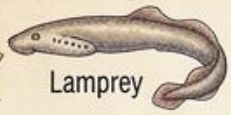
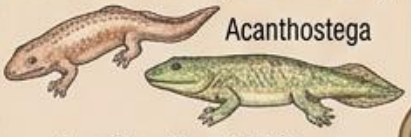
T.Y. B.Sc ZOOLOGY
PRACTICAL VI JOURNAL



GOGATE JOGALEKAR COLLEGE (AUTONOMOUS)
RATNAGIRI
DEPARTMENT OF ZOOLOGY

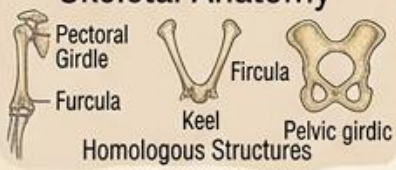
Corse code:- 25_USZOM 505
Topic:- Zoology Practical VI
Credit :- 02

Vertebrate Phyla Diversity



Agnatha, Chondrichthyes, Sarcopterygii, Tetrapods

Comparative Vertebrate Skeletal Anatomy



Homologous Structures

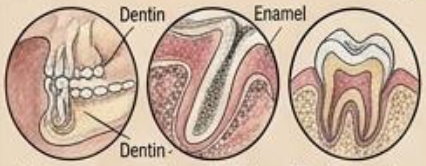
Vertebrate Defensive Strategies

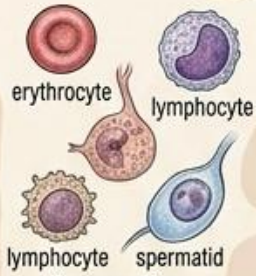
Camouflage, Structural Defense

Mammalian Histology (Basic Tissues)

Keys to Cells	A. Simple Squamous Epithelium (Alveolus)	B. Dense Connective Tissue (Tendon)	C. Skeletal Muscle (Myofibrils)	D. Nervous Tissue (Neuron with Glia)
			Detailed to cells	Detaled to cells

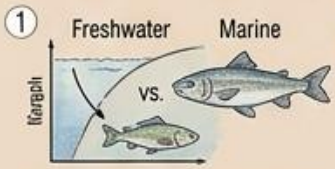
Mammalian Cranial Histology





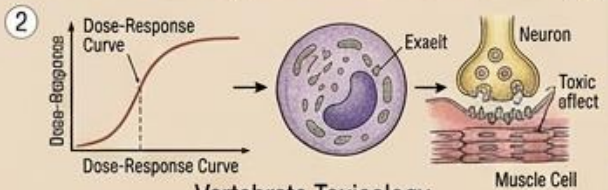
Comparative Vertebrate Cell Types

Animal Physiology & Toxicology



Vertebrate Osmoregulation

Vertebrate Toxicology



Vertebrate Toxicology

Comparative Vertebrate Embryology

Fish Bird Mammal

AUTHOR: DR. MADHURA MUKADAM

R. E. Society's

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AND

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NAAC Accredited 'A' Grade (4th Cycle)

Ratnagiri – 415612, Maharashtra, India

CERTIFICATE

This is to certify that Mr./Ms. _____ of T. Y. B. Sc. (Bachelor of Science) has satisfactorily completed and submitted the Practical Journal of Zoology (Major I/II) prescribed under the autonomous syllabus for Semester V/VI during the academic year 20 – 20 . The journal contains a record of practical work performed by the student according to the syllabus of the Bachelor of Science (B.Sc.) Programme of this college.

Course Teacher

Head, Department of Zoology

Place: Ratnagiri

Date: / / 20

1. COURSE OUTCOMES

After successful completion of the **T.Y. B.Sc. Zoology – Major II Practical Course**, students will be able to:

CO1: Apply knowledge of animal tissues and organ systems through microscopic examination and interpretation of histological preparations.

CO2: Understand the relationship between physiological and biochemical processes through the analysis of selected pathological and laboratory parameters.

CO3: Develop competence in biochemical techniques for the qualitative and quantitative estimation of important biological molecules and metabolites.

CO4: Apply principles of enzymology and biotechnology in practical studies involving enzyme activity, enzyme immobilisation and fermentation.

CO5: Analyse the effects of selected environmental agents and toxic substances using appropriate laboratory bioassays and interpret the results scientifically.

CO6: Develop scientific skills in observation, experimentation, data recording, calculation, interpretation and presentation of practical results while following appropriate laboratory safety and ethical practices.

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2. PRACTICAL LEARNING OUTCOMES

After completing the practical course, students will be able to:

1. Identify characteristic histological features of selected mammalian tissues and organs using permanent slides.
2. Understand the basic steps involved in microtomy and the preparation of histological sections.
3. Perform and understand the principles of histological staining techniques.
4. Interpret selected abnormal pathological reports and correlate abnormal parameters with possible physiological or histological changes.
5. Perform biochemical estimations of selected biological constituents using standard laboratory methods.
6. Prepare standard solutions, record absorbance values and determine the concentration of unknown samples using standard calibration curves.
7. Perform calculations and express biochemical results in appropriate units.
8. Determine enzyme activity and understand the principles of enzyme immobilisation.
9. Demonstrate the basic principles involved in microbial fermentation for ethanol production.
10. Conduct laboratory bioassays, where permitted by institutional guidelines, and record observations systematically.
11. Calculate and interpret experimental parameters obtained from toxicity and bioassay studies.
12. Prepare neat, labelled diagrams, tables, graphs and a systematic practical record.
13. Follow standard laboratory procedures, safety precautions and responsible scientific practices.

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3. GENERAL LABORATORY RULES

1. Students must enter the laboratory only with the permission of the teacher concerned.
2. Students should wear a clean laboratory coat or apron during practical sessions wherever required.
3. The practical journal and necessary stationery should be brought to every practical session.
4. Students should carefully listen to the instructions given before starting any experiment.
5. The laboratory bench should be kept clean and organised throughout the practical session.
6. All apparatus, instruments, slides and biological materials should be handled carefully.
7. Chemicals and reagents should be used only in the quantities prescribed for the experiment.
8. Labels on reagent bottles and chemical containers must be read carefully before use.
9. Unused reagents should not be returned to their original containers.
10. Mouth pipetting is strictly prohibited.
11. Eating and drinking inside the laboratory are strictly prohibited.
12. Mobile phones should not be used during practical sessions unless specifically permitted for academic purposes.
13. Microscopes and other optical instruments should be handled carefully and cleaned only with appropriate materials.
14. Glassware and laboratory instruments should be cleaned after use and returned to their designated places.
15. Any breakage, spill, injury or equipment malfunction should be reported immediately to the teacher or laboratory staff.
16. Students should not perform any experiment without proper instruction and supervision.
17. Laboratory waste should be disposed of only in designated containers and according to institutional procedures.
18. Students should wash their hands thoroughly after completing practical work.
19. Discipline, cleanliness and responsible behaviour must be maintained throughout the practical session.

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4. SAFETY PRECAUTIONS

1. Always follow the instructions provided by the teacher or laboratory staff.
2. Wear appropriate personal protective equipment, such as a laboratory coat, gloves and protective eyewear, whenever required.
3. Avoid direct contact with chemicals, stains, biological materials and laboratory samples.
4. Do not taste or intentionally smell chemicals or biological materials.
5. Never pipette liquids by mouth.
6. Handle acids, organic solvents and other hazardous reagents only under proper supervision and according to laboratory instructions.
7. Always add reagents carefully and use the prescribed quantities.
8. Handle concentrated acids and corrosive chemicals with particular care.
9. Use heating equipment, water baths and electrical instruments only after receiving proper instructions.
10. Keep flammable materials away from heat sources and open flames.
11. Allow heated apparatus to cool before handling.
12. Broken glass should not be picked up with bare hands.
13. Chemical spills should be reported immediately and managed according to institutional laboratory procedures.
14. Biological materials should be handled hygienically and disposed of appropriately.
15. Experiments involving toxicity testing or bioassays should be performed only according to the prescribed syllabus, institutional guidelines and applicable ethical requirements.

16. Students should not interpret laboratory observations or pathological reports as clinical diagnoses.
17. In case of an accident or injury, inform the teacher immediately.
18. Students should be familiar with the location of the first-aid box, fire extinguisher and emergency exits.
19. Wash hands thoroughly before leaving the laboratory.
20. Follow all institutional and departmental safety guidelines applicable to the laboratory.

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5. INSTRUCTIONS FOR MAINTAINING THE PRACTICAL JOURNAL

1. The practical journal should be maintained neatly and regularly throughout the academic year.
2. The student's name, class, division, roll number and academic year should be clearly written on the cover page.
3. An index of experiments should be maintained at the beginning of the journal.
4. Each experiment should be recorded according to the prescribed sequence.
5. Every experiment should include, wherever applicable:
    - Experiment Number and Title
    - Date
    - Aim
    - Principle
    - Requirements
    - Procedure
    - Observations
    - Calculations
    - Graphs
    - Diagrams
    - Result
    - Precautions
6. Observations should be recorded clearly and accurately during or immediately after the experiment.
7. Observation tables should be properly ruled, titled and labelled.
8. All calculations should include the relevant formula, substitution of values and appropriate units.
9. Standard curves and graphs should be plotted using suitable scales, with clearly labelled axes.
10. Histological and other biological diagrams should be neat, properly labelled and drawn with a sharp pencil.
11. Scientific names and technical terms should be written correctly.
12. Corrections should be made neatly, and excessive overwriting should be avoided.
13. Each completed practical should be submitted for checking and authentication within the prescribed time.
14. The journal should be updated regularly and should not be completed hurriedly at the end of the term.
15. The practical journal should serve as an authentic record of the practical work performed by the student.
16. The completed journal should be submitted for examination as per the instructions of the Department and applicable academic regulations.

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6. EVALUATION SCHEME

The practical performance of students may be assessed through **continuous internal assessment and end-semester practical examination**, as per the applicable academic regulations.

The evaluation may include the following components:

Component	Parameters for Assessment
Practical Performance	Understanding of the experiment, correct procedure and handling of apparatus and materials
Microscopic Observation/Identification	Identification and interpretation of histological slides, specimens or other practical materials
Observation and Recording	Accuracy, completeness and systematic presentation of observations
Calculations and Graphs	Correct formulae, calculations, units, graphical representation and interpretation
Biochemical Estimation	Proper preparation of standards, handling of instruments and interpretation of results
Practical Journal	Completeness, neatness, regularity and proper presentation of practical work
Viva-Voce	Understanding of principles, procedures, observations, calculations and applications
Laboratory Discipline and Safety	Regularity, responsible conduct, cleanliness and adherence to laboratory safety practices

General Guidelines for Assessment

1. Students should be assessed on both practical skills and conceptual understanding.
2. Greater importance should be given to the student's ability to perform, analyse and interpret an experiment rather than merely reproducing theoretical information.
3. The practical journal should be checked periodically during the academic term.
4. Students should demonstrate proper handling of laboratory instruments, specimens, slides, chemicals and other materials.
5. Viva-voce questions should assess understanding of the principle, procedure, observations, calculations and applications of the experiment.
6. Diagrams, observations, calculations, graphs and results should be assessed for accuracy and scientific presentation.
7. Experiments involving bioassays or toxicity studies should be assessed with due consideration to prescribed institutional safety and ethical guidelines.
8. The final distribution of marks should strictly follow the **applicable syllabus, examination pattern and academic regulations**.

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Experiment No. 1

STUDY OF MAMMALIAN TISSUES

Aim

To study the microscopic structure of mammalian tissues by examining the vertical section (V.S.) of a tooth and the transverse sections (T.S.) of the stomach, small intestine, and liver, and to identify their characteristic histological features and relate their structure to their functions.

Principle

Histology is the branch of biology that deals with the microscopic study of tissues and organs. In multicellular organisms, cells with similar structure and function are organized into tissues, and different tissues combine to form organs.

Each organ possesses a characteristic arrangement of tissues that enables it to perform specific physiological functions. For example:
The tooth possesses highly mineralised tissues for mastication.
The stomach contains glandular epithelium for digestion and secretion.
The small intestine has specialised absorptive structures called villi.
The liver consists of hepatic lobules adapted for metabolism, detoxification, and secretion.

Requirements

Permanent Histological Slides

1. Vertical Section (V.S.) of Mammalian Tooth
2. Transverse Section (T.S.) of Mammalian Stomach
3. Transverse Section (T.S.) of Mammalian Small Intestine
4. Transverse Section (T.S.) of Mammalian Liver

Apparatus

- Compound microscope
- Histological charts/models
- Lens

Procedure

1. General Procedure for Microscopic Observation
2. Clean the microscope lenses with lens paper.
3. Place the prepared slide on the microscope stage.
4. Secure the slide using stage clips.
5. Observe the section under the low-power objective (10X).
6. Adjust the focus using the coarse adjustment knob.
7. Identify the general organisation of the tissue.
8. Switch to the high-power objective (40X).
9. Use the fine adjustment knob to obtain a clear image.
10. Observe the arrangement of cells and tissue layers.
11. Identify characteristic structures.
12. Draw neat, labelled diagrams in the practical notebook.
13. Record observations and identify the specimen.

1. Vertical Section (V.S.) of Mammalian Tooth

A tooth is a hard calcified structure present in the oral cavity and is specialised for cutting, tearing, and grinding food. Histologically, the tooth consists of highly specialised connective tissues.

1. **Enamel**

Outermost covering of the crown.
Hardest substance in the body.
Composed mainly of calcium phosphate.
Contains no blood vessels or nerves.
Produced by ameloblast cells during development.
Function: Protects the tooth from wear and mechanical injury during mastication.

2. **Dentin**

Lies beneath the enamel.
Forms the major bulk of the tooth.
Less mineralised than enamel.
Contains microscopic dentinal tubules.
Function: Provides support and strength to the enamel.

3. **Pulp Cavity**

Central cavity of the tooth.
Contains connective tissue, blood vessels, lymph vessels, and nerves.
Function: Nourishes the tooth, provides sensation and participates in repair processes.

4**. Cementum**

Covers the root of the tooth.
Similar in composition to bone.
Function: Anchors the tooth to the periodontal ligament.

5. **Periodontal Ligament**

Fibrous connective tissue present between cementum and alveolar bone.
Function: Holds the tooth in the socket and acts as a shock absorber during chewing.

2. Transverse Section (T.S.) of Mammalian Stomach

The stomach is a muscular organ involved in temporary storage and digestion of food.
Layers of the Stomach
1. **Mucosa** Consists of: Simple columnar epithelium
Gastric pits Lamina propria
Gastric glands Muscularis mucosae
Function: Secretion of gastric juice and protection of stomach lining.

2. **Submucosa** Composed of: Dense connective tissue
Blood vessels and Nerves
Function: Supports the mucosa and supplies nutrients.

3. **Muscularis Externa** Composed of: Inner oblique layer
Middle circular layer
Outer longitudinal layer
Function: Churning and mixing of food.

4. **Serosa**
Thin outer covering composed of connective tissue and mesothelium.
Function: Reduces friction.

5. **Gastric Glands** Contain: Mucous cells -Secrete mucus.
Parietal cells - Secrete hydrochloric acid and intrinsic factor.
Chief cells - Secrete pepsinogen.

3. Transverse Section (T.S.) of Mammalian Small Intestine
The small intestine is the principal site of digestion and nutrient absorption.
Layers of the Small Intestine
1. **Mucosa** Contains: Villi
Columnar epithelium
Goblet cells
Crypts of Lieberkühn
2. **Submucosa Contains**: Connective tissue
Blood vessels
Nerve plexus
3. **Muscularis Externa** Contains: Inner circular layer
Outer longitudinal layer
4. **Serosa**
Outer connective tissue covering.
Structures Observed: Villi
Finger-like projections.
Function: Increase absorptive surface area.

Goblet Cells
Mucus-secreting cells.
Function: Lubrication and protection.

Crypts of Lieberkühn
Simple tubular glands.
Function: Secretion of intestinal juice.

4. Transverse Section (T.S.) of Mammalian Liver
The liver is the largest gland of the body and performs metabolic, secretory, and detoxification functions.
Structures Observed
1. **Hepatocytes -** Polygonal liver cells arranged in cords.
Function: Metabolism of carbohydrates, proteins, and lipids.
Bile secretion and Detoxification.
2. **Central Vein**
Located at the centre of the hepatic lobule.
Function: Collects blood from hepatic sinusoids.

3. **Hepatic Sinusoids**
Blood channels present between hepatic cords.
Function: Facilitate the exchange of substances between blood and hepatocytes.

4. Portal Triad

Located at the periphery of the lobule and consists of: a branch of the hepatic artery
Branch of the portal vein
Bile duct

Result

The vertical section of the tooth and transverse sections of the stomach, small intestine, and liver were studied successfully. Each tissue exhibited characteristic histological features related to its specific physiological functions.

Observation Table

Tissue	Important Structures Observed
Tooth	Enamel, dentin, pulp cavity, cementum
Stomach	Gastric pits, gastric glands, muscular wall
Small Intestine	Villi, goblet cells, crypts
Liver	Hepatocytes, central vein, sinusoids

Experiment No. 2

MICROTOMY AND PREPARATION OF HISTOLOGICAL SECTIONS

Aim

To learn the techniques involved in the preparation of permanent histological sections, including tissue preservation, fixation, dehydration, clearing, infiltration, embedding, sectioning, mounting, and staining for microscopic examination.

Principle

Histology is the branch of biology concerned with the microscopic study of tissues and organs. Since biological tissues are soft and contain a large amount of water, they cannot be examined directly under the microscope. Therefore, tissues must be processed and cut into extremely thin sections that allow light to pass.

Microtomy is the technique of preparing thin sections of biological tissues for microscopic examination. The process involves:

1. **Preservation of tissues** to prevent decomposition.
2. **Removal of water** from tissues.
3. **Replacement of water by paraffin wax** to provide support.
4. **Cutting thin sections** using a microtome.
5. **Staining** to differentiate various cellular components.

The thickness of histological sections usually ranges from **5 to 7 µm**, allowing light to pass through and reveal cellular details.

Requirements

Chemicals

- 10% Neutral Buffered Formalin (NBF)
- Ethanol (50%, 70%, 90%, and Absolute)
- Xylene
- Paraffin wax (melting point 56–58°C)
- Haematoxylin
- Eosin
- Distilled water
- DPX mountant
- Mayer's albumin (adhesive)

Apparatus

- Rotary microtome
- Embedding moulds
- Water bath (40–45°C)
- Hot plate
- Coplin jars
- Glass slides and coverslips
- Forceps
- Brushes
- Dissecting needles
- Staining rack
- Slide boxes
- Tissue cassette

Theory of Tissue Processing

1. Fixation - Fixation is the process of preserving tissues in a life-like condition by preventing autolysis and bacterial decomposition.

Purpose

- Preserves tissue architecture.
- Prevents enzymatic digestion.
- Hardens tissues.
- Maintains cellular details.

Common Fixative

10% Neutral Buffered Formalin

Composition

- Formaldehyde: 10%
- Distilled water: 90%
- Phosphate buffer

Duration

12–24 hours.

2. Dehydration -Removal of water from tissues using ascending grades of alcohol.
Since paraffin wax is insoluble in water, water must be removed before embedding.

Sequence

Solution	Duration
50% Ethanol	30 minutes
70% Ethanol	30 minutes
90% Ethanol	30 minutes
Absolute Alcohol I	30 minutes
Absolute Alcohol II	30 minutes

3. Clearing - Replacement of alcohol with a substance that is miscible with both alcohol and paraffin wax.

Clearing Agent - Xylene.

Purpose

- Removes alcohol.
- Makes tissues transparent.
- Facilitates paraffin infiltration.

Duration

30 minutes in two changes of xylene.

4. Infiltration - Replacement of clearing agent by molten paraffin wax.

Purpose

Provides rigidity and support to tissues for sectioning.

Conditions

Temperature: 58–60°C.

Duration

30–60 minutes in two or three changes.

5. Embedding - The process of enclosing tissues in molten paraffin wax and allowing it to solidify.

Purpose

- Provides support during section cutting.
- Maintains orientation of tissues.

6. Sectioning (Microtomy) - Cutting thin sections of paraffin-embedded tissues using a microtome.

Thickness of Sections

5–7 µm.

Purpose

Thin sections allow light to pass through the tissue.

7. Mounting - Transfer of tissue sections onto glass slides.

Purpose

To facilitate staining and microscopic observation.

8. Staining - Most tissues are colourless and therefore require staining.

Hematoxylin

- Basic dye
- Stains nuclei blue or purple.

Eosin

- Acidic dye
- Stains cytoplasm pink.

Procedure

A. Fixation

1. Cut tissue into small pieces (approximately 5 mm thick).
2. Immerse immediately in 10% neutral buffered formalin.
3. Use at least ten times the volume of fixative compared to tissue volume.
4. Leave for 24 hours.

B. Dehydration

Transfer tissue through ascending grades of alcohol:

50% → 70% → 90% → Absolute Alcohol I → Absolute Alcohol II

Keep tissue in each solution for approximately 30 minutes.

C. Clearing

1. Transfer tissue to xylene.
2. Keep for 30 minutes.
3. Repeat in fresh xylene.

Observation:

The tissue becomes translucent or transparent.

D. Infiltration

1. Transfer tissue into molten paraffin wax.
2. Maintain temperature at 58–60°C.
3. Change wax two to three times.

E. Embedding

1. Pour molten wax into an embedding mould.
2. Place tissue in proper orientation.
3. Allow wax to solidify.
4. Label the paraffin block.

F. Sectioning

1. Trim the paraffin block.

2. Mount it on the rotary microtome.
3. Adjust thickness to 5–7 µm.
4. Cut ribbon-like sections.
5. Collect sections using forceps.

G. Mounting

1. Float sections on a warm water bath (40–45°C).
2. Remove wrinkles.
3. Transfer sections onto slides coated with Mayer's albumin.
4. Dry the slides on a hot plate.

H. Staining (Haematoxylin and Eosin Method)

Deparaffinization

Xylene I – 5 minutes
Xylene II – 5 minutes

Hydration

Absolute Alcohol → 90% → 70% → 50% → Water

Staining

1. Stain with Haematoxylin for 5–7 minutes.
2. Wash under tap water.
3. Differentiate in acid alcohol if necessary.
4. Blue in alkaline water.
5. Counterstain with Eosin for 1–2 minutes.
6. Dehydrate through ascending grades of alcohol.
7. Clear in xylene.
8. Mount using DPX and place a coverslip.

Result

Permanent histological sections were successfully prepared by fixation, dehydration, clearing, infiltration, embedding, sectioning, mounting, and staining. The stained sections showed clear differentiation of cellular structures, with nuclei appearing blue and cytoplasm appearing pink.

Observations

Step	Observation
Fixation	Tissue preserved without decomposition
Dehydration	Water removed and tissue becomes firm
Clearing	Tissue becomes transparent
Infiltration	Tissue impregnated with paraffin wax
Embedding	Paraffin block formed
Sectioning	Thin ribbon-like sections obtained
Mounting	Sections adhered to slides
Staining	Nuclei blue and cytoplasm pink

Microscopic Observation

Tissue Component	Colour After H&E Staining
Nucleus	Blue-purple
Cytoplasm	Pink
Muscle fibres	Pink
Connective tissue	Light pink
Red blood cells	Bright pink

Precautions

1. Use fresh fixative.
2. Tissue pieces should be small.
3. Avoid incomplete dehydration.
4. Do not overheat paraffin wax.
5. Use sharp microtome blades.
6. Maintain proper section thickness.
7. Handle sections gently.
8. Avoid folds and wrinkles in sections.
9. Do not overstain or understain.
10. Ensure complete removal of xylene before mounting.

Experiment No. 3

HISTOLOGICAL STAINING TECHNIQUE (HEMATOXYLIN AND EOSIN STAINING)

Aim

To perform Haematoxylin and Eosin (H&E) staining and study the differentiation of tissue structures.

Principle

Different cellular components have varying affinities for dyes.

- Haematoxylin is a basic dye and stains nuclei blue-purple.
- Eosin is an acidic dye and stains cytoplasm and connective tissue pink.

H&E staining provides excellent contrast between different tissue components.

Requirements

- Tissue sections
- Haematoxylin
- Eosin
- Xylene
- Alcohol
- Distilled water
- Slides and coverslips

Procedure

1. Deparaffinize sections in xylene.
2. Hydrate through descending grades of alcohol.
3. Wash with distilled water.
4. Stain with haematoxylin for 5–10 minutes.
5. Wash and differentiate.
6. Counterstain with eosin for 1–2 minutes.
7. Dehydrate through alcohol.
8. Clear in xylene.
9. Mount with DPX.

Result

Different tissue components were successfully differentiated by H&E staining.

Observations

Tissue Component	Colour After H&E Staining
Nucleus	Blue-purple
Cytoplasm	Pink
Muscle fibers	Pink
Connective tissue	Light pink
RBCs	Bright pink

Precautions

1. Avoid overstaining.
2. Wash slides properly between steps.
3. Use fresh staining solutions.
4. Avoid air bubbles during mounting.

Experiment No. 4

Study of effect of Carbon tetrachloride (CCL4) on the level of Liver enzyme activity

A. Effect of CCl_4 on Aspartate Aminotransferase (AST/GOT) Activity

Aim

To study the *in vitro* effect of carbon tetrachloride (CCl_4) on the activity of aspartate aminotransferase (AST) in liver homogenate.

Materials Required

- Fresh goat/liver tissue
- 0.9% NaCl solution
- CCl_4 (analytical grade)
- Buffer (e.g., phosphate buffer, pH 7.4)
- Reagents for AST estimation (colourimetric/kit method)
- Test tubes, micropipettes, centrifuge, spectrophotometer

Principle

AST (GOT) is a transaminase enzyme present in hepatocytes. When exposed to CCl_4, hepatocytes undergo lipid peroxidation and membrane damage, leading to altered AST activity. Measuring AST levels in treated vs. control homogenates reflects CCl_4-induced hepatotoxicity. The amount of oxaloacetic acid formed depends on the quantity of enzymes; hence to assess enzyme activity is determined colorimetrically by the formation of 'hydrazone' with dinitrophenylhydrazine (DNPH) reagent. Hydrazone is a brown coloured complex formed in an alkaline medium. The amount of hydrazine formed is directly proportional to the intensity of the colour, which is measured colorimetrically at 540nm.

$$\text{Aspartic acid + alpha-ketoglutaric acid} \xrightarrow{\text{AST}} \text{Oacetic acid + Glutamic acid}$$

$$\text{Oxaloacetate + DNPH} \xrightarrow[\text{Alkaline medium}]{} \text{Hydrazone}$$

Procedure

1. Prepare 10% liver homogenate in chilled saline. Add 0.5 ml CCL_4/10gm and 0.5 ml coconut oil/10gm of tissue (1:1); keep it at RT for ½ an hour. Filter and centrifuge in an ice bath. Take supernatant as the treated sample.
2. Prepare 10% liver homogenate in chilled saline. Add 0.5 ml coconut oil/10gm of tissue (1:1) keep it at RT for ½ an hour. Filter and centrifuge in an ice bath. Take supernatant as the oil-treated sample.
3. Use both the samples and determine enzyme activity.
4. Estimate AST activity using the standard colourimetric method/kit.
5. Record absorbance and calculate enzyme activity (U/L).

Reagent	Blank	Standard	Test	Control
Reagent 1	0.25	0.25	0.25	0.25
Serum/Plasma			0.05	
Standard		0.05		
Mix well and incubate at 37 ^{0}C for 60 min				
Reagent 2	0.25	0.25	0.25	0.25
D/W	0.05			
Serum/Plasma				0.05
Mix well and allow to stand at room temp for 20 minutes				
Solution 1	2.5	2.5	2.5	2.5

Mix well and read the OD against D/W in a colorimeter using a green filter (490-550nm) within 15 minutes.

Result

AST(GOT) activity in IU/L - ---------- IU/L

CCl_4 exposure altered AST activity compared to the control, indicating hepatocellular injury.

Clinical significance:
Serum AST levels are elevated in viral and other forms of liver disease associated with hepatic necrosis. AST is present in liver cells in much higher concentrations than in any other organ.

Observation Table

Sample	Absorbance	AST Activity (U/L)
Blank		
Standard		
Test		
Control		

Calculation:

$$\text{AST activity} = \frac{\text{Absorbance of Test} - \text{Absorbance of Control}}{\text{Absorbance of Standard} - \text{Absorbance of Reagent Blank}} \times \text{Conc. of standard}$$

(Conc. Of standard – 114IU/L)

B. Effect of CCl_4 on Alanine Aminotransferase (ALT/GPT) Activity

Aim

To study the in vitro effect of carbon tetrachloride (CCl_4) on the activity of alanine aminotransferase (ALT) in liver homogenate.

Materials Required

- Fresh goat/liver tissue
- 0.9% NaCl solution
- CCl_4
- Buffer (pH 7.4)
- Reagents for ALT estimation (colorimetric/kit method)
- Test tubes, micropipettes, centrifuge, spectrophotometer

Principle

ALT (GPT) is a liver-specific enzyme involved in amino acid metabolism. CCl_4 induces hepatocyte necrosis and leakage of ALT into the extracellular medium. Measuring ALT activity helps assess CCl_4-induced liver toxicity. ALT catalyses the Transamination of L-alanine to alpha Ketoglutaric acid to form pyruvic acid and L-Glutamate as follows.

$$\text{L-alanine} \xrightarrow{\text{ALT/GPT}} \text{alpha-ketoglutarate} \xrightarrow{\text{ALT/GPT}} \text{L-glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + 2,4\ \text{DNPH} \xrightarrow{\text{Alkali}} \text{Hydrazon (Brown coloured complex)}$$

Procedure

1. Prepare 10% liver homogenate in chilled saline. Add 0.5 ml CCL4/10gm and 0.5 ml coconut oil/10gm of tissue (1:1); keep it at RT for ½ an hour. Filter and centrifuge in ice bath. Take supernatant as the treated sample.
2. Prepare 10% liver homogenate in chilled saline. Add 0.5 ml coconut oil/10gm of tissue (1:1) keep it at RT for ½ an hour. Filter and centrifuge in ice bath. Take supernatant as the oil-treated sample.
3. Use both the samples and determine enzyme activity.
4. Estimate ALT activity using the standard colourimetric method/kit.
5. Record absorbance and calculate enzyme activity (U/L).

Reagent	Blank	Standard	Test	Control
Reagent 1	0.25	0.25	0.25	0.25
Serum/Plasma			0.05	
Standard		0.05		
Mix well and incubate at 37 ^{0}C for 30 min				
Reagent2	0.25	0.25	0.25	0.25
D/W	0.05			
Serum/Plasma				0.05
Mix well and allow to stand at room temp. For 20 minutes				
Solution 1	2.5	2.5	2.5	2.5

Mix well and read the OD against D/W in a colourimeter using a green filter (490-550nm) within 15 minutes.

Result :
ALT (GOT) activity in IU/L - ---------- IU/L

CCl_4 treatment resulted in elevated ALT activity compared to control, confirming hepatotoxic effect.

Clinical significance:
Serum ALT levels are elevated in viral and other forms of liver disease associated with hepatic necrosis. ALT is present in liver cells in much higher concentrations than in any other organ.

Observation Table

Sample	Absorbance	AST Activity (U/L)
Blank		
Standard		
Test		
Control		

Calculation:

$$\text{ALT activity in IU/L} = \frac{\text{Absorbance of Test} - \text{Absorbance of Control}}{\text{Absorbance of Standard} - \text{Absorbance of Reagent Blank}} \times \text{Conc. of standard}$$

(Conc. Of standard – 150 IU/L)

C. Effect of CCl_4 on Alkaline Phosphatase (ALP) Activity

Aim

To study the in vitro effect of carbon tetrachloride (CCl_4) on the activity of alkaline phosphatase (ALP) in liver homogenate.

Materials Required

- Fresh goat/liver tissue
- 0.9% NaCl solution
- CCl_4
- Buffer (pH 7.4)
- Reagents for ALP estimation (colorimetric/kit method, e.g., p-nitrophenyl phosphate substrate)
- Test tubes, micropipettes, centrifuge, spectrophotometer

Principle

ALP is a membrane-bound enzyme. CCl_4 damages hepatocyte membranes, causing leakage and changes in ALP activity. Measuring ALP reflects membrane damage and cholestatic effects of CCl_4.

Procedure

1. Prepare 10% liver homogenate in chilled saline. Add 0.5 ml CCL4/10gm and 0.5 ml coconut oil/10gm of tissue (1:1) keep it at RT for ½ an hour. Filter and centrifuge in an ice bath. Take supernatant as the treated sample.
2. Prepare 10% liver homogenate in chilled saline. Add 0.5 ml coconut oil/10gm of tissue (1:1) keep it at RT for ½ an hour. Filter and centrifuge in an ice bath. Take supernatant as the oil-treated sample.
3. Use both the samples and determine enzyme activity.
4. Estimate ALP activity using standard colourimetric method/kit.
5. Record absorbance and calculate enzyme activity (IU/L).

Result :

ALP (GOT) activity in IU/L - ---------- IU/L

CCl_4 treatment resulted in elevated ALP activity compared to control, confirming hepatotoxic effect.

Clinical Significance

CCl_4 exposure caused significant changes in ALP activity, suggesting disruption of hepatocyte membrane integrity.

Observation Table

Sample	Absorbance	ALP Activity (IU/L)
Blank		
Standard		
Test		
Control		

Calculation:

$$\text{ALP activity in IU/L} = \frac{\text{Absorbance of Test} - \text{Absorbance of Control}}{\text{Absorbance of Standard} - \text{Absorbance of Reagent Blank}} \times \text{Conc. of standard}$$

(Conc. Of standard – 150 IU/L)

Experiment No. 5

Interpretation of Abnormal Pathological Reports

Aim

To study abnormal clinical pathological reports of blood (CBC), urine (routine), and stool (routine) tests and to record only the abnormal parameters in tabular form. The findings are to be correlated with possible histological and physiological changes.

Principle

z

Clinical laboratory reports help identify abnormalities in bodily functions. In this practical, students will examine pathological reports and note only those parameters that are outside the normal reference range.

Requirements

Abnormal CBC report

Abnormal urine routine report

Abnormal stool routine report

Reference normal values

Part A – Complete Blood Count (CBC)

Procedure

- Collect an abnormal CBC report.
- Compare each parameter with the normal reference range.
- Record only the abnormal parameters in the table below.

Normal CBC Values

Parameter	Normal Range
Haemoglobin (Hb)	Male: 13–17 g/dL Female: 12–15 g/dL
Total RBC count	4.5–5.9 million/µL
Total WBC count	4,000–11,000/µL
Platelets	1.5–4.5 lakh/µL
PCV / Haematocrit	36–50%
MCV	80–100 fL

Possible Correlation

- Anaemia
- Infection
- Inflammation
- Leukaemia
- Thrombocytopenia / thrombocytosis

Part B – Urine Routine Examination

Procedure

- Collect an abnormal urine routine report.
- Compare with normal values.
- Record only abnormal findings.

Normal Urine Findings

Parameter	Normal
Colour	Pale yellow
Appearance	Clear
Specific gravity	1.005–1.030
pH	4.5–8.0
Protein	Absent
Glucose	Absent
Ketones	Absent
RBC	0–2/HPF
Pus cells	0–5/HPF

Possible Correlation

- Diabetes mellitus
- Urinary tract infection
- Renal disease
- Jaundice
- Dehydration

Part C – Stool Routine Examination

Procedure

- Collect an abnormal stool report.
- Compare with normal findings.
- Record only abnormal parameters.

Normal Stool Findings

Parameter	Normal
Colour	Brown
Consistency	Formed
Mucus	Absent
Blood	Absent
Ova/Cysts	Absent
Pus cells	Absent
Parasites	Absent
Fat Globules	Absent

Possible Correlation

- Dysentery
- Amoebiasis
- Worm infestation
- Gastrointestinal bleeding
- Malabsorption

Result

Abnormal parameters from CBC, urine, and stool pathological reports were identified and recorded in tabular form. The findings were correlated with probable histological and physiological alterations.

Precautions

- Use properly labelled reports.
- Record only abnormal parameters.
- Compare with standard reference values.
- Maintain neat tabular presentation.

Observation Table

(CBC)

Parameter	Observed Value	Normal Range	High / Low	Abnormality
Haemoglobin				
RBC Count				
WBC Count				
Neutrophils				
Lymphocytes				
Platelets				
PCV / HCT				
MCV				
MCH				
MCHC				

(Urine)

Parameter	Observed Value	Normal	Abnormality
Colour		Pale yellow	
Appearance		Clear	
Specific Gravity		1.005–1.030	
pH		4.5–8.0	
Protein		Absent	
Glucose		Absent	
Ketones		Absent	
Bile pigments		Absent	
RBCs		0–2/HPF	
Pus Cells		0–5/HPF	
Epithelial Cells		Few	
Casts		Absent	
Crystals			

(Stool)

Parameter	Observed Value	Normal	Abnormality
Colour		Brown	
Consistency		Formed	
Mucus		Absent	
Blood		Absent	
Occult Blood		Negative	
Pus Cells		Absent	
Ova		Absent	
Cysts		Absent	
Parasites		Absent	
Fat Globules			

Experiment No. 6

ESTIMATION OF TOTAL PROTEIN BY THE LOWRY METHOD

Aim
To estimate the total protein concentration in a biological sample by Lowry's method.

Principle
The Lowry method is based on two sequential chemical reactions.

Step I – **Biuret Reaction**

In an alkaline medium, peptide bonds present in proteins react with cupric ions (Cu^{2+}) supplied by alkaline copper reagent to form a copper-protein complex.

Step II **– Reduction of Folin-Ciocalteu Reagent**

The copper-treated protein subsequently reduces the phosphomolybdic-phosphotungstic acid present in the Folin-Ciocalteu reagent. Amino acids such as tyrosine, tryptophan, and cysteine contribute significantly to this reduction, resulting in the formation of a blue-coloured complex.

The intensity of the blue colour is directly proportional to the protein concentration and is measured spectrophotometrically at 660 nm.

Requirements

Chemicals

- Protein sample (unknown)
- Bovine Serum Albumin (BSA) standard solution (1 mg/ml)
- Alkaline Copper Reagent (Reagent C)
- Folin-Ciocalteu Phenol Reagent (1N, diluted 1:1 before use)
- Distilled water

Apparatus

- Test tubes
- Micropipettes/Pipettes
- Test tube stand
- Vortex mixer (optional)
- Spectrophotometer/Colorimeter
- Cuvettes

Procedure
Preparation of Standard Curve

1. Label six test tubes as Blank, 1, 2, 3, 4 and 5.
2. Pipette BSA standard solution and distilled water according to the observation table.
3. Add 5 ml of freshly prepared Alkaline Copper Reagent to each tube.
4. Mix thoroughly.
5. Allow the tubes to stand for 10 minutes at room temperature.
6. Add 0.5 ml diluted Folin-Ciocalteu reagent rapidly to each tube.
7. Mix immediately.
8. Incubate the tubes for 30 minutes at room temperature in the dark.
9. Measure absorbance at 660 nm against the blank.

10. Plot a standard graph with protein concentration on the X-axis and absorbance on the Y-axis.

B. Estimation of Unknown Protein Sample

1. Take 1 ml of the unknown protein sample in a separate test tube.
2. Add 5 ml Alkaline Copper Reagent.
3. Incubate for 10 minutes.
4. Add 0.5 ml of diluted Folin-Ciocalteu reagent.
5. Mix thoroughly.
6. Allow colour to develop for 30 minutes.
7. Measure absorbance at 660 nm.
8. Determine the protein concentration from the standard graph.

Result

The total protein concentration of the given sample was found to be _____ mg/ml.

Precautions

1. Prepare the alkaline copper reagent freshly before use.
2. Use freshly diluted Folin-Ciocalteu reagent.
3. Add Folin reagent rapidly and mix immediately.
4. Maintain identical incubation times for all tubes.
5. Use clean and dry glassware.
6. Avoid contamination of reagents.
7. Take absorbance within the recommended time.
8. Prepare standards accurately.
9. Use the same cuvette throughout the experiment.
10. Calibrate the spectrophotometer before recording absorbance.

Observation Table

Preparation of Standard Curve

Tube	BSA Standard (ml)	Distilled Water (ml)	Protein (µg)	Alkaline Copper Reagent (ml)	Folin Reagent (ml)	O.D. at 660 nm
Blank	0.0	1.0	0	5.0	0.5	
1	0.2	0.8	200	5.0	0.5	
2	0.4	0.6	400	5.0	0.5	
3	0.6	0.4	600	5.0	0.5	
4	0.8	0.2	800	5.0	0.5	
5	1.0	0.0	1000	5.0	0.5	

Unknown Sample

Sample	Sample Volume (ml)	O.D. at 660 nm	Protein Concentration (mg/ml)
Unknown	1.0		

Graph
X-axis → Protein concentration (µg)
Y-axis → Absorbance at 660 nm

A straight-line graph is obtained because absorbance is directly proportional to protein concentration within the working range.

Formula

$$\text{Protein Concentration (mg/ml)} = \frac{\text{Protein obtained from the standard graph (µg)} \times \text{Dilution Factor}}{\text{Volume of sample taken (ml)} \times 1000}$$

- **Protein obtained from the standard graph (µg):**
  This is the amount of protein corresponding to the absorbance (O.D.) of your unknown sample.
  It is read directly from the standard curve.
  Unit: **micrograms (µg)**
- **Dilution Factor (DF):**
  If the sample was diluted before analysis, multiply by the dilution factor.
  If no dilution was made, **DF = 1**.
- **Volume of sample taken (ml):**
  This is the volume of the unknown sample used in the assay.
  Example: 0.2 ml, 0.5 ml, or 1.0 ml.
- **1000:**
  Since the standard graph gives the protein amount in **micrograms (µg)** and the final answer is required in **milligrams per millilitre (mg/ml)**, divide by **1000** because:

$$1000\ \mu g = 1\ mg$$

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RATNAGIRI

Experiment No. 7

ESTIMATION OF LIPID CONCENTRATION BY THE FOLIN–WU (PHOSPHOVANILLIN) METHOD

Aim
To estimate the total lipid concentration in a biological sample using the Folin–Wu (Phosphovanillin) colorimetric method.

Principle
Lipids present in the biological sample are first extracted using an appropriate solvent. The extracted lipids are treated with concentrated sulphuric acid, which causes dehydration and oxidation of unsaturated lipids to form carbonium ions.

These compounds subsequently react with phosphovanillin reagent to produce a stable pink coloured complex. The intensity of the colour formed is directly proportional to the amount of lipid present in the sample.

The absorbance of the coloured complex is measured spectrophotometrically at **540 nm**, and the lipid concentration is determined by comparing the absorbance with that of known lipid standards.

Requirements
Chemicals
- Lipid standard solution
- Biological sample extract
- Concentrated sulphuric acid (H_2SO_4)
- Phosphovanillin reagent
- Distilled water

Apparatus
- Test tubes
- Pipettes and micropipettes
- Test tube stand
- Boiling water bath
- Spectrophotometer
- Glass cuvettes
- Measuring cylinders
- Beakers

Preparation of Reagents
1. Lipid Standard Solution
Prepare a stock lipid standard of **1 mg/ml** using vegetable oil or cholesterol dissolved in chloroform (or as provided in the laboratory).
Working standards are prepared by appropriate dilution.

2. Phosphovanillin Reagent
Dissolve **0.6 g of vanillin** in **10 ml ethanol**.
Slowly add this solution to **90 ml of 85% phosphoric acid** with continuous stirring.

Store the reagent in an amber bottle at room temperature.

3. Concentrated Sulphuric Acid
Use analytical-grade concentrated H_2SO_4 carefully.

Procedure

Preparation of Standard Curve

1. Label six test tubes as Blank, S1, S2, S3, S4 and S5.
2. Pipette increasing volumes of lipid standard into each tube.
3. Make the volume up to **0.1 ml** with distilled water wherever necessary.
4. Add **2 ml concentrated sulphuric acid** carefully to each tube.
5. Mix thoroughly.
6. Place the tubes in a boiling water bath for **10 minutes**.
7. Remove the tubes and allow them to cool to room temperature.
8. Add **5 ml phosphovanillin reagent** to each tube.
9. Mix well.
10. Incubate at room temperature for **15 minutes**.
11. Measure the absorbance at **540 nm** against the reagent blank.

Estimation of Unknown Sample

1. Take **0.1 ml** of the lipid extract.
2. Add **2 ml concentrated sulphuric acid**.
3. Heat in a boiling water bath for **10 minutes**.
4. Cool to room temperature.
5. Add **5 ml phosphovanillin reagent**.
6. Mix thoroughly.
7. Incubate for **15 minutes**.
8. Measure the absorbance at **540 nm**.
9. Determine the lipid concentration from the standard graph.

Result

The lipid concentration of the given biological sample was found to be _____ **mg/ml**.

Precautions

1. Use clean, dry glassware throughout the experiment.
2. Handle concentrated sulphuric acid carefully by adding it slowly to the test tubes.
3. Heat all tubes for the same duration to ensure uniform colour development.
4. Allow the tubes to cool completely before adding phosphovanillin reagent.
5. Mix the contents thoroughly after adding each reagent.
6. Measure absorbance within the recommended incubation period.
7. Use the reagent blank to zero the spectrophotometer.
8. Prepare fresh phosphovanillin reagent for best results.

Observation Table

A. Preparation of Standard Solutions

Tube	Standard (ml)	Distilled Water (ml)	Lipid Amount (µg)
Blank	0.0	0.10	0
S1	0.02	0.08	20
S2	0.04	0.06	40
S3	0.06	0.04	60
S4	0.08	0.02	80
S5	0.10	0.00	100

(Using a standard concentration of 1 mg/ml)

B. Absorbance Readings

Tube	Lipid Amount (µg)	O.D. at 540 nm
Blank	0	
S1	20	
S2	40	
S3	60	
S4	80	
S5	100	
Unknown	—	

Graph

Draw a standard calibration curve using:

- **X-axis:** Lipid concentration (µg)
- **Y-axis:** Absorbance at 540 nm

Determine the concentration of the unknown sample from the graph.

Calculation

From the standard graph,

$$\text{Lipid concentration of unknown} = \frac{\text{Amount obtained from graph (µg)}}{\text{Volume of sample taken (ml)}}$$

If dilution was made,

$$\text{Final concentration} = \frac{\text{Amount from graph} \times \text{Dilution Factor}}{\text{Sample Volume}}$$

Express the result as **mg/ml**.

Experiment No. 8

ESTIMATION OF CHOLESTEROL LEVEL BY ABELL–LEVY–BRODIE–KING METHOD

Aim
To estimate the cholesterol concentration in a biological sample using the Abell–Levy–Brodie–King (ALBK) colorimetric method.

Principle
The Abell–Levy–Brodie–King (ALBK) method is based on the **Liebermann–Burchard reaction**. Cholesterol present in the biological sample is first extracted into chloroform. In the presence of **acetic anhydride** and **concentrated sulphuric acid**, cholesterol undergoes dehydration and oxidation to produce a stable **green coloured complex**.

The intensity of the green colour is directly proportional to the cholesterol concentration in the sample. The absorbance is measured spectrophotometrically at **620 nm**, and the cholesterol concentration is determined by comparing the absorbance of the unknown sample with that of standard cholesterol solutions.

Requirements
Chemicals
- Cholesterol standard solution
- Biological sample (serum or tissue extract)
- Chloroform
- Acetic anhydride
- Concentrated sulphuric acid (H_2SO_4)
- Distilled water

Apparatus
- Test tubes
- Pipettes and micropipettes
- Measuring cylinders
- Test tube stand
- Spectrophotometer
- Glass cuvettes
- Beakers

Preparation of Reagents
1. Cholesterol Standard Solution
Prepare a stock solution containing **1 mg/ml cholesterol** dissolved in chloroform.
Prepare working standards by suitable dilution.

2. Acetic Anhydride Reagent
Use analytical-grade acetic anhydride as supplied.

3. Concentrated Sulphuric Acid
Use analytical-grade concentrated sulphuric acid with proper safety precautions.

Procedure

Preparation of Standard Curve

1. Label six test tubes as Blank, S1, S2, S3, S4 and S5.
2. Pipette different volumes of cholesterol standard into the respective tubes.
3. Make the final volume **1 ml** with chloroform wherever required.
4. Add **2 ml acetic anhydride** to each tube.
5. Carefully add **0.1 ml concentrated sulphuric acid** down the side of each test tube.
6. Mix gently and thoroughly.
7. Allow the reaction mixture to stand for **15 minutes** at room temperature for complete colour development.
8. Measure the absorbance at **620 nm** against the reagent blank.

Estimation of Unknown Sample

1. Pipette **1 ml** of the sample extract into a test tube.
2. Add **2 ml acetic anhydride**.
3. Carefully add **0.1 ml concentrated sulphuric acid**.
4. Mix thoroughly.
5. Allow the mixture to stand for **15 minutes**.
6. Measure the absorbance at **620 nm**.
7. Determine the cholesterol concentration using the standard calibration curve.

Observation Table

A. Preparation of Standard Solutions

Tube	Cholesterol Standard (ml)	Chloroform (ml)	Cholesterol Amount (µg)
Blank	0.0	1.0	0
S1	0.20	0.80	200
S2	0.40	0.60	400
S3	0.60	0.40	600
S4	0.80	0.20	800
S5	1.00	0.00	1000

(Using a cholesterol standard concentration of 1 mg/ml)

B. Absorbance Readings

Tube	Cholesterol Amount (µg)	O.D. at 620 nm
Blank	0	
S1	200	
S2	400	
S3	600	
S4	800	
S5	1000	
Unknown	—	

Graph

Draw a standard calibration curve using:

- **X-axis:** Cholesterol concentration (µg)
- **Y-axis:** Absorbance at 620 nm

Determine the cholesterol concentration of the unknown sample from the graph.

Calculation

From the standard graph,

$$\text{Cholesterol concentration} = \frac{\text{Amount obtained from graph (µg)}}{\text{Volume of sample taken (ml)}}$$

If the sample was diluted,

$$\text{Final concentration} = \frac{\text{Amount from graph} \times \text{Dilution Factor}}{\text{Sample Volume}}$$

Express the result as **mg/dL** or **mg/ml**, depending on the sample and laboratory protocol.

Result

The cholesterol concentration of the given biological sample was found to be _____ **mg/dL**.

Precautions

1. Handle concentrated sulphuric acid and acetic anhydride with extreme care, as both are highly corrosive.
2. Perform the experiment in a well-ventilated area or fume hood.
3. Use clean, dry, and grease-free glassware.
4. Prepare fresh cholesterol standards before the experiment.
5. Add sulphuric acid slowly to avoid excessive heat generation.
6. Mix the reaction mixture uniformly after adding reagents.
7. Maintain identical incubation time for all tubes.
8. Measure absorbance immediately after complete colour development.
9. Use the reagent blank to calibrate the spectrophotometer.
10. Avoid contamination of reagents and samples.

Experiment No. 9

Estimation of Carbohydrates by Anthrone Method

Aim

To estimate the total carbohydrate content in a given biological sample using the Anthrone method.

Principle

Carbohydrates are dehydrated by concentrated sulphuric acid to form furfural or hydroxymethyl furfural derivatives. These compounds react with Anthrone reagent to produce a blue-green coloured complex. The intensity of the colour is directly proportional to the concentration of carbohydrates present and is measured colorimetrically at 620 nm.

Requirements

Apparatus

- Test tubes
- Pipettes / Micropipettes
- Water bath
- Colorimeter or spectrophotometer
- Test tube stand
- Measuring cylinder
-

Reagents

- Anthrone reagent: 0.2% Anthrone in concentrated H_2SO_4
- Standard glucose solution: 100 µg/mL
- Distilled water
-

Biological sample

- Tissue extract / plant extract / blood serum / other biological sample
-

Preparation of Standard Solutions

Take different volumes of glucose standard and make the final volume 1 mL with distilled water.

Tube	Glucose (mL)	Water (mL)	Glucose (µg)
B	0.0	1.0	0
S1	0.2	0.8	20
S2	0.4	0.6	40
S3	0.6	0.4	60
S4	0.8	0.2	80
S5	1.0	0.0	100

Procedure

- Take 1 mL of each standard solution in labelled test tubes.
- Take 1 mL of the biological sample in another test tube.
- Add 4 mL of freshly prepared Anthrone reagent to all tubes carefully.
- Mix thoroughly.
- Heat the tubes in a boiling water bath for 10 minutes.

- Cool the tubes to room temperature.
- Measure the absorbance at 620 nm against the blank.

Result

The total carbohydrate content of the given biological sample was estimated by the Anthrone method and found to be:

Carbohydrate content _____ **µg/mL (or mg/g tissue)**

Observation Table

Standard Glucose

Tube	Glucose (µg)	Absorbance at 620 nm
B	0	0.00
S1	20	
S2	40	
S3	60	
S4	80	
S5	100	

Sample

Sample	Absorbance
Unknown	

Standard Graph

Plot a graph with:

- X-axis: Amount of glucose (µg)
- Y-axis: Absorbance at 620 nm

Glucose standard curve (Anthrone method)
Illustrative standard curve showing absorbance increasing with glucose concentration.
00.20.40.60.8020406080100

Calculation

From the standard graph, determine the amount of carbohydrate corresponding to the absorbance of the unknown sample.

Formula

$$\text{Carbohydrate concentration} = \frac{\text{Amount from graph} \times \text{Dilution factor}}{\text{Volume of sample taken}}$$

Example

- Absorbance of sample = 0.38
- From graph, 0.38 corresponds to 60 µg glucose
- Volume of sample taken = 1 mL

$$\text{Carbohydrate} = \frac{60 \times 1}{1} = 60\ \mu g/mL$$

If the sample was diluted 10 times:

$$60 \times 10 = 600\ \mu g/mL$$

Experiment No. 10

QUANTIFICATION OF VITAMIN C USING DCPIP TITRATION

Aim
To determine the concentration of Vitamin C (ascorbic acid) in fruit juices or food samples by the **2,6-Dichlorophenol Indophenol (DCPIP) titrimetric method**.

Principle
Vitamin C (ascorbic acid) is a powerful reducing agent that readily reduces **2,6-dichlorophenol indophenol (DCPIP)**, a blue-redox dye, to its colourless leuco form.

In acidic medium, DCPIP appears blue in alkaline solution and pink in acidic solution. During titration, ascorbic acid reduces DCPIP until all the vitamin C is oxidized. The first permanent **light pink colour** persisting for **15 seconds** indicates the end point, signifying that all the ascorbic acid has reacted and excess DCPIP remains.

The volume of DCPIP required is directly proportional to the amount of Vitamin C present in the sample.

Requirements
Chemicals
- Standard ascorbic acid solution
- DCPIP solution
- Fruit juice or food extract
- Distilled water
- 4% Oxalic acid (or 3% metaphosphoric acid) for extraction and stabilization of Vitamin C

Apparatus
- Burette
- Pipettes
- Conical flasks (100 ml)
- Volumetric flasks
- Measuring cylinders
- Funnel
- Filter paper
- Beakers

Preparation of Reagents
1. Standard Ascorbic Acid Solution
Dissolve **100 mg of pure L-ascorbic acid** in **100 ml of 4% oxalic acid**.
Concentration = **1 mg/ml**
Prepare fresh before use.

2. DCPIP Solution
Dissolve **50 mg of 2,6-dichlorophenol indophenol** in distilled water and make the volume up to **200 ml**.

Store the solution in an amber bottle and prepare fresh every few days.

3. Oxalic Acid Solution (4%)
Dissolve **4 g oxalic acid** in distilled water and make the volume up to **100 ml**.
This solution prevents oxidation of Vitamin C during extraction.

Procedure
A. Standardization of DCPIP

1. Fill the burette with DCPIP solution.
2. Pipette **10 ml of standard ascorbic acid solution** into a conical flask.
3. Add **10 ml of 4% oxalic acid**.
4. Titrate with DCPIP until a **faint pink colour persists for 15 seconds**.
5. Record the titre value.
6. Repeat until concordant readings are obtained.
7. Calculate the standardization factor.

B. Estimation of Vitamin C in Sample

1. Filter the fruit juice if necessary.
2. Pipette **10 ml** of the sample into a conical flask.
3. Add **10 ml of 4% oxalic acid**.
4. Titrate against the standardized DCPIP solution.
5. Continue titration until a **light pink colour persists for 15 seconds**.
6. Record the titre value.
7. Repeat the titration and obtain concordant readings.
8. Calculate the Vitamin C concentration using the standard factor.

Result
The Vitamin C concentration in the given fruit juice/food sample was found to be _____ **mg/100 ml**.

Precautions

1. Prepare fresh DCPIP solution before use.
2. Perform titrations rapidly to minimize oxidation of Vitamin C by atmospheric oxygen.
3. Protect Vitamin C solutions from direct sunlight.
4. Use freshly prepared fruit juice or food extract.
5. Store reagents in amber-coloured bottles whenever possible.
6. Add oxalic acid or metaphosphoric acid to prevent oxidation of ascorbic acid.
7. Read the burette carefully to avoid parallax error.
8. The endpoint should be a faint pink colour persisting for **15 seconds**.
9. Use clean glassware free from detergent residues.
10. Obtain concordant titre values for accurate calculations.

Observation Table

A. Standardization of DCPIP

B.

Trial	Volume of Standard Ascorbic Acid (ml)	Initial Burette Reading (ml)	Final Burette Reading (ml)	DCPIP Used (ml)
I	10.0			
II	10.0			
III	10.0			

Average titre = _____ ml

B. Estimation of Sample

Trial	Sample Volume (ml)	Initial Burette Reading (ml)	Final Burette Reading (ml)	DCPIP Used (ml)
I	10.0			
II	10.0			
III	10.0			

Average titre = _____ ml

Calculation

Standardization Factor

$$\text{Standard Factor} = \frac{\text{Amount of standard Vitamin C (mg)}}{\text{Average DCPIP titre (ml)}}$$

Vitamin C Concentration

$$\text{Vitamin C (mg/100 ml)} = \frac{\text{Titre of Sample} \times \text{Standard Factor} \times 100}{\text{Volume of Sample Taken}}$$

where,

- Average titre of sample = _____ ml
- Standard factor = _____ mg/ml
- Sample volume = _____ ml

Experiment No. 11

Determination of Urease Activity

Aim

To determine the activity of the enzyme urease in a given sample by estimating the amount of ammonia released from urea.

Principle

Urease catalyses the hydrolysis of urea into ammonia and carbon dioxide.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

The ammonia produced reacts with Nessler's reagent to form a yellow to brown coloured complex. The intensity of the colour is proportional to the amount of ammonia released and hence to the urease activity. Absorbance is measured at 480 nm.

Requirements

Apparatus

- Test tubes
- Pipettes / Micropipettes
- Water bath (37°C)
- Colorimeter or spectrophotometer
- Test tube stand

Reagents

- Urea solution (2%)
- Phosphate buffer (pH 7.0)
- Nessler's reagent
- Ammonium sulphate standard solution
- Distilled water

Enzyme source

- Soybean extract / jack bean extract / bacterial culture / soil extract

Procedure

A. Preparation of Enzyme Extract

- Grind 1 g of soybean seeds with 10 mL phosphate buffer.
- Filter or centrifuge.
- Use the clear extract as the urease source.

B. Enzyme Assay

Prepare the reaction mixture as follows:

Component	Test	Blank
Phosphate buffer	1.0 mL	1.0 mL
Urea solution	1.0 mL	1.0 mL
Enzyme extract	0.5 mL	—
Distilled water	—	0.5 mL

- Mix the contents.

- Incubate at 37°C for 15 minutes.
- Stop the reaction by adding 1 mL Nessler's reagent.
- Make up the volume to 10 mL with distilled water.
- Measure absorbance at 480 nm against the blank.

Preparation of Ammonia Standard Curve

Tube	NH_4^+ Standard (mL)	Water (mL)	µg NH_3
B	0.0	1.0	0
S1	0.2	0.8	20
S2	0.4	0.6	40
S3	0.6	0.4	60
S4	0.8	0.2	80
S5	1.0	0.0	100

Add 1 mL Nessler's reagent to each tube and make up to 10 mL. Read absorbance at 480 nm.

Result

The urease activity of the given sample was determined by estimating the ammonia liberated from urea.

Urease activity

_____ µg NH_3 min^{-1} mL^{-1} enzyme extract

Observation Table

Standard Curve

NH_3 (µg)	Absorbance at 480 nm
0	0.00
20	
40	
60	
80	
100	

Test Sample

Sample Absorbance

Test

Standard Graph

Plot a graph with:

- X-axis: Amount of ammonia (µg)
- Y-axis: Absorbance at 480 nm

Ammonia standard curve for urease assay

Illustrative standard curve showing absorbance increasing with ammonia concentration.

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Calculation

From the graph, determine the amount of ammonia released by the enzyme.

Formula for Enzyme Activity

$$\text{Urease activity} = \frac{\mu g\ NH_3 \text{ released}}{\text{Time (min)} \times \text{Volume of enzyme (mL)}}$$

Example

- Absorbance of test = 0.32
- From graph = 60 µg NH_3
- Incubation time = 15 min
- Enzyme volume = 0.5 mL

$$\text{Activity} = \frac{60}{15 \times 0.5} = \frac{60}{7.5} = 8\ \mu g\ NH_3/min/mL$$

Experiment No. 12

Immobilization of Enzyme Using Encapsulation in Polymeric Materials

Aim

To immobilise an enzyme by encapsulation within a polymer matrix (such as calcium alginate beads) and study its enzymatic activity in comparison with the free enzyme.

Materials required

- Enzyme solution (e.g., invertase, amylase, or urease)
- Sodium alginate solution (2–3% w/v)
- Calcium chloride solution (0.1–0.2 M)
- Substrate solution (e.g., sucrose for invertase, starch for amylase, urea for urease)
- Distilled water
- Beakers, conical flasks
- Dropper or syringe
- Magnetic stirrer/glass rod
- Filter paper
- Test tubes and racks
- Water bath (for maintaining reaction temperature)
- pH indicator or colourimetric assay reagent (depending on enzyme used)

Principle

Enzyme immobilisation involves physically confining or localising enzymes in a support or matrix, while retaining their catalytic activity.

- In **encapsulation**, the enzyme solution is entrapped inside a polymeric matrix such as calcium alginate beads.
- Sodium alginate reacts with calcium ions (Ca^{2+}) to form insoluble calcium alginate, creating gel beads that entrap the enzyme.
- Immobilised enzymes can be easily separated from the reaction mixture and reused.
- The activity of immobilised enzymes can be studied by comparing the rate of substrate hydrolysis with that of free enzyme.

Procedure

1. **Preparation of alginate–enzyme mixture**
   - Mix equal volumes of enzyme solution with 2% sodium alginate solution.
   - Stir gently to avoid bubble formation.

2. **Bead formation (encapsulation step)**
   - Fill the mixture in a syringe or dropper.
   - Add dropwise into a beaker containing 0.1 M calcium chloride solution with gentle stirring.
   - Beads of calcium alginate will form instantly, entrapping the enzyme inside.

3. **Washing and storage**
   - Allow beads to harden for 15–20 minutes.
   - Collect beads using filter paper and wash thoroughly with distilled water to remove excess calcium chloride and unbound enzyme.

4. **Enzyme activity assay**
    - Transfer the beads into a test tube containing the substrate solution.
    - Incubate at a suitable temperature (e.g., 37 °C).
    - At intervals, test the solution for product formation (e.g., Benedict's test for glucose from sucrose hydrolysis, iodine test for starch hydrolysis, or Nessler's reagent for ammonia from urea).
    - Run a parallel experiment with free enzyme (no encapsulation) as a control.

Observations

Sample	**Substrate used**	**Product test used**	**Colour change/ Absorbance**	**Relative activity**
Free enzyme solution	Sucrose/Starch/Urea	Benedict's/Iodine/ Nessler's	Strong positive	High activity
Immobilised enzyme beads	Same as above	Same as above	Positive, but slower	Moderate activity

Results

Enzyme encapsulated in calcium alginate beads retained catalytic activity, as shown by the positive product test.

The activity was generally lower than that of the free enzyme, due to diffusion limitations inside the beads.

Immobilised enzymes have the advantage of reusability, stability, and easy separation from the reaction medium, making them useful in industrial bioprocesses.

Experiment No. 13

Fermentation Process for Ethanol Production

(To produce ethanol by yeast fermentation of glucose)

Aim

To produce ethanol by fermenting an aqueous glucose solution with baker's yeast and to monitor fermentation progress and estimate ethanol yield.

Materials required

- Baker's (*Saccharomyces cerevisiae*) yeast — active dry or fresh (5–10 g for lab-scale)
- Glucose (dextrose) or sucrose (table sugar) — analytical grade
- Distilled water
- Nutrient source (optional): ammonium sulfate or yeast extract (small amount, e.g. 0.5 g/L)
- Fermentation vessel (250–1000 mL Erlenmeyer flask or fermentation bottle) with airlock or rubber stopper + fermentation tube
- Sterile pipettes / measuring cylinder, spatula, beakers
- Hotplate and magnetic stirrer (or boiling setup)
- Thermometer
- pH paper or meter (optional)
- Hydrometer (specific gravity) or refractometer (to follow sugar consumption) — optional but recommended
- Stopwatch / timer
- Thermostatic incubator or water bath (settable to 25–35 °C) or warm place ~30 °C
- Sterile cloth/foil to cover if no airlock
- Safety equipment: gloves, goggles, lab coat, fire extinguisher (ethanol is flammable)

Optional: simple distillation apparatus / rotary evaporator (only under supervised lab conditions and where legal)

Principle

Yeast (*S. cerevisiae*) metabolises glucose anaerobically via glycolysis and alcoholic fermentation:

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$

Each mole of glucose (≈180.16 g) yields 2 moles of ethanol (≈92.14 g). The theoretical maximum yield of ethanol is therefore ≈0.512 g ethanol per g glucose (≈51.2% w/w). In practice, yields are lower due to biomass formation, side-products, and losses.

Fermentation proceeds fastest at moderate temperatures (≈25–35 °C) and under low-oxygen (anaerobic) conditions. CO_2 evolution indicates active fermentation.

Procedure

(student/lab-scale example — 1 L batch, adjust volumes proportionally)

A. Prepare medium (10% w/v glucose; adjust concentration as needed)

Weigh 100 g of glucose and dissolve it in ~800 mL of distilled water in a 1 L beaker.

(Optional) Add 1 g yeast extract or 0.5 g ammonium sulfate to provide nutrients.

Heat gently to ~80–90 °C and simmer briefly (2–5 min) to dissolve and sterilise (or autoclave if available).

Cool to ~30–35 °C. Check the temperature with a thermometer.

B. Prepare inoculum
Rehydrate active dry yeast in ~20–50 mL warm water (30–35 °C) for 5–10 min, or prepare fresh yeast slurry. Use approximately 5–10 g active dry yeast for 1 L medium (more for faster start).

C. Inoculation and setup
Transfer cooled medium to a sterile 1 L fermentation vessel (leave headspace). Top up to 1 L with distilled water if needed.

Measure and record initial specific gravity (SG) with a hydrometer or Brix with a refractometer (optional). Convert SG/Brix to approximate sugar concentration.

Inoculate with yeast slurry; swirl gently to mix. Fit the vessel with an airlock (filled with water) or attach a fermentation tube. If no airlock, loosely cover with sterile foil or cloth to allow CO_2 escape but limit oxygen entry.

Place at a controlled temperature of ~30 °C (range 25–32 °C is acceptable).

D. Fermentation monitoring
Monitor fermentation for 48–96 hours: observe CO_2 bubbling through airlock (active phase), note smell, clarity, and foam.

Record time to onset of vigorous bubbling and duration. Optionally take SG readings every 12–24 h until SG stabilises (no further drop).

When bubbling slows markedly, and SG stabilises for 24–48 h, fermentation is effectively complete.

E. Recovery (optional and supervised only)
To recover ethanol, the fermented broth can be clarified (remove solids) and concentrated/distilled under supervised lab conditions using proper glassware or a rotary evaporator. Do not distil at home.

Note: distillation concentrates ethanol but also concentrates impurities (and may be regulated).

Observations

Initial and final specific gravity (if hydrometer used).

Time to visible fermentation onset (CO_2 evolution).

Peak CO_2 evolution (approximate: vigorous/steady/slow).

Foam/ krausen formation (yes/no, height).

Clarity of ferment (turbid → clearer as yeast flocculates).

Odour (sweet → alcoholic).

Temperature during fermentation.

Any contamination signs (off-odours, pellicles, unexpected colours).

Sample observation table:

Time (h)	**Temp (°C)**	**SG/Brix**	**CO_2 activity (bubbles/min or qualitative)**	**Appearance**	**Notes**
0	30	1.070	0	Clear	Inoculated
12	30	1.045	Vigorous	Foamy	Active
24	30	1.010	Moderate	Turbid	
48	30	0.995	Slow	Clearing	Near completion
72	30	0.992	Very slow/none	Clearer	Fermentation complete

Experiment No. 14

Animal Toxicity Testing (LC_{50} Determination):

Aim

To determine the median lethal concentration (LC_{50}) of a specified chemical (e.g., pesticide, industrial toxin) for a chosen aquatic test organism by exposing groups to a range of concentrations and recording mortality over a fixed exposure period.

Materials required

- Test organisms (e.g., mosquito larvae or *Daphnia*), healthy and acclimated (specify species, size/age, N per replicate).
- Test chemical standard (analytical grade) and solvent (if needed).
- Dilution water (dechlorinated/culture water) matching the organism's habitat.
- Glass beakers/test chambers (identical volumes) or multi-chamber test system.
- Pipettes, volumetric flasks, balances for solution preparation.
- pH meter, thermometer, dissolved oxygen probe (or meters).
- Stopwatch/timer and data sheets.
- Personal protective equipment (gloves, goggles, lab coat).
- Waste containers and disinfectant for disposal.
- (Optional) Software or spreadsheet for LC_{50} calculation (or statistical packages for probit/Logit analysis).

Principle

Expose groups of organisms to a series of increasing concentrations of the test chemical for a defined exposure time (commonly 24, 48, 72 or 96 hours). Record mortality at defined intervals. From dose–response data, determine the concentration that produces 50% mortality (LC_{50}) by either interpolation between concentrations bracketing 50% mortality or by statistical methods (probit, logistic regression).

Experimental design (recommended for 24 h larval test)

Concentrations: At least five test concentrations that bracket 0–100% mortality (choose logarithmic or geometric spacing; e.g., 0.1, 0.3, 1.0, 3.0, 10.0 $mg \cdot L^{-1}$ depending on expected potency).

Replicates: 3–4 replicates per concentration.

Larvae per replicate: 20–25 larvae per replicate (commonly 25).

Controls: Negative control (water only). If solvent is used, include a solvent control at the same solvent fraction as treatments.

Environmental conditions: Temperature 25 ± 2 °C (or colony-maintenance temperature), photoperiod 12:12 L:D or constant low light, no feeding during exposure. Keep same water volume in each cup (e.g., 100 mL).

Definition of death: No movement when gently prodded with a fine brush and no signs of coordinated movement (no siphon movement/response). Record cumulative mortality at 24 h.

Procedure (outline)

- Larval selection & acclimation - Collect healthy, active third-instar larvae from the colony. Acclimate them to test water for 1–2 hours if transferred from different water. Do not feed during exposure.

Prepare stock solution - Accurately weigh the test chemical and dissolve it in the appropriate solvent to make a concentrated stock.

Prepare test concentrations - Make working dilutions (nominal concentrations) by diluting the stock into test water. Prepare fresh solutions just before exposure. Prepare a control and, if needed, solvent control.

Set up test vessels - Label cups/beakers with concentration and replicate number. Add an identical volume of test solution to each vessel (e.g., 100 mL). Gently introduce 20–25 third-instar larvae into each replicate using a pipette or paintbrush. Start the timer (t = 0) when the last replicate is set.

Exposure conditions - Keep test vessels undisturbed at constant temperature and light. No feeding. Avoid bubbling/aeration that can stress larvae; gentle aeration only if identical across treatments and necessary.

Record mortality at 24 hours -After exactly 24 hours, count dead larvae in each replicate. Gently probe to confirm death. Remove dead larvae after counting (optional) and record data.

Termination & disposal - After observations, kill surviving larvae ethically (e.g., immersion in 10% bleach) and dispose of all solutions and organisms per institutional biosafety procedures. Clean and disinfect glassware.

Observations (example table format)

Conc ($mg \cdot L^{-1}$)	Rep 1 (dead/total)	Rep 2	Rep 3	Rep 4 (optional)	Total dead	% Mortality
0 (control)	0/25	0/25	0/25	—	0/75	0.0
0.1	1/25	0/25	0/25	—	1/75	1.33
0.3	2/25	1/25	1/25	—	4/75	5.33
1.0	5/25	4/25	6/25	—	15/75	20.0
3.0	12/25	10/25	11/25	—	33/75	44.0
10.0	23/25	24/25	25/25	—	72/75	96.0

Data analysis

Control correction (Abbott's formula) — use when control mortality is between 0 and ~20% and guidelines permit correction:

$$\text{Corrected mortality (\%)} = \frac{\text{Observed mortality (\%)} - \text{Control mortality (\%)}}{100 - \text{Control mortality (\%)}} \times 100$$

Apply this to each treatment group before fitting dose-response, if required.

LC_{50} estimation

Use statistical methods such as probit analysis, logit (logistic regression), or nonlinear regression to estimate 24-h LC_{50} and 95% confidence limits. Recommended software: R (drc package), EPA Probit, GraphPad Prism, SPSS, or specialized ecotoxicology tools.

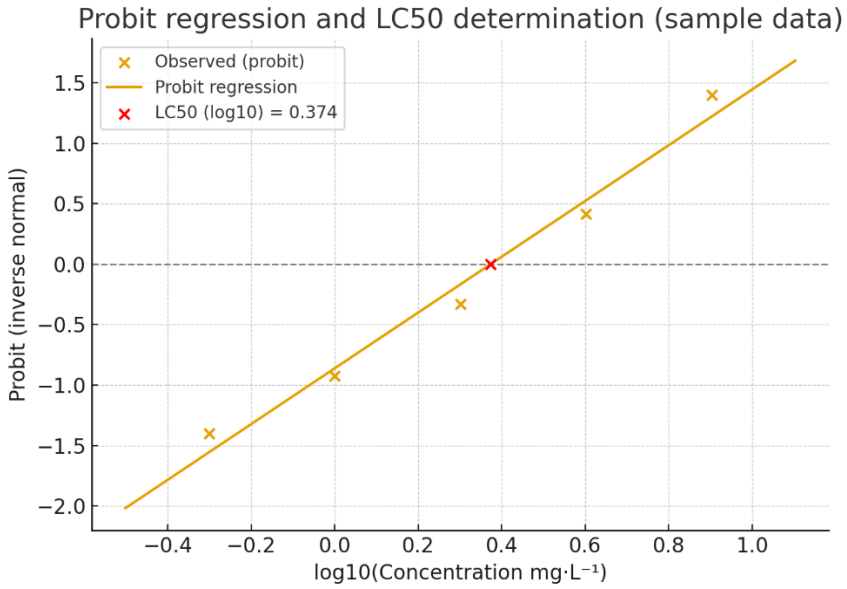
Input: concentration (preferably log-transformed) vs. proportion mortality (corrected if Abbott applied).

Report LC_{50} with 95% confidence intervals, slope ± SE, χ^2 goodness of fit (or p-value), and number of larvae tested.

Reporting statistical tests

Include number of replicates, larvae per replicate, whether mortality was corrected, software used, and test assumptions (e.g., binomial errors). If comparing treatments, consider LC_{50} ratio tests or overlap of CIs.

Concentration ($mg \cdot L^{-1}$)	Rep 1 (dead/25)	Rep 2	Rep 3	Total Dead (n=75)	% Mortality
0 (control)	0	0	0	0	0.0
0.1	1	0	0	1	1.33
0.3	2	1	1	4	5.33
1.0	5	4	6	15	20.0
3.0	12	10	11	33	44.0
10.0	23	24	25	72	96.0



Experiment No. 15

Bioassay for Pesticide Toxicity Using Brine Shrimp / Daphnia

Aim

To assess the toxicity of a pesticide by exposing Daphnia or brine shrimp (Artemia nauplii) to different concentrations of the pesticide and observing mortality and movement patterns.

Principle

A bioassay is a biological method used to determine the toxic effect of a chemical on living organisms. Aquatic organisms such as Daphnia and brine shrimp are highly sensitive to pollutants and pesticides. Increasing concentrations of a pesticide generally produce increased toxic effects, resulting in altered swimming behaviour and mortality. The concentration causing 50% mortality is termed LC_{50} (Lethal Concentration 50).

Requirements

Materials

- Brine shrimp nauplii / Daphnia (10 organisms per beaker)
- Artificial sea water or clean freshwater (as required)
- Pesticide stock solution
- Beakers or test tubes
- Dropper or micropipette
- Measuring cylinder
- Marker pen
- Hand lens / magnifying lens

Preparation of Test Solutions

Prepare a series of pesticide concentrations from the stock solution.

Example concentrations:

Beaker	Pesticide concentration
A	Control (0 ppm)
B	5 ppm
C	10 ppm
D	20 ppm
E	40 ppm

Add equal volumes (e.g., 50 mL) of each solution to separate beakers.

Procedure

- Label five beakers as Control, 5 ppm, 10 ppm, 20 ppm, and 40 ppm.

- Add 50 mL of the respective pesticide solution to each beaker.
- Introduce 10 active brine shrimp / Daphnia into each beaker.
- Observe the organisms immediately and after 15 min, 30 min, 1 h, 6 h, and 24 h.
- Record:
    - Number of dead organisms
    - Swimming activity
    - Response to light or touch
    - Abnormal movements (jerky, slow, spiralling, immobile)
- Calculate percentage mortality after 24 h.

Observation Table

A. Mortality Record

Concentration (ppm)	No. of organisms	Dead after 24 h	% Mortality
0 (Control)	10		
5	10		
10	10		
20	10		
40	10		

Formula

$$\% \text{ Mortality} = \frac{\text{Number dead}}{\text{Total organisms}} \times 100$$

B. Movement Pattern Observation

Concentration	Swimming speed	Abnormal movement	Response to stimulus
Control			
5 ppm			
10 ppm			
20 ppm			
40 ppm			

Example of Expected Results

Concentration	% Mortality
0 ppm	0%
5 ppm	10%
10 ppm	30%
20 ppm	60%
40 ppm	100%

Graph

Plot a graph with:

- X-axis: Pesticide concentration (ppm)
- Y-axis: Percentage mortality

Pesticide concentration vs mortality

Illustrative bioassay response showing increasing mortality with higher pesticide concentration.

Interpretation

- Control: Normal active swimming, no mortality.
- Low concentration: Slight reduction in movement.
- Moderate concentration: Slow swimming, irregular movements, some mortality.
- High concentration: Severe impairment, loss of balance, immobility, high mortality.

The pesticide shows a dose-dependent toxic effect.