PANDIT SUNDARLAL SHARMA (OPEN) UNIVERSITY CHHATTISGARH
BILASPUR

LABORATORY MANUAL

Bachelor of Science

Zoology

(B.Sc. Ist Year)

Department of Zoology

PANDIT SUNDARLAL SHARMA (OPEN) UNIVERSITY CHHATTISGARH, BILASPUR

VERIFIED

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MANUAL FOR ZOOLOGY LABORATORY BSC FIRST YEAR

PRACTICAL SCHEME

SNo	PARTICULARS	MARKS
1	Major Dissection	10
2	Minor Dissection	05
3	Exercise based on Adaptation	04
4	Cytological Preparation	05
5	Spotting 08 (Permanent slides-4, and specimens of Invertebrates-2)	16
6	Sessional Marks	10
	Total	50

PRACTICAL WORK

The practical work will, in general be based on the syllabus prescribed in theory and the candidates will be required to show knowledge of the following:

I. Dissection of Earthworm, Cockroach, Palaemon and Pila under the major dissection.
II. Minor Dissection: Appendages of Prawn and Hastate plate. Mouth parts of Insects, Redula of Pila (Alernative methods such as clay/thermacol/drawing model etc)
III. Museum specimens of invertebrates
IV. Slides: Invertebrates, Frog embryology, chick embryology and cytology.
V. Study of limbs, girdles and vertebral column of Frog, Varanus, Fowl and Rabbit.

INSTRUCTIONS

1. Students are advised to enter the laboratory with prior knowledge and permission of the instructor/ Lab incharge.

2. Do not use any laboratory equipment without instruction and authorization from the instructor. Report any damaged or broken equipment to your instructor immediately.

3. Never engage in any rowdy, playful, or unprofessional activities in the laboratory.

4. Use all chemicals with caution. Do not taste or inhale and avoid direct touch to your skin. In case of any chemicals splashing in eyes or skin, immediately go to nearest sink, flush and wash affected place.

5. Report any and all accidents, spills, breakages, or injuries to the instructor.

6. Objects like Scalpels and Razors should be used only after getting proper handling instructions and authorization from instructor.

7. Do not keep unnecessary books, backpacks and other personal items on laboratory benches.

8. Avoid open long hair, flowing clothing, open-toed shoes in laboratory.

9. Pregnant or immunocompromised student must inform the instructor. Pregnant students will not be allowed to do dissections or work with any body fluids without having a doctor's note for permission.

10. Before laboratory, wash hands thoroughly and line the work area with clean paper towels. After laboratory, wipe down work area with disinfectant and wash hands thoroughly.

11. Dispose of used slides, chemicals, any tissue wastes or hazardous wastes in proper disposal container. Follow the instructor's instruction before disposal of anything in the laboratory.

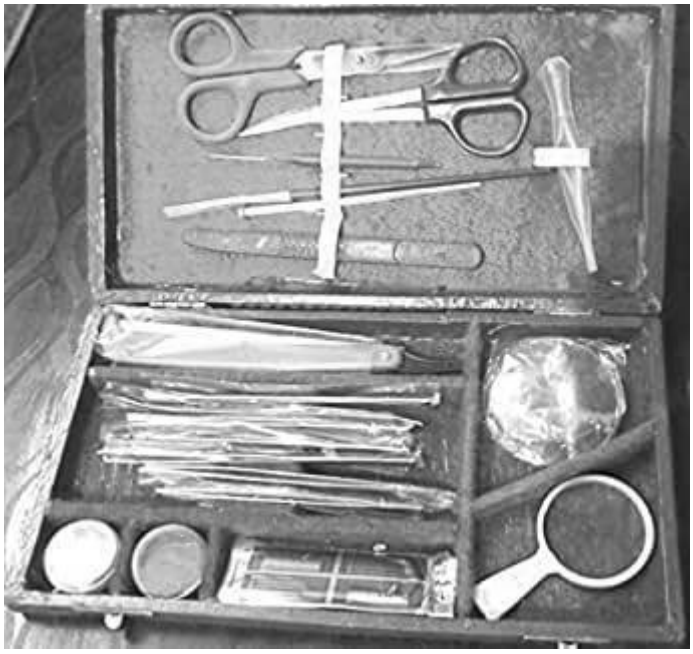
Leave the laboratory in better condition than you entered. Put all microscopes, glass and plastic materials or others back in place properly. Clean laboratory benches, wash glass wares, slides, trays or any reusable things of such kind. Dispose specimen and others things properly.

COMMONLY USED INSTRUMENTS IN ZOOLOGY LABORATORY

Dissection Box

It is a box which contains all the necessary equipment to perform various activities in a laboratory It mainly consists of 1.needle 2.forceps (blunt and sharp) 3.sissors 4.brush 5.coverslips 6.watch glass 7.blade 8.slide 9.dropper

A biology student must have a dissection box with him to carry out experiments in Zoology laboratory. The needle and forcep help in mounting material with coverslip easily and accurately. The main purpose of using this box is to carry out dissections(opening the body of animal with blade), hence it is known as dissection box. Forcep helps in holding the materials. Needle helps in mounting. Dropper helps in taking the liquids. Blades help in dissection.



Dissection Box

Now days dissection boxes of various companies are available in market in affordable prices. We can also get the individual parts separately.

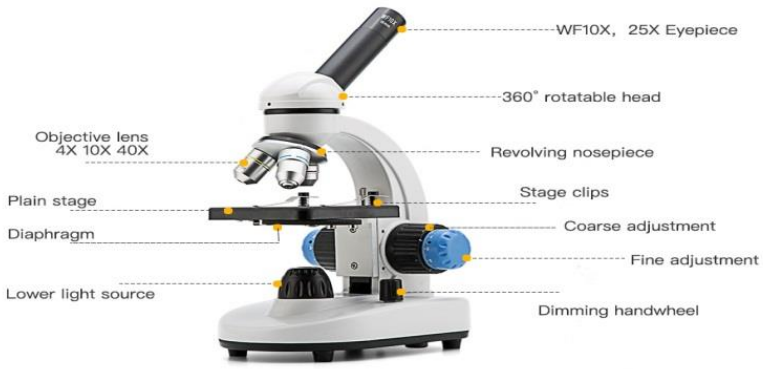
Simple Dissecting microscope

A dissecting microscope is used to view three-dimensional objects and larger specimens, with a maximum magnification of 100x. This type of microscope might be used to study external features on an object or to examine structures not easily mounted onto flat slides.

Dissecting Microscope

Compound Microscope

This is a high power light microscope with multiple lenses i.e. the objective lens (typically 4x, 10x, 40x or 100x) is compounded (multiplied) by the eyepiece lens (typically 10x) to obtain a high magnification of 40x, 100x, 400x and 1000x. This microscope has multiple use in biological laboratories. Learners can observe biological slides or thinly cut sections of any object by this tool. Histological slides, bacteria, protozoa etc. can be studied under this microscope. Up-right microscope is basically used in research purpose. Here, the source of transmitted light and the condenser are located below the stage, pointing up and the objective is placed on the top of the stage pointing down. The specimen is observed from the top. This is used for observing the living cells or samples that are squeezed between a slide and coverslip.



Compound Microscope

Both microscopes have similar features. Each microscope has an eyepiece as well as a light source. Light microscopes usually have eyepieces that are magnified 10x plus multiple objective lenses that are magnified between 4x and 100x (SF Fig. 2.2 A). The total magnification is calculated by multiplying the eyepiece magnification (10x) by the objective lens magnification (e.g., 40x), for a total magnification would be 400x. On a light microscope, a sample is placed on a glass slide and light is passed through the sample from underneath the stage. This type of microscope shows great detail of small, thin objects. To view thicker objects, the tissue sample must be thinly sliced and prepared on a glass slide in two-dimensions. With a dissecting microscope whole objects can be viewed in three dimensions. Samples do not need to be sliced, and larger, live animals can be observed. Light can be passed through from underneath the sample, but also from the top or side using an external light source. However, the magnification power of the dissecting microscope is less powerful than that of a light microscope.

Incubator

Generally, any incubator is used to maintain optimal temperature, humidity and other conditions such as the CO (CO2) and oxygen content of the atmosphere inside a device, which is used to grow and maintain microbiological cultures or cell cultures. BOD incubators are used in determining the Biological Oxygen Demand (BOD) as this tool helps to maintain

a favourable temperature (200 C) inside. BOD incubator is also helpful to measure molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron.

Incubator

pH meter

This instrument is used to measure the hydrogen-ion activity in water-based solutions, indicating its acidity or alkalinity expressed as pH. Electric potential between a pH electrode and a reference electrode is measured to detect the pH of any solution and so the pH meter is sometimes referred to as potential pH meter. The voltage between the two electrodes converted into pH values and displayed. This instrument is very common in use in the biological and chemical laboratories to prepare different chemicals, reagents, buffers with specific pH.

pH Meter

Some Laboratory Protocols in Zoology (BSc First Year)

Preparation Techniques: Wet Mount and Squash method, Staining

The main methods of placing samples onto microscope slides are wet mount, dry mount, smear, squash and staining but students use to prefer the squash methods for the preparation of mitosis cell division slides.

Wet Mount:

Used for aquatic samples, living organisms and natural observations, wet mounts suspend specimens in fluids such as water, brine, glycerin and immersion oil. A wet mount requires a liquid, tweezers, pipette and paper towels.

To prepare the slide:

- Place a drop of fluid in the center of the slide
- Position sample on liquid, using tweezers
- At an angle, place one side of the cover slip against the slide making contact with outer edge of the liquid drop
- Lower the cover slowly, avoiding air bubbles
- Remove excess water with the paper towel

Although wet mounts can be used to prepare a significantly wide range of microscope slides, they provide a transitory window as the liquid will dehydrate and living specimens will die. Organisms such as protozoa may only live 30 minutes under a wet mount slide; applying petroleum jelly to the outer edges of the cover slip creates a seal that may extend the life of the slide up to a few days. In addition, larger protozoan such as paramecium may be too large and/or move too quickly under the wet mount. In these circumstances, adding ground pieces of cover glass to the water before the slip layer will create added space and chemicals or strands of cotton can be added to slow the movement of paramecium, amoeba and ciliates.

Squash Slides: Designed for soft samples, squash slides begin by preparing a wet mount; place lens tissue over the cover glass; gently press down, careful not to destroy the sample or break the cover glass, and squash the sample; remove excess water.

Staining: A variety of methods exist for staining microscope slides, including non-vital or in vitro stains of non-living cells and vital or in vivo stains of living tissue. Staining provides contrast through color that reveals structural details undetected in other slide preparations.

Staining solutions such as iodine, methylene blue and crystal violet can be added to wet or dry mounts.

A simple staining method:

- Add a drop of staining solution on the edge of one side of the cover slip
- Position the edge of a paper towel on the opposite end
- Allow dye to be pulled across the specimen

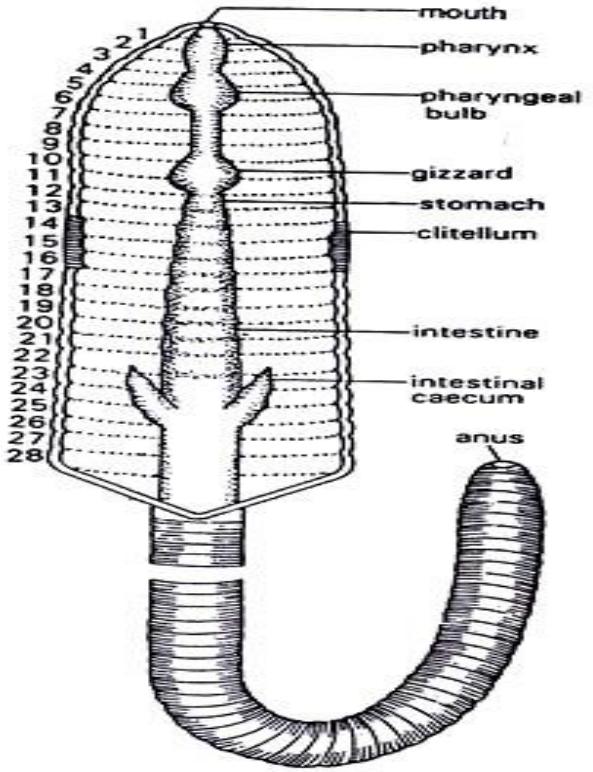
Stains are especially useful in the fields of histology, virology and pathology, allowing researchers to study and diagnose diseases, identify gram positive and negative bacteria as well as examine detailed attributes of a variety of cells.

Major Dissection – Earthworm

- Wash the live specimens with water to get rid of mucus. Drop them in a petri dish containing 30% alcohol. Take out the specimens immediately after ceasation of movements and put in tap water in a large beaker.
- Place the specimen on the fingers of your left hand. Insert the tip of one of the blades of a pair of fine scissors through the skin above the dorsal blood vessel at about 30th segment of the body. Hold the scissors almost in a horizontal position keeping the lower arm just below the body wall and cut the skin anteriorly for about 2 cm.
- Lay the worm dorsal side up. Pin the cranial and caudal ends.
- Incise beyond the clitellum, then extend the cut to both ends, from the middle out. Take great care to cut no more than 1/16 of an inch deep into the worm.
- With forceps, grasp the edges of the skin carefully. Pull the skin back so that it can be pinned down revealing the internal anatomy of the worm. Take care not to tear or pin any internal organs.
- Put the worm on the dissecting tray, keeping the dorsal surface upwards and fix it in a straight line on the wax with a few pins passing through the skin of the lateral sides and one at each of the anterior and the posterior end. Care should be taken not to damage the nerve ring at the anterior end and the anal region at the posterior end.
- Starting from the initial incision cut the skin along the mid-dorsal line, proceeding anteriorly or posteriorly or both as required for dissection. Hold the skin with a pair of fine forceps and free it from septa with a fine needle. Care must be taken not to damage the gut or other organs. Pin down the loose flaps of the skin and proceed for dissection of organ systems.

The Alimentary System:

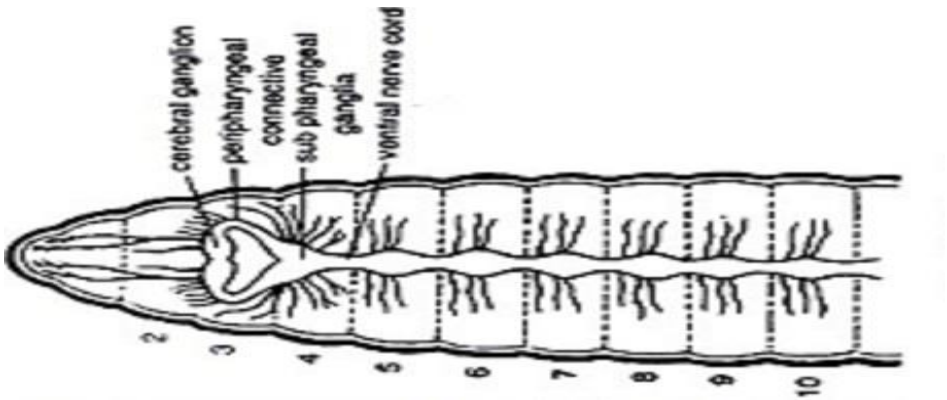
The alimentary canal is a straight tube running from the mouth to the anus. It is exposed when the skin is cut open. It runs along the whole length of the body. Remove the seminal vesicles in 10-12 segments to fully expose the gut.



Alimentary Canal of Earthworm

Dissection of Nervous System:

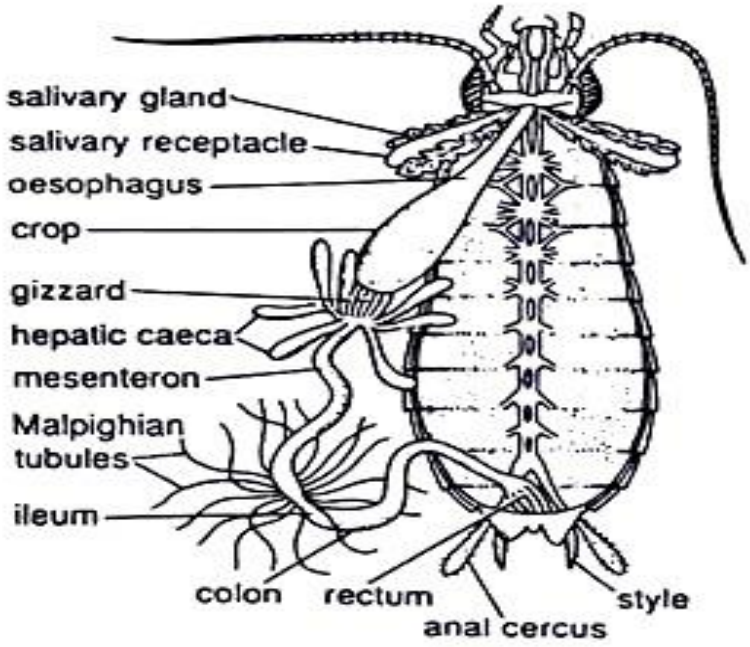
It is located on the ventral surface except the anterior end, which is oriented vertically. Cut open the skin along the whole length of the body taking care that the nerve ring, which is dorsoventral in orientation, is not damaged. Separate the gut from the body wall. The anterior and posterior ends of the alimentary canal should be carefully detached from the body wall to which it is attached. Cut the oesophagus and carefully pull out the anterior end of the gut from behind. The nerve ring is exposed. Remove the rest of the gut. The ventral nerve cord is clearly seen.



Nervous System of Earthworm

The Alimentary System of Cockroach:

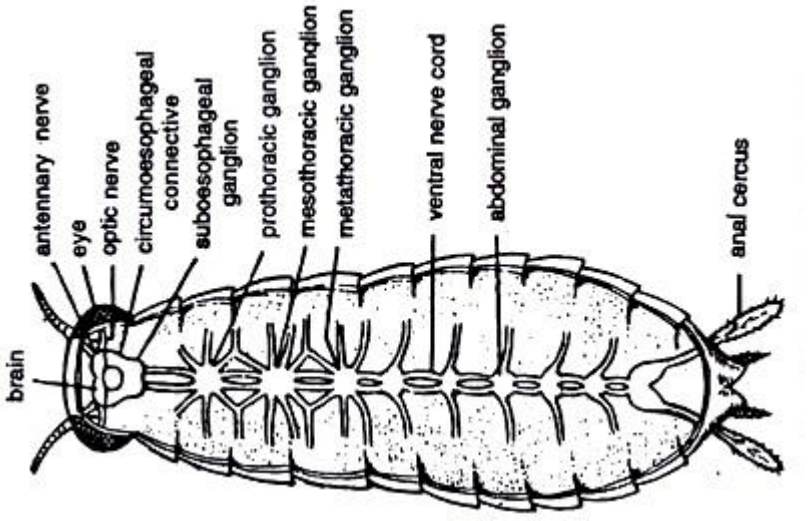
Carefully uncoil the intestine and stretch the alimentary canal to one side. Prevent it from coming back to the original position by pushing down a pin in the wax between the gut and the specimen.



Digestive System of Cockroach

Dissection of Nervous System of Cockroach:

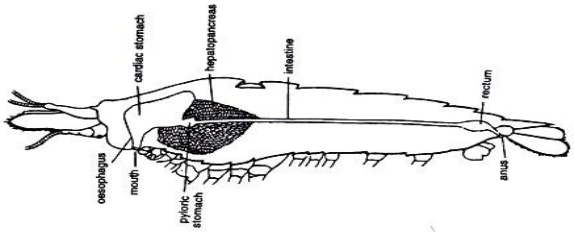
Fix the head of the specimen by pinning through the mandibles. The rest of the body should be fixed to the wax of the dissecting tray in the way already described. Carefully remove the epicranial plate of the head capsule and expose the cerebral ganglia. Cut the pharynx and pull it out with the oesophagus. Remove the viscera and the ventral nerve cord is exposed. Expose the roots of the circumoesophageal connectives on the lateral sides of the brain and trace them to the points where they meet the sub-oesophageal ganglia.



Nervous System of Cockroach

Dissection of Alimentary System of Prawn:

Remove the dorsal wall of the cephalothorax, pericardial sinus, gonads and the renal sac. The major part of the cardiac stomach, the pyloric stomach and the anterior part of the mid gut (intestine) are embedded in the hepato-pancreas. Give a superficial incision with a pair of fine scissors along the mid-dorsal line of the abdomen up to its posterior end. A narrow groove is exposed in which lies the intestine. The intestine blends with the surrounding muscles in colour but very often it stands out prominently due to its contents. The muscles of one side at the base of the telson should be cut to expose the rectum.

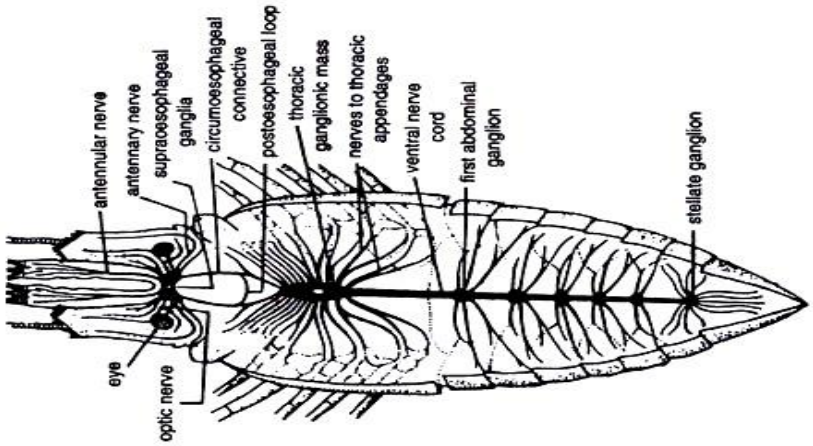


Alimentary Canal of Prawn

Dissection of Nervous System of Prawn:

Remove the carapace and the terga with pleura. The brain is located below the base of the rostrum. Remove the chitinous endo-pharyngeal skeletal plates overlying the brain. The brain is covered by a thick layer of fat. Remove the fat to expose the brain. Remove the hepatopancreas. Cut the oesophagus and pull it out with the stomach. Expose the nerves arising from the brain and follow their courses. Trace the two circumoesophageal connectives running downwards from the lateral sides of the brain to meet the ventral sub-oesophageal ganglionic mass. To expose the ventral nerve cord cut the large flexor muscles of the

abdomen along the middle line with a scalpel. Press the muscles laterally and pin them down to the wax of the dissecting tray. The nerve cord with its ganglia is clearly visible.



Nervous System of Prawn

Minor Dissection –

Mouth Parts of Cockroach: Remove the mouth parts one by one as described below and place them in a drop of water on a clean slide in a manner. Hold cockroach on the neck region with your hand with ventral surface facing vental. Locate the lower lip (labium) of mouth which is a flattened plate on the floor. Hold it from its base with the help of a pair of forceps and pull it out. This is labium. A. Next, remove maxillae from the sides and then the mandibles underneath them. Below the labium is present hypopharynx in the middle line. Finally take out upper lip called labrum. Observations

1. Mouth-parts mandibular type, i.e, biting and chewing type.

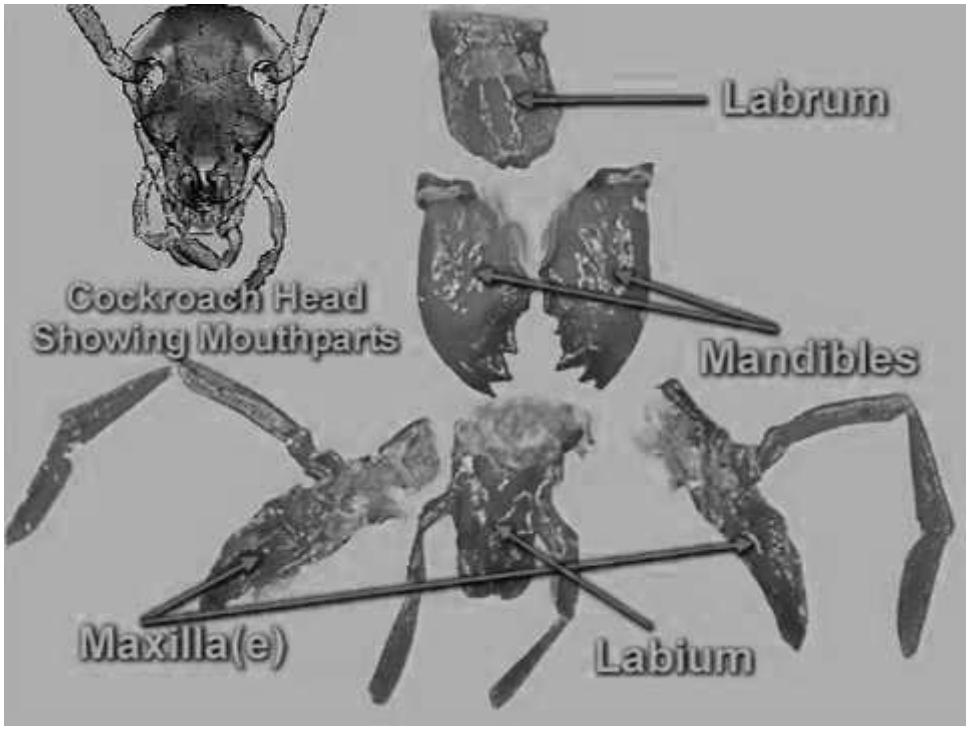
2. Mandibles plate-like and broad. The inner margins are serrated (toothed) adapted for biting the food.

3. Maxillae consist of: i) Protopodite - basal part, formed of cardo and stipes ii) Exopodite - outer part, forming maxillary palp. iii) Endopodite - inner part, formed of lacina and galea

4. Labium which forms the lower lip is composed of: i) basal part having submentum, mentum and prementum. ii) paired labial palps which represent the outer part. iii) inner part which is formed of glossae and paraglossae.

5. Hypopharynx tongue like with opening of salivary glands.

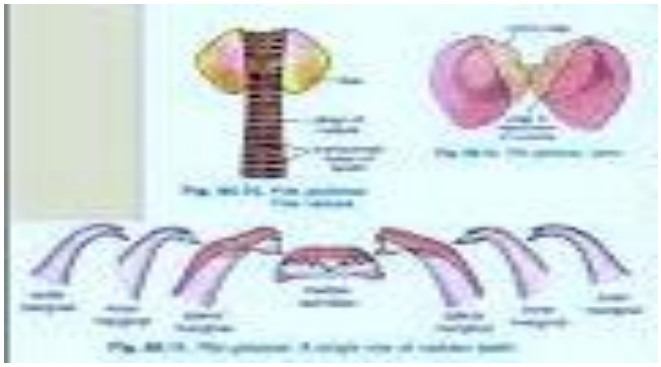
6. Upper lip formed by labrum and epipharynx.



Redula of Pila:

The Radula is the organ of Pila, which is used to cut the food in srnall pieces. The radula is a flat ribbon-like structure found inside the buccal cavity of Pila. Radula is brownish in colour and quite hard. In this exercise follow the procedure given below to take out the radula from the Pila.

Radula is characterized by having transverse rows of minute liorny teeth Eacli row has seven curving teeth. The one ill the center is large and is called tlie median rachidian tooth. This is followed by one lateral and two marginals on each side. Posterior end of radula lies in a t radular sac wliicli constarltly secretes tlie teetli as they are beiiig worn out on the , anterior end. Tlie cells, wliicli secrete the teetli, are called odontoblasts.



Redula of Pila

Procedure

1. To take out tlie radula, remove the skill over tlie liead region and you will see a routided structure, tlie buccnl mass. This is liiglily muscular structure, wliicli encloses tlie buccal cavity.

2. If you now cut away tlie top layers of tliis buccal mass, you will see tlie radula in tlie buccal cavity. 3. Pick up tlie radula with a forceps and tlieri cut it at its points of attachment.

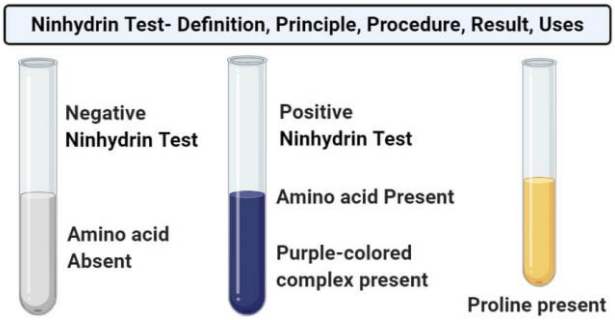
4. Place it in a watcli glass and cover it with water.

Biochemical Test

1. Millon's Test:

Chemical reagents required: 2 ml protein solution and 1 ml Millon's reagent.

Procedure: Take 2 ml of protein solution in the test tube and then add 1 ml of Millon's reagent. Mix thoroughly and bring to boil gradually.



Observation:

Appearance of white precipitate, which will turn into red as it will coagulate by absorbing heat. In presence of peptone, there will be less number of precipitate. Then the whole solution becomes re as the precipitate gets mixed in the solution. Chemical basis of the reaction: Amino acid, tyrosine is present in almost all the proteins. In presence of Millon's reagent, tyrosine reacts with the mercuric and mercuric nitrate and due to the presence of phenol group in tyrosine, red colored mercuric (II) compound is produced.

2.Biuret test

Chemical reagents required: 2 ml protein solution, 2 ml 10% NaOH solution, 1% copper sulphate solution.

Procedure • Add 2 ml protein solution and 2 ml 10% NaOH solution. Mix it thoroughly. • Then add 2 drops of cupper sulphate solution. Mix the whole solution properly.

Observation A purple color develops slowly. Chemical basis of the reaction In basic medium Cu2 + gets attached with the peptide bonds and forms a purple complex.

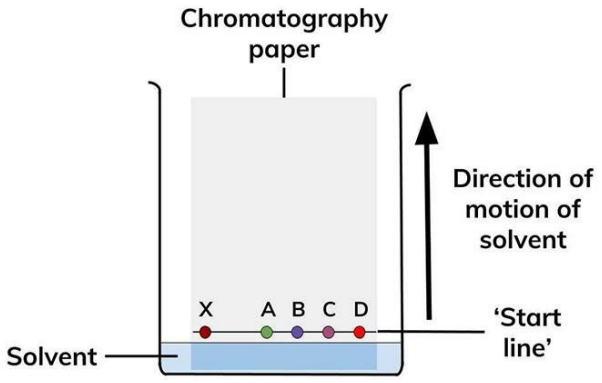
Chromatography

Chromatography is a method of physical separation in which components of mixture gets separated on two phases. One of the phase is the immobile porous bed bulk liquid which is called stationary phase and the other phase is the mobile fluid that flows over the stationary phase under gravity. During the movement of the sample, a separated result is formed by the repeated desorption and sorption in the direction of the mobile phase migration. Several key factors are responsible on the separation process like partition between liquid-liquid, affinity between molecular weight and characteristics related to liquid-solid adsorption. An interaction between the molecules are physical and involves weak chemical bonds like dipole-dipole interaction and hydrogen bond formation and adhere to the stationary components. Components that adhere strongly to the stationary phase moves slowly than those who adhere weakly. There are number of chromatography separation taking place in the industries but for laboratory syllabus paper chromatography is used frequently.

Paper chromatography:

In a paper chromatography, separation of the mixture is performed on a paper strip which is a stationary phase and a liquid solvent acts as a mobile phase.

How it works?



Paper chromatography

- A drop of mixture is placed on one end of the paper and dried. Then the paper is dipped into the solvent up to the spot.
- In the paper chromatography, component separates in two ways:

In **paper adsorption chromatography,** stationary phase and mobile phase molecules act based on the degree of interaction. Higher affinity molecules are adsorbed for a long time where movement of speed is decreased. However, low affinity molecules move faster thus, molecules are separated.

==========##########==========

Procedure

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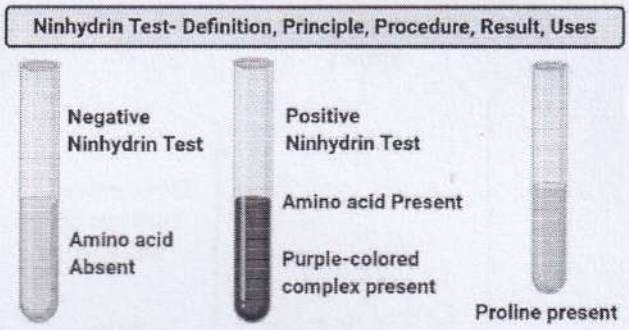

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VERIFIED

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MANUAL FOR ZOOLOGY LABORATORY BSC SECOND YEAR

PRACTICAL SCHEME

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2	Exercise based on evolution	05
3	Exercise based on applied Zoology	05
4	Exercise based on Animal Behavior	04
5	Spotting (Permanent slides-4, bones-2 and specimens of chordate-2)	16
6	Viva voice	05
7	Sessional Marks	05
	Total	50

PRACTICAL WORK

The practical work in general shall be based on syllabus prescribed in theory. Therefore students are advised to do all experiments accordingly.

The students will be required to show knowledge of the following:

I. Study of the representative examples of the different chordates.
II. Dissection of various systems of Scoliodon-Afferent and Efferent branchial, Cranial nerves and Internal Ear.
III. Simple microscopic technique through unstained or stained permanent mounts.
IV. Study of prepaired slides histological as per theory papers.
V. Study of limbs, girdles and vertebral column of Frog, Varanus, Fowl and Rabbit.
VI. Identification of species and individuals of Honey Bee
VII. Life cycle of Honey Bee and Silkworm.
VIII. Exercise based on Evolution and Animal behaviour

INSTRUCTIONS

1. Enter the laboratory with prior knowledge and permission of the instructor/ Lab incharge with proper dress and required equipments.

2. Never use any laboratory equipment without instruction and authorization from the instructor. Report any damaged or broken equipment to your instructor immediately.

3. Do not engage in any rowdy, playful, or unprofessional activities in the laboratory.

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A biology student must have a dissection box with him to carry out experiments in biology(boring,zoology) laboratory. The needle and forcep help in mounting material with coverslip easily and accurately. The main purpose of using this box is to carry out dissections(opening the body of animal with blade), hence it is known as dissection box. Forcep helps in holding the materials. Needle helps in mounting. Dropper helps in taking the liquids. Blades help in dissection.

Dissection Box

Now a days dissection boxes of various companies are available in market in affordable prices. We can also get the individual parts separately.

Simple Dissecting microscope

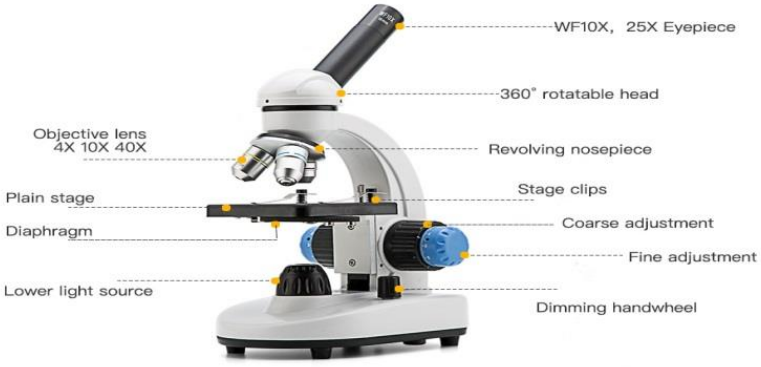
A dissecting microscope is used to view three-dimensional objects and larger specimens, with a maximum magnification of 100x. This type of microscope might be used to study external

features on an object or to examine structures not easily mounted onto flat slides.

Dissecting Microscope of Laboratory Work

Compound Microscope

This is a high power light microscope with multiple lenses i.e. the objective lens (typically 4x, 10x, 40x or 100x) is compounded (multiplied) by the eyepiece lens (typically 10x) to obtain a high magnification of 40x, 100x, 400x and 1000x. This microscope has multiple use in biological laboratories. Learners can observe biological slides or thinly cut sections of any object by this tool. Histological slides, bacteria, protozoa etc. can be studied under this microscope. Up-right microscope is basically used in research purpose. Here, the source of transmitted light and the condenser are located below the stage, pointing up and the objective is placed on the top of the stage pointing down. The specimen is observed from the top. This is used for observing the living cells or samples that are squeezed between a slide and coverslip.



Compound Microscope for Laboratory Work

Both microscopes have similar features. Each microscope has an eyepiece as well as a light source. Light microscopes usually have eyepieces that are magnified 10x plus multiple

objective lenses that are magnified between 4x and 100x (SF Fig. 2.2 A). The total magnification is calculated by multiplying the eyepiece magnification (10x) by the objective lens magnification (e.g., 40x), for a total magnification would be 400x. On a light microscope, a sample is placed on a glass slide and light is passed through the sample from underneath the stage. This type of microscope shows great detail of small, thin objects. To view thicker objects, the tissue sample must be thinly sliced and prepared on a glass slide in two-dimensions. With a dissecting microscope whole objects can be viewed in three dimensions. Samples do not need to be sliced, and larger, live animals can be observed. Light can be passed through from underneath the sample, but also from the top or side using an external light source. However, the magnification power of the dissecting microscope is less powerful than that of a light microscope.

Microtome

Microtome (Greek mikros, meaning “small”, and temnein, meaning “to cut”) is a tool which is used to cut several materials into extremely thin slices. Based on the mechanism, microtomes are of different types i.e. Rocking, Rotary, Base-sledge, Sliding, Freezing, Vibrating, Saw, Cryostat. Generally, in biological laboratories Rotary Microtomes are commonly used. Different sized Knife blades are used to cut any desired item and microtome sections with thickness between 50 nm and 100 µm, can be produced. In biological science this tool is used to study the histology of organisms i.e., tissue sections from different organs of the animals. Tissues to be studied, are cut into thin sections by the microtome, processed, stained and observed under microscope.

Microtome for Laboratory Work

Hot Plate

These are electrically controlled plate which are used to dry slides or straighten the paraffinized tissue sections. Metalized surface of the tool gets heated by the electric coils underneath. Also such instrument is used in heating solutions and preparing reagents etc.

Hot Plate

BOD Incubator

Generally, any incubator is used to maintain optimal temperature, humidity and other conditions such as the CO (CO2) and oxygen content of the atmosphere inside a device, which is used to grow and maintain microbiological cultures or cell cultures. BOD incubators are used in determining the Biological Oxygen Demand (BOD) as this tool helps to maintain a favourable temperature (200 C) inside. BOD incubator is also helpful to measure molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron.

pH meter

This instrument is used to measure the hydrogen-ion activity in water-based solutions, indicating its acidity or alkalinity expressed as pH. Electric potential between a pH electrode and a reference electrode is measured to detect the pH of any solution and so the pH meter is sometimes referred to as potential pH meter. The voltage between the two electrodes converted into pH values and displayed. This instrument is very common in use in the biological and chemical laboratories to prepare different chemicals, reagents, buffers with specific pH.

pH meter

Some Laboratory Protocols in Zoology (BSc Final Year)

Preparation Techniques: Dry Mounts, Wet Mount, Squash, Staining

The main methods of placing samples onto microscope slides are wet mount, dry mount, smear, squash and staining.

Dry Mount:

The dry mount is the most basic technique: simply position a thinly sliced section on the center of the slide and place a cover slip over the sample.

Dry mounts are ideal for observing hair, feathers, airborne particles such as pollens and dust as well as dead matter such as insect and aphid legs or antennae. Opaque specimens require very fine slices for adequate illumination.

Since they are used for primarily inorganic and dead matter, dry mounts can theoretically last indefinitely.

Wet Mount: Used for aquatic samples, living organisms and natural observations, wet mounts suspend specimens in fluids such as water, brine, glycerin and immersion oil. A wet mount requires a liquid, tweezers, pipette and paper towels.

To prepare the slide:

- Place a drop of fluid in the center of the slide
- Position sample on liquid, using tweezers
- At an angle, place one side of the cover slip against the slide making contact with outer edge of the liquid drop
- Lower the cover slowly, avoiding air bubbles
- Remove excess water with the paper towel

Although wet mounts can be used to prepare a significantly wide range of microscope slides, they provide a transitory window as the liquid will dehydrate and living specimens will die. Organisms such as protozoa may only live 30 minutes under a wet mount slide; applying petroleum jelly to the outer edges of the cover slip creates a seal that may extend the life of the slide up to a few days. In addition, larger protozoan such as paramecium may be too large and/or move too quickly under the wet mount. In these circumstances, adding ground pieces of cover glass to the water before the slip layer will create added space and chemicals or strands of cotton can be added to slow the movement of paramecium, amoeba and ciliates.

Squash Slides: Designed for soft samples, squash slides begin by preparing a wet mount; place lens tissue over the cover glass; gently press down, careful not to destroy the sample or break the cover glass, and squash the sample; remove excess water.

Staining: A variety of methods exist for staining microscope slides, including non-vital or in vitro stains of non-living cells and vital or in vivo stains of living tissue. Staining provides contrast through color that reveals structural details undetected in other slide preparations.

Staining solutions such as iodine, methylene blue and crystal violet can be added to wet or dry mounts.

A simple staining method:

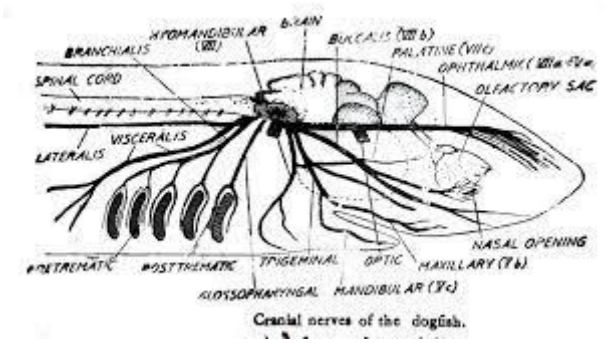
- Add a drop of staining solution on the edge of one side of the cover slip
- Position the edge of a paper towel on the opposite end
- Allow dye to be pulled across the specimen

Stains are especially useful in the fields of histology, virology and pathology, allowing researchers to study and diagnose diseases, identify gram positive and negative bacteria as well as examine detailed attributes of a variety of cells.

Major Dissection – Cranial nerves of Scoliodon

Scoliodon exhibit ten pair of cranial nerves which arises from the brain. The may be sensory, motor or mixed type according to their functions. The twelve pairs of cranial nervers present in chordates are given below:

#	Name	Nerve type	Function
I	Olfactory	Sensory	Smell
II	Optic	Sensory	Vision
III	Oculomotor	Motor	Most eye movement
IV	Trochlear	Motor	Moves eye
V	Trigeminal	Both	Face sensation, mastication
VI	Abducens	Motor	Abducts the eye
VII	Facial	Both	Facial expression, taste
VII	Vestibulocochlear	Sensory	Hearing, balance
IX	Glossopharyngeal	Both	Taste, gag reflex
X	Vagus	Both	Gag reflex, parasympathetic innervation
XI	Accessory	Motor	Shoulder shrug
XII	Hypoglossal	Motor	Swallowing, speech

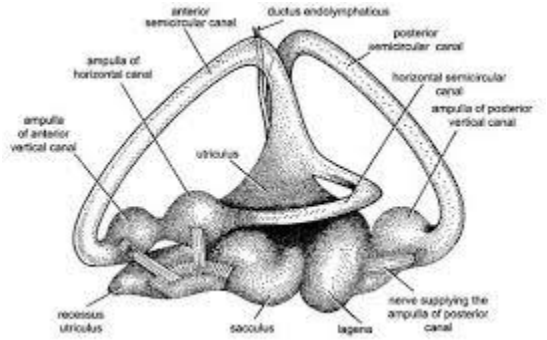


Cranial Nervous of Scoliodon

Minor Dissection – Internal Ear of Scoliodon:

They are internal ear of scoliodon is responsible for the equilibrium and hearing. The internal ear is a complex structure. It is called the membranous labyrinth. It is present in the auditory capsule of the cranium. It is covered by cartilaginous labyrinth. A space is present between the membranous and cartilaginous labyrinths. It is called peri lymphatic space. It contains peri lymph. It protects and transmits vibrations to the membranous labyrinth. The connective tissues extends from the Cartilaginous to the membranous labyrinth. The membranous labyrinth has laterally compressed vestibule which is divided into two chambers known as, the upper narrow utricles and Lower wide sacculus.

The two chambers are connected with each other by a wide passage, the sacculoutricular duct. The sacculus gives off a conical projection from its lower side, called lagena. A narrow canalarisesfrom the dorsal side of the sacculus called ductus endolymphaticus. It pierces through the roof of the cranium and open out by a minute pore. Before it opens out the duct dilates into saccus endolymphaticus. Three semicircular ducts are present. They are called the external, anterior and posterior semicircular canals. . The external canal is horizontal and the other two are vertical in position. All the semicircular anals open into the utriculus at both of their ends. One end of each canal is dilated into oval ampulla. The ampullae of the anterior and the external canals lie close together towards the anterior end of the ear.



Internal Ear of Scoliodon

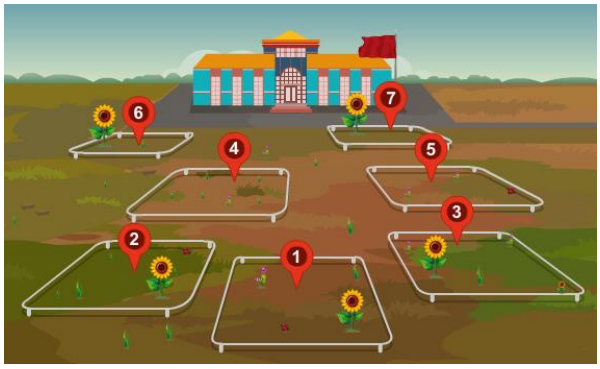
Study of plant population density by quadrat method

A population is a group of individuals of the same species which inhabit a particular space at a particular time. The number of individuals in a population never remains constant. It may increase or decrease due to many factors like birth rate, death rate and migration. The number

of individuals of the species in any unit area at a given time is its population density. The unit area may be as small as 5 square centimeters to as large as 10 square metres, depending on the size and nature of the plant community under study.

Let's understand the Quadrat Method.

Counting all individuals in a population is the most accurate way to determine its size. However, this approach is not usually feasible, especially for large populations or extensive habitats. Scientists usually calculate plant populations with the quadrat method. A quadrat is a square that encloses an area within a habitat. For herbaceous vegetation, a metre square quadrat is normally used.



Once analyzed, the sample data enables the scientist to calculate the population size and population density for the entire population. Population density is calculated by counting all the individuals present at a given time in a given space, divided by the number of units of area or space.

Population density is calculated as follows:
Density = (Total no.of individuals of the species in all the sampling unit (S))/(Total number of sampling units studied (Q))

Learning Outcomes:

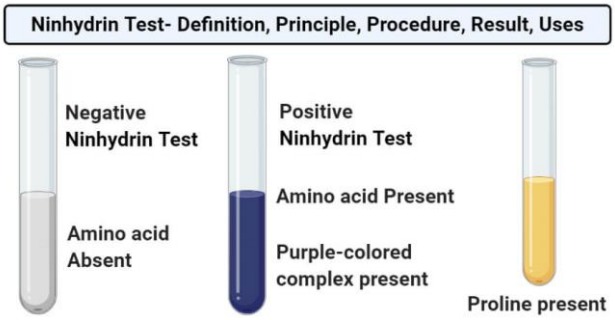
- Students understand the term quadrat method.
- Students understand how to calculate plant population density.
- Students do the experiment better in the real lab having gone through the animation and simulation.

Biochemical Test

1. Millon's Test:

Chemical reagents required: 2 ml protein solution and 1 ml Millon's reagent.

Procedure: Take 2 ml of protein solution in the test tube and then add 1 ml of Millon's reagent. Mix thoroughly and bring to boil gradually.



Observation: Appearance of white precipitate, which will turn into red as it will coagulate by absorbing heat. In presence of peptone, there will be less number of precipitate. Then the whole solution becomes re as the precipitate gets mixed in the solution. Chemical basis of the reaction: Amino acid, tyrosine is present in almost all the proteins. In presence of Millon's reagent, tyrosine reacts with the mercuric and mercuric nitrate and due to the presence of phenol group in tyrosine, red colored mercuric (II) compound is produced.

2.Biuret test

Chemical reagents required: 2 ml protein solution, 2 ml 10% NaOH solution, 1% copper sulphate solution.

Procedure • Add 2 ml protein solution and 2 ml 10% NaOH solution. Mix it thoroughly. • Then add 2 drops of cupper sulphate solution. Mix the whole solution properly.

Observation A purple color develops slowly. Chemical basis of the reaction In basic medium Cu2 + gets attached with the peptide bonds and forms a purple complex.

Chromatography

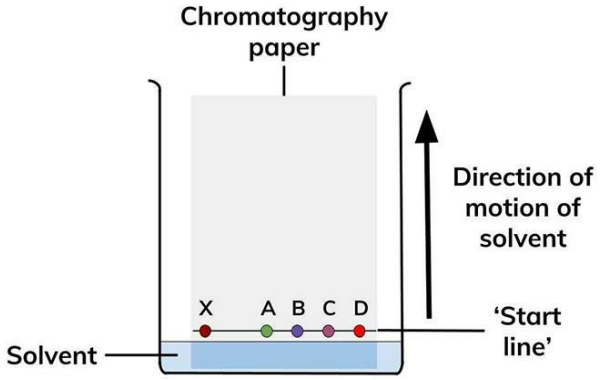
Chromatography is a method of physical separation in which components of mixture gets separated on two phases. One of the phase is the immobile porous bed bulk liquid which is called stationary phase and the other phase is the mobile fluid that flows over the stationary

phase under gravity. During the movement of the sample, a separated result is formed by the repeated desorption and sorption in the direction of the mobile phase migration. Several key factors are responsible on the separation process like partition between liquid-liquid, affinity between molecular weight and characteristics related to liquid-solid adsorption. An interaction between the molecules are physical and involves weak chemical bonds like dipole-dipole interaction and hydrogen bond formation and adhere to the stationary components. Components that adhere strongly to the stationary phase moves slowly than those who adhere weakly. There are number of chromatography separation taking place in the industries but for laboratory syllabus paper chromatography is used frequently.

Paper chromatography:

In a paper chromatography, separation of the mixture is performed on a paper strip which is a stationary phase and a liquid solvent acts as a mobile phase.

How it works?



Paper Chromatography

Paper chromatography

- A drop of mixture is placed on one end of the paper and dried. Then the paper is dipped into the solvent up to the spot.
- In the paper chromatography, component separates in two ways:

In **paper adsorption chromatography,** stationary phase and mobile phase molecules act based on the degree of interaction. Higher affinity molecules are adsorbed for a long time where movement of speed is decreased. However, low affinity molecules move faster thus, molecules are separated.

==========##########==========

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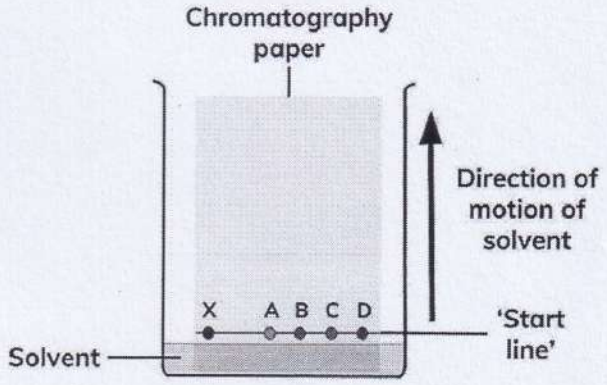

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PANDIT SUNDARLAL SHARMA (OPEN) UNIVERSITY CHHATTISGARH
BILASPUR

LABORATORY MANUAL

Bachelor of Science

Zoology

(B.Sc. IIIrd Year)

Department of Zoology

PANDIT SUNDARLAL SHARMA (OPEN) UNIVERSITY CHHATTISGARH, BILASPUR

VERIFIED

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PSSOU, CG Bilaspur

REGISTRAR
Pt. Sunderlal Sharma (Open)
University Chhattisgarh
BILASPUR (C.G.)

MANUAL FOR ZOOLOGY LABORATORY BSC FINAL YEAR

PRACTICAL SCHEME

SNo	PARTICULARS	MARKS
1	Haematological experiments	08
2	Ecological experiments	06
3	Staining of gram+ve and gram-ve bacteria	05
4	Biochemical Experiments	06
5	Chromatography	05
6	Spotting Permanent slides of parasites, pH meter, calorimeter, centrifuge, compound microscope, dissecting microscope.	10
7	Viva voice	05
8	Sessional	05
	Total	50

PRACTICAL WORK

The practical work in general shall be based on syllabus prescribed in theory. Therefore students are advised to do all experiments accordingly.

The students will be required to show knowledge of the following:

I. Detection of blood group, RBCs counting and WBCs counting
II. Estimation of population density, percentage frequency and relative density.
III. Analysis of producers and consumers in grassland
IV. Detection of gram positive and gram negative bacteria
V. Biochemical detection of protein, carbohydrates and lipids.
VI. Chromatography paper or gel
VII. Calorimetre estimation of Hb
VIII. Study of permanent slides of parasites based on theory paper
IX. Working principle of pH meter, colorimeter, centrifuge and microscope.

INSTRUCTIONS

1. Do not enter and work alone in the laboratory without prior knowledge and permission of the instructor.

2. Never use any laboratory equipment without instruction and authorization from the instructor. Report any damaged or broken equipment to your instructor immediately.

3. Do not engage in any rowdy, playful, or unprofessional activities in the laboratory.

4. Use all chemicals with caution. Do not taste or inhale and avoid direct touch to your skin. In case of any chemicals splashing in eyes or skin, immediately go to nearest sink, flush and wash affected place.

5. Report any and all accidents, spills, breakages, or injuries to the instructor.

6. Any sharp objects like Scalpels and Razors should be used only after getting proper handling instructions and authorization from instructor.

7. Do not keep unnecessary books, backpacks and other personal items on laboratory benches.

8. Avoid open long hair, flowing clothing, open-toed shoes in laboratory.

9. Pregnant or immunocompromised student must inform the instructor. Pregnant students will not be allowed to do dissections or work with any body fluids without having a doctor's note for permission.

10. Before laboratory, wash hands thoroughly and line the work area with clean paper towels. After laboratory, wipe down work area with disinfectant and wash hands thoroughly.

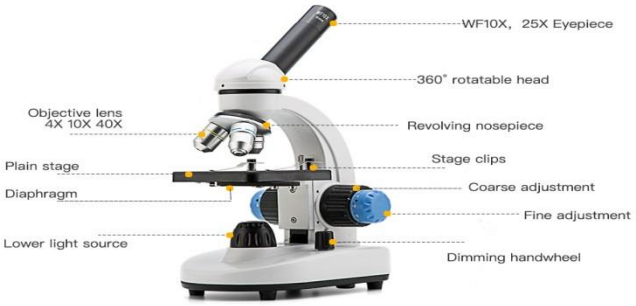
11. Dispose of used slides, chemicals, any tissue wastes or hazardous wastes in proper disposal container. Follow the instructor's instruction before disposal of anything in the laboratory.

Leave the laboratory in better condition than you entered. Put all microscopes, glass and plastic materials or others back in place properly. Clean laboratory benches, wash glass wares, slides, trays or any reusable things of such kind. Dispose specimen and others things properly.

COMMONLY USED INSTRUMENTS IN ZOOLOGY LABORATORY

Compound Microscope

This is a high power light microscope with multiple lenses i.e. the objective lens (typically 4x, 10x, 40x or 100x) is compounded (multiplied) by the eyepiece lens (typically 10x) to obtain a high magnification of 40x, 100x, 400x and 1000x. This microscope has multiple use in biological laboratories. Learners can observe biological slides or thinly cut sections of any object by this tool. Histological slides, bacteria, protozoa etc. can be studied under this microscope. Up-right microscope is basically used in research purpose. Here, the source of transmitted light and the condenser are located below the stage, pointing up and the objective is placed on the top of the stage pointing down. The specimen is observed from the top. This is used for observing the living cells or samples that are squeezed between a slide and coverslip.



Compound Microsocpe

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Microtome for Laborotories

Hot Plate

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Hot Plate

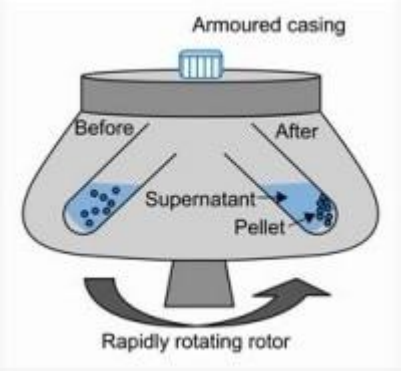
BOD Incubator

Generally, any incubator is used to maintain optimal temperature, humidity and other conditions such as the CO (CO2) and oxygen content of the atmosphere inside a device, which is used to grow and maintain microbiological cultures or cell cultures. BOD incubators are used in determining the Biological Oxygen Demand (BOD) as this tool helps to maintain a favourable temperature (200 C) inside. BOD incubator is also helpful to measure molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron.

BOD Incubator

Centrifuge

This tool is useful to separate different components of any solution based on their molecular size, weight and concentration. This machine puts an object in rotation around a fixed axis (spins it in circle), applying a force perpendicular to the axis of spin (outward) that can be very strong. This equipment works on the basis of sedimentation principle. Putting the object in centrifuge tubes, the machine can be set to centrifuge at required rate. This tool has a wide application in biological laboratories to differentiate different components of the tissue, cell etc. biochemical components with different molecular weights can also be differentiae via this machine.



Centrifugal Machine

pH meter

This instrument is used to measure the hydrogen-ion activity in water-based solutions, indicating its acidity or alkalinity expressed as pH. Electric potential between a pH electrode and a reference electrode is measured to detect the pH of any solution and so the pH meter is sometimes referred to as potential pH meter. The voltage between the two electrodes converted into pH values and displayed. This instrument is very common in use in the biological and chemical laboratories to prepare different chemicals, reagents, buffers with specific pH.

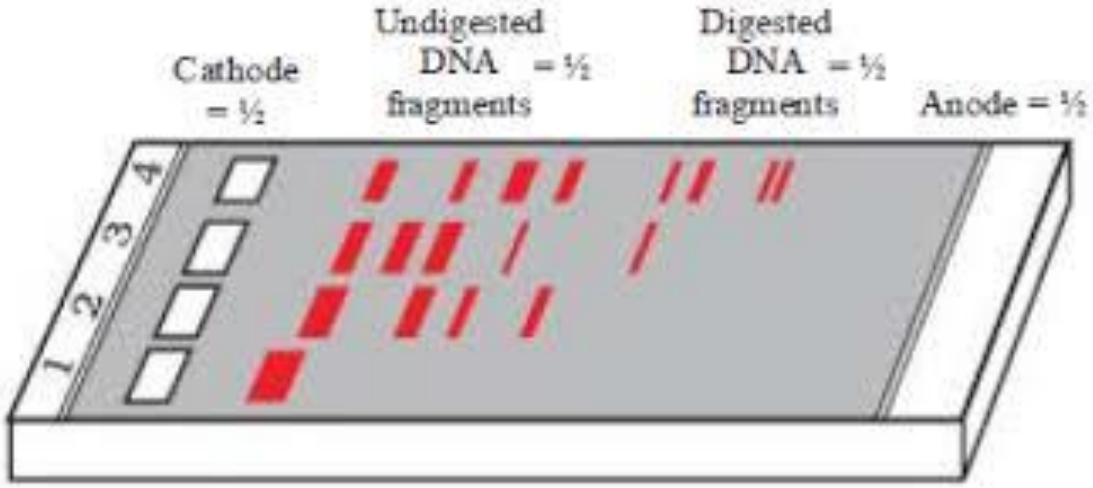
pH Meter

Gel Electrophoresis

System Gel electrophoresis is used in separation of nucleic acids and proteins based on their size. This apparatus is composed of a porous gel matrix. Nucleic acids or proteins pass through these porous structure and forms bands at different parts forming gradient according to their comparative size. The gel box features a cathode at one end and an anode at the other. Ionic buffer fills the box and creates electric field when charge is applied. Electrophoresis is used by laboratories studying vaccines, medications, forensics, DNA profiling or other life science applications. Gel electrophoresis can be done in two ways i.e. horizontal or vertical orientation.

a. Horizontal Gel Electrophoresis: In this case the gel is casted in horizontal orientation and submerged by running buffer in the gel box. The gel box is divided into two compartments, with agarose gel separating the two.

b. Vertical Gel Electrophoresis: This apparatus utilizes a discontinuous buffer system with two chambers in the gel box. The bottom of the gel is submerged in buffer in one chamber and the top is submerged in buffer in another chamber. A small amount of buffer comes through the gel from the top to the bottom chamber. Unlike Horizontal system acrylamide can be used in vertical gel electrophoresis.



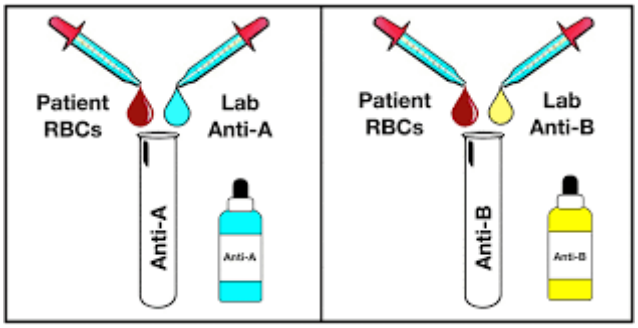
Gel Electrophoresis

Some Laboratory Protocols in Zoology (BSc Final Year)

Haematological Experiments (ABO Blood group system)

The ABO grouping system is based on the type of antigens that live on the surface of the red blood cells (RBCs). If the word ‘antigen’ rings a rusty bell, we may recollect that these are protein molecules that stimulate an immune response from the white blood cells (WBCs) in the body. These need not necessarily be foreign bodies: our systemgenerates ‘autoantigens’ on its own as well. The type of autoantigens that our RBCs carry determines our blood group in the following manner:

- Blood group A has type A antigens (and type B antibodies)
- Blood group B has type B antigens (and type A antibodies)
- Blood group AB has type A as well as type B antigens (and no antibodies)
- Blood group O has no antigens at all (and both types of antibodies)
- (Antibodies are also protein molecules launched by the body to counter the effect of antigens.)



ABO Blood Group System

Rh Blood Types

A further classification is possible based on the Rh (Rhesus) factor. Named after the Rhesus monkey that was mistakenly thought to have the same antigen, this protein – which is also an antigen – is found on the cell membrane of RBCs. The determination of the various types within the Rh factor itself is a complex science, but as far as blood groups are concerned, the classification is based, quite simply, on their presence or absence. Blood that lacks Rh antigens is Rh-negative, and the other kind is Rh-positive. Accordingly, a plus or a minus sign is attached to the ABO classification, giving rise to these types: A+, A-, B+, B-, AB+, AB-, O+, and O-.

Significance of blood groups

The problem and the answer both lie in the antigens. Since these substances trigger immune responses from the body, it is of utmost importance to ensure compatibility before blood transfusions and donations are made. If, for instance, a person of blood group B receives blood of type A, the recipient's body will label the A-type antigens as foreign bodies, and resist the transfusion. It is for this reason that group AB can receive blood from any type (since it contains and recognises both types of antigens) but group O can only take from type O. The same rule applies for the highly immunogenic Rh-factor as well. Rh-negative mothers who first had an Rh-positive baby might develop antibodies that may harm an Rh-positive baby during the second pregnancy, leading to severe anaemia. It is not surprising, therefore, that many documents – ranging from university applications to employee ID-cards ask for the blood group to be specified.

Testing Procedure

The same principle mentioned above is applied in the laboratory.

In the first step, known as forward typing, antibodies that attack type A and type B blood are introduced into the blood sample, and the reaction is observed. If the target blood cells group together, it means that the blood sample has reacted with the antibodies. For instance, anti-A antibodies induce agglutination (grouping or clumping) in type A blood.

The second step is called reverse typing, and is performed on the serum left behind after RBCs and WBCs are removed. Blood of a predetermined type (either A or B) is added and its behaviour in the serum is observed – and this should confirm the result of forward typing.

The Rh-factor test also follows a similar pattern. Antibodies to Rh are mixed with the blood sample, and if there is a clumping reaction, the blood is Rh-positive.

Preparation and Accessibility

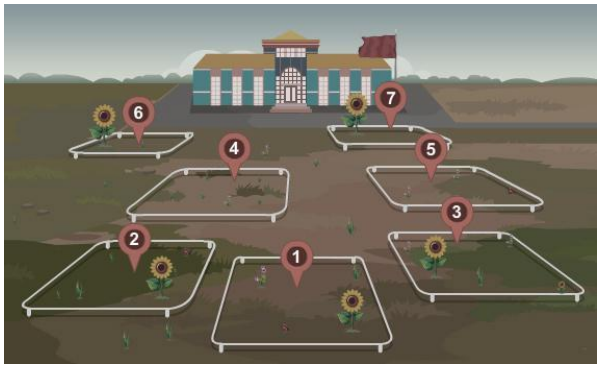
No prior preparation is required for a blood group test, other than the extraction of a blood sample. Any diagnostic centre will have a laboratory equipped to perform the test on this sample, but it is always safer to consult an established and reliable organization. Apollo Diagnostics gives the assurance of accurate testing and quick, authentic results. Since a lot depends on this basic test, the earlier it is done, the better.

Study of plant population density by quadrat method

A population is a group of individuals of the same species which inhabit a particular space at a particular time. The number of individuals in a population never remains constant. It may increase or decrease due to many factors like birth rate, death rate and migration. The number of individuals of the species in any unit area at a given time is its population density. The unit area may be as small as 5 square centimeters to as large as 10 square metres, depending on the size and nature of the plant community under study.

Let's understand the Quadrat Method.

Counting all individuals in a population is the most accurate way to determine its size. However, this approach is not usually feasible, especially for large populations or extensive habitats. Scientists usually calculate plant populations with the quadrat method. A quadrat is a square that encloses an area within a habitat. For herbaceous vegetation, a metre square quadrat is normally used.



Quadrates of Grassland

Once analyzed, the sample data enables the scientist to calculate the population size and population density for the entire population. Population density is calculated by counting all the individuals present at a given time in a given space, divided by the number of units of area or space.

Population density is calculated as follows:

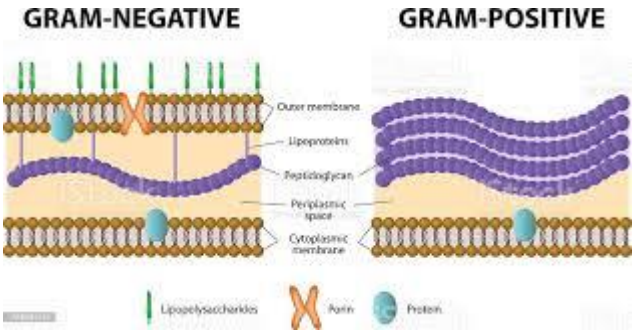
Density = (Total no.of individuals of the species in all the sampling unit (S))/(Total number of sampling units studied (Q))

Learning Outcomes:

- Students understand the term quadrat method.
- Students understand how to calculate plant population density.
- Students do the experiment better in the real lab having gone through the animation and simulation.

Gram staining of Bacteria

Danish microbiologist and physician Hans Christian Gram in 1884, developed a staining procedure by which bacteria can be classified in two groups as Gram positive and Gram negative bacteria. Most of the bacteria is encased by a strong cell wall in which a carbohydrate matrix is cross-linked by short polypeptide chains. Gram positive bacteria (encased by thick layer of peptidoglycan- 90% of cell wall) retain the color of the stain and the Gram-negative bacteria (thin layer of peptidoglycan- 10% of cell wall and high lipid content) don't retain the color of the stain, when the bacteria is subjected to it. After the staining, Gram-positive bacteria will appear as violet or blue-black and the Gram-negative bacteria will appear as red/pink.



Materials required for the experiment

Methanol, aqueous solution (0.5%) of crystal violet, Gram's iodine, acetone alcohol (1:1), safranin (1% aqueous solution), glass slide, Bunsen burner, wire loop, forceps, distilled water in wash bottle, blotting paper, bacterial culture on plate and compound microscope.

Protocol

- At first clean the glass slide and take a wire loop. Then allow the wire loop to cool after faming it on Bunsen burner.
- Dip the wire loop very quickly into the bacterial colony and transfer the bacteria in the loop of the glass slide.
- Then spread the bacteria gently on the glass slide after mixing it properly.
- After that the bacterial source needs to be fixed on the glass slide by using methanol. The fixation can also be done by holding the slide horizontally with forceps and passing it quickly over the flame of the Bunsen burner.
- Flood the slide with crystal violet stain and leave it for about 30 seconds. All the cells turn into blue or violet.

• Then pour the iodine solution over the bacterial smear and keep it for another 30 seconds. All the cell still appears blue.

• Decolorize the cells by using organic solvents such as acetone or alcohol by keeping it on slide for not more than 2- 5 seconds. Decolorization step is helpful to distinguish the Gram-positive bacteria from Gram-negative bacteria.

• Then counter stain the slide by using red dye safranin and keep it for 1-2 minutes.

• Wash with water and blot dry.

Observations

Under compound microscope the Gram-positive bacteria will appear in violet or blue-black and the Gram-negative bacteria will appear in red or pinkish color.

Precautions

• Don't over-stain the bacterial smear either by crystal-violet or safranin stain.

• Don't delay in collecting the sample on glass slide from the bacterial colony.

• Glass slide needs to be properly cleaned.

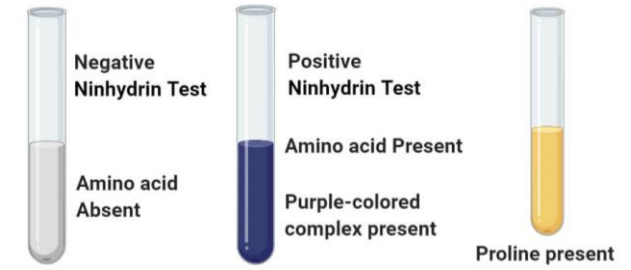
• Correct thickness of bacterial smear is very important.

• Don't wash the slide by acetone or alcohol for longer time unnecessarily.

1. Millon's Test:

Chemical reagents required: 2 ml protein solution and 1 ml Millon's reagent.

Procedure: Take 2 ml of protein solution in the test tube and then add 1 ml of Millon's reagent. Mix thoroughly and bring to boil gradually.



Millon's Test

Observation: Appearance of white precipitate, which will turn into red as it will coagulate by absorbing heat. In presence of peptone, there will be less number of precipitate. Then the whole solution becomes re as the precipitate gets mixed in the solution. Chemical basis of the reaction: Amino acid, tyrosine is present in almost all the proteins. In presence of Millon's reagent, tyrosine reacts with the mercuric and mercuric nitrate and due to the presence of phenol group in tyrosine, red colored mercuric (II) compound is produced.

2.Biuret test

Chemical reagents required: 2 ml protein solution, 2 ml 10% NaOH solution, 1% copper sulphate solution.

Procedure • Add 2 ml protein solution and 2 ml 10% NaOH solution. Mix it thoroughly. • Then add 2 drops of cupper sulphate solution. Mix the whole solution properly.

Observation A purple color develops slowly. Chemical basis of the reaction In basic medium Cu2 + gets attached with the peptide bonds and forms a purple complex.

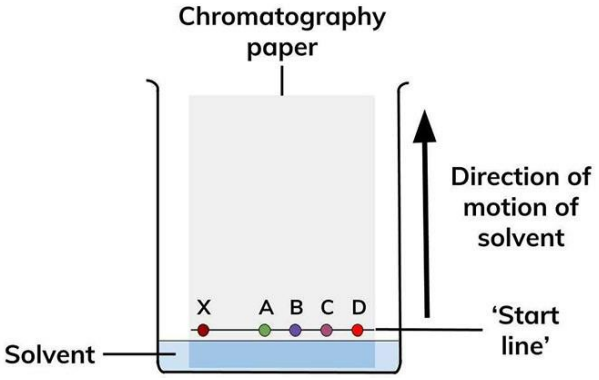
Chromatography

Chromatography is a method of physical separation in which components of mixture gets separated on two phases. One of the phase is the immobile porous bed bulk liquid which is called stationary phase and the other phase is the mobile fluid that flows over the stationary phase under gravity. During the movement of the sample, a separated result is formed by the repeated desorption and sorption in the direction of the mobile phase migration. Several key factors are responsible on the separation process like partition between liquid-liquid, affinity between molecular weight and characteristics related to liquid-solid adsorption. An interaction between the molecules are physical and involves weak chemical bonds like dipole-dipole interaction and hydrogen bond formation and adhere to the stationary components. Components that adhere strongly to the stationary phase moves slowly than those who adhere weakly. There are number of chromatography separation taking place in the industries but for laboratory syllabus paper chromatography is used frequently.

Paper chromatography:

In a paper chromatography, separation of the mixture is performed on a paper strip which is a stationary phase and a liquid solvent acts as a mobile phase.

Mechanism



Paper Chromatography

Paper chromatography

- A drop of mixture is placed on one end of the paper and dried. Then the paper is dipped into the solvent up to the spot.
- In the paper chromatography, component separates in two ways:

In **paper adsorption chromatography,** stationary phase and mobile phase molecules act based on the degree of interaction. Higher affinity molecules are adsorbed for a long time where movement of speed is decreased. However, low affinity molecules move faster thus, molecules are separated.

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VERIFIED

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