- Block making. Make blocks either in metal L-shaped angles or in paper boat or in cavity blocks. 'L' pieces are preferred. First apply little glycerin on their internal surface. Pour melted wax at bottom of rectangular cavity formed by two 'L! pieces. Then add tissue and more melted wax to fully cover the tissue. Keep L pieces in a trough. Add water around them. As the melted wax is solidified, flood it with tap water. After cooling, the block comes out from 'L' pieces or remove L pieces. Because of glycerine 'L' pieces don't stick with wax. During block- making, see that no air bubble comes. If there are some air bubbles remove them with hot spatula.
- Trimming of blocks. Trim the wax around the embedded material and make a perfect rectangular block. On one side keep sufficient space in the block for fixing it on a block holder. Apply half an inch wax layer over block holder.
- Section cutting. The blocks are cut either by rocking microtome or rotatory microtome at 6 microns (\x) thickness. Ribbons are kept over Mayer's albumen coated slides. Keep-clean slides ready. Apply pin head Mayer's albumen over the slide and rub it by last finger. Mayer's albumen helps in sticking the sections over glass surface. Keep 2 to 4 rows of sections depending upon the breadth of the sections. Ribbons should be kept upto more than half of the slide. Space should be left for putting labels over the slide. Flatten the section over a hot brass plate. The temperature of the hot plate should be nearly 40-45 degree. Add few drops of water below ribbons. As the water is heated ribbons become expanded by semi-melting of the wax. Sections should not be separated. Never do flattening over spirit lamp as in most of the cases wax melts and sections are burnt After all the sections and ribbons become flattened, drain off water and leave the slide at room temperature overnight for drying the ribbons. Mark the ribbon side by glass marking pencil.
- Staining, dehydration, clearing and mounting. Double staining is applied. Haematoxylin and eosin stain nucleus and cytoplasm of the cells

What is the purpose of microscope slides?

Self-Assessment Exercises

Which type of slides are the easiest to prepare?

A. Dry mount
B. Wet mount
C. Prepared mount
D. Electron

4.4 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.

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4.6 Possible Answers to Self-Assessment Exercises

1. A

UNIT 5 MICROTOMY AND HAND SECTIONING

Unit Structure

5.1 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.

5.2 Intended Learning Outcomes (ILOs)

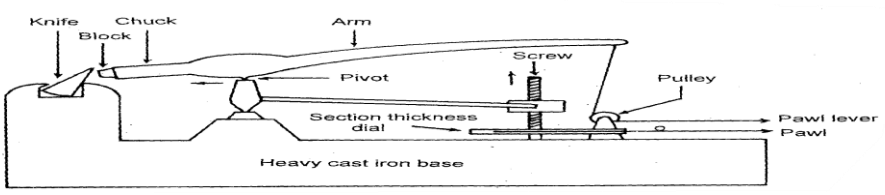
At the end of this topic, students should be able to;

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

5.3 Main Content

Microtomes

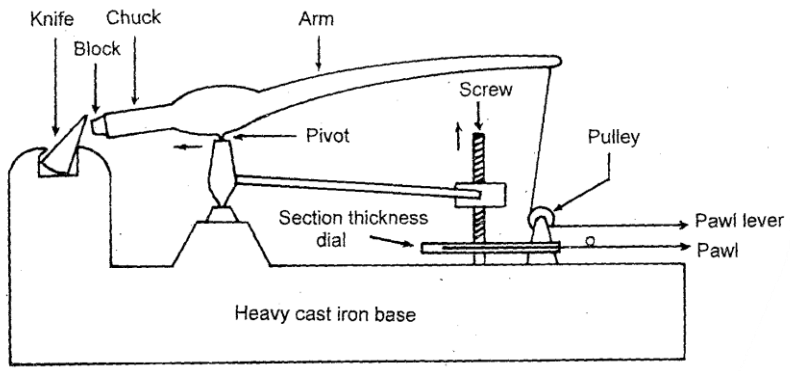
You will come across microtomes in a biology lab. The mechanical cutting of plant/animal materials are done by the aid of microtomes and the process is commonly known as microtomy. Mircrotomes can be divided into four basic groups: Rocking, Rotary, Sledge (sliding) and



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.

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. 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.

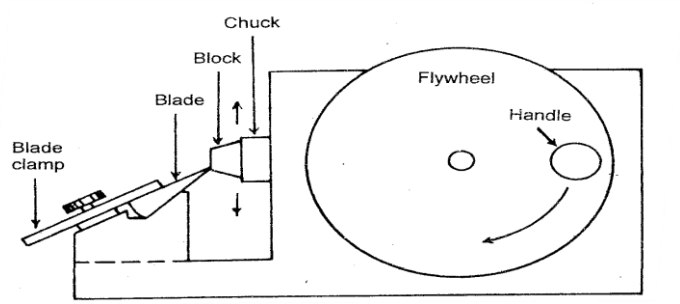
The block for sectioning is fixed to the chuck which is rocked up and down about the *pivot* by the action of the *pawl lever* on the arm. With each rocking movement the chuck passes the *knife* which is only a little heavier than a cut throat razor, and which has its cutting edge upward and a slide from the block on the downward stroke. On the upward stroke the *pawl* itself is engaged and moves the chuck forward by and 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.

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. Rotary microtomes enable sectionsof 5 microns thickness to be cut.



Sledge Microtomes

The sledge microtome is a devise 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.

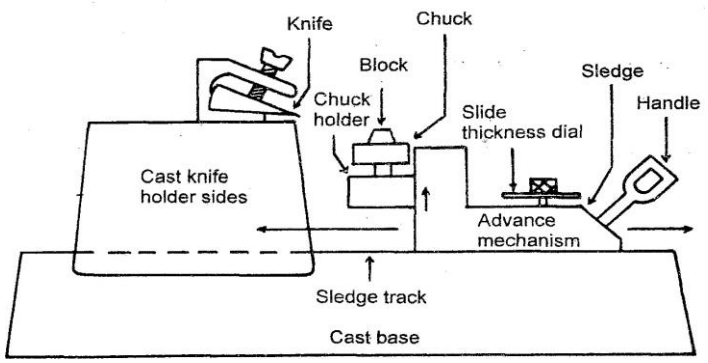


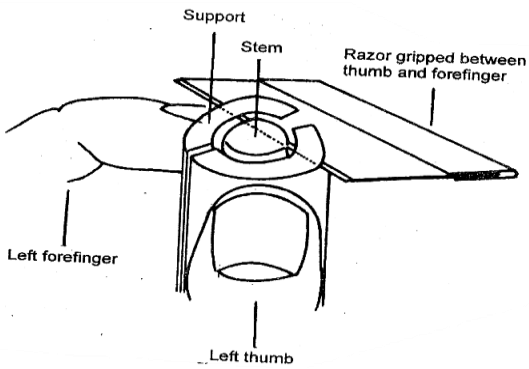
Fig 2.3: A sledge microtome.

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 CO_2 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 CO_2 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.



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). 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.
Fig 2.4: Microtome Knives

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 bladesmay 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 hunkof 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 aboutfifty times. This is how they come from the manufacturer! The job of a microtomist is to keep it that way.

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 recentsubstitute (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 (Fig 2.5). The single hollow ground 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 iteasier 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 mounting. The commonest rocking microtome is called?

Self-Assessment Exercises

What is Rotary Microtomes?

5.4 Summary

Microtomes as instruments used for cutting plants or animals inthe 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. The important points to note in this unit are:

- Microtomy is the mechanical cutting of plant / animal materials inthe laboratory.
- The Rocking, Rotary, Sledge / Sliding and the Freezing are the fourbasic groups of microtomes.
- The actual cutting is done with microtome knives or sharp razorblades.
- 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 theobject preferably only one cut thick for microscopic viewing.

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5.6 Possible Answers to Self-Assessment Exercises

- Rotary Microtomes are larger and heavier instruments even more heavily damped to prevent vibrations
- 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.
- Rotary microtomes enable sections of 5 microns thickness to be cut.

Glossary

- **Arm**- The part that serves as both the support of the body tube and its lens systems. The part held when the microscope is carried. Also sometimes referred to as limb.
- **Base**- The weighted bottom portion of the microscope which gives it both balance and resistance to unexpected movement or vibration.
- **Condenser**- The lens system between the illuminator and the specimen which condenses the light onto the specimen.
- **Eyepiece**- The lens system closest to the eye. Also referred to as "oculars"

- **Illuminator** - The source of light which illuminates the object or specimen to be observed. It may have fixed intensity or variable intensity via a control knob.
- **Microscope**- A high precision optical instrument which uses light to observe objects. It is capable of high magnification and resolution and is used for making minute details visible.
- **Stage**- The platform which holds the specimen. Types include: plain, mechanical, motorized, heated, etc.

End of the module Questions

1. What are the types of microscopes?
2. What is a simple microscope?
3. What are the various types of compound microscopes?
4. The magnifying power of the compound microscope is the product of the magnification of the objective lens and _____.

   a. Eyepiece
   b. Arm
   c. Reflector
   d. Body tube

5. The use of a single convex lens or groups of lenses is found in _____.
   a. Telescopes
   b. Fluorescent lamps
   c. Magnifying glass
   d. Option A) and C)

6. _____ is the metallic platform that is fitted to the lower part of the arm with a hole in the centre.
   a. Base
   b. Drawer tube
   c. Stage
   d. Automatic Stop

7. Stereo microscope provides _____.
   a. One-dimensional image
   b. Two-dimensional image
   c. Three-dimensional image
   d. None of the options
8. Light can be focused by adjusting the _____.
   a. Base
   b. Condenser
   c. Stage
   d. Automatic Stop

9. What magnification does the ocular lens provide?
   a. 2x
   b. 3x
   c. 15x
   d. 50x
10. _____ eyepieces are seen in the binocular head.
   a. Two
   b. Three
   c. Four
   d. Five
11. What is Microtome?

Answers

1. Two types of microscopes are simple microscopes and compound microscopes.
2. It is an optical device that uses the optical power of a single lens for magnification.
3. Different types of compound microscopes are:
   Biological microscopes
   Stereo microscopes
   Phase contrast microscopes
   Fluorescence microscopes
   Polarizing microscopes
   Petrographic microscopes
4. A
5. D
6. C
7. C
8. B
9. C
10. A
11. A microtome is a mechanical device used to slice biological specimens into extremely thin segments for microscopic examination.

MODULE 2

UNIT 1 CALORIMETRY

Unit Structure

1.1 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 $1^{o}C$ and *'metry'* that is, measurement (from the word *meter* which is a device used for making measurements). You are familiar with the metalliccontainer usually used for measuring the heat content ofsubstances 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 bodyto another as a result of the temperature gradient between them. Naturally, heat flows from high to low temperature parts of a system (body).

1.2 Intended Learning Outcomes (ILOs)

At the end of this topic, students should be able to;

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

1.3 Main Content

Calorimetry is the process of measuring the amount of heat released or absorbed during a chemical reaction. By knowing the change in heat, it can be determined whether or not a reaction is exothermic (releases heat) or endothermic (absorbs heat). Calorimetry also plays a large part of everyday life, controlling the metabolic rates in humans and consequently maintaining such functions like body temperature.

a. Constant Pressure Calorimetry

Because calorimetry is used to measure the heat of a reaction, it is a crucial part of thermodynamics. In order to measure the heat of a reaction, the reaction must be isolated so that no heat is lost to the environment. This is achieved by use of a calorimeter, which insulates the reaction to better contain heat. Coffee cups are often used as a quick and easy to make calorimeter for constant pressure. More sophisticated bomb calorimeters are built for use at constant volumes.

b. Constant Volume Calorimetry

Constant Volume (bomb) calorimetry, is used to measure the heat of a reaction while holding volume constant and resisting large amounts of pressure. Although these two aspects of bomb calorimetry make for accurate results, they also contribute to the difficulty of bomb calorimetry. Here, the basic assembly of a bomb calorimeter will be addressed, as well as how bomb calorimetry relates to the heat of reaction and heat capacity and the calculations involved in regards to these two topics.

c. Differential Scanning Calorimetry

Differential scanning calorimetry is a specific type of calorimetry including both a sample substance and a reference substance, residing in separate chambers. While the reference chamber contains only a solvent, the sample chamber contains an equal amount of the same solvent in addition to the substance of interest, of which the ΔH is being determined. The ΔH due to the solvent is constant in both chambers, so any difference can be attributed to the presence of the substance of interest.

One technique we can use to measure the amount of heat involved in a chemical or physical process is known as **calorimetry**. Calorimetry is used to measure amounts of heat transferred to or from a substance. To do so, the heat is exchanged with a calibrated object (calorimeter). The

temperature change measured by the calorimeter is used to derive the amount of heat transferred by the process under study. The measurement of heat transfer using this approach requires the definition of a **system** (the substance or substances undergoing the chemical or physical change) and its **surroundings** (all other matter, including components of the measurement apparatus, that serve to either provide heat to the system or absorb heat from the system).

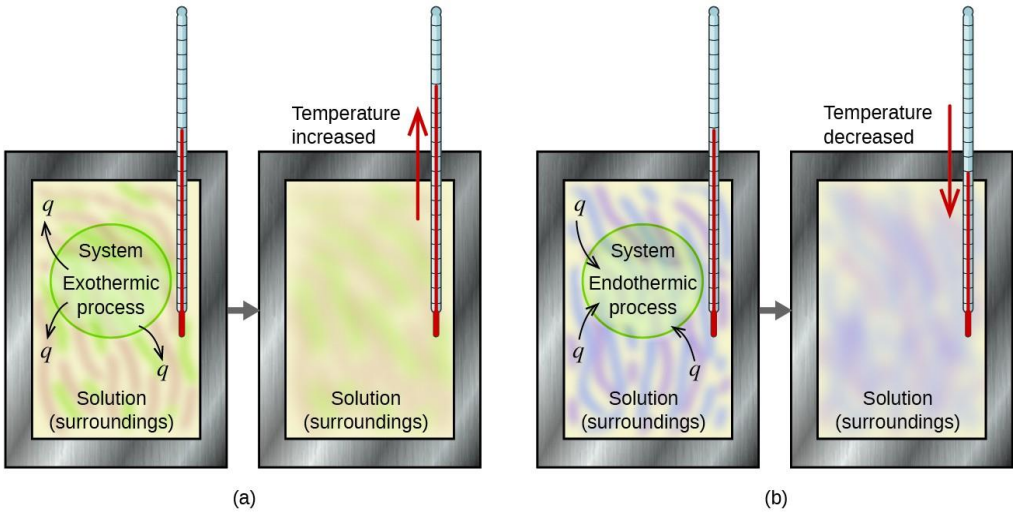


Figure 11: In a calorimetric determination, either (a) an exothermic process occurs and heat, q, is negative, indicating that thermal energy is transferred from the system to its surroundings, or (b) an endothermic process occurs and heat, q, is positive, indicating that thermal energy is transferred from the surroundings to the system.

A **calorimeter** is a device used to measure the amount of heat involved in a chemical or physical process. For example, when an exothermic reaction occurs in solution in a calorimeter, the heat produced by the reaction is absorbed by the solution, which increases its temperature. When an endothermic reaction occurs, the heat required is absorbed from the thermal energy of the solution, which decreases its temperature. The temperature change, along with the specific heat and mass of the solution, can then be used to calculate the amount of heat involved in either case.

Scientists use well-insulated calorimeters that all but prevent the transfer of heat between the calorimeter and its environment, which effectively limits the "surroundings" to the nonsystem components of the calorimeter (and the calorimeter itself.) This enables the accurate determination of the heat involved in chemical processes, the energy content of foods, and so on. General chemistry students often use simple calorimeters constructed from polystyrene cups. These easy-to-use

"coffee cup" calorimeters allow more heat exchange with their surroundings, and therefore produce less accurate energy values.

Commercial solution calorimeters are also available. Relatively inexpensive calorimeters often consist of two thin-walled cups that are nested in a way that minimizes thermal contact during use, along with an insulated cover, handheld stirrer, and simple thermometer. More expensive calorimeters used for industry and research typically have a well-insulated, fully enclosed reaction vessel, motorized stirring mechanism, and a more accurate temperature sensor (Fig 1.3).

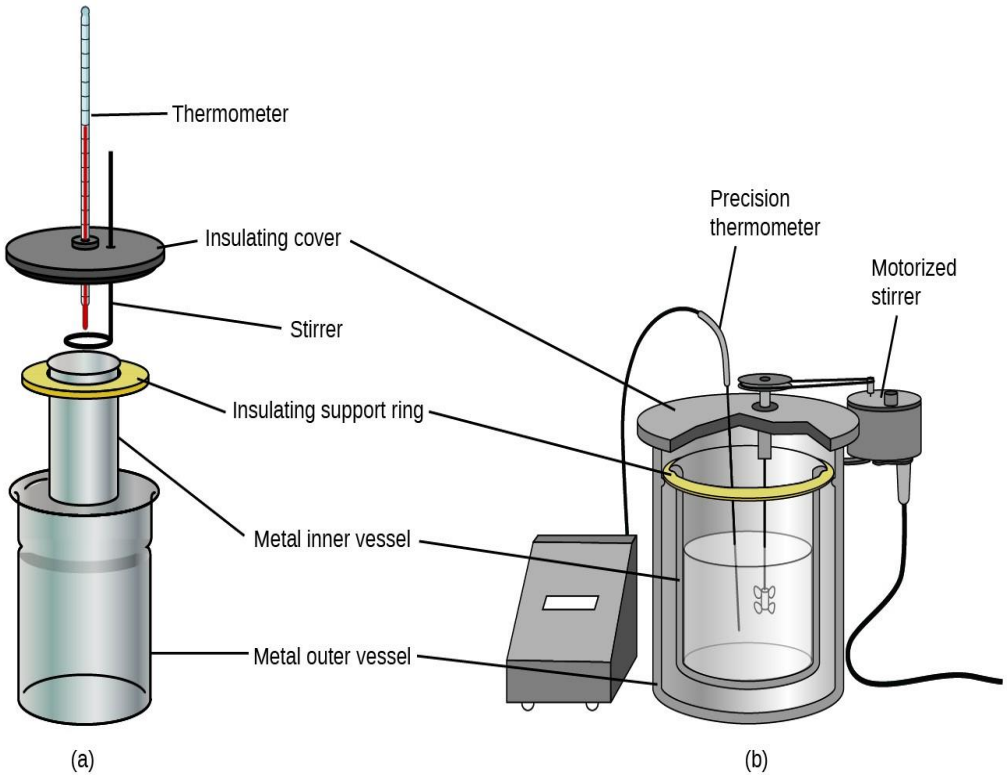


Fig 1.3. Commercial solution calorimeters range from (a) simple, inexpensive models for student use to (b) expensive, more accurate models for industry and research.

Before we practice calorimetry problems involving chemical reactions, consider a simpler example that illustrates the core idea behind calorimetry. Suppose we initially have a high-temperature substance, such as a hot piece of metal (M), and a low-temperature substance, such as cool water (W). If we place the metal in the water, heat will flow from M to W. The temperature of M will decrease, and the temperature of W will increase, until the two substances have the same temperature—that is, when they reach thermal equilibrium (Fig 1.4). If this occurs in a calorimeter, ideally all of this heat transfer occurs between the two substances, with no heat gained or lost by either the

calorimeter or the calorimeter's surroundings. Under these ideal circumstances, the net heat change is zero:
q substance M+q substance W=0$\diamondsuit$ substance M+$\diamondsuit$ substance W=0
This relationship can be rearranged to show that the heat gained by substance M is equal to the heat lost by substance W:
q substance M=−q substance W$\diamondsuit$ substance M=−$\diamondsuit$ substance W

The magnitude of the heat (change) is therefore the same for both substances, and the negative sign merely shows that q substance M$\diamondsuit$ substance M and q substance W$\diamondsuit$ substance W are opposite in direction of heat flow (gain or loss) but does not indicate the arithmetic sign of either *q* value (that is determined by whether the matter in question gains or loses heat, per definition). In the specific situation described, q substance M$\diamondsuit$ substance M is a negative value and q substance W$\diamondsuit$ substance W is positive, since heat is transferred from M to W.

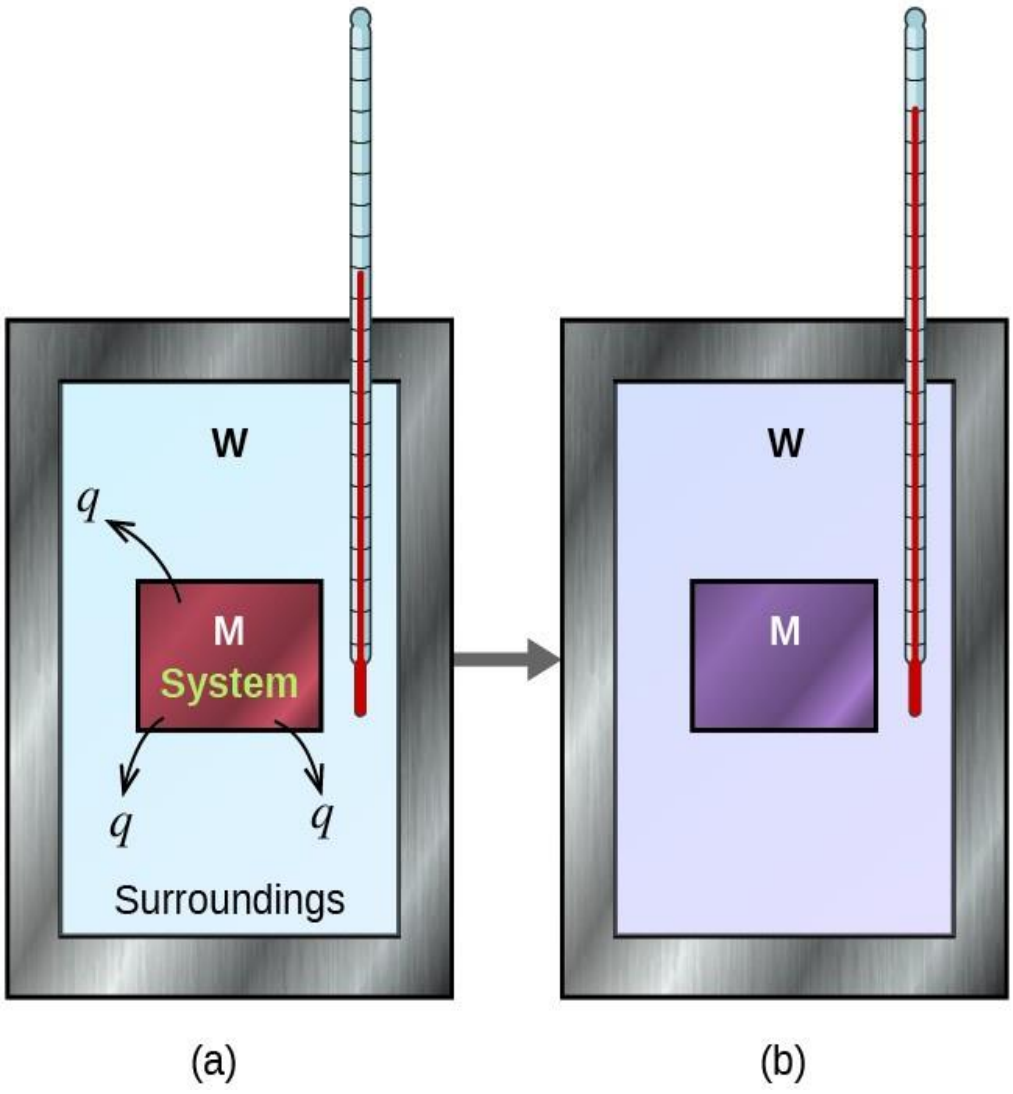


Fig 1.4. In a simple calorimetry process, (a) heat, q, is transferred from the hot metal, M, to the cool water, W, until (b) both are at the same temperature.

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 *cal*-4.184*j* 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 Celsus 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.

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 1^0C. The amount of heat required to raise the temperature of a substance by 1^0C 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:

$$\textit{Specific heat capacity } (J/kg^0C) = \frac{\text{amount of heat absorbed or given out by a body}}{\text{mass of substances}} \text{ x change in temperature}$$

Note that for a substance of any mass, we can define the *heat* or *thermal* capacity as *thermal capacity = mass of substance x specific heat capacity in* J/^{o}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 determinationof 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 intemperature 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 dependson 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:

$$\textit{Specific} \text{ latent heat } (\textit{in J/kg}) = \frac{\text{amount of heat absorbed or given out by a body}}{\text{mass of substances}}$$

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.

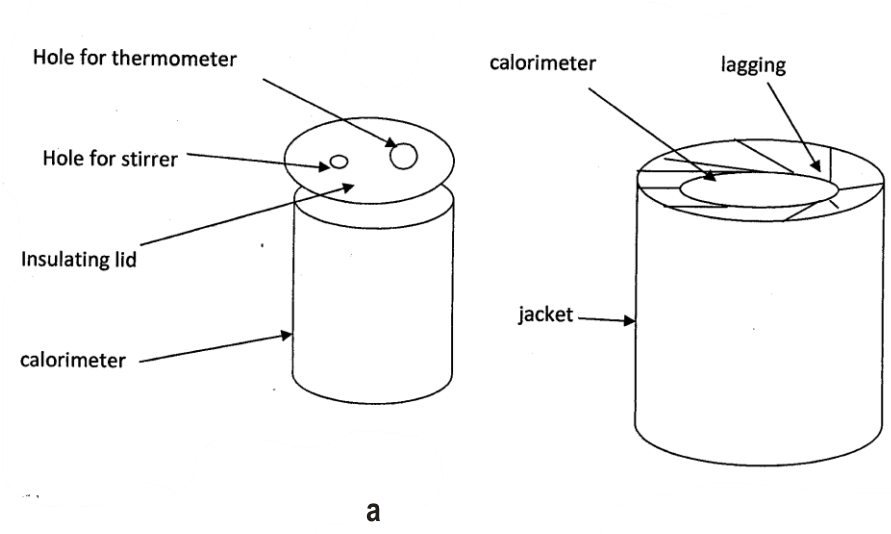
Experimental Methods of Calorimetry

Method of Mixtures and Heat Exchanges

The methods depend 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 thisprinciple 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 alwaysbe held in a container. Thus, part of the heat lost by the hot objectis 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.



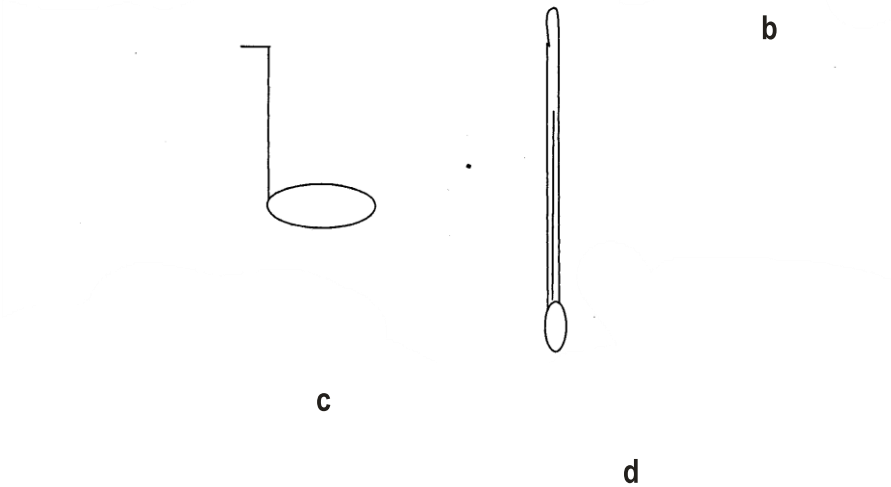


Fig 3.1:a, a calorimeter; b, a lagged calorimeter; c, a stirrer and d, a thermometer

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 Sokid by the Method of Mixtures

We determine the mass m_X and temperature T_X of a solid substance
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:

We use a solid of known specific heat capacity
Use the liquid whose specific heat capacity is required instead of water.
The same procedure and calculations as before can then be carried out.

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 agiven 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 flowsin 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.

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.

Uses of Calorimetry

It is well known now that matter always obeys the principle of lowest energy i.e. given the option, the matter will exist in the lowest energy state possible. Despite this, matter can have a variety of energetic states. Uranium atoms, for example, are a powerhouse.

The energy of matter has a profound effect on its natural occurrence and its reactivity etc. If we can unravel the relationship between them, then we can predict the natural occurrence, reactivity and physical properties based on the energy measurements we make through calorimetry. Understanding the thermodynamic properties of a substance will inevitably yield answers to structure and other properties.

Types of Calorimeters

Different types of calorimeters are given below:

Adiabatic Calorimeters
Reaction Calorimeters
Bomb Calorimeters (Constant Volume Calorimeters)
Constant Pressure Calorimeters
Differential Scanning Calorimeter
What is Calorimetry?

Self-Assessment Exercises

Itemize the different types of calorimeters.

1.4: Summary

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.

The important points to note in this unit are:

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

1.5 References/Further Reading/Web Sources

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1.6 Possible Answers to Self-Assessment Exercises

Adiabatic Calorimeters
Reaction Calorimeters
Bomb Calorimeters (Constant Volume Calorimeters)
Constant Pressure Calorimeters
Differential Scanning Calorimeter

UNIT 2 PHOTOMETRY

Unit Structure

2.1 Introduction

Photometry is the science of the measurement of light, in terms ofits *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.

2.2 Intended Learning Outcomes (ILOs)

At the end of this topic, students should be able to;

a. define Photometry?
b. explain Photometry with its units of measurement
c. compare Photometric and Radiometric quantities

2.3 Main Content

The measurement of the intensity of brightness of light that can be perceived by the human eye is called photometry. Meaning of photometry is different from radiometry. Radiometry measures the levels of optical radiations. In 1924, the Commission Internationale de l'Eclairage (CIE) decided to make photometry a part of modern science. They also defined the response of the average human eye. The commission experimented and measured light-adapted eyes for several people and put down the data. The curve of the data revealed that people

strongly responded to green color and were less sensitive to extreme ends like red and violet.

The modern photometer meaning 'is the radiant power of every wavelength', measured by a luminosity function as per the human's sensitivity to brightness. Photometry is the science behind the measurement of light, which is perceived in terms of brightness to the human eye. It should not be confused with radiometry which refers to the measurement of radiant energy in terms of absolute power.

Photometry is a branch of science which concerns light in terms of colour which is experienced by the eye from physical stimulation that exerts influence on photons inside the eye and also the response with the brain. It is used to describe and measure the propagation of light through materials and space.

Only light having a wavelength between 360 and 800 nm is responded to by the human eye. Radiometry mainly deals with electromagnetic radiation at all frequencies and wavelengths while photometry mainly deals with visible light - part of the electromagnetic spectrum which is responsible for the stimulation of vision in the human eye.

There are Two Weighting Functions:

- Photopic sensitivity function (for light conditions) and
- Scotopic function (for dark conditions).

The photopic sensitivity function is used in this condition, but sometimes the scotopic sensitivity function is also used in the same way.

Meaning of Photometer

A photometer is a device that is used to measure light. The root word "photo," means light. For example, photosynthesis is a word that describes how plants produce their own food by using light energy. A photon is a particle of light, a photograph is an image which is made from light-sensitive film or light-sensitive device.

A photometer is a device that measures the strength of electromagnetic radiation in the range of infrared radiation to ultraviolet radiation, including the visible part of the electromagnetic spectrum. Usually, a photometer converts light into electric current by using a photoresistor, photomultiplier, and photodiode.

Photometers Measure the Following Parameters

- Illuminance
- Light absorption
- Irradiance
- Reflection of light
- Scattering of light
- Fluorescence
- Luminescence
- Phosphorescene

Types of Photometry

The observer will be ready to perform photometry on an image when the image bias is subtracted and flat-fielded. To understand the meaning of photometry, one must know the types of photometry; which are: Differential, and Absolute

A. **In Differential Photometry:** The object under the study of differential photometry is seen beside other related comparison stars. The count for both objects are then compared to find the difference between them; this difference is used to derive the difference in brightness. In this manner, the relative brightness of the object under study as compared to another object in consideration is measured.

B. **Absolute Photometry:** The object under study in absolute photometry is observed without reference or comparison with any nearby star, and then the ADU count is analyzed to measure the actual brightness of the object. Calculation of the actual brightness of an object by an absolute photometry method is much more complicated as compared to differential photometry.

Principles of Photometers

Photometers examine how light interacts with reflective materials. The array of photometers describes the working principle of photometry in different fields of study. Some instruments use the principle of photometry to observe how light absorbs and/or reflects wavelengths. Some instruments and devices measure light by converting it into electric current and measuring the intensity of electric current produced by the light. There are also certain photometers that shoot white light at a surface to measure the amount of light that is reflected at the instrument.

When a light is passed through a colored solution, lights of certain wavelengths are selectively absorbed by the solution, which gives a plot of the absorption spectrum of the solution. The wavelength of light at which maximum absorption occurs is called an absorption maximum (λmax) of that solution. Some wavelengths of light are not absorbed by the solution and are transmitted through it, giving the solution its color. Photometric instruments measure transmittance, and it is defined as the ratio of the intensity of emergent light to that of the intensity of incident light, mathematically:

Transmittance (T) = Intensity of the emergent (or transmitted) light / Intensity of the incident light = l_e / l_O
Transmittance is expressed in a range of 0 to 100%.

Photometry Applications

- Highly sensitive photometers are used to evaluate the contrast ratios of cathode-ray tubes, flat panel displays, and liquid crystal displays.
- Goniophotometers are used to characterize the contrast and luminance of flat panel displays over a huge array of angles.
- Photometers are applied for speedy and accurate testing of automotive dashboards and cockpit displays.
- The illuminance of theater screens, transmittance of filters, uniformity of projection systems, and reflectance of paper, ceramics, and textiles are some other common uses of photometers.

Working of Single Beam Photometer

A source produces light. The light from this source is subjected to a solution. A part of the light is observed by the solution, and the remaining part of the light is transmitted. The transmitted light falls on detectors, which produce photocurrent, which is proportional to the intensity of incident light. This photocurrent is passed through a galvanometer where readings are displayed.

The instrument is operated in the following steps:

Initially, the detector is darkened, and galvanometer reading is mechanically adjusted to zero.
Now a reference solution is kept in the sample holder.
Light is transmitted from this reference solution.
An intensity control circuit is present to adjust the intensity of light emitted by the source, in such a way that the galvanometer shows 100% transmission.

After calibration, the readings of the standard sample (Q_s) and the unknown sample (Qa) are taken. The concentration of unknown samples is found using the following formula.

$Q_a = Q_S * I_Q/I_S$
Where,
Q_a = Concentration of the unknown sample,
Q_S = Concentration of the reference sample,
I_Q = Unknown reading, and
I_S = Reference reading.

Photometry is the science behind the measurement of light, which is perceived in terms of brightness to the human eye. It should not be confused with radiometry which refers to the measurement of radiant energy in terms of absolute power. Photometry is a branch of science which concerns light in terms of colour which is experienced by the eye from physical stimulation that exerts influence on photons inside the eye and also the response with the brain. It is used to describe and measure the propagation of light through materials and space.

Only light having a wavelength between 360 and 800 nm is responded to by the human eye. Radiometry mainly deals with electromagnetic radiation at all frequencies and wavelengths while photometry mainly deals with visible light - part of the electromagnetic spectrum which is responsible for the stimulation of vision in the human eye.

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 themeasured 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 responsesas a function of wavelength when it is adapted to light conditions (photopic vision) and dark conditions (scotopic vision). Photometryis 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.

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 *luminous power*
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				

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:

a. Luminance (photometric) and radiance (radiometric)
b. Luminous flux (photometric) and radiant flux (radiometric)
c. 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 alight 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

Quantity	*Symbol*	*SI unit*	*Abbr.*	*Notes*
Radiant energy	*Q*	Joule	J	energy
Radiant flux	□	Watt	W	radiant energy per unit time, also called *radiant power*
Radiant intensity	*I*	watt per steradian	$W \cdot sr^{-1}$	power per unit solid angle
Radiance	*L*	watt per steradian per square metre	$W \cdot m^{-2} \cdot sr^{-1}$	power per unit solid angle per unit *projected* source area. called *intensity* in some other fields of study.
Irradiance	*E,I*	watt per square metre	$W.m^{-2}$	power incident on a surface. sometimes confusingly called "intensity".
Radiant exitance /Radiant emittance	*M*	watt per square metre	$W.m^{-2}$	power emitted from a surface
Radiosity	J or $J_□$	watt per square metre	$W.m^{-2}$	Emitted plus reflected power leaving a surface

Spectral radiance	L_{Ω} or L_{ν}	watt per steradian per metre^3 or watt per steradian per square metre per hertz	$W.sr^{-1}.m^{-3}$ or $W.sr^{-1}.m^{-2}.Hz^{-1}$	Commonly measured in $W.s^{-1}.r^{-2}.nm^{-1}.m$
Spectral irradiance	E_{Ω} or $E\nu$	watt per metre^3 or watt per square metre per hertz	$W \cdot m^{-3}$ Or $W \cdot m^{-2} \cdot Hz^{-1}$	Commonly measured in $W \cdot m^{-2} \cdot nm^{-1}$

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 usuallythey 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 60watt 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 thestandard 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.

Photometric Measurement Techniques

Photometric measurement is based on photodetectors, devices (of several types) that produce and 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 changethe orientation of the luminaire with respect to the photocell. In either case, luminous intensity is tabulated from this data and used in lighting design. Define Photometry?

Self-Assessment Exercises

Enumerate photometric applications

2.4 Summary

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. The important points to note in this unit are:

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

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2.6 Possible Answers to Self-Assessment Exercises

- Highly sensitive photometers are used to evaluate the contrast ratios of cathode-ray tubes, flat panel displays, and liquid crystal displays.
- Goniophotometers are used to characterize the contrast and luminance of flat panel displays over a huge array of angles.
- Photometers are applied for speedy and accurate testing of automotive dashboards and cockpit displays.
- The illuminance of theater screens, transmittance of filters, uniformity of projection systems, and reflectance of paper, ceramics, and textiles are some other common uses of photometers.

UNIT 3 CHROMATOGRAPHY AND CONDUCTOMETRY

Unit Structure

3.1 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 analyzed using any of the techniques known.

3.2 Intended Learning Outcomes (ILOs)

At the end of this topic, students should be able to;

a. Explain the principle on which chromatography as a separation technique is based.
b. Describe the different methods of chromatography available.
c. Mention some applications of chromatography
d. Discuss the concept of Conductometry
e. Describe the measuring units involved in Conductometry
f. List some applications of Conductometry

3.3 Main Content

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 streeming 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 chromatography is based on their different distribution between twonon-miscible phases.

Types of Chromatography

a. 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 fibersof the paper and moving liquid in developing solvent. It uses a strip of paper and capillary action is used pull the solvents up throughthe paper to separate the solutes. A small concentrated spot of solution that contains the sample is applied to a strip ofchromatography paper about 2 cm away from the base of the plate, usually using a capillary tube for maximum precision. This sampleis 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, Rf, due to their differing interactions with the stationary and mobile phases.

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

This method has been largely replaced by thin layer chromatography

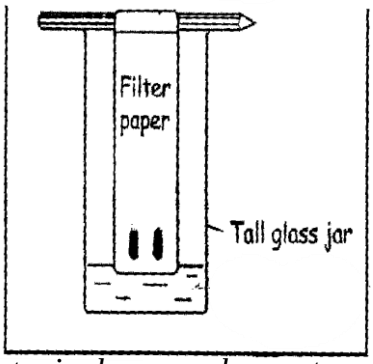

Fig. 4.1: Set up of a typical paper chromatography tank.

b. Thin-layer Chromatography: The surface of the plate consists of a very thin layer of silica gel on a plastic or Aluminum 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.

c. 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 organicsolvent 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.

d. 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:

- Pneumatic system –gas supply (flow control and measurement).
- Injection system – heated injector port, where the sample is vaporized if necessary
- Column – where the separation occurs
- Oven – the coiled column is wholly contained in athermostatically controlled oven.
- Detector – integral detector or link to a mass spectrometer

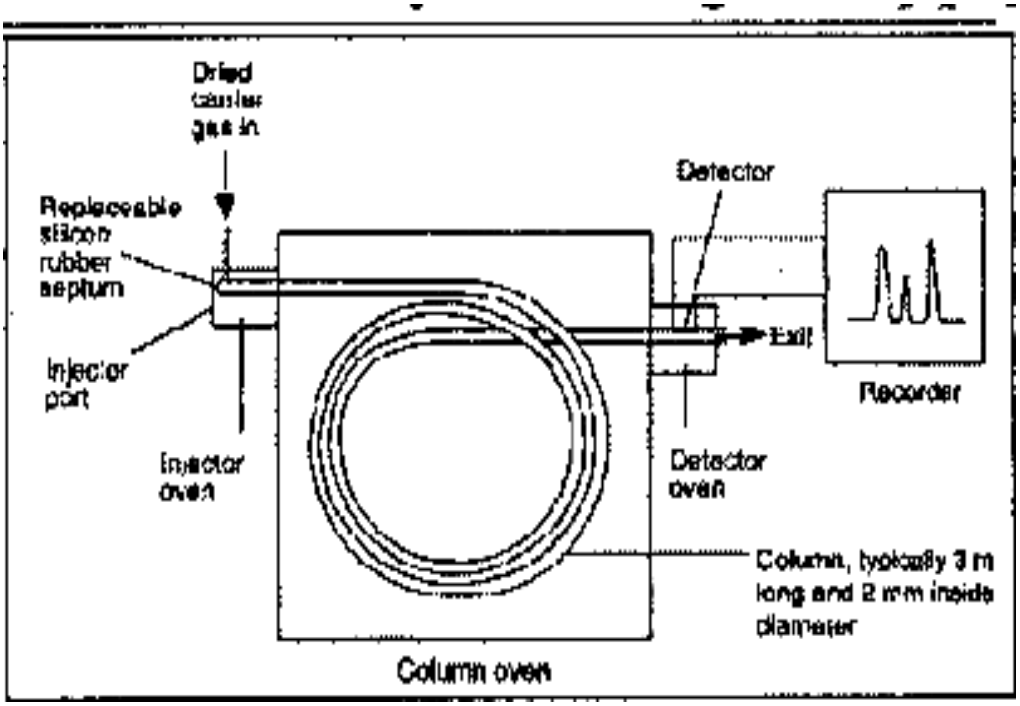

Fig. 4.2: set up of a typical Gas Chromatography.

How does Gas Chromatography Work?

A carrier gas, examples of which are Helium and Neon flows through the system. A valve controls the flow rate. A sample of the volatile mixture is injected into the carrier gas.The sample is vaporized in the heated injector port. 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.

The coiled column is contained in the thermostatically controlled oven. 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. Two types of detectors can be used:

- thermal conductivity detectors which respond to changes in the thermal conductivity of the gas leaving the column and
- 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

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

High Performance Liquid Chromatography

Basic Components:

a. **Solvent Reservoir.**
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 cm^3 min^{-1}

b. **The Injector System:** The sample to be separated is injected into the liquid phase at this point. 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.

c. **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.

Applications of Chromatography

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

b. Gas Chromatography is used to analyze 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 of 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.

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;

a. the number of ions. The more ions a solution contain, the higher its conductivity.
b. 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 CI^-. If an ion is surrounded by water molecules (hydratization) and therefore becomes larger, its conductivity decreases.
- the solvent: the more polar a solvent, the mor 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 noionization.
- 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 deceases.

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 thermostatic, 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 □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.

Applications of Conductometry

a. 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.
b. They can also be used to determine the many physical quantities such as degree of dissociation and dissociation constants of weak acids and bases.
c. They can be sued to determine the ionic product of water, solubility and solubility products of sparingly soluble salts.
d. They form the basis for conductometric titration methods.
Itemize the different types of chromatography.

Self-Assessment Exercises

Enumerate the applications of conductometry.

3.4 Summary

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. 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.

3.5 References/Further Reading/Web Sources

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3.6 Possible Answers to Self-Assessment Exercises

a. 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.
b. They can also be used to determine the many physical quantities such as degree of dissociation and dissociation constants of weak acids and bases.
c. They can be sued to determine the ionic product of water, solubility and solubility products of sparingly soluble salts.
d. They form the basis for conductometric titration methods.

UNIT 4 EVALUATING ORIGINAL RESEARCH PAPERS & INTERNET TECHNOLOGY

Unit Structure

4.1 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.

4.2 Intended Learning Outcomes (ILOs)

At the end of this topic, the students should be able to;

a. Distinguish an original research paper.
b. List the criteria for evaluating an original research paper.
c. Evaluate an original research paper state if any, the contributions of a research paper to knowledge.

4.3 Main Content

Quality

Quality refers to the standard of something when it iscompared to other things like it. When you have a research paper to evaluate, and you compare it with others you have read before orhad a knowledge of, will it be able to compare favourably withthem? 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.

Originality

Ancillary to above 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.

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 in depth on superficial study? Is the study directed at a goal? This is all what a focused research paper tends to address.

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

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 valueto knowledge.

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.

Internet Technology

In the present age of information Technology, use of Internet is becoming quite popular for accessing information on any topic of your interest. It also provides tremendous opportunities to students, researchers and professionals for getting information on matters related to academic and professional topics and lot more. In the present world, most of the people who have computers around themselves use Internet to access information from the World Wide Web, exchange messages & documents and e-services.

Internet is a global system of interconnected computer networks that use the standard Internet protocol suite (TCP/IP) to serve billions of users worldwide. It is a *network of networks* that consists of millions of private, public, academic, business, and government networks, of local to global scope, that are linked by a broad array of electronic, wireless and optical networking technologies.

The Internet carries a vast range of information resources and services, such as the inter linked hypertext documents of the World Wide Web (www) and the infrastructure to support electronic mail.

History of Internet

The Internet is a global network of networks that enables computers of all kinds to directly and transparently communicate and share services throughout the world. In 1969 the precursor of Internet is born: ARPA net. ARPA = Advanced Research Projects Agency sponsored by the American Department of Defense (DOD). Designed to connect military research centers. Distributed computer system able to survive a nuclear attack. Problem: ARPAnet could connect only networks of the same type.

In 1970, ARPA starts developing the Transmission Control Protocol / Internet Protocol (TCP/IP), a technology for connecting networks of different types (produced by different companies). Other networks appear, such as CSNET and BITNET. The Internet = a network of

networks. People around the world share ideas, information, comments, and stories.

Uses of Internet

Internet has been the most useful technology of the modern times which helps us not only in our daily lives, but also our personal and professional lives developments. For the students and educational purposes, the internet is widely used to gather information so as to do the research or add to the knowledge of various subjects. Even the business professionals and the professionals like doctors, access the internet to filter the necessary information for their use. The internet is therefore the largest encyclopedia for everyone, in all age categories. The internet has served to be more useful in maintaining contacts with friends and relatives who live abroad permanently.

Advantages of Internet

a. E-mail: Email is now an essential communication tools in business. With e-mail you can send and receive instant electronic messages, which works like writing letters. Your messages are delivered instantly to people anywhere in the world, unlike traditional mail that takes a lot of time. Email is free, fast and very cheap when compared to telephone, fax and postal services.
b. Information: Information is probably the biggest advantage internet is offering. There is a huge amount of information available on the internet for just about every subject, ranging from government law and services, trade fairs and conferences, market information, new ideas and technical support.
c. Online Chat: You can access many ‘chat rooms’ on the web that can be used to meet new people, make new friends, as well as to stay in touch with old friends.
d. Services: Many services are provided on the internet like net banking, job searching, purchasing tickets, hotel reservations, guidance services on array of topics engulfing every aspect of life.
e. Communities: Communities of all types have sprung up on the internet. Its a great way to meet up with people of similar interest and discuss common issues.
f. E-commerce: Along with getting information on the Internet, you can also shop online. There are many online stores and sites that can be used to look for products as well as buy them using your credit card.
g. Entertainment: Internet provides facility to access wide range of Audio/Video songs, plays films. Many of which can be downloaded.

h. Software Downloads: You can freely download innumerable, software like utilities, games, music, videos, movies, etc from the Internet.

Limitations of Internet

Services of Internet

Email: Email is the fastest means of communication. With email one can also send software and certain forms of compressed digital image as an attachment. E-mail or Electronic mail is a paperless method of sending messages, notes or letters from one person to another or even many people at the same time via Internet. E-mail is very fast compared to the normal post. E-mail messages usually take only few seconds to arrive at their destination. One can send messages anytime of the day or night, and, it will get delivered immediately.

Advantages

a. The biggest advantage of using email is that it is cheap, especially when sending messages to other states or countries and at the same time it can be delivered to a number of people around the world.
b. It allows you to compose note, get the address of the recipient and send it. Once the mail is received and read, it can be forwarded or replied. One can even store it for later use, or delete.
c. In e-mail even the sender can request for delivery receipt and read receipt from the recipient.

Features of E-mail

a. One-to-one or one-to-many communications
b. Instant communications
c. Physical presence of recipient is not required
d. Most inexpensive mail services, 24-hours a day and seven days a week
e. Encourages informal communications

Discussion groups: News groups or discussion groups facilitate Internet user to join for various kinds of debate, discussion and news sharing.

Long-distance computing: Long-distance computing was an original inspiration for development of ARPANET and does still provide a very useful service on Internet. Programmers can maintain accounts on distant, powerful computers and execute programs.

File transfers: File transfer service allows Internet users to access remote machines and retrieve programs, data or text. File Transfer Protocol (FTP), is an Internet utility software used to uploaded and download files. It gives access to directories or folders on remote computers and allows software, data and text files to be transferred between different kinds of computers. FTP works on the basis of same principle as that of Client/Server. FTP "Client" is a program running on your computer that enables you to communicate with remote computers. The FTP client takes FTP command and sends these as requests for information from the remote computer known as FTP servers. To access remote FTP server it is required, but not necessary to have an account in the FTP server. When the FTP client gets connected, FTP server asks for the identification in terms of User Login name and password of the FTP client. If one does not have an account in the remote FTP server, still he can connect to the server using anonymous login. Using anonymous login anyone can login in to a FTP server and can access public archives; anywhere in the world, without having an account. One can easily Login to the FTP site with the username anonymous and e-mail address as password.

Objectives of FTP:

Provide flexibility and promote sharing of computer programs, files and data
Transfer data reliably and more efficiently over network
Encourage implicit or indirect use of remote computers using Internet
Shield a user from variations in storage systems among hosts.

Types of Internet Connections

Dial up Connection: Dial-up refers to an Internet connection that is established using a modem. The modem connects the computer to standard phone lines, which serve as the data transfer medium. When a user initiates a dial-up connection, the modem dials a phone number of an Internet Service Provider (ISP) that is designated to receive dial-up calls. The ISP then establishes the connection, which usually takes about ten seconds and is accompanied by several beepings and a buzzing sound. After the dial-up connection has been established, it is active until the user disconnects from the ISP. Typically, this is done by selecting the "Disconnect" option using the ISP's software or a modem utility program. However, if a dial-up connection is interrupted by an incoming phone call or someone picking up a phone in the house, the service may also be disconnected.

Advantages

a. Low Price
b. Secure connection – your IP address continually changes
c. Offered in rural areas – you need a phone line

Disadvantages

a. Slow speed.
b. Phone line is required.
c. Busy signals for friends and family members.

Leased Connection: Leased connection is a permanent telephone connection between two points set up by a telecommunications common carrier. Typically, leased lines are used by businesses to connect geographically distant offices. Unlike normal dial-up connections, a leased line is always active. The fee for the connection is a fixed monthly rate. The primary factors affecting the monthly fee are distance between end points and the speed of the circuit. Because the connection doesn't carry anybody else's communications, the carrier can assure a given level of quality.

Advantage

a. Secure and private: dedicated exclusively to the customer
b. Speed: symmetrical and direct
c. Reliable: minimum down time
d. Wide choice of speeds: bandwidth on demand, easily upgradeable
e. Leased lines are suitable for in-house office web hosting

Disadvantages

a. Leased lines can be expensive to install and rent.
b. Not suitable for single or home workers
c. Lead times can be as long as 65 working days
d. Distance dependent to nearest POP
e. Leased lines have traditionally been the more expensive access option. A Service Level Agreement (SLA) confirms an ISP's contractual requirement in ensuring the serviceis maintained. This is often lacking in cheaper alternatives.

DSL connection: Digital Subscriber Line (DSL) is a family of technologies that provides digital data transmission over the wires of a local telephone network. DSL originally stood for *digital subscriber loop*. In telecommunications marketing, the term DSL is widely understood to mean Asymmetric Digital Subscriber Line (ADSL), the most commonly installed DSL technology. DSL service is delivered simultaneously with wired telephone service on the same telephone line. This is possible because DSL uses higher frequency bands for data separated by filtering. On the customer premises, a DSL filter on each

outlet removes the high frequency interference, to enable simultaneous use of the telephone and data.

Advantages:

a. **Security:** Unlike cable modems, each subscriber can be configured so that it will not be on the same network. In some cable modem networks, other computers on the cable modem network are left visibly vulnerable and are easily susceptible to break in as well as data destruction.
b. Integration: DSL will easily interface with ATM and WAN technology.
c. High bandwidth
d. Cheap line charges from the phone company.
e. Good for "bursty" traffic patterns

Disadvantages

a. No current standardization: A person moving from one area to another might find that their DSL modem is just another paperweight. Customers may have to buy new equipment to simply change ISPs.
b. Expensive: Most customers are not willing to spend more than $20 to $25 per month for Internet access. Current installation costs, including the modem, can be as high as $750.
c. Distance Dependence: The farther you live from the DSLAM (DSL Access Multiplexer), the lower the data rate. The longest run lengths are 18,000 feet, or a little over 3miles.

Cable Modem Connection: A cable modem is a type of Network Bridge and modem that provides bi-directional data communication via radio frequency channels on a HFC and RFoG infrastructure. Cable modems are primarily used to deliver broadband Internet access in the form of cable Internet, taking advantage of the high bandwidth of a HFC and RFoG network. They are commonly deployed in Australia, Europe, Asia and Americas.

Advantages

a. Always Connected: A cable modem connection is always connected to the Internet. This is advantageous because you do not have to wait for your computer to "log on" to the Internet; however, this also has the disadvantage of making your computer more vulnerable to hackers.

Broadband: Cable modems transmit and receive data as digital packets, meaning they provide high-speed Internet access. This makes cable modem connections much faster than traditional dial-up connections.

Bandwidth: Cable modems have the potential to receive data from their cable provider at speeds greater than 30 megabits per second; unfortunately, this speed is rarely ever realized. Cable lines are shared by all of the cable modem users in a given area; thus, the connection speed varies depending upon the number of other people using the Internet and the amount of data they are receiving or transmitting.

File Transfer Capabilities: Downloads may be faster, but uploads are typically slower. Since the same lines are used to transmit data to and from the modem, priority is often given to data traveling in one direction.

Signal Integrity: Cable Internet can be transmitted long distances with little signal degradation. This means the quality of the Internet signal is not significantly decreased by the distance of the modem from the cable provider.

Routing: Cable routers allow multiple computers to be hooked up to one cable modem, allowing several devices to be directly connected through a single modem. Wireless routers can also be attached to your cable modem.

Rely on Existing Connections: Cable modems connect directly to preinstalled cable lines. This is advantageous because you do not need to have other services, such as telephone or Internet, in order to receive Internet through your cable modem. The disadvantage is that you cannot have cable internet in areas where there are no cable lines.

Disadvantages

Cable internet technology excels at maintaining signal strength over distance. Once it is delivered to a region, however, such as a neighborhood, it is split among that regions subscribers. While increased capacity has diminished the effect somewhat, it is still possible that users will see significantly lower speeds at peak times when more people are using the shared connection.

Bandwidth equals money, so cable’s advantage in throughput comes with a price. Even in plans of similar speeds compared with DSL, customers spend more perMb with cable than they do with DSL.

It’s hard to imagine, but there are still pockets of the United States without adequate cable television service. There are far fewer such pockets without residential land-line service meaning cable internet is on balance less accessible in remote areas.

VSAT: Short for very small aperture terminal, an earthbound station used in satellite communications of data, voice and video signals, excluding broadcast television. A VSAT consists of two parts, a transceiver that is placed outdoors in direct line of sight to the satellite and a device that is placed indoors to interface the transceiver with the end user's communications device, such as a PC. The transceiver receives or sends a signal to a satellite transponder in the sky. The satellite sends and receives signals from a ground station computer that acts as a hub for the system. Each end user is interconnected with the hub station via the satellite, forming a star topology. The hub controls the entire operation of the network. For one end user to communicate with another, each transmission has to first go to the hub station that then retransmits it via the satellite to the other end user's VSAT.

Advantages

a. Costs Insensitive to Distance
b. Single Platform service delivery (one-stop-shop)
c. Flexibility
d. Upgradeable
e. Low incremental costs per unit

Disadvantages

a. High start-up costs (hubs and basic elements must be in place before the services can be provided)
b. Higher than normal risk profiles
c. Severe regulatory restrictions imposed by countries that prevent VSAT networks and solutions from reaching critical mass and therefore profitability
d. Some service quality limitations such the high signal delays(latency)
e. Natural availability limits that cannot be mitigated against
f. Lack of skills required in the developing world to design, install and maintain satellite communication systems adequately

World Wide Web (WWW)

The World Wide Web allows computer users to locate and view multimedia-based documents (i.e., documents with text, graphics, animations, audios or videos) on almost any subject. Even though the Internet was developed more than three decades ago, the introduction of the World Wide Web is a relatively recent event. In 1990, Tim Berners-Lee of CERN (the European Laboratory for Particle Physics) developed the World Wide Web and several communication protocols that form the backbone of the Web. The Internet and the World Wide Web surely will be listed among the most important and profound creations of humankind. In the past, most computer applications executed on "stand-

alone" computers (i.e., computers that were not connected to one another).

Self-Assessment Exercises

Explain the questions to be answered in the application of evaluation of an original paper

4.4 Summary

The important points to note in this unit are:

a. The quality of a research paper will determine its acceptability.
b. The originality and focus of such paper are of paramount importance.
c. The method for the study must be standard and the study should be able to contribute useful information to knowledge now or latter.
d. It will also be good if the paper can be of useful application now or thereafter.

4.5 References/Further Readings/Web Sources

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4.6 Possible Answers to Self-Assessment Exercises

- 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?

UNIT 5 SCIENTIFIC METHODS OF INVESTIGATION

Unit Structure

5.1 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 is 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.

5.2 Intended Learning Outcomes (ILOs)

At the end of this topic, students should be able to;

a. Enumerate the qualities / attributes of a good Scientist
b. Justify the use of instruments for research.
c. Explain the procedure of experiments, predict and interpret scientific result
d. Explain the conduct of experiment and keep record.
e. Report your investigations as done universally and communicate your scientific findings.

5.3: Main Content

An investigation is the heart of Science. It is how scientists do research. Investigations provide an answer to questions and help us resolve

problems. Even if the evidence doesn't provide solutions, it may still be useful, leading to new issues for investigation. Science strides, as more knowledge is gathered. In this article, we will walk you through the scientific investigation, a logical problem-solving approach used by scientists.

How to Make a Scientific Investigation?

At the heart of all sciences, there lies a problem-solving approach known as the scientific method. The scientific method has basic procedures as follows:

a. Make an observation

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.

Observation

- Seeds are produced in fruits
- If seeds taken from a fruit are sown they will grow into plants ifconditions are right.
- These plants will be similar to those from which the fruits were taken and will produce similar fruits.

b. Ask a question.
c. Form a hypothesis.
d. Make predictions based on the hypothesis.
e. Test the prediction.
f. Iterate: Use a new hypothesis or prediction.

Scientists in different fields of Sciences ask different questions and perform different tests. However, the same core approach is used by scientists to find answers that are logical and supported by evidence.

Scientific Investigation Example

Let us better understand scientific investigation by applying its steps to solve everyday problems.

1. Make an observation

Suppose you switch on your television at home to watch your favorite movie. However, your television doesn't turn on.

2. Ask a question

Why didn't my television switch on?

3. Propose a hypothesis

A potential answer to a question that can be tested is known as a hypothesis. The hypothesis, in this case, can be that television didn't work because of the broken electrical outlet.

4. Make predictions

A prediction is an outcome we would expect to see if the hypothesis is correct. In this case, we would predict that if the electrical outlet is broken, then plugging the television into a different outlet should solve the problem.

5. Test the predictions

To test the hypothesis, we need to perform an experiment associated with the forecast. For instance, in this case, we would plug the television into a different outlet and see if it works.

If the television switches on, then the hypothesis is likely to be correct.

If the television doesn't switch on, then the hypothesis is likely to be wrong.

Unless there was a flaw in the test, a contradicting result implies that we should reject the hypothesis and look for a new one.

6. Iterate

The last step is to reflect on our results and use them to guide our next steps.

If the hypothesis was correct, then we might have to perform a further test to confirm it to be more explicit. For example, why did the outlet break?

If it were not supported, then we would have to develop a new hypothesis. For example, there is a broken circuit on television.

In most circumstances, scientific investigation is an iterative process. It is a cycle rather than a straight line

Interpretation

- After making such observations about seeds from different kinds of plants you might come to certain conclusions:
- Seeds are produced only in fruits
- There is something in a seed that can develop into a plant
- All plants grown from seed are similar to those from which the fruitwas taken and they produced similar seeds.

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 allof the following:
- Identification and classification of the problem your set out to solve or shed some light. Is it on plant, animal, soil or their components?

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.

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.

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.

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 ofany kind recorded as words or numbers (Numerical data) as you count and measure. Counting and measuring enable scientists to be precise. 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 anything 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.

Writing a report of Scientific Investigation

a. Introduction: What was the purpose of your investigation?
b. Materials: List the materials you used
c. Methods: What did you do and how did you do them?
d. Results: State concisely your findings. These may be presented mainlyin tables, graphs or other diagrams.
e. Discussion: How did you interpret your results?
f. 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 makingfood) 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 noneis given off, this will be validated by your observations hence your results.

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:

a. 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.
b. Journals: New research which does not have to be kept secret for commercial or defense 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.
c. 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.
d. 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.
e. Trade Literature: Trade literature-advertisements, catalogues and company magazines – is a well – used sources of information, particularly in engineering and construction.
f. Other media of publication are Books, Reviews, Abstracts, Indexes, References and Databases.

What is Scientific Research?

It is a process of rigorous reasoning based on interactions among theories methods and findings. It is built on understanding derived from the objective testing of models or theories; it accumulation of scientific knowledge is laborious, plodding, circuitous, and indirect; Scientific knowledge is developed and honed through critique contested findings,

replication, and convergence; Scientific knowledge is developed through sustained efforts; Scientific inquiry must be guided by fundamental principles.

- A study is deemed to be "scientific" when:
- There are a clear set of testable questions underlying the design;
- The methods are appropriate to answer the questions and falsify competing hypotheses and answers;
- The study is explicitly linked to theory and previous research;
- The data are analyzed systematically and with the appropriate tools;
- The data are made available for review and criticism.

Fundamental Principles of Scientific Research

i. Ask significant questions that can be answered empirically.
   - "The formulation of a problem is often more essential than its solution, which may be merely a matter of mathematical or experimental skill. To raise new questions, new possibilities, to regard old questions from a new angle, requires creative imagination and marks real advance in science" (Einstein & Infeld, 1938);
   - The research questions must be asked in a way that allows for empirical investigation.

ii. Link research to relevant theory.
   - Scientific research can be guided by a conceptual framework model, or theory that generates questions to be asked or answers to the questions posed;
   - Theory drives the research question, the use of methods, and the interpretation of results.

iii. Select and apply research designs and methods that permit direct investigation of the question.
   - The trustworthiness of any research study is predicated initially on several major elements:
   - The suitability of the proposed research design or methodology to address the specific questions posed by the study;
   - The scientific rigor by which the methodology is applied;
   - The trustworthiness of any research study is predicated initially on several major elements (cont'd):

   The link between question and methodology must be clear and justified;
   - Detailed description of the method, measures, data collection procedures, data analyses, and subjects must be available to permit replication.

iv. Provide a coherent and explicit chain of reasoning that can be replicated.
- What assumptions underlying the inferences were made? Were they clearly stated and justified?
- How was evidence judged to be relevant?
- How were alternative, competing hypotheses, and explanations identified, considered, and accounted for (accepted or discarded)?

v. Provide a coherent and explicit chain of reasoning that can be replicated (cont'd).
- How were the links between data and the conceptual or theoretical framework made?
- The chain of reasoning depends upon the design which depends on the type of question:
- Description – what is happening?
- Cause – is there a systematic effect?
- Process/mechanism- why or how does the effect occur?

vi. Replicate and generalize across studies.
- Internal Validity: The observations made are consistent and generalize from one observer to another, from one task to a parallel task from one measurement occasion to another occasion.
- Statistical methods – e.g. correlation;
- Non-statistical methods – e.g. triangulation, comparative analysis.
- External Validity: The extent to which the treatment conditions and participant population reflect the "world" to which generalization is desired.

vii. Report research publicly to encourage professional scrutiny, critique and replication.
- Criticism is essential to scientific progress;
- The extent to which new findings can be reviewed contested, and accepted or rejected by scientific peers depends upon accurate, comprehensive, and accessible records of:
- Data
- Methods
- Inferential reasoning

Concept of hypotheses formulation and testing

a. "It is a tentative prediction about the nature of the relationship between two or more variables."

b. "A hypothesis can be defined as a tentative explanation of the research problem, a possible outcome of the research, or an

educated guess about the research outcome." (Sarantakos, 1993: 1991)

c. "Hypotheses are always in declarative sentence form, and they relate, either generally or specifically, variables to variables."

d. "An hypothesis is a statement or explanation that is suggested by knowledge or observation but has not, yet, been proved or disproved." (Macleod Clark J and Hockey L 1981)

Nature of Hypothesis

a. The hypothesis is a clear statement of what is intended to be investigated. It should be specified before research is conducted and openly stated in reporting the results. This allows to: Identify the research objectives; Identify the key abstract concepts involved in the research Identify its relationship to both the problem statement and the literature review

b. A problem cannot be scientifically solved unless it is reduced to hypothesis form

c. It is a powerful tool of advancement of knowledge, consistent with existing knowledge and conducive to further enquiry

d. It can be tested – verifiable or falsifiable

e. Hypotheses are not moral or ethical questions

f. It is neither too specific nor to general

g. It is a prediction of consequences

h. It is considered valuable even if proven false. This statement satisfies both criteria for experimental hypotheses. It is a

a) **Prediction:** It predicts the anticipated outcome of the experiment

b) **Testable:** Once you have collected and evaluated your data.

The importance of hypotheses may be summarized as under:

a. Hypotheses facilitate the extension of knowledge in an area. They provide tentative explanations of facts and phenomena, and can be tested and validated. It sensitizes the investigator to certain aspects of situations which are relevant from the standpoint of the problem in hand.

b. Hypotheses provide the researcher with rational statements, consisting of elements expressed in a logical order of relationships which seek to describe or to explain conditions or events, that have not yet been confirmed by facts. The hypotheses enable the researcher to relate logically known facts to intelligent guesses about unknown conditions. It is a guide to the thinking process and the process of discovery. It is the investigator's eye – a sort of guiding light in the work of darkness.

c. Hypotheses provide direction to the research. It defines what is relevant and what is irrelevant. The hypotheses tell the researcher specifically what he needs to do and find out in his study. Thus, it prevents the review of irrelevant literature and the collection of useless or excess data. Hypotheses provide a basis for selecting the sample and the research procedures to be used in the study. The statistical techniques needed in the analysis of data, and the relationships between the variables to be tested, are also implied by the hypotheses. Furthermore, the hypotheses help the researcher to delimit his study in scope so that it does not become broad or unwieldy.

d. Hypotheses provide the basis for reporting the conclusions of the study. It serves as a framework for drawing conclusions. The researcher will find it very convenient to test each hypothesis separately and state the conclusions that are relevant to each. On the basis of these conclusions, he can make the research report interesting and meaningful to the reader. It provides the outline for setting conclusions in a meaningful way.

e. Hypothesis has a very important place in research although it occupies a very small pace in the body of a thesis. It is almost impossible for a research worker not to have one or more hypotheses before proceeding with his work.

Sources Of Hypothesis:

a. Review of similar studies in the area or of the studies on similar problems;

b. Examination of data and records, if available, concerning the problem for possible trends, peculiarities and other clues;

c. Discussions with colleagues and experts about the problem, its origin and the objectives in seeking a solution.

d. Exploratory personal investigation which involves original field interviews on a limited scale with interested parties and individuals with a view to secure greater insight into the practical aspects of the problem.

e. Intuition is often considered a reasonable source of research hypotheses -- especially when it is the intuition of a well-known researcher or theoretician who “knows what is known”

f. Rational Induction is often used to form “new hypotheses” by logically combining the empirical findings from separate areas of research

g. Prior empirical research findings are perhaps the most common source of new research hypotheses, especially when carefully combined using rational induction

h. Thus hypothesis are formulated as a result of prior thinking about the subject, examination of the available data and material including related studies and the council of experts.

Types of Hypotheses

a. Null Hypotheses

Designated by: H0 or HN. Pronounced as "H oh" or "H-null". The **null hypothesis** represents a theory that has been put forward, either because it is believed to be true or because it is to be used as a basis for argument, but has not been proved. It has serious outcome if incorrect decision is made!

b. Alternative Hypotheses

Designated by: H1 or HA. The **alternative hypothesis** is a statement of what a hypothesis test is set up to establish. Opposite of Null Hypothesis is only reached when if H_0 is rejected. Frequently "alternative" is actual desired conclusion of the researcher!

c. Working Hypothesis

The working or trail hypothesis is provisionally adopted to explain the relationship between some observed facts for guiding a researcher in the investigation of a problem. A Statement constitutes a trail or working hypothesis (which) is to be tested and conformed, modifies or even abandoned as the investigation proceeds.

Formulating Hypothesis

There are no precise rules for formulating hypotheses and deducing consequences from them that can be empirically verified. However, there are certain necessary conditions that are conducive to their formulation. Some of them are:

a. *Richness of background knowledge*. A researcher may deduce hypotheses inductively after making observations of behaviour, noticing trends or probable relationships. Background knowledge, however, is essential for perceiving relationships among the variables and to determine what findings other researchers have reported on the problem under study. New knowledge, new discoveries, and new inventions should always form continuity with the already existing corpus of knowledge and, therefore, it becomes all the more essential to be well versed with the already existing knowledge. Hypotheses may be

formulated correctly by persons who have rich experiences and academic background, but they can never be formulated by those who have poor background knowledge.

b. *Versatility of intellect*: Hypotheses are also derived through deductive reasoning from a theory. Such hypotheses are called deductive hypotheses. A researcher may begin a study by selecting one of the theories in his own area of interest. After selecting the particular theory, the researcher proceeds to deduce a hypothesis from this theory through symbolic logic or mathematics. This is possible only when the researcher has a versatile intellect and can make use of it for restructuring his experiences. Creative imagination is the product of an adventure, sound attitude and agile intellect. In the hypotheses formulation, the researcher works on numerous paths. He has to take a consistent effort and develop certain habits and attitudes. Moreover, the researcher has to saturate himself with all possible information about the problem and then think liberally at it and proceed further in the conduct of the study.

c. *Analogy and other practices*. Analogies also lead the researcher to clues that he might find useful in the formulation of hypotheses and for finding solutions to problems. The researcher, however, should use analogies with caution as they are not fool proof tools for finding solutions to problems. At times, conversations and consultations with colleagues and expert from different fields are also helpful in formulating important and useful hypotheses.

Characteristics of a Good Hypothesis

a. Hypothesis should be clear and precise. If the hypothesis is not clear and precise, the inferences drawn on its basis cannot be taken as reliable.

b. Hypothesis should be capable of being tested. Some prior study may be done by researcher in order to make hypothesis a testable one. A hypothesis "is testable if other deductions can be made from it which, in turn, can be confirmed or disproved by observation."

c. Hypothesis should state relationship between variables, if it happens to be a relational hypothesis.

d. Hypothesis should be limited in scope and must be specific. A researcher must remember that narrower hypotheses are generally more testable and he should develop such hypotheses.

e. Hypothesis should be stated as far as possible in most simple terms so that the same is easily understandable by all concerned. But one must remember that simplicity of hypothesis has nothing to do with its significance.

f. Hypothesis should be consistent with most known facts i.e. it must be consistent with a substantial body of established facts. In other words, it should be one which judges accept as being the most likely.

g. *The hypotheses selected should be amenable to testing within a reasonable time*. The researcher should not select a problem which involves hypotheses that are not agreeable to testing within a reasonable and specified time. He must know that there are problems that cannot be solved for a long time to come. These are problems of immense difficulty that cannot be profitably studied because of the lack of essential techniques or measures.

h. Hypothesis must explain the facts that gave rise to the need for explanation. This means that by using the hypothesis plus other known and accepted generalizations, one should be able to deduce the original problem condition. Thus hypothesis must actually explain what it claims to explain, it should have empirical reference.

Errors in Hypotheses

a. **Type I:** A type I error occurs when the null hypothesis (H0) is wrongly rejected. For example, A type I error would occur if we concluded that the two drugs produced different effects when in fact there was no difference between them.

b. **Type II:** A type II error occurs when the null hypothesis H0, is not rejected when it is in fact false.
For example: A type II error would occur if it were concluded that the two drugs produced the same effect, that is, there is no difference between the two drugs on average, when in fact they produced different ones.

Scientific Report Writing

Science is the orderly collection of scientific records—i.e., observations about the natural world made via well-defined procedures—and scientific records are archived in a standardized form, the scientific research paper. A research project has not contributed to science until its results have been reported in a standard paper, the observations in which are accompanied by complete recipes. Therefore, to be a contributing scientist, you must write scientific papers. Begin with the all-important recipes, the *Materials and Methods*. Next, collect your data and draft the *Results*. As your experiments end, formulate the outlines of a *Discussion*. Then write a working *Conclusion*. Now, go back and write the historical context, the *Introduction*. Only after all else has been written and tidied up, will you have sufficient perspective to write the *Title* and the *Abstract*.

a. **TITLE**: Titles should be specific, using the fewest possible words to adequately *describe* the contents of the paper. Include specifics such as the name of the organism studied.

b. The introduction has two functions:

a) to provide the context for your investigation

b) to state the question asked and the hypothesis tested in the study.

Begin the introduction by reviewing the background information (include citations!) that will enable the reader to understand the objective of the study and the significance of the question. The background information should make it clear *why* you did the experiment. Follow the background information with a clear statement of the question, goal(s), or objective or hypothesis of your investigation. Briefly describe the experiment performed (one or two sentences only) and the outcome you predicted. These items are usually at the end of the introduction. Always briefly state what organisms and variables were studied in the introduction. The introduction may be short (2-3 paragraphs) depending on your paper topic.

c. **Materials And Methods:** The materials and methods section explains what was done clearly and completely. Another scientist should be able to read it and repeat your experiment. This section should be a narrative description that integrates the materials and procedures used in the investigation. Do not make lists of the materials or an outline of the steps of the procedure. Rather, write the materials and methods section concisely in paragraphs that describe each step of the experiment. Use past tense, since this section describes how you *did* your study. Sometimes this information is covered in a laboratory manual or handout, and if so, it is may **NOT** be necessary to "copy" this procedural information into your report. Instead, simply inform the reader with a citation that you used the materials and methods specified in your laboratory manual or other source by making a proper reference to the resource. The complete citation for the resource should be included in the "Literature Cited" section of your report. Any statistical tests used to analyze your data should also be described here, citing a source for the statistical tests, perhaps a statistics book or your lab manual.

d. Results: The results section may consist of at least four components:

a) one or two sentences reminding the reader about the nature of the research,

b) one or more paragraphs that *describe* the results in narrative,

c) figures (graphs, diagrams, pictures), and

d) tables.

The text of your results section should clearly *describe* what was found, and not require the reader to interpret data from figures and tables. Generally, results clearly *summarize* and describe trends in the data and observations obtained in the study. All statements must be supported with reference to data. All figures and tables included in your paper must be cited in the textual description of your results. Most importantly, the results section should be free of *interpretation* of the data (interpretation belongs in the discussion section). Once again, use past tense as this section describes what you found. When reporting quantitative results, be sure to include both a measure of central tendency (e.g. average or median) and a measure of variability (e.g. range or standard error). Means (averages) should always be followed by an indication of variability around that mean such as the standard error, the units, and the number of replicate data points used to determine the mean. Tables and figures may be embedded in your text, or it is often easier to put them on individual sheets either inserted in the page after they are referred to or all attached at the end of the report. Tables and figures (see examples that follow) typically present *summaries* of data, not all the raw data points. Sketches are considered "figures" and should be accompanied by magnification if a microscope is used. Both tables and figures should have an **informative caption** containing the word "Figure" or "Table" followed by the figure or table number in the order referred to in the written portion of your results, and then be followed by a descriptive title. This "title" is a statement that explains the content (but not trends or results) of the figure or table clear enough so they "stand alone" without needing reference to the written portion of your results section. Table captions are always placed above a table, while figure captions are always placed below a graph, picture, or sketch.

e. The discussion section is your chance to interpret your results and put them into context regarding your original objectives or experimental question. The discussion should also relate your findings to the present state of knowledge (cite sources) and possible future needs for research. Make sure this is genuinely interpretive (explains your results, i.e. answering *why*?), and not just a restatement of the introduction or results sections. You might want to start with restating your experimental question or predictions and how your results did or did not confirm your question or prediction. Are there any possible alternative explanations for your results? It is very important that each conclusion that you make is justified by results of your experiment(s) and that you make the connection between the results and your conclusions. Any statements of fact or opinion

must be supported by references to the literature, to your data, or specific examples. You should discuss the broader significance of your results, i.e. do they have implications for our understanding of the organism(s) studied or can they be applied to problems such as public health or maintaining biodiversity? You may also wish to suggest additional hypotheses that could be tested. Your discussion may include problems or limitations you encountered with experimental design, data collection or analysis. It should address any unusual or unexpected findings logically. When appropriate, identify any possible sources of error, and how such difficulties might be avoided. As in other sections, when discussing your data and comparing it with previous studies, you must cite or acknowledge all outside sources of information.

f. **Literature Cited:** In scientific writing, you take ideas and conclusions from other work to provide background information and to support your work. The sources of these ideas must be acknowledged with citations using the "author, date" method (see "Citing references in the text" below). In scientific writing you put these ideas *in your own words* and *do not use quotes*. Full reference information of only those references cited in your paper are listed in the Literature Cited section. This is an alphabetical list by author and year of all printed materials referred to in your report. Items you read but did not cite in the text of your report should *not* be included. When putting together your literature cited section, the following author/year format from the Council for Biological Editors (CBE) style manual should be used: Citations from scientific research journals (by one, two, or three or more authors). What is scientific method?

Ans: The Scientific method is a process with the help of which scientists try to investigate, verify, or construct an accurate and reliable version of any natural phenomena. They are done by creating an objective framework for the purpose of scientific inquiry and analysing the results scientifically to come to a conclusion that either supports or contradicts the observation made at the beginning.

Self-Assessment Exercises

What is hypothesis?

5.4 Summary

The scientific methods of investigation involve 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.

5.5 References/Further Reading/Web Sources

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5.6 Possible Answers to Self-Assessment Exercises

A hypothesis is an assumption that is made based on some evidence. This is the initial point of any investigation that translates the research questions into predictions. It includes components like variables, population and the relation between the variables. A research hypothesis is a hypothesis that is used to test the relationship between two or more variables

Glossary

Chromatography: The separation of a mixture by passing it through a medium in which its components move at different rates.

Column: The component of the chromatography system where the separation of the sample occurs. Can be an actual column or a loop of tubing.

Column packing: The solid material, usually a porous solid with or without a chemically interactive surface placed inside or on the walls of a column, used to differentially retain analytes; also referred to as the stationary phase.

Eluate: The combination of mobile phase and solute exiting the column; also called effluent.

Eluent: The mobile phase used to carry out a separation.

Gas chromatography is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition

High-performance liquid chromatography (HPLC): A chromatographic technique used to separate, identify and quantify sample components in a liquid mixture, often characterized by high pressure.

L**igand**: An ion or molecule that binds to another (usually larger) molecule.

End of the module Questions

1. What is a calorimeter?
2. What is the principle behind a calorimeter?

3. What is the formula for heat transfer?
4. What are the common types of calorimeters?
5. What are the main components of a calorimeter?
6. What is a bomb calorimeter?
7. Inside a closed system, the heat energy lost by a hot object is equivalent to the total heat energy _____ by the cold body.
8. The concept of calorimetry is built around the law of _____.
9. Paper Chromatography is a separatory technique that is used to separate_____
   a. SIMPLE mixtures
   b. Complex mixtures
   c. Viscous mixtures
   d. Metals
10. Which of the following is used as a spraying reagent in paper chromatography?
   a. Concentrated Hydrochloric Acid Solution
   b. Sodium Chloride Solution
   c. Ninhydrin Solution
   d. Copper Sulphate Solution
11. Which of the following is not a development technique in paper chromatography?
   a. High-Pressure Liquid Chromatography
   b. Ascending Chromatography
   c. Descending Chromatography
   d. Two-Dimensional Chromatography
12. The pattern on the paper in Paper chromatography is called_____
   a. Chroma
   b. Chromatograph
   c. Chroming
   d. Chromatogram
13. A Combination Of paper chromatography and electrophoresis involves_____
   a. Electrical mobility of the ionic species
   b. Partition chromatography
   c. Both (a) and (b)
   d. None of the above
14. What is paper chromatography?
15. What are the applications of chromatography?
16. What is Rf value?
17. What are the moving and stationary phases in paper chromatography?
18. What are the advantages of paper chromatography?
19. What are the disadvantages of paper chromatography?
20. How does the liquid rise through a filter paper in paper chromatography?

21. What are the various factors that affect the Rf value of a compound?
22. What are the fundamental features of the compound used as a developer?
23. In which of the following type of paper chromatography does the mobile phase move horizontally over a circular sheet of paper?
a. Ascending – descending chromatography
b. Ascending paper chromatography
c. Descending paper chromatography
d Radial paper chromatography
24. What are the steps of the scientific method?
25. What is the aim of scientific methods?
26. Explain the step: Analysis and Conclusion.

Answer

1. A calorimeter is an apparatus used for calculating the heat developed during a chemical, mechanical or electrical reaction. It also helps to measure the heat capacity of various materials.
2. When two objects of different temperatures (ideally a liquid or a solid) come in contact with each other, then the heat is transmitted from the warmer object to the colder object, until a state of thermal equilibrium is reached between them. The object at a higher temperature dissipates heat, while the object at a lower temperature absorbs heat energy. The concept of calorimetry is built around the law of conservation energy. Inside a closed system, the heat energy lost by a hot object is equivalent to the total heat energy absorbed by the cold body.
3. The heat transfer in a system is calculated using the formula, $q=mc\Delta t$ Where
q is the value of heat transfer
m is the object's mass
c is the object's specific heat
Δt is the change in the temperature
4. Accelerated Rate Calorimeters, Differential Scanning Calorimeters, Titration Calorimeters, and Isothermal Micro Calorimeters are the most common types of calorimeters.
5. A standard calorimeter simply comprises a thermometer connected to a metal vessel full of water hanging above a combustion chamber.
6. A bomb calorimeter is one among the widespread calorimeters. It is made of an enclosure in which the interactions occur, enveloped by a fluid such as water that sucks the heat of the reaction, and consequently increases in temperature. The value of this temperature rise, the quantity of the weight, and the heat

properties of the liquid and container allows the total amount of heat produced to be calculated.

7. absorbed
   Explanation: Inside a closed system, the heat energy lost by a hot object is equivalent to the total heat energy absorbed by the cold body.
8. conservation energy
   Explanation: The concept of calorimetry is built around the law of conservation energy.
9. B: Paper Chromatography is a separatory technique that is used to separate complex mixtures.
10. C: Ninhydrin is used as a spraying reagent in paper chromatography.
11. A: High-Pressure Liquid Chromatography is not a development technique in paper chromatography.
12. D: The pattern on the paper in Paper chromatography is called chromatogram.
13. C: A combination of paper chromatography and electrophoresis involves electrical mobility of the ionic species and partition chromatography.
14. Paper chromatography is an analytical method used to separate dissolved chemical substances by taking the benefit of different migration rates across sheets of paper.
15. There are miscellaneous applications of paper chromatography.

- It is used to study the process of fermentation and ripening.
- It is used to specify drugs and dopes.
- It is used to detect impurities.
- It is used to check the purity of medicines.
- It is used to review makeup and cosmetics.

16. Rf value is the ratio of the distance travelled by the solute to the distance travelled by the solvent. Rf = Distance travelled by the solute / Distance travelled by the solvent.
17. Water absorbed on the cellulose, constituting the paper, works as a stationary phase while the organic solvent works as a moving phase in the paper chromatography.

18. There are miscellaneous advantages of paper chromatography.

- It is affordable and relatively easier than other methods.
- It is time-efficient.
- It needs a nominal quantity of samples for testing.
- It is relatively effortless to set up and handle.
- It is susceptible.
- It can separate both organic and inorganic substances.

19. There are miscellaneous disadvantages of paper chromatography.
- It is tough to separate the complex mixtures by paper chromatography.
- It is incompatible for testing large quantities of samples.
- It cannot separate volatile compounds.
- It is not suitable for quantitative analysis.
- It is not accurate as high-pressure liquid chromatography and thin-layer chromatography.
- We cannot separate chromatograms for a long time period.

21. The liquid rise through a filter paper in paper chromatography by the mean of the capillary action.
22. Rf value of a compound depends on the following factors.
    - Temperature of the surroundings
    - Nature of the compound
    - Nature of the solvent.
23. The fundamental features of the compound used as a developer are enlisted below.
    - The compound should not react with the substances that are being separated.
    - The compound should be volatile.
    - The compound must impart colour at separate spots.

23. D: In radial paper chromatography, the mobile phase move horizontally over a circular sheet of paper.
24. Observation, data collection, testing and analysing.
25. The aim of all the scientific methods is to analyze the observation made at the beginning but there are various steps adopted as per the requirement of any given observation.
26. This step involves the use of proper mathematical and other scientific procedures to determine the results of the experiment. Based on the analysis, the future course of action can be determined. If the data found in the analysis is consistent with the hypothesis, it is accepted. If not, then it is rejected or modified and analyzed again.