SCHEME : J

Name : _____

Roll No. : _____ Year : 20___ 20___

Exam Seat No. : _____

LABORATORY MANUAL FOR
BIOCHEMISTRY & CLINICAL PATHOLOGY (20058)

SECOND YEAR D.PHARMACY

MAHARASHTRA STATE BOARD OF TECHNICAL EDUCATION, MUMBAI
(Autonomous) (ISO 9001: 2015) (ISO/IEC 27001:2013)

VISION

To ensure that the Diploma level Technical Education constantly matches the latest requirements of technology and industry and includes the all-round personal development of students including social concerns and to become globally competitive, technology led organization.

MISSION

To provide high quality technical and managerial manpower, information and consultancy services to the industry and community to enable the industry and community to face the challenging technological & environmental challenges.

QUALITY POLICY

We, at MSBTE are committed to offer the best-in-class academic services to the students and institutes to enhance the delight of industry and society. This will be achieved through continual improvement in management practices adopted in the process of curriculum design, development, implementation, evaluation and monitoring system along with adequate faculty development programmes.

CORE VALUES

MSBTE believes in the following

- Skill development in line with industry requirements
- Industry readiness and improved employability of Diploma holders
- Synergistic relationship with industry
- Collective and Cooperative development of all stake holders
- Technological interventions in societal development
- Access to uniform quality technical education

Laboratory Manual of

BIOCHEMISTRY & CLINICAL PATHOLOGY (20058)

Second Year

Diploma in Pharmacy (PH)

Maharashtra State
Board of Technical Education, Mumbai.
(Autonomous)
(ISO 9001:2015) (ISO/IEC27001:2013)

PCI ER-2020/'J' Scheme Curriculum

Maharashtra State Board of Technical Education

(Autonomous) (ISO 9001:2015) (ISO/IEC 27001:2013)

4th Floor Government Polytechnic Building, 49, Kherwadi,
Bandra (East), Mumbai- 400051

(Printed On July,2024)

MAHARASHTRA STATE BOARD OF TECHNICAL EDUCATION

CERTIFICATE

This is to certify that, Mr. /Ms. _____

Roll No. _____ of Second Year Diploma in Pharmacy has studying at (Institute)_____ has completed the practical work satisfactorily in Biochemistry and Clinical Pathology (20058) for the academic year 20____to 20____ as prescribed in the PCI ER 2020 syllabus.

Date: _____ Enrolment No.: _____

Place: _____ Exam Seat No.: _____

Course Teacher **Principal**

External Examiner

Seal of the Institute

MAHARASHTRA STATE BOARD OF TECHNICAL EDUCATION MUMBAI

PROGRAM OUTCOMES

1. **Pharmacy knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy.
2. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of the limitations.
3. **Leadership skills:** Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and wellbeing.
4. **Professional identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).
5. **Pharmaceutical ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.
6. **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.
7. **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.
8. **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
9. **Life-long learning:** Recognize the need for and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

COMPETENCIES FOR THE INDIAN D. PHARM HOLDERS

Competency is defined as "A distinct composite of knowledge, skill, attitude and value that is essential to the practice of the profession in real life contexts".

The candidates who successfully complete the Diploma in Pharmacy (D. Pharm) program of Education Regulations 2020 (ER-2020), from the institutions approved by the Pharmacy Council of India are expected to attain the following professional competencies.

1. Review Prescriptions: The student should receive and handle prescriptions in a professional manner and be able to check for their completeness and correctness. Also, the prescribers should be contacted for any clarifications & corrections in the prescriptions with suggestions if any.

2. Dispense Prescription / Non-Prescription Medicines: The student should be able to dispense the various scheduled drugs / medicines as per the implications of the Drug & Cosmetic Act and Rules thereunder. Also, the non-prescription medicines (over-the-counter drugs) should be dispensed judicially to the patients as required.

3. Provide Patient Counselling / Education: The student should be able to effectively counsel / educate the patients / caretakers about the prescription / non-prescription medicines and other health related issues. Effective communication includes using both oral and written communication skills and various communication techniques.

4. Hospital and Community Pharmacy Management: The student be able to manage the drug distribution system as per the policies and guidelines of the hospital pharmacy, good community pharmacy practice and the recommendations of regulatory agencies. Also, be able to manage the procurement, inventory, and distribution of medicines in hospital / community pharmacy settings.

5. Expertise on Medications: The student should be able to provide an expert opinion on medications to health care professionals on safe and effective medication – use, relevant policies and procedures based on available evidence.

6. Proficiency on Pharmaceutical Formulations: The student should be able to describe the chemistry, characteristics, types, merits and demerits of both drugs and excipients used in pharmaceutical formulations based on her/his knowledge and scientific resources.

7. Entrepreneurship and Leadership: The student should be able to acquire the entrepreneurial skills in the dynamic professional environments. Also, be able to achieve leadership skills through teamwork and sound decision-making skills.

8. Deliver Primary and Preventive Healthcare: The student should be able to contribute to various healthcare programs of the nation including disease prevention initiatives to improve public health. Also contribute to the promotion of national health policies.

9. Professional, Ethical, and Legal Practice: The student should be able to deliver professional services in accordance with legal, ethical, and professional guidelines with integrity.

10. Continuing Professional Development: The student should be able to recognize the gaps in the knowledge and skills in the effective delivery of professional services from time to time and be self-motivated to bridge such gaps by attending continuing professional development programs.

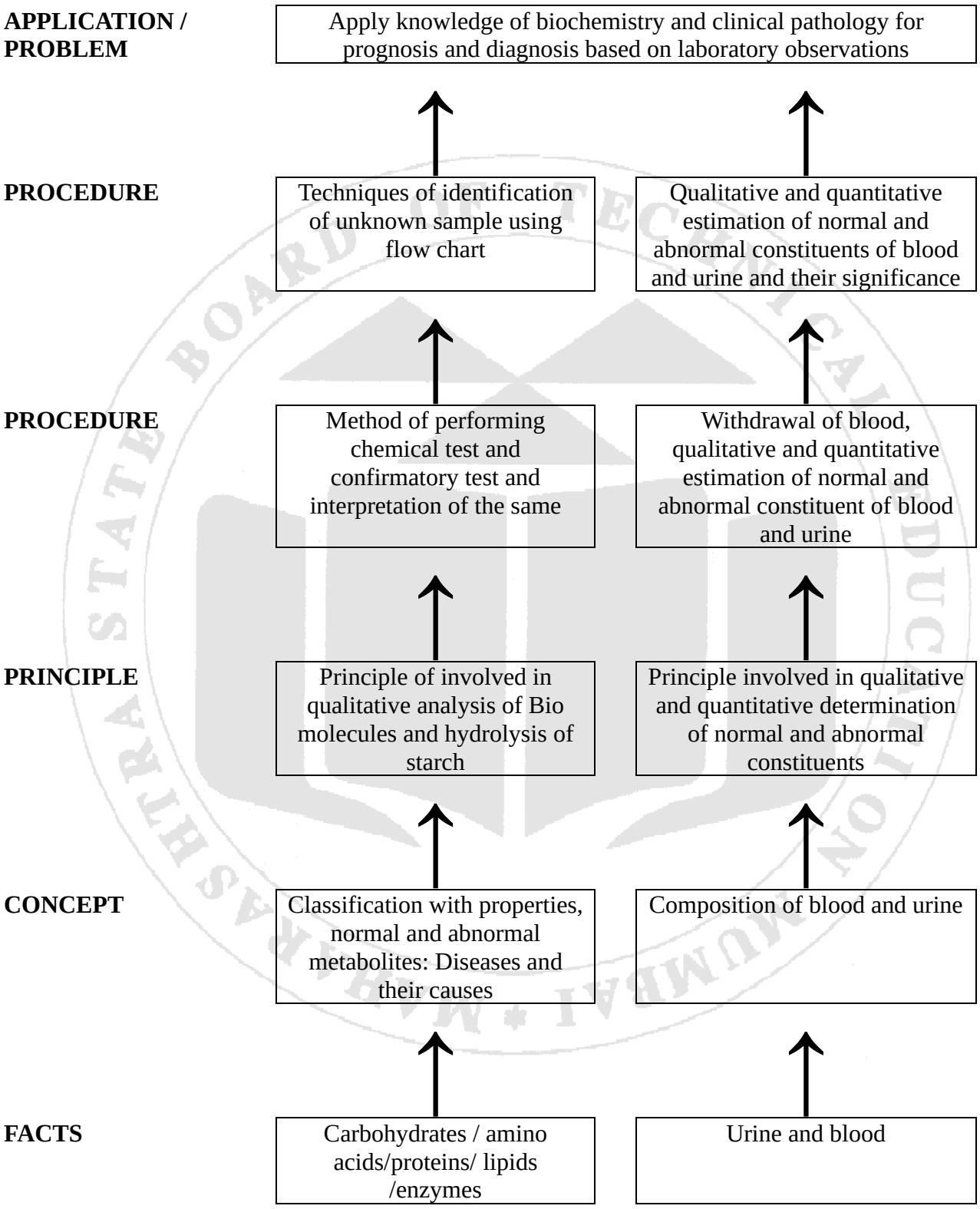
COMPETENCY MAPPING WITH THE COURSE

Competencies	Biochemistry & Clinical Pathology
1.Review Prescriptions	✓
2. Dispense Prescription / Non-Prescription Medicines	✓
3.Provide Patient Counselling / Education	✓
4.Hospital and Community Pharmacy Management	
5.Expertise on Medications	✓
6.Proficiency on Pharmaceutical Formulations	
7.Entrepreneurship and Leadership	
8.Deliver Primary and Preventive Healthcare	✓
9.Professional, Ethical, and Legal Practice	
10.Continuing Professional Development	

GRAPHICAL STRUCTURE OF SUBJECT AREA

Second Year Diploma in Pharmacy

BIOCHEMISTRY AND CLINICAL PATHOLOGY (20058)



BIOCHEMISTRY AND CLINICAL PATHOLOGY (PRACTICAL)

Course Code: ER20-23P/20058 **50 Hours (2 Hours / Week)**

Scope: This course is designed to train the students in the qualitative testing of various biomolecules and testing of biological samples for determination of normal and abnormal constituents.

Course Objectives: This course will train and provide hands –on experiences on the following.

1. Qualitative determination of biomolecules / metabolites in simulated biological samples.
2. Determination of normal and abnormal constituents of simulated blood and urine samples.

Course Outcomes: Upon successful completion of this course, the students will be able to

1. Qualitatively determine the biomolecules / metabolites in the given biological samples.
2. Determine the normal and abnormal constituents in blood and urine samples and interpret the result of such testing.

Practicals

1. Qualitative analysis of carbohydrates (4 experiments)
2. Qualitative analysis of Proteins and amino acids (4 experiments)
3. Qualitative analysis of lipids (2 experiments)
4. Qualitative analysis of urine for normal and abnormal constituents (4 experiments)
5. Determination of constituents of urine (Glucose, creatinine, chlorides) (2experiments)
6. Determination of constituents of blood / serum (simulated) (Creatine, glucose, cholesterol, Calcium, Urea, SGOT/ SGPT) (5 experiments)
7. Study the hydrolysis of starch from acid and salivary amylase enzyme.

Assignments

The students shall be asked to submit written assignments on various Pathology Lab Reports (One assignment per student per sessional period. i.e., a minimum of THREE assignments per student)

Strategy for Implementation:

It is suggested that 32-35% of experiments shall be completed before every sessional exam.

INSTRUCTIONS TO TEACHERS

Teachers shall explain the following points to the students before starting of the practicals:

1. Objectives of Learning: To foster better understanding of the subject and to inculcate the skills and attitude related to practicals.
2. Graphical Structure: In graphical structure, topics and subtopics are organized in a systematic way to achieve the ultimate purpose of learning the subject. This is arranged in the form of facts, concepts, principles, procedures, applications, and problems.
3. Elementary Guide to work in Laboratory: The methods and other finer details of the equipment including equipment specifications should be explained to avoid equipment breakages, create conducive environment for proper organizing of the practical work with the time schedule.
4. Teachers should verify and check the work conditions of the equipment and request the students to follow the standard operating procedures (SOP).
5. Before starting the practical, Teachers should explain the strategies of the experiment.
6. Teachers should ensure the active participation of students while performing the experiment.
7. Observations should be checked individually, and each student should be given a chance to perform the experiment.
8. Teachers should ask the students to complete the questions which are given at the end of the experiment accordingly.
9. Assessment of manuals should be done according to the assessment norms. Proper marks should be distributed according to the performance of the individuals.
10. Teachers should explain the competencies that students should achieve, in detail with their importance to students after completion of their course.
11. Apart from the syllabus, teachers should provide and cover extra topics which are beneficial for the students.
12. Explanations about various equipment with some interesting videos, reagents, chemicals, glassware's should be given to students prior to commencing the practical.
13. Teachers should observe the students when students are performing practicals in groups, proper contributions of the individual student should be there, and record of observation should be noted by all of them.
14. Teachers should also organize a visit to the pharmaceutical industries where students get a brief idea about the manufacturing processes of common dosage forms such as tablets, capsules, liquid orals, injectables, etc.
15. Teachers should also ask them to gather information about each type of dosage form, their generic name, branded names, and label contents.
16. Teachers may suggest the students refer to sources of information such as literature, research papers, books, attending conferences, seminars for the updating of knowledge.
17. According to the professional competencies given by PCI, teachers should develop the professional skills of the students.
18. Teachers should construct different types of sessions for students such as quizzes, group discussions projects on different topics, etc.
19. Teachers should ensure that revised CIAAN - 2017 norms or the latest norms given by MSBTE are followed simultaneously and implemented.
20. Teachers should follow the guidelines given by PCI & MSBTE from time to time.

BLOOMS TAXONOMY LEVELS

1 Knowledge

Define, Identify, Describe, Recognize, Tell, Explain, Recite, Memorize, Illustrate, Quote

2 Understand

Summarize, Interpret, Classify, Compare, Contrast, Infer, Relate, Extract, Paraphrase, Cite

3 Apply

Solve, Change, Relate, Complete, Use, Sketch, Teach, Articulate, Discover, Transfer

4 Analyze

Contrast, Relate, Devise, Distill, Correlate, Illustrate, Conclude, Categorize, Connect, Take apart

5 Evaluate

Criticize, Reframe, Judge, Defend, Appraise, Value, Prioritize, Plan, Grade,

6 Create

Design, Modify, Role-play, Develop, Rewrite, Pivot, Modify, Collaborate, Invent, Write

INSTRUCTIONS TO STUDENTS

Students should follow the instructions given below for better understanding of the subject from a theoretical and practical concept of view.

1. As per the instructions, the students should wear an apron, cap, mask, gloves and slippers before entering the lab.
2. The students should keep their important things in the locker which is provided by the college.
3. While entering the laboratory, the students should carry manuals, rough book and practical requirements as instructed.
4. Students should attend the practical regularly throughout the year, so as to understand the subject properly, and to develop the skills for performing the experiments and attaining the competencies.
5. The students should carry out the experiment individually and perform the experiment at the allotted specific work area.
6. The practical applications of every experiment should be noted by the students.
7. Students should answer the questions asked in the practicals and should ask the teacher about their difficulties without any hesitation.
8. After completion of practicals students should write the answers of the question given at the end of the experiment.
9. Students should develop different types of competencies to become competent Pharmacists.
10. Students should actively participate in group discussions, activities, etc. and strive to achieve the knowledge, skills, and attitude.
11. Students should submit the manual for assessing regularly on the scheduled date.
12. After completing the practical, the student should clean the platform and glassware that he has used.

LABORATORY MANUAL OF BIOCHEMISTRY AND CLINICAL PATHOLOGY

MAPPING OF COURSE OUTCOMES

Exp. No.	Title of Experiment	CO1	CO2
1	Introduction to Biochemistry and Clinical Pathology Laboratory	✓	✓
2	Qualitative analysis of carbohydrate (C1)	✓	
3	Qualitative analysis of carbohydrate (C2)	✓	
4	Qualitative analysis of carbohydrate (C3)	✓	
5	Qualitative analysis of carbohydrate (C4)	✓	
6	Qualitative analysis of carbohydrate (C5)	✓	
7	Qualitative analysis of protein (P1)	✓	
8	Qualitative analysis of protein (P2)	✓	
9	Qualitative analysis of a sample containing amino acid (AA1)	✓	
10	Qualitative analysis of a sample containing amino acid (AA2)	✓	
11	Qualitative analysis of lipid	✓	
12	Identification tests of cholesterol	✓	
13	Qualitative analysis of urine sample for normal constitutes (UN1)		✓
14	Qualitative analysis of urine sample for normal constitutes (UN2)		✓
15	Qualitative analysis of urine sample for abnormal constitutes (UA1)		✓
16	Qualitative analysis of urine sample for abnormal constitutes (UA2)		✓
17	Qualitative analysis of urine sample for abnormal constitutes (UA3)		✓
18	Estimation of glucose in urine sample		✓
19	Estimation of creatinine in urine sample		✓
20	Estimation of creatinine in human blood		✓
21	Estimation of glucose in human blood		✓
22	Estimation of cholesterol in human blood		✓
23	Estimation of calcium in human blood		✓
24	Estimation of urea in human blood		✓
25	Hydrolysis of starch	✓	

LIST OF EXPERIMENTS AND RECORD OF PROGRESSIVE ASSESSMENT

Exp. No.	Title of Experiment	Page No	Date of Performance	Date of Submission	Assessment Marks 10	Teacher's Signature
1	Introduction to Biochemistry and Clinical Pathology Laboratory					
2	Qualitative analysis of carbohydrate (C1)					
3	Qualitative analysis of carbohydrate (C2)					
4	Qualitative analysis of carbohydrate (C3)					
5	Qualitative analysis of carbohydrate (C4)					
6	Qualitative analysis of carbohydrate (C5)					
7	Qualitative analysis of protein (P1)					
8	Qualitative analysis of protein (P2)					
9	Qualitative analysis of a sample containing amino acid (AA1)					
10	Qualitative analysis of a sample containing amino acid (AA2)					
11	Qualitative analysis of lipid					
12	Identification tests of cholesterol					
13	Qualitative analysis of urine sample for normal constitutes (UN1)					
14	Qualitative analysis of urine sample for normal constitutes (UN2)					
15	Qualitative analysis of urine sample for abnormal constitutes (UA1)					
16	Qualitative analysis of urine sample for abnormal constitutes (UA2)					
17	Qualitative analysis of urine sample for abnormal constitutes (UA3)					
18	Estimation of glucose in urine sample					
19	Estimation of creatinine in urine sample					
20	Estimation of creatinine in human blood					
21	Estimation of glucose in human blood					

Exp. No.	Title of Experiment	Page No	Date of Performance	Date of Submission	Assessment Marks 10	Teacher's Signature
22	Estimation of cholesterol in human blood					
23	Estimation of calcium in human blood					
24	Estimation of urea in human blood					
25	Hydrolysis of starch					

I) PRACTICAL RECORD MARKS*:

Sessional Exam	Experiment No.		Total no. of experiment conducted	Average marks obtained for the experiment conducted. (out of 10)	Teacher's Signature
	From	To			
First Sessional					
Second Sessional					
Third Sessional					

*Sessional wise marks should be considered for internal assessment of practical sessional examinations (out of 10M)

II) ASSIGNMENT MARKS#:

Sr. No.	Title of Assignment	Marks out of 10#	Assignment Marks (Average of three)	Teacher's Signature
1				
2				
3				

#Marks should be transferred from Appendix -1 A typical format for assessment of an assignment.

Average Sessional Marks out of 10	Assignments Marks out of 10 (Average of three)	Total Marks out 20	Teacher's Signature

Experiment No. 1
Introduction to Biochemistry and Clinical Pathology Laboratory

1. Aim

To get introduced to Biochemistry and Clinical Pathology laboratory.

2. Practical Significance

The Biochemistry and Clinical Pathology laboratory plays a vital role in healthcare by analyzing blood, urine, and other body fluids using various chemicals, reagents, and equipment. These analyses help us to understand the biochemical changes and physiological alterations in the human body caused by diseases. Biochemical parameters not only aid in diagnosing diseases but also in managing them. However, for accurate treatment of the underlying disease, the test results must be highly precise. Therefore, it's crucial for students to understand the proper use of various laboratory tools, including colorimeters, spectrophotometers, and centrifuges. In this experiment, students will gain hands-on experience handling this equipment and collecting blood, urine, and other body fluid samples effectively.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle and working of colorimeter and centrifuge.	CO 2	BTL2
2	Handle the equipment: colorimeter, spectrophotometer, centrifuge.	CO 1 & 2	BTL2
3	State various types of biological specimens.	CO 1	BTL1
4	Collect the specimens of blood and urine.	CO1 & 2	BTL3

4. Relevant Theoretical Background

A. Handling of Equipment

In this laboratory, the equipment used for estimation of various biochemical parameters are: colorimeter, spectrophotometer and centrifuge.

a. Colorimeter:

Colorimetry is based on measurement of the intensity of the color. Quantitative estimation of substances can be achieved by measuring the intensity of their colored solutions. This technique is known as colorimetric analysis or colorimetry.

Many substances of biological and medical interest are colored but the colorless constituents can be converted into the colored compounds by subjecting them to the specific chemical reactions so that they can be detected by the Colorimeter. Colored substances present in a solution absorb light in a visible region of the spectrum; the amount of light absorbed depends on the intensity of the color. A high concentration of the substance indicates the high intensity of the color.

Beer-Lambert law states that the absorbance of a solution at a specific wavelength is directly proportional to its concentration and the distance of the light travels through the solution (path length). This law allows us to measure the concentration of an unknown (test) solution using a specific formula.

$$\text{Concentration of the test} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

The basic components of a photometer and spectrophotometer are

i. A light source
ii. Monochromator or filter
iii. Cuvette
iv. Photodetector and
v. Galvanometer.

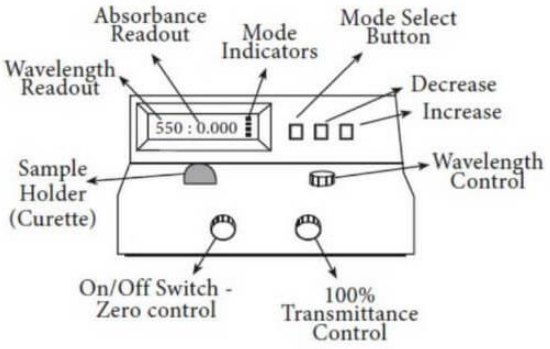


Fig 1.1: Colorimeter

Distinguishing Photometers, Spectrophotometers, and Colorimeters:

Photometers: These instruments use light filters to select a specific wavelength for photometric measurements.

Spectrophotometers: In contrast, spectrophotometers employ a prism or diffraction grating with a slit to achieve monochromatic light across a wider range of wavelengths.

Colorimeters: Colorimeters are a type of photometer equipped with colored filters typically ranging from 400 to 700 nanometers (nm). These filters allow them to isolate specific wavelengths:

- Blue filter: transmits light around 425 nm (appears blue)
- Green filter: transmits light around 525 nm (appears green)
- Red filter: transmits light around 690 nm (appears red)

By using these colored filters, colorimeters can perform basic analyses based on the intensity of light absorbed at specific wavelengths.

b. Spectrophotometer:

When incident light travels through a sample solution, a spectrophotometer, like a colorimeter, measures the absorbance of light intensity. The basic principle of a colorimeter and a spectrophotometer is the same. The spectrophotometer also covers the ultraviolet (UV) region range (200–400 nm) in addition to the visible range. Unlike absorbance readings at a fixed wavelength in a colorimeter, the absorbance in a spectrophotometer can be recorded over a wide range of wavelengths, even at every 5 or 10 nm intervals. It provides a data spectrum instead of a specific absorbance reading.

Spectrophotometers come in two types: single beam and double beam. The operation of a single beam spectrophotometer is the same as that of a colorimeter. In a double beam spectrophotometer, the light from the source is split into two beams. One beam illuminates the reference or blank sample, and the other beam passes through the sample under

consideration. The sample cuvette used in a spectrophotometer is made of quartz instead of a glass cuvette as in a colorimeter.

c. Centrifuge:

A centrifuge is a device that uses centrifugal force to separate components of blood or other fluids. The denser particles will settle at the bottom, while lightweight particles will be on the top. Before using the centrifuge, make sure the blood sample is collected in a tube with an appropriate anticoagulant, then properly close it with the lid and place it in one of the cavities of the centrifuge. To maintain balance in the centrifuge, fill the opposite side cavity with a test tube containing other samples or distilled water. Then, allow the centrifuge to rotate at a constant speed, typically 4000-5000 rpm, for a specified time.

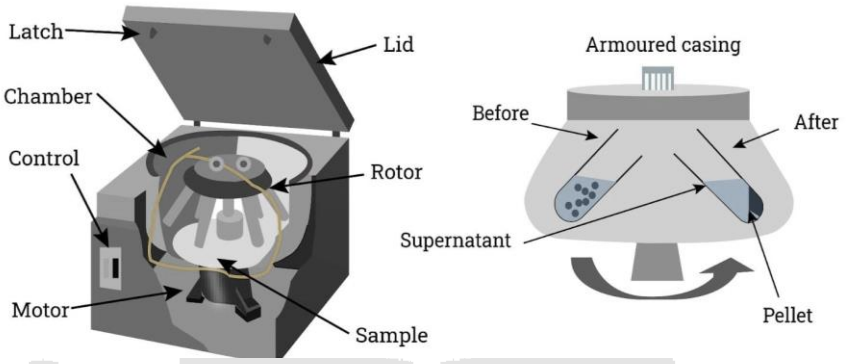


Fig 1.2: Centrifuge

B. Collection of specimens for analysis:

The important biological specimens collected for biochemical estimation include:

a. Blood (Whole blood, plasma, serum)
b. Urine
c. Faeces
d. Cerebrospinal fluid
e. Other Body fluids like saliva, etc.

A. **Blood / Plasma / Serum - Specimen Collection:**

A whole blood sample contains plasma, red blood cells (RBCs), white blood cells (WBCs), and platelets. Typically, 3–5 mL of blood with an appropriate anticoagulant is required for many investigations. Commonly used anticoagulants include EDTA, heparin, sodium fluoride, sodium or potassium oxalate, etc. Special tubes used for blood collection are called Vacutainers.

Plasma is blood minus cells. Blood is added to plasma-separating tubes containing ethylenediaminetetraacetic acid (EDTA), mixed properly, and allowed to stand for 10 minutes. Then, it is centrifuged at 200 rpm; the supernatant liquid is plasma.

Serum is blood minus the cells and clotting factors. To collect serum, blood is drawn from the patient and added to a vacutainer without anticoagulant. After 20-30 minutes, a blood clot forms, and it is centrifuged at 3000 rpm; the supernatant liquid is serum.

Commonly used anticoagulants:

Anticoagulant	Requirement	Mechanism
Heparin	2mg/10mL	Heparin inhibits conversion of prothrombin to thrombin.
EDTA	2-10mg/mL	Calcium chelation.
Oxalate salts	2-3mg/mL	Calcium precipitation.

B. **Urine - Specimen Collection:**

To obtain accurate analytical results, care must be taken in the collection and transportation of urine to the laboratory. Urine should be collected in clean, sterile containers with tightly fitting lids to avoid spillage. The best urine specimen is the first voided urine in the morning, as it is the most concentrated. A midstream specimen should be collected.

5. Requirements

Centrifuge, Colorimeter, Spectrophotometer, Cuvettes, Beakers, Pipettes, Test Tubes, Common laboratory reagents.

6. Requirements used

_____

_____

7. Precautions to be taken

A. Handling the Centrifuge:

a. Balance the load each time the centrifuge is used.
b. Do not overfill the centrifuge tubes.
c. Avoid opening the lid while the rotor is moving.
d. Follow the manufacturer's instructions for safe operating speeds; do not run a rotor beyond its maximum rated speed.

B. Handling the Calorimeter:

a. Avoid vigorous stirring of the liquid, as it can cause an increase in temperature.
b. Dry the cuvette from the outside before placing it in the calorimeter.
c. Turn off power sources, clean up any spills or debris, and secure the equipment according to laboratory protocols.

C. Handling the Spectrophotometer:

a. Use clean, clear cuvettes for samples, making sure they are free of bubbles, scratches, and fingerprints.
b. Always measure a blank sample to set a baseline for accurate readings.
c. Turn off the spectrophotometer after use and cover it with a dust cover to protect it from dust and contaminants.
d. Always follow the manufacturer's instructions and guidelines for the specific spectrophotometer model being used.

8. Procedure

a. Observe the demonstration given by the teacher about various equipment and record the information in the observation table.
b. Take a round in the laboratory with the teacher and understand the general workings in the laboratory.
c. Observe the charts and diagrams displayed in the laboratory.

d. Understand the general precautions to be followed while working in the laboratory.
e. Seek a demonstration of the fire extinguisher mounted on the wall of the laboratory from the teacher.

9. Observations

a. Name the parts of a Photoelectric colorimeter and write its functions.

Sr. No.	Name of Part	Function
1		
2		
3		

b. Name the parts of the Centrifuge and write its functions.

Sr. No.	Name of Part	Function
1		
2		
3		

c. Name and write formula of common laboratory reagents.

Sr. No.	Name of Common laboratory reagent	Formula
1		
2		
3		
4		
5		
6		
7		
8		

10. References

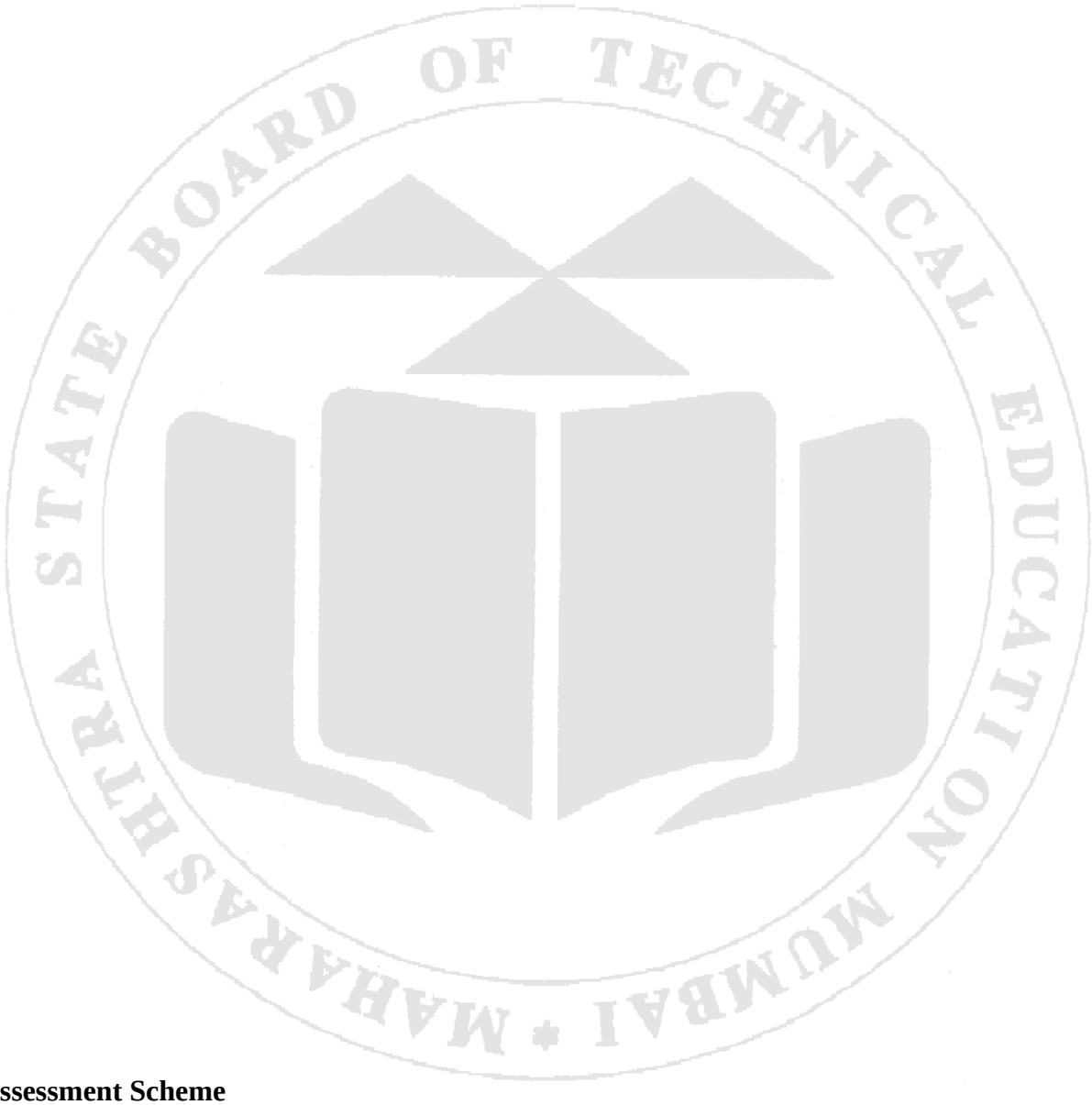
a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (P) Ltd, First edition.
b. Basic Concepts in Clinical Biochemistry: A Practical Guide, Vijay Kumar, Kiran Dip Gill, Springer Nature Singapore Pte Ltd. 2018.

11. Related questions

a. Name the different types of balances available in the laboratory for weighing.
b. Write the applications of colorimeter and centrifuge.
c. State the difference between a colorimeter and a spectrophotometer.
d. Give the difference between plasma and serum.

e. Enlist the anticoagulants used in blood collection.

(Space for Answers)



12. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 2
Qualitative Analysis of Carbohydrate (C1)

1. Aim
To identify the given sample of carbohydrates by qualitative test (C1)

2. Practical Significance
The qualitative analysis of carbohydrate is a significant tool for identifying and classifying them. This analysis depends on color changes observed following specific chemical reactions. Carbohydrates are organic biomolecules with the general formula $C_n(H_2O)_n$. They are composed primarily of three elements: carbon, hydrogen, and oxygen, with a 2:1 ratio of hydrogen to oxygen. Qualitative analysis of carbohydrate involves using specific reagents to detect their presence based on the reaction between the test sample and the reagent. This reaction typically produces a characteristic color, allowing us to identify the presence of carbohydrates. After completing this experiment, students will be able to identify and confirm the presence of specific carbohydrate in a given sample.

3. Practical Outcomes (PrO)
The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind the specific test	CO 1	BTL2
2	Write analytical reports systematically.	CO 1	BTL2
3	Observe and distinguish the colour while performing various chemicals tests.	CO 1	BTL2
4	Identify and confirm the specific carbohydrate present in the given sample.	CO1	BTL4

4. Relevant Theoretical Background
Carbohydrates are naturally occurring organic compounds containing carbon, hydrogen, oxygen elements. Chemically, carbohydrates may be defined as polyhydroxy-aldehydes or ketones or compounds which produce units of such type on hydrolysis. Carbohydrates are also referred to as saccharides which is a Greek word that means sugar because nearly all carbohydrates have a sweet taste.

Classification based on chemical composition:

A. **Monosaccharides** $C_n(H_2O)_n$ (Cannot be hydrolyzed)
   a. Aldose (Contain Aldehyde group): Glucose.
   b. Ketose (Contain Ketone group): Fructose.

B. **Disaccharides** $C_n(H_2O)_{n-1}$ (Hydrolyzed to two units of monosaccharides): Sucrose, Lactose, Maltose.

C. **Oligosaccharides** (Hydrolyzed to three units of monosaccharides): Raffinose.

D. **Polysaccharides** $(C_6H_{10}O_5)_x$ (Hydrolyzed to three to ten units of monosaccharides)
   a. Homopolysaccharide (Hydrolyzed to only one type of monosaccharide): Starch, Glycogen.
   b. Heteropolysaccharides (Hydrolyzed to two or more type of monosaccharides): Agarose.

5. Requirements

Equipment: Microscope, Electric water bath.

Glassware: Test tubes, Test tube holder, Beakers, Funnel, Graduated Pipettes, Tripod Stand, Filter Paper, Watch Glass, Slide.

Chemicals: Molisch's reagent, Conc. H_2SO_4, 0.02N Iodine solution, Fehling's solution A, Fehling's solution B, Benedict's reagent, Tommer's reagent, Barfoed's reagent, Seliwanoff's reagent, α-naphthol solution (1% in alcohol), Conc. HCl, Conc. HNO_3, Phenyl hydrazine hydrochloride, Acetate buffer (pH 5.0), Sodium hydroxide solution, Methylene blue solution, Ammoniacal silver nitrate solution, Fougler's reagent.

Reagents Used for Qualitative Analysis

a. Molisch reagent – 1% α-naphthol in alcohol.
b. Fehling's solution A – 6.92% of copper sulphate in distilled water.
c. Fehling's solution B – 250 g sodium hydroxide and 320 g sodium potassium tartarate in 500 mL water.
d. Benedict's reagent – Dissolve 173 g sodium citrate and 100 g anhydrous sodium carbonate in about 800 mL water, separately dissolve 17.3 g copper sulphate in 100 mL water, mix both the solution and make volume to 1000 mL with water.
e. Barfoed's reagent - 13.3 g copper acetate in 200 mL water, filter and then add to it 1.8 mL glacial acetic acid.
f. Seliwanoff's reagent - Dissolve 50 mg of resorcinol in 33 mL concentrated hydrochloric acid and dilute it to 1000 mL with water.
g. Fougler's reagent - Dissolve 40 g urea in 80 mL of 40% w/w sulphuric acid, add to it 2 g stannous chloride and boil till clear solution is obtained. Cool make volume to 40 mL with 40% w/w sulphuric acid.
h. Tommer's reagent - 5 % copper sulphate in water and equal volume of sodium hydroxide solution.

6. Requirements used

_____

_____

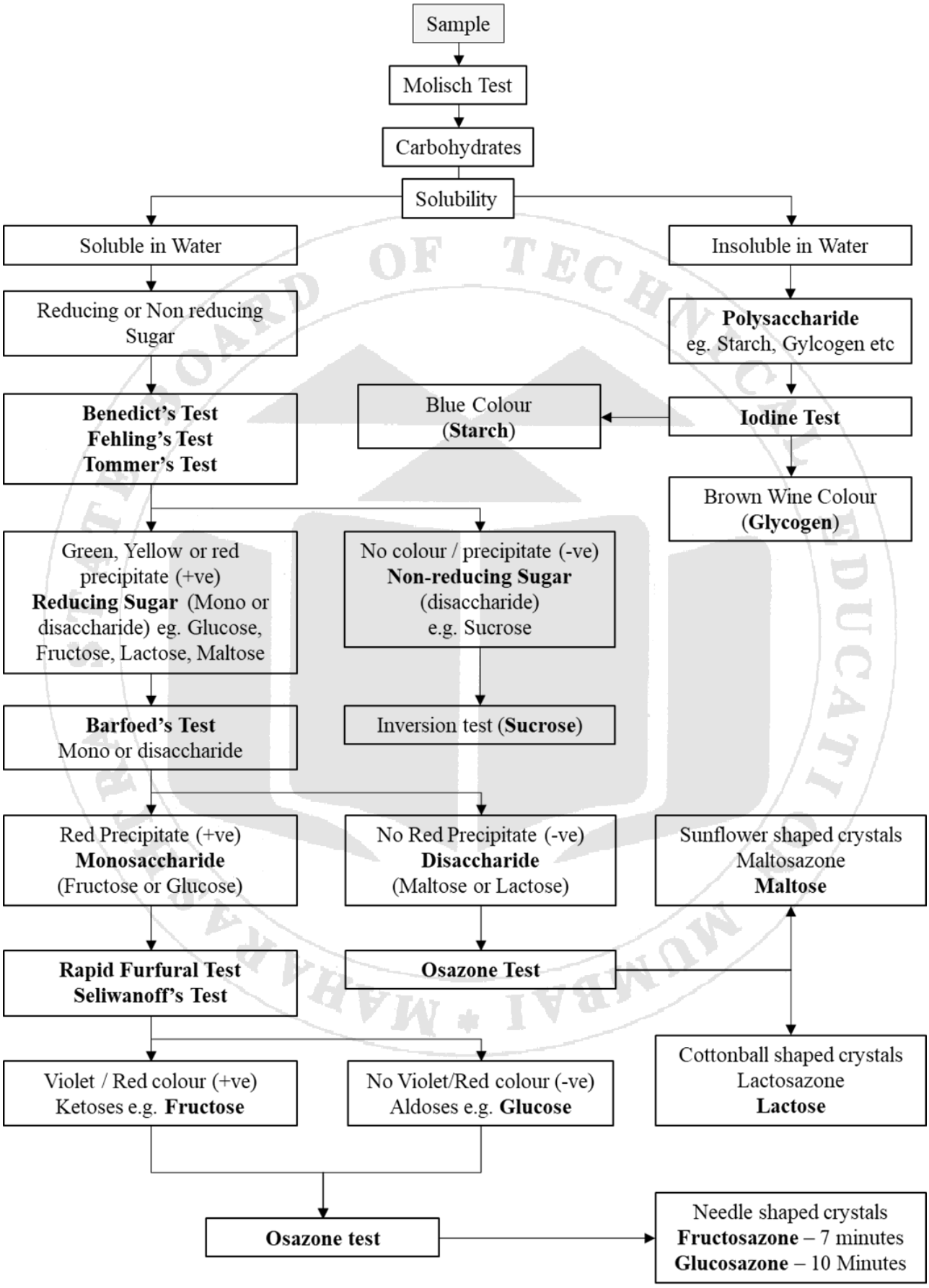
7. Precautions to be taken

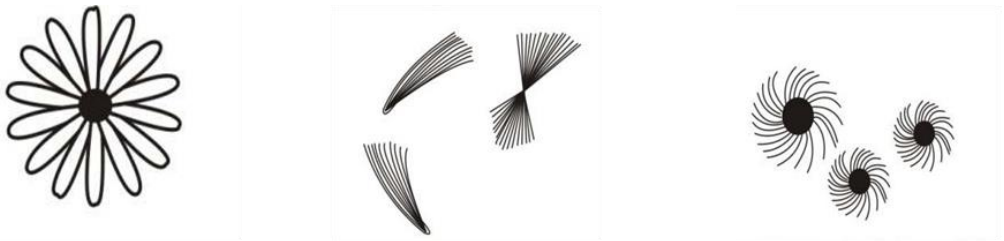
a. The solutions/reagents used for this experiment should be prepared by using distilled water.
b. Do not suck acids or other chemicals by mouth; use a pipette aid or suction bulb.
c. When mixing acid and water, add concentrated acid to water dropwise while stirring.

8. Procedure

a. Follow the general scheme outlined for qualitative analysis of carbohydrates.
b. **Sugar / Sample solution:** Dissolve 1 g of the given sample of carbohydrate in 20 ml of water. This sugar solution will be used to perform chemical tests for the identification of carbohydrates.
c. Perform a step-by-step chemical test on an unknown carbohydrate sample using a chart to identify the given sample.
d. Write your findings for the identification of the unknown carbohydrate sample in a given observation table.

General scheme for qualitative analysis of carbohydrates





Sunflower like Crystals (Maltosazone crystals)

Needle like Crystals (Glucosazone or Fructosazone crystals)

Cotton ball like Crystals (Lactosazone crystals)

Fig 2.1: Osazone crystals in Microscope

General scheme for qualitative analysis of carbohydrates

Test	Observation	Inference
1. Molisch's Test 2 mL of given sample solution + 3-5 drops of Molisch's reagent. Mix and incline the test tube slightly and add conc. Sulphuric acid (2 mL). (Do not shake the test tube, while adding the acid).	A violet / purple ring at the junction of two liquids	Carbohydrates present.
2. Solubility Test Carbohydrate sample + Water.	Soluble	Mono and Disaccharides present.
	Insoluble	Polysaccharides present.
3. Iodine Test (For insoluble sample) Add a few drops of N/50 iodine solution to 2 mL of the sample solution and mix.	Blue colour appears	Starch is present.
	Brown wine colour appears	Glycogen is present.
4. Fehling's Test Mix 1 mL each of Fehling's solution A and B in a test tube. Add a few drops of test sample/solution and mix. Heat the tube in a boiling water bath for 5 to 10 min.	Appearance of yellow or brick red color.	Reducing sugar is present.
5. Benedict's Test Add a 8-10 drops of test sample solution to 5 mL of Benedict's qualitative reagent taken in a test tube and place the tube in boiling water for 5 to 10 minutes. Allow it to cool.	Appearance of green/ yellow /orange/ red color precipitate.	Reducing sugar is present.
6. Tommer's Test Add 2 mL of test sample solution to 2 mL of Tommer's reagent and place the tube in boiling water for 2 minutes. Allow it to cool.	Appearance of red / yellow precipitate.	Reducing sugar is present.
7. Barfoed's Test 2 mL of the Barfoed's reagent taken	Appearance of brick red precipitate at the bottom of	Presence of reducing monosaccharide. (If the

Test	Observation	Inference
in a test tube, add 2 mL of the test sample solution and heat the contents in a boiling water bath. Allow it to cool	the test tube within 5 minutes.	time taken for the formation of the precipitate is more, it is suggestive of a reducing disaccharide.)
8. Seliwanoff's Test Add a 2 mL of test sample solution to 3 mL of the Seliwanoff's reagent taken in a test tube and place the tube in a boiling water bath for 5 minutes. Allow it to cool.	Appearance of red colour or red precipitate.	Presence of ketose sugar like fructose.
9. Rapid furfural Test Add 2 mL of test sample solution to 1 ml of α-naphthol solution (1% in alcohol) and add 5 mL conc HCl. Heat the content in a boiling water bath.	Appearance of deep purple colour.	Presence of ketose sugar like fructose.
10. Osazone Test Take 5 mL of the sugar solution in a test tube, add 0.5 g of phenyl hydrazine hydrochloride, add 0.1 g of sodium acetate and 8-10 drops of glacial acetic acid. The contents are mixed well and placed in a boiling water bath. Cool the solution to room temperature and transfer a few crystals onto a glass slide, cover it with a cover slip and observe the shape of the crystals under a light microscope.	Needle shaped yellow crystals of fructosazone are formed within 5 – 7 minutes.	Presence of fructose.
	Needle shaped yellow crystals of glucosazone are formed within 10 minutes.	Presence of glucose.
	Sunflower shaped crystals of maltosazone are formed in 30 minutes.	Presence of maltose.
	Cotton ball shaped crystals of lactosazone formed.	Presence of lactose.

Confirmatory Tests for Carbohydrates

A. Confirmatory test for Glucose

Test	Observation	Inference
a) Osazone test	Needle shaped yellow crystals formed in 10 minutes	Glucose confirmed
b) Add 2 mL of test sample solution to 2 mL of 5% NaOH solution.	Appearance of brown resinous ppt	Glucose confirmed
c) Take 3 mL of water, add a few drops of methylene blue solution, 1 mL of 5% NaOH and 2 mL test sample solution and boil.	Solution is decolorized	Glucose confirmed
d) Add 3 mL of test sample solution to 1 ml of picric acid solution and 1 mL of 5% NaOH and boil.	Appearance of red colour	Glucose confirmed

B. Confirmatory test for Lactose

Test	Observation	Inference
a) Osazone test	Cotton ball shaped crystals	Lactose confirmed

Test	Observation	Inference
b) Mucic acid test: To 5 mL of the test sample solution in a test tube, add 2 mL of mucic acid reagent (conc. Nitric acid.) Heat the test tube in a water bath until the volume of solution is reduced to 2-3 mL. Allow it to cool. Let the test tube sit overnight before collecting the results.	Formation of crystal at the bottom of the tube	Lactose confirmed

C. Confirmatory test for Sucrose

Inversion test (Sucrose hydrolysis test) - Take 5 mL of test sample solution in a test tube. Add 1mL of Conc. HCl and boil it for 3 minutes. Cool it. Neutralize it with 20% Sodium carbonate solution by drop wise addition. Now this neutralized solution should be used for performing following test.

Test	Observation	Inference
a) Benedict's Test	Appearance of green/ yellow/ orange/ red color precipitate	Sucrose confirmed
b) Fehling's Test	Appearance of yellow or brick red color	Sucrose confirmed

D. Confirmatory test for Fructose

Test	Observation	Inference
a) Osazone test	Needle shaped yellow crystals formed in 7 minutes	Fructose confirmed
b) Fougler's Test To 3 mL Fougler's reagent, add 0.5 mL of test sample solution. Boil it for 1 minute and allow it to cool	Appearance of blue colour	Fructose confirmed

E. Confirmatory test for Starch

Test	Observation	Inference
Half Saturation Test: Take 5 mL of starch solution in the test tube. Add 5 mL of saturated ammonium sulphate solution. Shake it vigorously and allow it to stand for 5 minutes.	Appearance of white precipitate.	Starch confirmed
Filter it and perform an iodine test on filtrate.	No Blue colour	Starch confirmed

F. Confirmatory test for Maltose

Test	Observation	Inference
a) Osazone test	Sunflower shaped crystals of maltosazone formed in 30 minutes	Maltose confirmed

9. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

Test	Observation	Inference

10. Result

The given carbohydrates sample was found to be _____. It is _____ (Monosaccharide / Disaccharide / Polysaccharide).

11. Conclusion

The given sample of carbohydrate is identified by qualitative analysis.

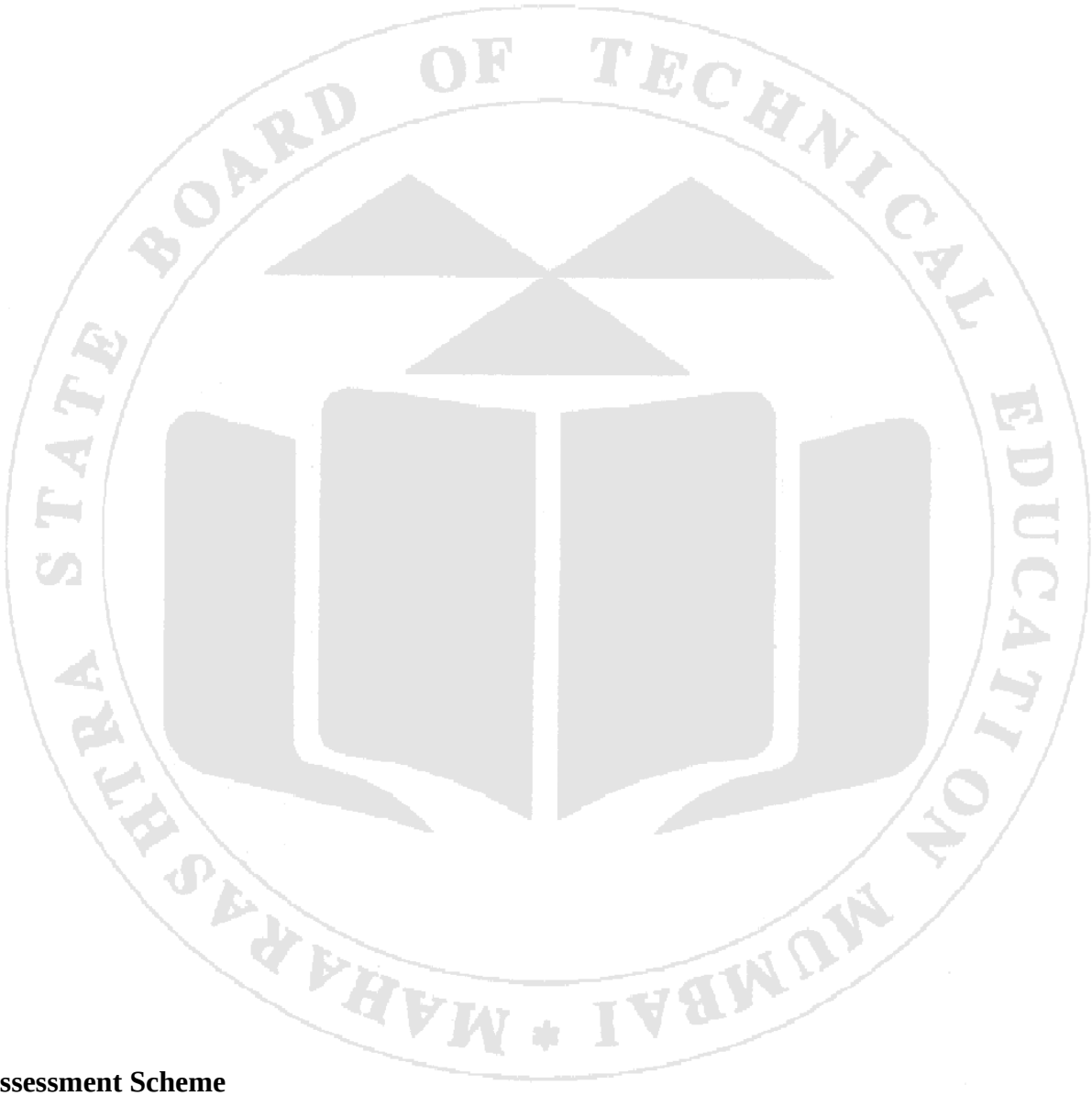
12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.
b. Practical Biochemistry, G. Rajagopal, B.D. Toora, Ahuja publishing house, fourth edition.
c. Indian Pharmacopoeia 2018

13. Related questions

a. Give two differences between Mono and Disaccharides?
b. Write the principle of the Molisch test.
c. What is the significance of the Iodine test?
d. Write reactions involved in the Osazone test

(Space for Answers)



14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 3
Qualitative Analysis of Carbohydrate (C2)

1. Aim

To identify the given sample of carbohydrate by qualitative tests (C2)

2. Practical Significance

The qualitative analysis of carbohydrate is a significant tool for identifying and classifying them. This analysis depends on color changes observed following specific chemical reactions. Carbohydrates are organic biomolecules with the general formula $C_n(H_2O)_n$. They are composed primarily of three elements: carbon, hydrogen, and oxygen, with a 2:1 ratio of hydrogen to oxygen. Qualitative analysis of carbohydrates involves using specific reagents to detect their presence based on the reaction between the test sample and the reagent. This reaction typically produces a characteristic color, allowing us to identify the presence of carbohydrates. After completing this experiment, students will be able to identify and confirm the presence of specific carbohydrate in a given sample.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind the specific test	CO 1	BTL2
2	Write analytical reports systematically.	CO 1	BTL2
3	Observe and distinguish the colour while performing various chemicals tests.	CO 1	BTL2
4	Identify and confirm the specific carbohydrate present in the given sample.	CO1	BTL4

4. Relevant Theoretical Background

Qualitative analysis of carbohydrate

There are various tests used in the qualitative analysis of carbohydrates, such as the Molisch test, Benedict's test, Tommer’s test, Iodine test, Barfoed's test, and the Seliwanoff test (resorcinol test). In qualitative analysis, the results are obtained based on the formation of specific complexes due to reactions. These complexes are detected by observing changes in the color of the sample solution.

A. Molisch test

The Molisch test is used to distinguish between carbohydrates and non-carbohydrates. It is a preliminary test used to detect the presence of carbohydrates in a sample. Strong H_2SO_4 hydrolyzes carbohydrates (poly- and disaccharides) to liberate monosaccharides. These monosaccharides undergo dehydration to form furfural (from pentoses) or hydroxymethylfurfural (from hexoses), which then condense with α-naphthol to form a violet-colored complex.

B. Benedict’s test:

The Benedict test is used to identify reducing sugars that have a free ketone or aldehyde functional group. This test specifically targets reducing sugars, which predominantly form enediols under alkaline conditions. The enediol forms of sugars reduce cupric ions (Cu^{2+}) from copper sulfate to cuprous ions (Cu+), resulting in the formation of either a yellow precipitate of cuprous hydroxide or a red precipitate.

C. Fehling's test

Fehling's test is a chemical test used to detect the presence of reducing sugars, particularly aldehyde functional groups in monosaccharides and some disaccharides. The test involves two separate solutions, Fehling's A (containing copper sulfate) and Fehling's B (containing potassium sodium tartrate and sodium hydroxide). When these solutions are mixed and heated with a reducing sugar, such as glucose, the aldehyde group of the sugar reduces the copper ions in Fehling's solutions, leading to the formation of a reddish-brown precipitate of copper(I) oxide. This color change indicates a positive result for the presence of a reducing sugar.

D. Iodine test

The iodine test is used to detect starch in a given sample and to differentiate between starch, glycogen, and other carbohydrates. Polysaccharides react with iodine to form a colored complex. Starch specifically gives a blue color, while dextrins give a red color when combined with iodine.

E. Barfoed’s test

Barfoed’s test is primarily used for detecting the presence of monosaccharides or disaccharides in a given sample. The principle of this test is similar to that of Benedict’s test, except that the reduction occurs in a mildly acidic medium. Since an acidic environment is not conducive to reduction, only strong reducing sugars (monosaccharides) yield a positive result in this test. Therefore, Barfoed's test is a crucial reaction for distinguishing between monosaccharides and disaccharides.

F. Seliwanoff’s test

The Seliwanff’s, also known as the Resorcinol test, is used to differentiate between sugars containing a ketone group (ketoses) and those containing an aldehyde group (aldoses). This test specifically targets ketohexoses. Concentrated hydrochloric acid dehydrates ketohexoses to form furfural derivatives, which then condense with resorcinol to produce a cherry red complex.

G. Rapid furfural test

The rapid furfural test is used specifically for the detection of ketohexoses. In this test, ketohexoses are converted to furfural derivatives upon treatment with hydrochloric acid (HCl). These furfural derivatives then react with α-naphthol, forming a distinctive, purple-colored complex. This color change indicates a positive result for the presence of ketohexoses in a sample.

H. Foulger's test

Foulger’s is a chemical test used to detect the presence of ketohexoses. In this test, the ketohexoses react with urea in the presence of stannous chloride, leading to the formation of a blue-colored complex. This color change serves as an indicator for the presence of ketohexoses in the sample.

I. Inversion test (Sucrose hydrolysis test):

The inversion test, also known as the sucrose hydrolysis test, is a chemical method used to detect the presence of sucrose and evaluate a substance's ability to break down sucrose into glucose and fructose. Since sucrose is a non-reducing sugar, it does not yield positive results in Benedict's and Barfoed's tests. However, sucrose can be hydrolyzed by concentrated HCl to convert it into glucose and fructose, which reduce monosaccharides that respond to the

reducing reactions. Following sucrose hydrolysis, the medium needs to be made alkaline by adding Na_2CO_3 for an effective reduction process.

J. Osazone test:

The osazone test is a chemical method utilized for identifying and characterizing reducing sugars, particularly monosaccharides and some disaccharides. This test involves the reaction of the sugar with phenylhydrazine hydrochloride in the presence of sodium acetate under heat. The resulting reaction forms osazones, which are crystalline derivatives of sugar and phenylhydrazine. The distinct morphology of osazone crystals aids in determining the specific type of sugar in the sample. This test is crucial in differentiating sugars based on their structural variations and finds common use in carbohydrate analysis and biochemistry laboratories.

When phenylhydrazine in acetic acid is boiled with reducing sugars, osazones are formed, with the involvement of the first two carbons (C_1 and C_2). Sugars differing in configuration at these carbons produce the same type of osazones due to their interaction with phenylhydrazine. For instance, glucose, fructose, and mannose yield needle-shaped osazones. In contrast, reducing disaccharides like maltose form sunflower-shaped osazones, while lactose results in powder-puff-shaped osazones, demonstrating the specificity of osazone morphology in sugar differentiation

5. Requirements

Equipment: Microscope, Electric water bath.

Glassware: Test tubes, Test tube holder, Beakers, Funnel, Graduated Pipettes, Tripod Stand, Filter Paper, Watch Glass, Slide.

Chemicals: Molisch's reagent, Conc. H_2SO_4, 0.02N Iodine solution, Fehling's solution A, Fehling's solution B, Benedict's reagent, Tommer's reagent, Barfoed's reagent, Seliwanoff's reagent, α-naphthol solution (1% in alcohol), Conc. HCl, Conc. HNO_3, Phenyl hydrazine hydrochloride, Acetate buffer (pH 5.0), Sodium hydroxide solution, Methylene blue solution, Ammoniacal silver nitrate solution, Fougler's reagent.

6. Requirements used

_____

_____

7. Precautions to be taken

a. The solutions/reagents used for this experiment should be prepared by using distilled water.
b. Do not suck acids or other chemicals by mouth; use a pipette aid or suction bulb.
c. When mixing acid and water, add concentrated acid to water dropwise while stirring.

8. Procedure

a. Follow the general scheme outlined for qualitative analysis of carbohydrates.
b. **Sugar / Sample solution:** Dissolve 1 g of the given sample of carbohydrate in 20 ml of water. This sugar solution will be used to perform chemical tests for the identification of carbohydrates.
c. Perform a step-by-step chemical test on an unknown carbohydrate sample using a chart to identify the given sample. (Refer Experiment No. 2)

d. Write your findings for the identification of the unknown carbohydrate sample in a given observation table.

9. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

10. Result

The given carbohydrates sample was found to be _____. It is _____ (Monosaccharide / Disaccharide / Polysaccharide).

11. Conclusion

The given sample of carbohydrate is identified by qualitative analysis.

12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.
b. Practical Biochemistry, G. Rajgopal, B.D. Torra, Ahuja's publishing house, fourth edition.
c. Indian Pharmacopoeia 2018

13. Related questions

a. Name the reducing disaccharides and give its hydrolysis product.
b. Draw the structure of Sucrose and Fructose.
c. What is the significance of Seliwanoff's test?
d. Name the test used to distinguish between monosaccharides and disaccharides. Write its procedure.

(Space for Answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 4
Qualitative Analysis of Carbohydrate (C3)

1. Aim

To identify the given sample of carbohydrate by qualitative tests (C3)

2. Practical Significance

The qualitative analysis of carbohydrate is a significant tool for identifying and classifying them. This analysis depends on color changes observed following specific chemical reactions. Carbohydrates are organic biomolecules with the general formula $C_n(H_2O)_n$. They are composed primarily of three elements: carbon, hydrogen, and oxygen, with a 2:1 ratio of hydrogen to oxygen. Qualitative analysis of carbohydrates involves using specific reagents to detect their presence based on the reaction between the test sample and the reagent. This reaction typically produces a characteristic color, allowing us to identify the presence of carbohydrates. After completing this experiment, students will be able to identify and confirm the presence of specific carbohydrate in a given sample.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind the specific test	CO 1	BTL2
2	Write analytical reports systematically.	CO 1	BTL2
3	Observe and distinguish the colour while performing various chemicals tests.	CO 1	BTL2
4	Identify and confirm the specific carbohydrate present in the given sample.	CO1	BTL4

4. Relevant Theoretical Background

Structure of Carbohydrates

Carbohydrates are organic molecules composed of carbon (C), hydrogen (H), and oxygen (O) atoms, typically with the general formula $(CH_2O)_n$. They are classified based on their structure and complexity into monosaccharides, disaccharides, oligosaccharides, and polysaccharides.

A. Monosaccharides

Monosaccharides are the simplest form of carbohydrates consisting of a single sugar molecule. They are classified based on the number of carbon atoms and the type of carbonyl group (aldehyde or ketone).

Glucose: An aldohexose with the molecular formula $C_6H_{12}O_6$. It has an aldehyde group (-CHO) and six carbon atoms.

Fructose: A ketohexose with the molecular formula $C_6H_{12}O_6$. It has a ketone group (C=O) and six carbon atoms.

B. Disaccharides

Disaccharides consist of two monosaccharide units linked by a glycosidic bond.

Sucrose: Composed of glucose and fructose units. The glycosidic bond is between the α1 carbon of glucose and the β2 carbon of fructose.

Lactose: Composed of glucose and galactose units. The glycosidic bond is between the β1 carbon of galactose and the β4 carbon of glucose.

C. Oligosaccharides

Oligosaccharides consist of 3 to 10 monosaccharide units linked by glycosidic bonds. They play important roles in cell recognition and signaling.

Example: Raffinose (a trisaccharide) is composed of galactose, glucose, and fructose units.

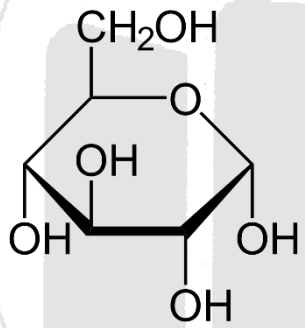
D. Polysaccharides

Polysaccharides are complex carbohydrates composed of many monosaccharide units linked by glycosidic bonds. They serve as energy storage and structural components.

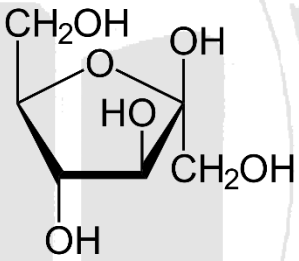
Starch: A storage polysaccharide in plants composed of amylose (unbranched) and amylopectin (branched) chains of glucose.

Glycogen: A storage polysaccharide in animals, highly branched and composed of glucose units.

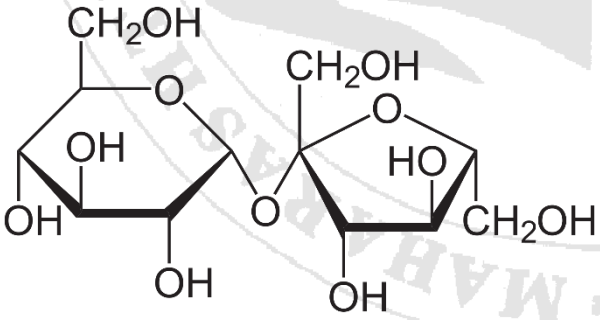
Cellulose: A structural polysaccharide in plant cell walls composed of β-glucose units linked by β1→4 glycosidic bonds.



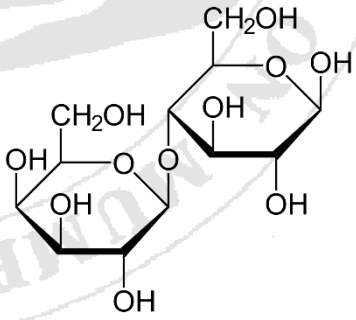
Glucose



Fructose



Sucrose



Lactose

5. Requirements

Equipment: Microscope, Electric water bath.

Glassware: Test tubes, Test tube holder, Beakers, Funnel, Graduated Pipettes, Tripod Stand, Filter Paper, Watch Glass, Slide.

Chemicals: Molisch's reagent, Conc. H_2SO_4, 0.02N Iodine solution, Fehling's solution A, Fehling's solution B, Benedict's reagent, Tommer's reagent, Barfoed's reagent, Seliwanoff's reagent,

α-naphthol solution (1% in alcohol), Conc. HCl, Conc. HNO_3, Phenyl hydrazine hydrochloride, Acetate buffer (pH 5.0), Sodium hydroxide solution, Methylene blue solution, Ammoniacal silver nitrate solution, Fougler's reagent.

6. Requirements used

_____

_____

7. Precautions to be taken

a. The solutions/reagents used for this experiment should be prepared by using distilled water.
b. Do not suck acids or other chemicals by mouth; use a pipette aid or suction bulb.
c. When mixing acid and water, add concentrated acid to water dropwise while stirring.

8. Procedure

a. Follow the general scheme outlined for qualitative analysis of carbohydrates.
b. **Sugar / Sample solution:** Dissolve 1 g of the given sample of carbohydrate in 20 ml of water. This sugar solution will be used to perform chemical tests for the identification of carbohydrates.
c. Perform a step-by-step chemical test on an unknown carbohydrate sample using a chart to identify the given sample. (Refer Experiment No. 2)
d. Write your findings for the identification of the unknown carbohydrate sample in a given observation table.

9. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

10. Result

The given carbohydrates sample was found to be _____. It is _____ (Monosaccharide / Disaccharide / Polysaccharide).

11. Conclusion

The given sample of carbohydrate is identified by qualitative analysis.

12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.
b. Practical Biochemistry, G. Rajgopal, B.D. Torra, Ahuja's publishing house, fourth edition.
c. Indian Pharmacopoeia 2018

13. Related questions

a. How is the rapid furfural test used to detect specific types of sugars?
b. Define two diseases related to abnormal metabolism of glucose.
c. How does the osazone test help in identifying specific types of sugars?
d. What is the purpose of the iodine test in carbohydrate qualitative analysis?

(Space for Answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 5
Qualitative Analysis of Carbohydrate (C4)

1. Aim

To identify the given sample of carbohydrate by qualitative tests (C4)

2. Practical Significance

The qualitative analysis of carbohydrate is a significant tool for identifying and classifying them. This analysis depends on color changes observed following specific chemical reactions. Carbohydrates are organic biomolecules with the general formula $C_n(H_2O)_n$. They are composed primarily of three elements: carbon, hydrogen, and oxygen, with a 2:1 ratio of hydrogen to oxygen. Qualitative analysis of carbohydrates involves using specific reagents to detect their presence based on the reaction between the test sample and the reagent. This reaction typically produces a characteristic color, allowing us to identify the presence of carbohydrates. After completing this experiment, students will be able to identify and confirm the presence of specific carbohydrate in a given sample.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind the specific test	CO 1	BTL2
2	Write analytical reports systematically.	CO 1	BTL2
3	Observe and distinguish the colour while performing various chemicals tests.	CO 1	BTL2
4	Identify and confirm the specific carbohydrate present in the given sample.	CO1	BTL4

4. Relevant Theoretical Background

Refer Experiment No. 2, 3 and 4.

5. Requirements

Equipment: Microscope, Electric water bath.

Glassware: Test tubes, Test tube holder, Beakers, Funnel, Graduated Pipettes, Tripod Stand, Filter Paper, Watch Glass, Slide.

Chemicals: Molisch's reagent, Conc. H_2SO_4, 0.02N Iodine solution, Fehling's solution A, Fehling's solution B, Benedict's reagent, Tommer's reagent, Barfoed's reagent, Seliwanoff's reagent, α-naphthol solution (1% in alcohol), Conc. HCl, Conc. HNO_3, Phenyl hydrazine hydrochloride, Acetate buffer (pH 5.0), Sodium hydroxide solution, Methylene blue solution, Ammoniacal silver nitrate solution, Fougler's reagent.

6. Requirements used

_____

_____

7. Precautions to be taken

a. The solutions/reagents used for this experiment should be prepared by using distilled water.
b. Do not suck acids or other chemicals by mouth; use a pipette aid or suction bulb.
c. When mixing acid and water, add concentrated acid to water dropwise while stirring.

8. Procedure

a. Follow the general scheme outlined for qualitative analysis of carbohydrates.
b. **Sugar / Sample solution:** Dissolve 1 g of the given sample of carbohydrate in 20 ml of water. This sugar solution will be used to perform chemical tests for the identification of carbohydrates.
c. Perform a step-by-step chemical test on an unknown carbohydrate sample using a chart to identify the given sample. (Refer Experiment No. 2)
d. Write your findings for the identification of the unknown carbohydrate sample in a given observation table.

9. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

10. Result

The given carbohydrates sample was found to be _____. It is _____ (Monosaccharide / Disaccharide / Polysaccharide).

11. Conclusion

The given sample of carbohydrate is identified by qualitative analysis.

12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.
b. Practical Biochemistry, G. Rajgopal, B.D. Torra, Ahuja's publishing house, fourth edition.
c. Indian Pharmacopoeia 2018

13. Related questions

a. What is the inversion test, and why is it important in evaluating sugar composition?
b. What is the significance of Seliwanoff's test?
c. How does Benedict’s test differentiate between reducing and non-reducing sugars?
d. Justify the non-reducing property of sucrose.

(Space for Answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 6
Qualitative Analysis of Carbohydrate (C5)

1. Aim

To identify the given sample of carbohydrate by qualitative tests (C5)

2. Practical Significance

The qualitative analysis of carbohydrate is a significant tool for identifying and classifying them. This analysis depends on color changes observed following specific chemical reactions. Carbohydrates are organic biomolecules with the general formula $C_n(H_2O)_n$. They are composed primarily of three elements: carbon, hydrogen, and oxygen, with a 2:1 ratio of hydrogen to oxygen. Qualitative analysis of carbohydrates involves using specific reagents to detect their presence based on the reaction between the test sample and the reagent. This reaction typically produces a characteristic color, allowing us to identify the presence of carbohydrates. After completing this experiment, students will be able to identify and confirm the presence of specific carbohydrate in a given sample.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind the specific test	CO 1	BTL2
2	Write analytical reports systematically.	CO 1	BTL2
3	Observe and distinguish the colour while performing various chemicals tests.	CO 1	BTL2
4	Identify and confirm the specific carbohydrates present in the given sample.	CO1	BTL4

4. Relevant Theoretical Background

Refer Experiment No. 2, 3 and 4.

5. Requirements

Equipment: Microscope, Electric water bath.

Glassware: Test tubes, Test tube holder, Beakers, Funnel, Graduated Pipettes, Tripod Stand, Filter Paper, Watch Glass, Slide.

Chemicals: Molisch's reagent, Conc. H_2SO_4, 0.02N Iodine solution, Fehling's solution A, Fehling's solution B, Benedict's reagent, Tommer's reagent, Barfoed's reagent, Seliwanoff's reagent, Sodium hydroxide solution, α-naphthol solution (1% in alcohol), Conc. HCl, Conc. HNO_3, Phenyl hydrazine hydrochloride, Acetate buffer (pH 5.0), Methylene blue solution, Ammoniacal silver nitrate solution, Fougler's reagent.

6. Requirements used

_____

_____

7. Precautions to be taken

a. The solutions/reagents used for this experiment should be prepared by using distilled water.
b. Do not suck acids or other chemicals by mouth; use a pipette aid or suction bulb.
c. When mixing acid and water, add concentrated acid to water dropwise while stirring.

8. Procedure

a. Follow the general scheme outlined for qualitative analysis of carbohydrates.
b. **Sugar / Sample solution:** Dissolve 1 g of the given sample of carbohydrate in 20 ml of water. This sugar solution will be used to perform chemical tests for the identification of carbohydrates.
c. Perform a step-by-step chemical test on an unknown carbohydrate sample using a chart to identify the given sample. (Refer Experiment No. 2)
d. Write your findings for the identification of the unknown carbohydrate sample in a given observation table.

9. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

10. Result

The given carbohydrates sample was found to be _____. It is _____ (Monosaccharide / Disaccharide / Polysaccharide).

11. Conclusion

The given sample of carbohydrate is identified by qualitative analysis.

12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.
b. Practical Biochemistry, G. Rajgopal, B.D. Torra, Ahuja's publishing house, fourth edition.
c. Indian Pharmacopoeia 2018

13. Related questions

a. What are some common chemical tests used in the qualitative analysis of carbohydrates?
b. What is the role of phenylhydrazine in the qualitative analysis of carbohydrates?
c. Explain the procedure and significance of Fehling's test in carbohydrate analysis.
d. Draw the structure of Maltosazone.

(Space for Answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

QUALITATIVE ANALYSIS OF PROTEINS AND AMINO ACIDS

Amino Acids:

Amino acids are organic compounds that serve as the building blocks of proteins. They are composed of an amino group (-NH2), a carboxyl group (-COOH), a hydrogen atom, and a side chain (R group) that varies in structure and properties among different amino acids. There are 20 standard amino acids that are commonly found in proteins, each distinguished by its specific side chain. Amino acids play crucial roles in biological processes such as protein synthesis, enzyme catalysis, cell signaling, and structural support. They are also involved in metabolic pathways and serve as precursors for various molecules in the body.

Protein:

A protein is a large and complex organic molecule made up of one or more chains of amino acids. Proteins are essential components of all living organisms and play crucial roles in various biological processes. They are involved in structural support, enzymatic reactions, transportation of molecules, immune responses, and cell signaling, among other functions. The sequence and arrangement of amino acids determine the structure and function of a protein, allowing for a wide range of diversity and specificity in biological activities.

A peptide bond is a type of covalent bond that links two amino acids together in a protein chain. It forms between the carboxyl group (-COOH) of one amino acid and the amino group ($-NH_2$) of another amino acid, resulting in the release of a water molecule (H_2O) in a condensation reaction.

To identify proteins and amino acids, two types of reactions are commonly performed:

A. Precipitation reactions (For proteins):

Proteins are macromolecules that are mostly hydrophilic and carry electrical charges distributed on their surface. Therefore, proteins can be precipitated for identification by dehydrating them or neutralizing their electrical charge. Neutralization can be achieved by adjusting the acidity or alkalinity of the protein solution, causing them to precipitate. Inorganic salts like ammonium sulfate act as dehydrating agents, removing water of hydration from proteins and causing them to precipitate.

B. Colour reactions (For proteins and amino acids):

Proteins and amino acids react with various reagents due to the presence of peptide bonds and different amino acid side chains (-R groups). Specific color reactions are used to identify the side chain in the amino acid, which is helpful for both qualitative and quantitative determination of proteins. The qualitative determination of proteins using color reactions is significant for:

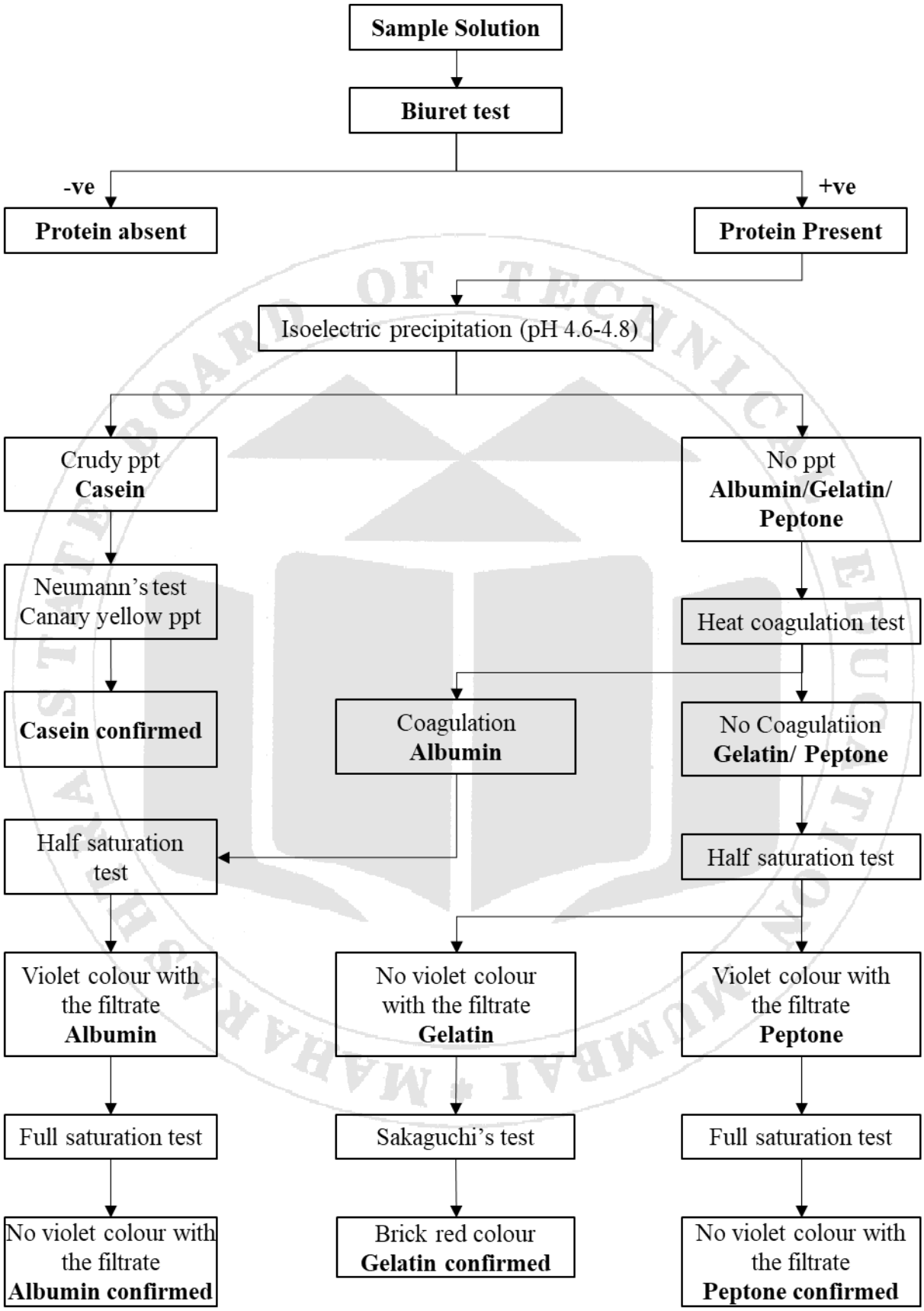
a. Diagnosis of aminoaciduria (presence of amino acids in urine).

b. Nutritional assessment.

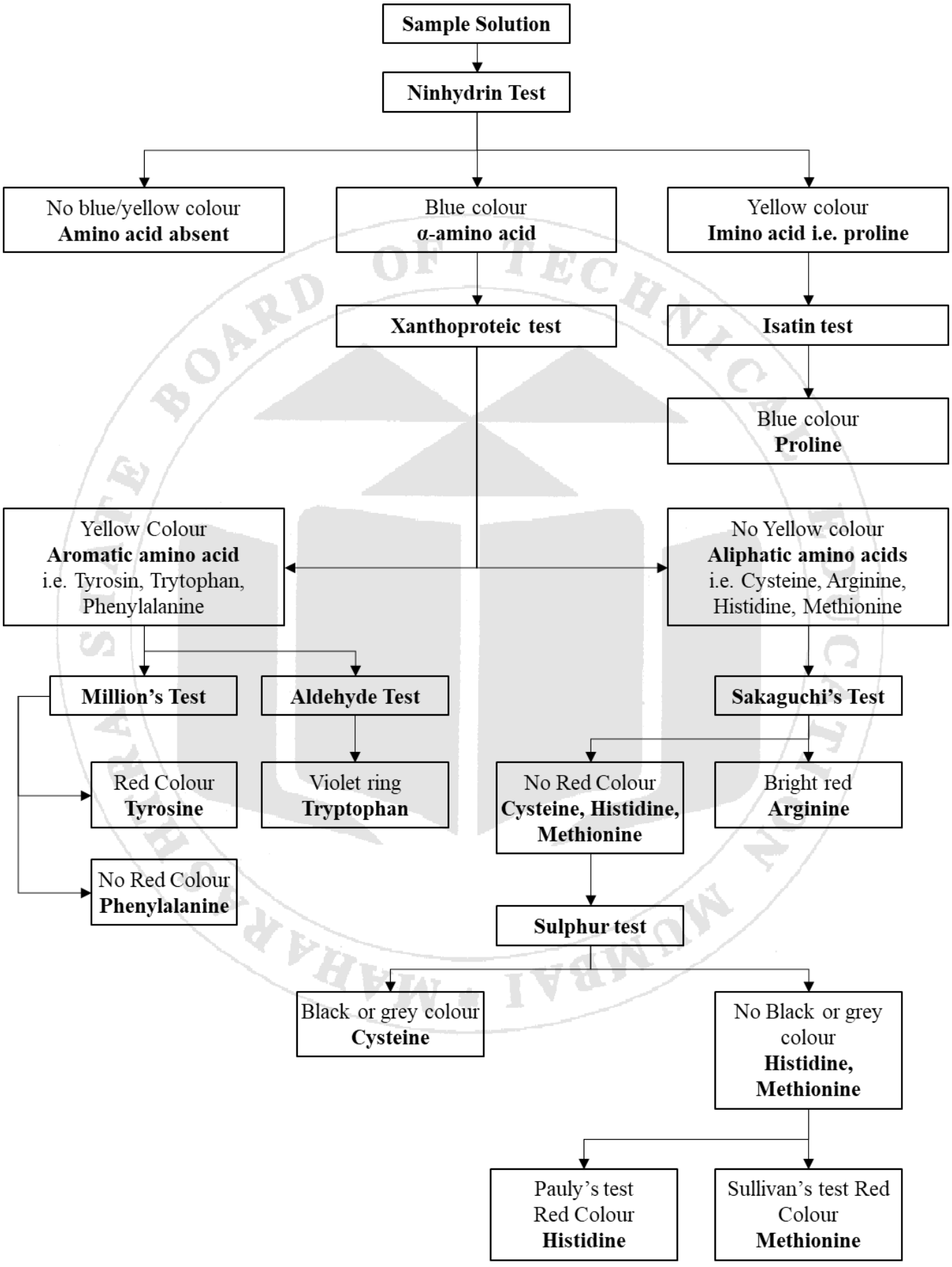
c. Detection of proteins or amino acids in biological fluids of unknown concentrations.

The identification of unknown samples of proteins and amino acids typically follows Scheme-I and Scheme-II, respectively, using these methods.

Scheme I: Qualitative Analysis of proteins:



Scheme II: Qualitative analysis of amino acids



A. Qualitative Analysis of proteins

Test	Observation	Inference	Remark
1. Biuret test 2 mL of the sample solution + 2 mL 40 % NaOH and 1% $CuSO_4$ solution, 2-5 drops.	Pink or violet colour	Proteins or Peptide bonds (2 or more) are present	Excess of copper sulphate solution causes formation of blue coloured copper hydroxide and that will mask the violet colour. Colour depends on nature of protein Proteoses – Purple Peptones – Pink
2.Ninhydrin test 1 mL of sample solution + 2-3 drops of freshly prepared 0.1% ninhydrin solution. Boil for 2 min and allow to cool.	Bluish purple colour	Amino acid Present	Proteins show a faint blue colour with ninhydrin.
3. Physical Properties			
Appearance f solution	Turbid	Albumin/Casein may be present.	
	Clear Solution	Gelatin/ Peptone may be present.	
Colour of solution	Milky	Albumin/Casein may be present.	
	Faint Yellow	Peptone may be present.	
	Faint Brown	Gelatin may be present.	
Smell	Egg like	Albumin may be present.	
	Milk like	Casein may be present.	
	Meat like	Gelatin/ Peptone may be present.	
Litmus test	Neutral	Albumin/Gelatin may be present.	
	Acidic	Peptone may be present.	
	Alkaline	Casein may be present.	
4. Isoelectric precipitation 3 mL of sample solution + drop of bromocresol green solution, observe the blue colour, then add 2% acetic acid till the colour changes to light green (pH 4.6-4.8).	Curdy white ppt	Casein present	Only casein is ppt out at pH 4.6-4.8. Perform Neumann's test if casein precipitates out.
	No ppt	Albumin/Gelatin/ Peptone may be present.	
5. Heat coagulation test Take a test tube and fill a sample solution up to two thirds. Heat the upper one third portion of the sample solution. Note whether any precipitate has appeared. Irrespective of the presence or absence of the appearance of the precipitate add 2% acetic acid drop by drop. Note whether the precipitate formed earlier (if any) has intensified or appeared upon adding acetic acid.	On heating ppt is formed and becomes dense on addition of acetic acid.	Albumin present	Heating causes coagulation of albumin, addition of acetic acid lowers pH of medium towards pI (isoelectric pH) of albumin and enhances precipitation.
	No ppt	Gelatin/Peptone may be present	

Test	Observation	Inference	Remark
6. Half saturation test 3 mL sample solution + 3 mL of saturated ammonium sulphate solution, shake vigorously for 2 min, keep aside for 5 min, filter and collect the filtrate. Perform biuret test with the filtrate. To 2 mL of the above filtrate, add 2 mL 40 % NaOH + 1% $CuSO_4$ solution (2-5 drops).	Pink or violet colour	*Albumin or Peptone present (*Write only one based on heat coagulation test, refer scheme-I)	Albumin being relatively small in size is not completely precipitated by saturated ammonium sulphate solution and hence go into the filtrate and gives a positive biuret test.
	No pink or violet colour	Gelatin present	
7. Full saturation test 5 mL of sample solution + ammonium sulphate crystals, shake well till some crystals remain at the bottom of the tube. Keep aside for 5 minute and filter. Collect the filtrate. Perform biuret test with the filtrate. 2 mL of the above filtrate + 2 mL 40 % NaOH and 1% $CuSO_4$ solution (2-5 drops).	No pink or violet colour	Albumin present	
	Pink or violet colour	Gelatin present	
8. Neumann's test 5 mL of sample solution + 0.5 mL of 40 % NaOH. Heat for one minute and then cool + 0.5 mL of conc HNO_3 + 1mL of saturated ammonium molybdate solution.	Canary yellow ppt	Casein confirmed	This test is specific for presence of phosphorus in casein. Casein is digested by heating with NaOH, and phosphorus is liberated.
9. Sakaguchi's test 5 mL of sample solution + 5 drops of 5% sodium hydroxide, Shake well + 2-4 drops of Molisch's reagent + 4-5 drops of 10% sodium hypochlorite or 10 drops of bromine water.	Brick red colour	Gelatin confirmed	This test detects the presence of guanidine groups. Care shall be taken while handling bromine solution

B. Qualitative Analysis of Amino acids

Test	Observation	Inference	Remark
1. Ninhydrin test 1 mL of sample solution + 2-3 drops of freshly prepared 0.1% ninhydrin solution. Boil for 2 min and allow to cool.	Blue or yellow colour	Amino acid Present	α-aminoacids – blue colour. iminoacid (proline) – yellow colour. Proteins show a faint blue colour with ninhydrin.
2. Xanthoproteic test Add 1 mL of conc HNO_3 to 2-3 mL of sample solution. Heat to boil. Cool, then transfer half of the solution into another test tube. (One test tube is kept as control and the other as a test.) To one tube add 40% NaOH or ammonia solution in excess.	A white ppt is formed by adding nitric acid that turns to yellow colour after heating. Orange to red colour appears after adding NaOH or ammonia solution.	Aromatic amino acids such as tyrosine or tryptophan may be present.	Phenylalanine even though it contains a benzene ring does not give this test positive.
	No yellow colour	Aliphatic amino acids may be such as cysteine, arginine, histidine may present	
3. Millon's test 2 mL of sample solution + 2 mL of modified millon's reagent, boil for 30 seconds, a precipitate may form at this stage. Cool on ice + a few drops of 1% $NaNO_2$ and gently warm in the water bath.	Red colour	Tyrosine confirmed	Modified millon's reagent minimises interference from inorganic salts.
4. Aldehyde test (Hopkins-Cole test) 2-3 mL sample solution + 2 drops of 1/500 formaldehyde + 1 drop of 10% mercuric sulfate (in 10 % sulfuric acid). Mix well, + 3 mL of concentrated sulfuric acid through the sides of the test tube	A purple ring develops at the junction of two layers.	Tryptophan confirmed	Mercuric sulphate (in sulphuric acid) act as an oxidizing agent and it oxidizes the indole ring of tryptophan, Formaldehyde reacts with the oxidized indole ring to form a purple colour complex.
5. Ehrlich's test 1 mL of Sample solution + 2-3 drops of Ehrlich's reagent and mix well.	Purple color	Tryptophan confirmed	This test is a specific test for aldehyde or tryptophan.
6. Sakaguchi test 5 mL of sample solution + 5 drops of 5% sodium hydroxide, Shake well + 2-4	Carmine red colour	Arginine confirmed	This test detects the presence of guanidine groups. Care shall be taken while handling
	No red colour	Cysteine,	

Test	Observation	Inference	Remark
drops of Molisch's reagent + 4-5 drops of 10% sodium hypochlorite or 10 drops of bromine water.		Histidine, Methionine may present	bromine solution
7. Sulphur test 2-3 mL sample solution + 3 mL of 40% NaOH and boil for 3 minutes. Cool, +1 mL of lead acetate solution.	Black or grey colour	Cysteine confirmed	This test is positive for -SH containing amino acids i.e. cysteine and cystine but not methionine because of the placement of -S- in the thio ether linkage.
	No Black or grey colour	Histidine, Methionine may present	
8. Sodium nitroprusside test 1 mL of sample solution, add 2-3 drops of sodium nitroprusside solution and mix well. Add few (4-5) drops of ammonia solution and mix well.	Red colour	Cysteine confirmed	
9. Sullivan's test 1 mL sample solution + 2 drops of 2% glycine + 2 drops of 10% sodium nitroprusside solution, mix well, warm in water bath for 5 min, cool in ice cold solution + 0.5 mL dil HCl solution, allow to stand 15 min at room temperature.	Red colour	Methionine confirmed	This test is used for the detection of methionine either in a free form or in proteins.
10. Pauly's test 0.5 mL of sulphanilic acid + 0.5 mL of sodium nitrite solution allow to stand for 1 min + 1 mL of sample solution, mix well + 1 mL dil sodium carbonate solution.	Cherry red color	Histidine confirmed	
11. Isatin test Apply a drop of sample solution on a Whatman No. 1 filter paper strip. Dry the spot, apply a drop of isatin reagent on to the dried spot of the sample, dry again.	Blue color spot	Proline confirmed	This test is also positive for hydroxyproline.

Reagents Used for Qualitative Analysis of Proteins and Amino acids

Albumin Solution: Dissolve 5 g of albumin in 500 mL of water. Prepare it on the day of experiment or one day before and store it in the fridge. Or prepare 5% solution of egg white in water.

Casein Solution: Dissolve 5 g of casein in 500 mL of 0.1 N NaOH. Warm gently if necessary.

Gelatin Solution: Dissolve 5 g of gelatin in 500 mL of boiling water.

Peptone solution: Dissolve 5 g of peptone in 500 mL water, add glacial acetic acid dropwise till the turbidity disappears.

Isatin reagent: Dissolve 2 g of isatin powder in 200 mL dil acetic acid solution.

Modified Millon's reagent: Dissolve 10 g of mercuric sulphate in 10 % 100 mL H_2SO_4.

Ehrlich's reagent: 0.5 to 2.0 g of p-dimethyl amino benzaldehyde in 50 mL of 95% ethanol. To this, 50 mL 10% H_2SO_4 is added.

Hypobromite reagent (to be prepared freshly): Dissolve 5 g of NaOH in 50 mL of distilled water and add one commercially available elemental bromine vial to it.

Ammonium molybdate solution: Dissolve 100 g molybdic acid in 145 mL of ammonium hydroxide solution and add 270 mL of distilled water. Pour this solution gently in a mixture of 490 mL of nitric acid and 1150 mL of distilled water. Keep this solution in a warm place, decant the solution from any sediment and keep in glass stoppered bottles.

Amino acid solution: Dissolve 0.1g of the individual amino acid (standard amino acid samples are commercially available) in 100 mL of distilled water.

1/500 Formaldehyde: Dissolve 1 mL of formaldehyde in 500 mL of distilled water.

Bromocresol Green (pH range 3.8-5.4; colour range – yellow to green): Add 0.1 g of bromocresol green and 3 mL of 0.05 N NaOH to 250 mL of distilled water.

Ninhydrin solution (0.1%): 100 mg of ninhydrin in 100 mL acetone.

1% Sodium Nitrite ($NaNO_2$): Dissolve 1 g sodium nitrite in 100 mL of water.

Sodium nitroprusside solution: Dissolve 5 g of sodium nitroprusside in 100 mL of water.

Experiment No. 7
Qualitative Analysis of Proteins (P1)

1. Aim

To identify the unknown protein sample by qualitative analysis (P1).

2. Practical Significance

The qualitative analysis of proteins and amino acids aids in diagnosing metabolic disorders such as proteinuria, phenylketonuria, tyrosinemia, homocystinuria, non-ketotic hyperglycinemia, and maple syrup urine disease, among others. Elevated or reduced levels of proteins and amino acids beyond certain thresholds in biological samples indicate a disease or disorder. Protein deficiency in the body primarily leads to Kwashiorkor disease and Marasmus, while increased protein levels can signify infection, chronic inflammation, and heart diseases. In this practical, students will learn to analyze a given sample of protein and draw conclusions from their findings.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind the specific test	CO 1	BTL2
2	Perform the qualitative tests for proteins	CO 1	BTL5
3	Write analytical reports systematically.	CO 1	BTL2
4	Observe and distinguish the colour while performing various chemicals tests.	CO 1	BTL2
5	Identify and confirm the specific protein present in the given sample.	CO1	BTL4

4. Relevant Theoretical Background

Proteins are complex molecules essential for numerous biological functions, including structure, function, and regulation of tissues and organs in the body. They are composed of amino acids linked together by peptide bonds, forming long chains that fold into specific three-dimensional shapes.

Types of Proteins

A. Simple Proteins

Simple proteins consist only of amino acids or their derivatives. They do not contain any non-protein components. These proteins can be further divided into:

a. **Globular Proteins**: These proteins are usually soluble in water and have a spherical shape. Examples include enzymes, hormones, and antibodies. Examples: Myoglobin, Hemoglobin.

b. **Fibrous Proteins**: These proteins are usually insoluble in water and have long, fibrous structures. They provide structural support and strength to tissues. Examples: Collagen, Keratin, Elastin.

B. Conjugated Proteins

Conjugated proteins consist of a simple protein combined with a non-protein component known as a prosthetic group. The prosthetic group can be a metal ion, a vitamin, a lipid, a carbohydrate, or a nucleic acid. Based on the type of prosthetic group, conjugated proteins are classified as:

a. **Nucleoproteins**: Proteins combined with nucleic acids. Example: Chromatin.

b. **Glycoproteins**: Proteins combined with carbohydrates. Example: Mucin.
c. **Lipoproteins**: Proteins combined with lipids. Example: Low-density lipoprotein (LDL).
d. **Phosphoproteins**: Proteins combined with phosphate groups. Example: Casein.
e. **Hemoproteins**: Proteins combined with heme groups. Example: Hemoglobin, Cytochromes.
f. **Metalloproteins**: Proteins combined with metal ions. Example: Ferritin, which stores iron.

C. Derived Proteins

Derived proteins are those that are formed from simple or conjugated proteins through physical or chemical means. These can be the result of protein degradation or partial hydrolysis. Derived proteins are usually less complex and have undergone some form of modification. They are classified into two categories:

a. **Primary Derived Proteins**: These are formed by the initial steps of protein hydrolysis. They are still relatively complex. Examples: Proteoses, Peptones.
b. **Secondary Derived Proteins**: These are formed by further hydrolysis or decomposition of primary derived proteins, leading to simpler substances. Examples: Peptides, Free Amino Acids.

5. Requirements

Glassware: Test tubes, Test tube holder, Beaker, Measuring cylinder, Graduated pipette, Water bath, Wire gauze, Tripod stand.

Chemicals: Albumin, Casein, Gelatin, Bromocresol green solution, 2% Acetic acid, Ammonium sulphate solution, Sodium hydroxide Solution (40%), Copper sulphate solution (1%), Ammonium sulphate crystals, Ninhydrin reagents, Conc. Nitric acid, Molisch reagents, Bromine water or Sodium hypobromite solution.

6. Requirements used

_____

_____

7. Precautions to be taken

a. The solutions/reagents used for this experiment should be prepared by using distilled water.
b. Do not suck acids or other chemicals by mouth; use a pipette aid or suction bulb.
c. When mixing acid and water, add concentrated acid to water dropwise while stirring.

8. Procedure

a. Refer to the general scheme for qualitative analysis of proteins.
b. Prepare solution of given protein sample.
c. Perform the stepwise chemical tests on an unknown protein sample according to a scheme to identify the given sample.
d. Report your analysis for the identification of a given protein sample in an observation table.
e. Record the results and interpret the analysis.

9. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

10. Result

The given unknown protein sample was found to be _____. It is _____ (Simple / Conjugated / Derived) protein.

11. Conclusion

The unknown sample of protein was identified by qualitative analysis.

12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.
b. Practical Biochemistry, G. Rajgopal, B.D. Torra, Ahuja's publishing house, fourth edition.
c. Indian Pharmacopoeia 2018.

13. Related questions

a. Define protein, amino acids, and peptide bond.
b. What is the role of Biuret reagents in protein analysis?
c. Write the major sources of albumin, gelatin, and casein.
d. Describe the principle involved in the full saturation test.

(Space for Answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 8
Qualitative Analysis of Proteins (P2)

1. Aim

To identify the unknown protein sample by qualitative analysis (P2).

2. Practical Significance

The qualitative analysis of proteins and amino acids aids in diagnosing metabolic disorders such as proteinuria, phenylketonuria, tyrosinemia, homocystinuria, non-ketotic hyperglycinemia, and maple syrup urine disease, among others. Elevated or reduced levels of proteins and amino acids beyond certain thresholds in biological samples indicate a disease or disorder. Protein deficiency in the body primarily leads to Kwashiorkor disease and Marasmus, while increased protein levels can signify infection, chronic inflammation, and heart diseases. In this practical, students will learn to analyze a given sample of protein and draw conclusions from their findings.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind the specific test	CO 1	BTL2
2	Perform the qualitative tests for proteins	CO 1	BTL5
3	Write analytical reports systematically.	CO 1	BTL2
4	Observe and distinguish the colour while performing various chemicals tests.	CO 1	BTL2
5	Identify and confirm the specific protein present in the given sample.	CO1	BTL4

4. Relevant Theoretical Background

Principles of Tests used in qualitative analysis of proteins:

A. **Biuret Test:** The Biuret test is based on the ability of peptide bonds in proteins to complex with copper ions under alkaline conditions. When proteins or peptides (with two or more peptide bonds) react with Biuret reagent, which contains copper sulfate in an alkaline solution, a violet-colored complex forms. This color change indicates the presence of proteins.

B. **Heat Coagulation Test:** The Heat Coagulation test is based on the principle that proteins denature and coagulate upon heating. When a protein solution is heated, the protein molecules unfold and aggregate, leading to the formation of a precipitate. The coagulation occurs due to the disruption of non-covalent interactions (such as hydrogen bonds and hydrophobic interactions) that maintain the protein's native structure.

C. **Half Saturation Test:** The Half Saturation test involves the use of ammonium sulfate to partially precipitate proteins from a solution. Ammonium sulfate increases the ionic strength of the solution, leading to the precipitation of proteins due to the "salting out" effect. In this test, the protein solution is half-saturated with ammonium sulfate, causing less soluble proteins to precipitate while more soluble proteins remain in solution. This method helps to fractionate proteins based on their solubility.

D. **Full Saturation Test:** The Full Saturation test also uses ammonium sulfate but to completely precipitate proteins from a solution. When the protein solution is fully saturated with ammonium sulfate, all proteins precipitate out of the solution due to the high ionic strength and

salting out effect. This method is useful for the total precipitation and recovery of proteins from a solution.

E. **Neumann's Test:** Neumann's test is used to detect the presence of hydroxyproline, an amino acid found in collagen and gelatin. In this test, hydroxyproline is oxidized to produce a red-colored compound in the presence of a specific reagent. The formation of the red color indicates the presence of hydroxyproline, which is a marker for collagenous proteins.

F. **Sakaguchi Test:** The Sakaguchi test is specific for detecting the amino acid arginine. In this test, arginine reacts with α-naphthol and an oxidizing agent (such as sodium hypobromite or sodium hypochlorite) to form a red-colored complex. The presence of this red color indicates the presence of arginine in the protein sample.

5. Requirements

Glassware: Test tubes, Test tube holder, Beaker, Measuring cylinder, Graduated pipette, Water bath, Wire gauze, Tripod stand.

Chemicals: Albumin, Casein, Gelatin, Bromocresol green solution, 2% Acetic acid, Ammonium sulphate solution, Sodium hydroxide Solution (40%), Copper sulphate solution (1%), Ammonium sulphate crystals, Ninhydrin reagents, Conc. Nitric acid, Molisch reagents, Bromine water or Sodium hypobromite solution.

6. Requirements used

_____

_____

7. Precautions to be taken

a. The solutions/reagents used for this experiment should be prepared by using distilled water.
b. Do not suck acids or other chemicals by mouth; use a pipette aid or suction bulb.
c. When mixing acid and water, add concentrated acid to water dropwise while stirring.

8. Procedure

a. Refer to the general scheme for qualitative analysis of proteins.
b. Prepare solution of given protein sample.
c. Perform the stepwise chemical tests on an unknown protein sample according to a chart to identify the given sample.
d. Report your analysis for the identification of a given protein sample in an observation table.
e. Record the results and interpret the analysis.

9. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

Test	Observation	Inference

10. Result

The given unknown protein sample was found to be _____. It is _____ (Simple / Conjugated / Derived) protein.

11. Conclusion

The unknown sample of protein was identified by qualitative analysis.

12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.
b. Practical Biochemistry, G. Rajagopal, B.D. Torra, Ahuja's publishing house, fourth edition.
c. Indian Pharmacopoeia 2018.

13. Related questions

a. Explain the principle involved in the isoelectric precipitation test.
b. Describe the principle involved in the heat coagulation test.
c. What are examples of Simple, conjugated and derived protein?
d. Describe Kwashiorkor and Marasmus disease.

(Space for Answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 9
Qualitative Analysis of Amino acids (AA1)

1. Aim
To identify the unknown amino acid sample by qualitative analysis (AA1).

2. Practical Significance
The qualitative analysis of proteins and amino acids aids in diagnosing metabolic disorders such as proteinuria, phenylketonuria, tyrosinemia, homocystinuria, non-ketotic hyperglycinemia, and maple syrup urine disease, among others. Elevated or reduced levels of proteins and amino acids beyond certain thresholds in biological samples indicate a disease or disorder. Protein deficiency in the body primarily leads to Kwashiorkor disease and Marasmus, while increased protein levels can signify infection, chronic inflammation, and heart diseases. In this practical, students will learn to analyze a given sample of amino acid and draw conclusions from their findings.

3. Practical Outcomes (PrO)
The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind the specific test.	CO 1	BTL2
2	Perform the qualitative tests for amino acids.	CO 1	BTL5
3	Write analytical reports systematically.	CO 1	BTL2
4	Observe and distinguish the colour while performing various chemicals tests.	CO 1	BTL2
5	Identify and confirm the specific amino acid present in the given sample.	CO1	BTL4

4. Relevant Theoretical Background
Amino acids:

Amino acids are the building blocks of proteins and play a crucial role in various biological processes. Each amino acid consists of a central carbon atom (alpha carbon) bonded to a hydrogen atom, an amino group ($-NH_2$), a carboxyl group (-COOH), and a distinctive side chain (R group) that determines its unique properties. There are 20 standard amino acids, which can be classified based on the characteristics of their side chains.

Classification of Amino Acids

A. **Based on Nutritional Requirements**:

a. **Essential Amino Acids**: These amino acids cannot be synthesized by the human body and must be obtained through the diet. Examples: Valine, Leucine, Isoleucine, Phenylalanine, Tryptophan, Threonine, Methionine, Lysine, and Histidine (essential for children).

b. **Non-Essential Amino Acids**: These amino acids can be synthesized by the human body. Examples: Alanine, Asparagine, Aspartic acid, Glutamic acid, Serine.

B. Classification Based on Structure

a. **Aliphatic Amino Acids:** These amino acids have non-aromatic hydrocarbon side chains. They are typically nonpolar and hydrophobic.

i. Glycine (Gly, G): The simplest amino acid with a single hydrogen atom as its side chain.

ii. Alanine (Ala, A): Has a methyl group ($-CH_3$) as its side chain.

iii. Valine (Val, V): Has a branched-chain structure with a -$CH(CH_3)_2$ side chain.
iv. Leucine (Leu, L): Similar to valine but with an extra -CH_2 group in the side chain.
v. Isoleucine (Ile, I): An isomer of leucine with a different branching in the side chain.

b. **Hydroxyl-Containing Amino Acids**: These amino acids have side chains containing hydroxyl (-OH) groups, making them polar and capable of forming hydrogen bonds.
i. Serine (Ser, S): Has a hydroxyl group (-CH_2OH) in its side chain.
ii. Threonine (Thr, T): Contains a hydroxyl group and a methyl group on a single carbon (-$CH(OH)-CH_3$).

c. **Sulfur-Containing Amino Acids:** These amino acids contain sulfur in their side chains.
i. Cysteine (Cys, C): Contains a thiol group (-CH_2-SH) which can form disulfide bonds.
ii. Methionine (Met, M): Contains a sulfur atom in its side chain (-$CH_2-CH_2-S-CH_3$).

d. **Aromatic Amino Acids:** These amino acids have aromatic rings in their side chains, which can participate in stacking interactions.
i. Phenylalanine (Phe, F): Contains a benzyl side chain (-$CH_2-C_6H_5$).
ii. Tyrosine (Tyr, Y): Contains a hydroxyl group attached to a benzyl side chain (-$CH_2-C_6H_4OH$).
iii. Tryptophan (Trp, W): Contains a larger aromatic side chain with a fused double ring (indole).

e. **Basic Amino Acids:** These amino acids have side chains that are positively charged at physiological pH due to the presence of amino groups.
i. Lysine (Lys, K): Has a long aliphatic chain ending in an amino group (-$(CH_2)_4-NH_2$.
ii. Arginine (Arg, R): Contains a guanidinium group (-$(CH_2)_3$-NH-$C(NH_2)_2$.
iii. Histidine (His, H): Contains an imidazole ring in its side chain.

f. **Acidic Amino Acids and their Amides:** These amino acids have side chains that are negatively charged at physiological pH due to the presence of carboxyl groups, or their amides which are uncharged but polar.
i. Aspartic Acid (Asp, D): Contains a carboxyl group (-CH_2-COOH).
ii. Glutamic Acid (Glu, E): Similar to aspartic acid but with an extra -CH_2 group (-CH_2-CH_2-COOH).
iii. Asparagine (Asn, N): The amide form of aspartic acid (-CH_2-$CONH_2$).
iv. Glutamine (Gln, Q): The amide form of glutamic acid (-CH_2-CH_2-$CONH_2$).

g. **Imino Acid:** This category includes amino acids with a unique cyclic structure that involves the amino group.
i. Proline (Pro, P): Unique cyclic structure that limits its flexibility and affects protein folding.

5. Requirements

Glassware: Test tubes, Test tube holder, Beaker, Measuring cylinder, Graduated pipette, Water bath, Wire gauze, Tripod stand.

Chemicals: Sodium hydroxide solution (40%), Copper sulphate solution (1%), Ninhydrin solution, Conc. HNO_3, Ammonium molybdate solution, Modified Millon's reagent, Sodium nitrite, Formaldehyde solution (1/500), Mercuric Sulphate (10%), Ehrlich's reagent, Molisch's reagent, Sodium hypochlorite, Bromine water, Lead acetate, Sodium nitroprusside, Ammonia solution, Conc. H_2SO_4, Dil HCl, Sulphanilic acid, Sodium carbonate, Isatin reagent.

6. Requirements used

_____

_____

7. Precautions to be taken

a. The solutions/reagents used for this experiment should be prepared by using distilled water.
b. Do not suck acids or other chemicals by mouth; use a pipette aid or suction bulb.
c. When mixing acid and water, add concentrated acid to water dropwise while stirring.

8. Procedure

a. Refer to the general scheme for qualitative analysis of amino acids.
b. Prepare solution of given amino acid sample.
c. Perform the stepwise chemical tests on an unknown amino acid sample according to a chart to identify the given sample.
d. Report your analysis for the identification of a given amino acid sample in an observation table.
e. Record the results and interpret the analysis.

9. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

Test	Observation	Inference

10. Result

The given unknown amino acid sample was found to be _____. It is_____ (acidic / basic / neutral) amino acid.

11. Conclusion

The unknown sample of amino acid was identified by qualitative analysis.

12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.
b. Practical Biochemistry, G. Rajagopal, B.D. Torra, Ahuja's publishing house, fourth edition.
c. Indian Pharmacopoeia 2018.

13. Related questions

a. Explain the principle involved in Neumann’s test.
b. Describe the significance of the ninhydrin test.
c. Write the principle involved in the xanthoproteic test.
d. Enlist the essential amino acids.
e. How would you differentiate between proline and tyrosine?

(Space for Answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 10
Qualitative Analysis of Amino acids (AA2)

1. Aim
To identify the unknown amino acid sample by qualitative analysis (AA2).

2. Practical Significance
The qualitative analysis of proteins and amino acids aids in diagnosing metabolic disorders such as proteinuria, phenylketonuria, tyrosinemia, homocystinuria, non-ketotic hyperglycinemia, and maple syrup urine disease, among others. Elevated or reduced levels of proteins and amino acids beyond certain thresholds in biological samples indicate a disease or disorder. Protein deficiency in the body primarily leads to Kwashiorkor disease and Marasmus, while increased protein levels can signify infection, chronic inflammation, and heart diseases. In this practical, students will learn to analyze a given sample of amino acid and draw conclusions from their findings.

3. Practical Outcomes (PrO)
The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind the specific test.	CO 1	BTL2
2	Perform the qualitative tests for amino acids.	CO 1	BTL5
3	Write analytical reports systematically.	CO 1	BTL2
4	Observe and distinguish the colour while performing various chemicals tests.	CO 1	BTL2
5	Identify and confirm the specific amino acid present in the given sample.	CO1	BTL4

4. Relevant Theoretical Background
Principles of Tests used in qualitative analysis of proteins:

A. **Ninhydrin test:** This test is an identification test for the presence of amino acids. In this reaction amino acids react with ninhydrin (a powerful oxidizing agent) reagent to give a purple-coloured complex (Ruhemann's purple) while, imino acids (proline and hydroxyproline) react with ninhydrin to produce yellow colour.

B. **Xanthoproteic test:** This test is an identification test for the presence of aromatic amino acids. In this reaction aromatic amino acids (tyrosine or tryptophan or proteins with aromatic amino acids) undergo nitration in the presence of nitric acid to produce nitro-derivatives that are yellow in colour. At the end of the reaction addition of NaOH (alkaline pH), the colour changes to orange due to the ionization of the phenolic group.

C. **Ehrlich's test**: This is an identification test for the presence of amino acid Tryptophan. In this reaction indole ring of tryptophan reacts with paradimethylamino benzaldehyde under acidic conditions to give a purple colour.

D. **Hopkins-Cole test**: This test is a confirmatory test for the presence of amino acid tryptophan. In this reaction indole moiety of tryptophan condenses with aldehydes under acidic environment to yield.

E. **Lead sulphide test**: This test is an identification test for sulphur containing amino acids. This test mainly depends on formation of inorganic sulphide from organic sulphur. The sulphur containing amino acids, (cysteine and cystine) upon boiling with sodium hydroxide (hot alkali)

produce sodium sulphide. This can be detected by precipitating inorganic sulphide to lead sulphide(black), using lead acetate solution purple- or violet-coloured compounds.

F. **Sodium nitroprusside test**: Sodium nitroprusside reacts with the thiol group of the cysteine under alkaline condition to yield an intense purple coloured compound, which fades after few minutes

G. **Sakaguchi's test**: α-naphthol (1-hydroxy naphthalene) reacts with a guanidine group containing amino acid like arginine under alkaline condition, which upon treatment with hypobromite or hypochlorite, produces a characteristic red colour.

H. **Millon's test**: This test is specific to phenolic group containing amino acid such as tyrosine. Tyrosine reacts with mercuric ions in acidic condition in the presence of sodium nitrite, to give a red colour complex (Millon's red).

I. **Isatin test**: Imino acids such as proline and hydroxyl proline condense with isatin under acidic conditions to yield a blue coloured adduct.

5. Requirements

Glassware: Test tubes, Test tube holder, Beaker, Measuring cylinder, Graduated pipette, Water bath, Wire gauze, Tripod stand.

Chemicals: Sodium hydroxide solution (40%), Copper sulphate solution (1%), Ninhydrin solution, Conc. HNO_3, Ammonium molybdate solution, Modified Millon's reagent, Sodium nitrite, Formaldehyde solution (1/500), Mercuric Sulphate (10%), Ehrlich's reagent, Molisch's reagent, Sodium hypochlorite, Bromine water, Lead acetate, Sodium nitroprusside, Ammonia solution, Conc. H_2SO_4, Dil HCl, Sulphanilic acid, Sodium carbonate, Isatin reagent.

6. Requirements used

_____

_____

7. Precautions to be taken

a. The solutions/reagents used for this experiment should be prepared by using distilled water.
b. Do not suck acids or other chemicals by mouth; use a pipette aid or suction bulb.
c. When mixing acid and water, add concentrated acid to water dropwise while stirring.

8. Procedure

a. Refer to the general scheme for qualitative analysis of amino acids.
b. Prepare solution of given amino acid sample.
c. Perform the stepwise chemical tests on an unknown amino acid sample according to a chart to identify the given sample.
d. Report your analysis for the identification of a given amino acid sample in an observation table.
e. Record the results and interpret the analysis.

9. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

10. Result

The given unknown amino acid sample was found to be _____. It is_____ (acidic / basic / neutral) amino acid.

11. Conclusion

The unknown sample of amino acid was identified by qualitative analysis.

12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.
b. Practical Biochemistry, G. Rajagopal, B.D. Torra, Ahuja's publishing house, fourth edition.
c. Indian Pharmacopoeia 2018.

13. Related questions

a. Draw the structures of tyrosine and tryptophan.
b. Enlist non-essential amino acids.
c. How would you differentiate between cysteine and methionine?
d. Draw the chemical structure of optically inactive amino acids.
e. Explain the role of modified Millon's reagent.

(Space for Answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No 11
Qualitative analysis of Lipids

1. Aim:

To perform various qualitative tests for lipids.

2. Practical significance:

The tests performed to identify lipids are specific to their chemical nature and the functional groups present in them. Qualitative analysis of lipids helps us to determine their presence or absence based on color changes. This analysis plays a significant role in identifying adulteration in edible oils, making it vital in both the industry and the health sector. In this experiment, students will learn to analyze a given sample of lipids and draw conclusions from their findings.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Enlist various tests used for identification of lipids.	CO 1	BTL1
2	Perform the qualitative tests for lipids.	CO 1	BTL5
3	Write analytical reports systematically.	CO 1	BTL2
4	Observe and distinguish the colour while performing various chemical tests.	CO 1	BTL2

4. Relevant Theoretical Background

The term 'Lipid' was coined by the German chemist Bloor in 1943. Lipids are hydrophobic biomolecules, insoluble in water but soluble in organic solvents such as benzene, chloroform, hexane, and diethyl ether. They are known for their high energy content and are classified as simple, conjugated, and derived lipids.

Classification of Lipids

A. Simple Lipids: Esters of fatty acids with alcohol.
   a. **Fats and oils**- Triacylglycerol (Esters of fatty acids with glycerol).
   b. **Waxes**- Esters of fatty acids with alcohol other than glycerol.

B. Compound Lipids: Esters of fatty acids with alcohol containing additional groups.
   a. **Phospholipids**- Individual molecules with a phosphate group and a nitrogenous base.
      i. Glycerophospholipids: Phospholipids contain glycerol. E.g. Lecithin, cephalin
      ii. Sphingophospholipids: Phospholipids contain Sphingosine. E.g. Sphingomyelin
   b. **Glycolipids:** Individual molecules with a carbohydrate (sugar) attached to a lipid. E.g. Glycosphingolipids
   c. **Lipoproteins:** Macromolecular complexes of lipids with proteins.
   d. **Other complex lipids**: Sulfolipids, aminolipids and lipopolysaccharides.

C. Derived Lipids: Derived by modification, condensation or hydrolysis of simple and compound lipids. E.g. Monoacylglycerols and diacylglycerols, steroid hormones, lipid soluble vitamins

In addition to serving as an energy source, lipids have diverse functions such as forming plasma membranes, creating micelles, influencing hormonal action, and acting as thermal insulators. Despite their importance, the presence of free-moving lipids in the bloodstream and their accumulation in various vital organs can contribute significantly to lifestyle disorders like cardiovascular diseases (CVS) and obesity. In this experiment, we will focus on different tests used

to identify the chemical nature of lipids, distinguishing between saturated and unsaturated types, and determining the presence or absence of sterols.

Principles of specific tests:

A. Solubility test:

This is a most important step to know the chemical nature of the given sample of lipid. Due to hydrophobic of nature of lipids they are insoluble in water and are soluble in organic solvents.

B. Emulsification test:

The emulsion test is used to detect the presence of lipids. This process stabilizes the water and oil emulsion by using emulsifying agents. When fat or oil is mixed with water, it forms a supernatant layer. Emulsifying agents such as bile salts or soap reduce the surface tension of water, creating small lipid droplets suspended in the solution, visible as an emulsion.

C. Saponification test:

The alkaline hydrolysis of triacylglycerols to produce glycerol and fatty acids. Latter sodium or potassium ions combines with fatty acids to form soap (foam). Hence, this is known as saponification reaction.

D. Acrolein test:

Acrolein test is used to detect the presence of fat and glycerol. This test is based on the dehydration reaction, in which the water molecules are removed from the glycerol by adding reagent potassium hydrogen sulphate. The reaction between glycerol and potassium hydrogen sulphate results in acrolein (acrylic aldehyde) formation, which has characteristic pungent odour.

E. Hubl's iodine test (Tests for unsaturation):

This test is mainly helpful to evaluate the unsaturation in the given oil sample. The two principles that will explain the reaction mechanism are given below.

a. When bromine is reacted with unsaturated fatty acids, produces di-halo adducts. Consumption of more bromine indicates a higher percentage of unsaturation.

b. Decolorization of alkaline potassium permanganate is also an indirect measure of unsaturation in fatty acids. (Unsaturated fatty acids undergo incomplete oxidation)

5. Requirements

Glassware: Test tubes, Test tube holder, beakers, water bath, funnel, graduated pipettes, tripod stand, filter paper

Test sample: Oil samples like Coconut oil, Ghee, Palm oil, and Butter.

Chemicals: Ether, Benzene, Chloroform, Hexane, Ethanol, Bile salt solution (1%), Alcoholic-KOH (2% w/v KOH in ethyl alcohol), Potassium bisulphate, Dil $KMnO_4$ solution (0.05% w/v), Bromine water, Hubl's Iodine Solution.

Preparation of Reagents:

a. **Alcoholic-KOH (2% w/v)** - 2 g of potassium hydroxide (KOH) in 100 mL of ethyl alcohol.

b. **Dil $KMnO_4$ solution** (0.05% w/v) - 50 mg of $KMnO_4$ in 100 mL of distilled water.

c. **Hubl's Iodine Solution** - To prepare this (a) dissolve 2.6 g of iodine in 40 mL of ethanol (95% v/v), (b) dissolve 6.0 g of mercuric chloride in 40 mL of ethanol (95% v/v). Transfer both solutions 'a' and 'b' into a beaker and make up the volume to 100 mL with same ethanol.

6. Requirements Used

_____

_____

7. Procedure:

Perform following tests on given sample of lipid, observe and interpret as mentioned in the table:

Test	Observation	Inference
1. Solubility Test a) Add 1 mL fat /oil sample in 5 mL of water.	Immiscible (Found small floating droplets on water).	Presence of fat or oil
b) To small quantities of fat/oil taken in a separate test tube add the different organic solvents separately (ether, benzene, chloroform, hexane)	Soluble	Presence of fat or oil
2. Translucent Spot Test Take a filter paper. Add one drop of water at one end and a drop of sample at the other end.	Appearance of a translucent (semitransparent)/ greasy spot.	Presence of fat or oil
3. Emulsification Test a) To 1 mL alcoholic solution of fat/oil add 3 mL of water. Shake it vigorously.	A homogenous emulsion is formed which breaks on standing by separating oil phase in form of tiny droplets of oils.	Presence of fat or oil
b) To 1 mL alcoholic solution of fat/oil add 5 mL 1% bile salt solution and 3 mL of water. Shake it vigorously.	A homogenous emulsion is formed which remains stable on standing.	Presence of fat or oil
4. Saponification Test Take a sample of oil /fat in a test tube. Add 3 mL alcoholic-KOH and mix well. Place the test tube in a boiling water bath for 10-15 min.	Appearance of froth/foam.	Presence of fat or oil
5. Acrolein Test Take 1 ml of oil /fat sample in a test tube. and add a pinch of potassium bisulphate/ potassium hydrogen sulphate and mix well. Heat the solution for a few minutes.	A pungent smell.	Presence of glycerol
6. Unsaturation Test Take 1 mL of oil/ fat solution in a test tube, add 2 mL of chloroform/ether and dissolve completely. To this add potassium permanganate solution or bromine water drop by drop.	Decolourisation of potassium permanganate or bromine water.	Presence of unsaturated fatty acids.
7. Hubl's iodine test Take 1 mL of oil/ fat solution in a test tube, add 2 mL of chloroform/ether and dissolve completely. To this add Hubl's reagent drop wise mixing the contents	Decolourisation of iodine solution.	Presence of unsaturated fatty acids.

8. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

9. Result

The given sample of fat / oil _____ (complies / does not complies) physical and chemical test.

10. Conclusion

The qualitative tests for lipids were performed for a sample of fat / oil.

11. References

a. https://biologyreader.com/qualitative-analysis-of-lipids.html.
b. https://egyankosh.ac.in//handle/123456789/68529.

12. Related questions

a. How can one distinguish between saturated and unsaturated lipids using a simple qualitative test?
b. What role do emulsifying agents play in lipid tests, and why are they necessary? Name the emulsifying agent.
c. Discuss the significance of the iodine test in identifying the presence of lipids. What color changes are observed during this test?
d. Give the principle of Acrolein Test.
e. Define saponification of lipids.

(Space for Answers)

13. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No 12
Identification tests for cholesterol

1. Aim:

To perform identification tests for cholesterol.

2. Practical significance:

The tests performed to identify cholesterol are specific to its chemical nature and the functional groups present in it. Qualitative analysis of cholesterol helps us to determine its presence or absence based on color changes. Cholesterol deposition on artery walls leads to the destruction of their normal elasticity, a condition known as atherosclerosis. The qualitative analysis of cholesterol plays a significant role in the health sector. In this experiment, students will learn to analyze a given sample of cholesterol and record their conclusions.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind the specific test.	CO 1	BTL2
2	Perform the qualitative tests for cholesterol.	CO 1	BTL5
3	Write analytical reports systematically.	CO 1	BTL2
4	Observe and distinguish the colour while performing various chemical tests.	CO 1	BTL2
5	Enlist various tests used for identification of cholesterol.	CO1	BTL1

4. Relevant Theoretical Background

Cholesterol, the most abundant animal sterol, consists of a cyclopentano perhydro phenanthrene nucleus with 27 carbon atoms, as depicted in Fig 12.1. It is an amphipathic molecule, featuring a polar head group (the hydroxyl group at C-3) and a nonpolar hydrocarbon body (comprising the steroid nucleus and the hydrocarbon side chain at C-17).

All animal cells synthesize cholesterol, and it is also present in foods from animal sources like egg yolks, meat, and cheese. It serves as an essential structural component of animal cell membranes and acts as a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D. However, excessive cholesterol in the blood can lead to artery clogging, potentially resulting in heart attacks or strokes.

Cholesterol in the bloodstream associates with proteins to form lipoproteins, facilitating its transport. There are two main types of cholesterol:

- LDL (low-density lipoprotein), also known as 'bad cholesterol,' can combine with other substances to form plaque in arteries, leading to atherosclerosis and coronary artery disease.
- HDL (high-density lipoprotein), or 'good cholesterol,' removes cholesterol from blood vessels and transports it back to the liver for breakdown and elimination.

High levels of LDL and low levels of HDL increase the risk of heart disease.

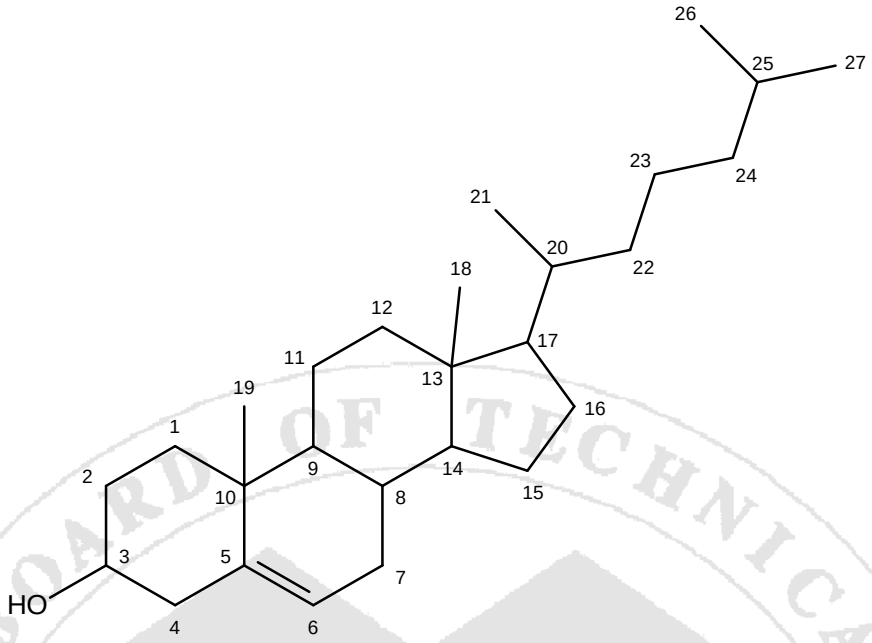


Fig 12.1: Structure of Cholesterol

Properties and reactions of Cholesterol

Cholesterol appears as a yellowish crystalline solid, with colourless and rhombic-shaped crystals that display a distinctive notch appearance under a microscope. It is insoluble in water but soluble in organic solvents like chloroform, benzene, and ether. For qualitative identification and quantitative estimation of cholesterol, several reactions are employed, including Salkowski's test and the Liebermann-Burchard reaction.

Salkowski's test is significant for detecting cholesterol based on distinct color changes produced during the dehydration of cholesterol with sulfuric acid, yielding clear colors.

Liebermann-Burchard reaction involves the use of a reagent that reacts with cholesterol, resulting in a deep green coloration. Initially, the color starts as purplish-pink and progresses to light green before turning very dark green. This color change occurs due to the reaction between the hydroxyl group (-OH) of cholesterol and the reagents, sulfuric acid, and acetic anhydride, enhancing the conjugation of unsaturation in adjacent fused rings. Acetic anhydride serves as a solvent and dehydrating agent, while sulfuric acid acts as both a dehydrating and oxidizing agent. This reaction is valuable for the colorimetric estimation of cholesterol in blood.

5. Requirements

Glassware: Test tubes, Test tube holder, beakers, water bath, funnel, graduated pipettes, tripod stand, filter paper.

Chemicals: Cholesterol, Ether, Chlorobenzene, Chloroform, Conc. H_2SO_4, Acetic anhydride

6. Requirements Used

_____

_____

7. Procedure:

Perform following tests on given sample of lipid, observe and interpret as mentioned in the table.

Test	Observation	Inference
1. **Solubility Test** a) Add 0.5 g cholesterol in 5 mL of water	Insoluble	Presence of cholesterol
b) To small quantities of cholesterol taken in a separate test tube add the different organic solvents separately (ether, chlorobenzene, chloroform)	Soluble	Presence of cholesterol
2. **Microscopic examination**	White shining rhombic crystals	Presence of cholesterol
3. **Salkowski test** Take 2 mL of cholesterol in a clean dry test tube and add 2 mL chloroform. To this add equal volume of concentrated sulphuric acid along the sides of the test tube and mix.	Appearance of reddish violet colour in upper chloroform layer and a yellow colour with green fluorescence in the lower acid layer	Presence of cholesterol
4. **Liebermann-Burchard Test** Take 2 mL of cholesterol in chloroform solution in a clean dry test tube. Add 10 drops of acetic anhydride and few drops of concentrated sulphuric acid and mix well.	Appearance of reddish violet colour which rapidly changes to deep blue and then emerald green.	Presence of cholesterol

8. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

9. Result

The given sample of cholesterol _____ (complies / does not complies) physical and chemical test.

10. Conclusion

The identification tests for cholesterol were performed and reported.

11. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.
b. https://egyankosh.ac.in//handle/123456789/68529.

12. Related questions

a. Discuss the Liebermann-Burchard reaction and its significance in detecting cholesterol.
b. What is the normal cholesterol level in blood?
c. Discuss the role of cholesterol in biological systems and its implications for health and disease.
d. Explain the principle behind Salkowski's test for cholesterol identification.

(Space for Answers)

13. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No 13
Qualitative analysis of Urine for Normal Constituents (UN1)

1. Aim:

To detect normal constituents in a given sample of urine by qualitative tests (UN1).

2. Practical significance:

Urine is an excretory product of the body produced by the kidneys. Analysis of urine provides valuable information about the condition of the human body and its metabolic state. Examination of urine is one of the basic procedures in clinical biochemistry that significantly contributes to the diagnostic process, as well as monitoring the disease course and effects of therapy. In this experiment, students will learn to analyze the given sample of urine for normal constituents.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Differentiate between organic and inorganic constituents of urine.	CO 2	BTL3
2	Perform the qualitative analysis of urine for normal constituents.	CO 2	BTL5
3	Write analytical reports systematically.	CO 2	BTL2
4	Interpret the result and diagnose the various renal and systemic diseases	CO 2	BTL6

4. Relevant Theoretical Background

Urine is an excretory product produced by the kidneys. Most waste products are excreted through urine. Urine contains a variety of organic and inorganic substances. The main organic nitrogen-containing waste products (besides proteins) are urea, uric acid, creatinine, and ethereal sulfates. Chlorides, sulfates, phosphates, sodium, potassium, calcium, and magnesium are the chief inorganic constituents of urine.

Physical and Chemical Properties of Normal Urine

Parameter	Specification
Colour	Straw Colour
Turbidity	Clear
pH	6.0
Specific gravity	1.015 – 1.025
Volume / Day	1.5 liter
Inorganic Constituents	
Sodium Chloride	10 – 12 g/day
Calcium	0.1 – 0.3 g/day
Phosphates	0.8 – 1.3 g/day
Sulphates	0.7 – 1.0 g/day
Potassium	3.0 g/day
Organic Constituents	
Urea	15 – 30 g/day
Uric acid	0.5 – 1.0 g/day
Ammonia	0.6 – 0.7 g/day
Creatinine	1 – 2 g/day

5. Requirements

Glassware: Beakers, Test tubes, Test tube stands, Burner, Glass rod, Filter paper.

Chemicals: Dil. HCl, Dil. H_2SO_4, Conc. HNO_3, Silver Nitrate solution, Ammonium molybdate (1N), Barium chloride solution, NaOH (40%), Phenolphthalein indicator, Strong ammonia, Acetic acid solution (1%), Potassium oxalate solution, Sodium hypobromite solution, Urease powder, Sodium carbonate (anhydrous), Sodium nitroprusside solution, Picric acid solution (saturated), Ehrlich reagent, Benedict's Uric acid reagent

6. Requirements Used

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7. Procedure:

A. Collection of Urine Sample

For qualitative urine analysis, begin by voiding a small amount of urine first. Then, collect 15-20 ml of midstream urine in a clean glass or plastic container.

B. Preparation of Simulated Urine Sample (If a urine sample is not available, a simulated urine sample should be used.)

Dissolve a sufficient quantity of some normal constituents (Urea**,** Creatinine**,** Sodium Chloride**,** Calcium Chloride**,** Potassium Chloride**,** Magnesium Sulfate**,** Sodium Bicarbonate**,** Sodium Phosphate etc) in 1 liter of distilled water. Adjust the pH to around 6.0 using dilute hydrochloric acid (HCl) or sodium hydroxide (NaOH) if necessary.

C. Physical Properties of Normal Urine

Test	Observation	Inference
Appearance	Clear and Transparent	Normal
	Turbid	Abnormal due to presence of pus cells or increased excretion of phosphates in alkaline urine
Colour	Straw or amber yellow colour	Normal
	Deep yellow	Abnormal due to presence of bile pigment (Jaundice).
	Reddish	Abnormal due to hematuria
	Reddish brown	Abnormal due to haemoglobinuria.
	Milky white	Abnormal due to chyluria.
	Black on standing	Abnormal due to presence of homogenic acid.
Odour	Aromatic Odour	Normal
	Putrid odour	Abnormal – Bacterial decomposition.
	Fruity odour	Abnormal – Diabetic ketoacidosis.
	Mousy odour	Abnormal – Phenylketonuria.
	Burnt sugar	Abnormal – maple syrup urine disease
Volume	800 – 2000 ml/ day	Normal
	More than 2000 mL/day	Abnormal – Polyuria – Increase volume due to more water intake, disease state like diabetes, high protein diet, diuretic

Test	Observation	Inference
		drugs.
	Less than 800 mL/day	Oliguria – Decreased volume due to excessive fluid loss due to vomiting, diarrhea, etc.
	No urine	Anuria – Total absence of urine due to acute tubular necrosis, shock, blood transfusion reaction.
pH	6 – 7.5	Normal
	Acidic (below 6)	Normally seen after high meat diet. Abnormally seen in acidosis.
	Alkaline (Above 8)	Alkalosis

D. Tests for Normal Inorganic constituents in Urine

Test	Observation	Inference
1. Test for chloride 3 mL urine + 0.5 mL conc. HNO_3 + 1 mL of 3% Silver nitrate solution.	A white precipitate of silver chloride appeared and soluble in ammonium hydroxide solution	Chloride is Present
2. Test for Biocarbonate 3 mL urine + dil. HCl or dil H_2SO_4	Effervescence of CO_2 gas	Bi-carbonate present
3. Test for Sulphate 5 mL urine + few drops of Conc. HCl + 5 mL 10% barium chloride	A white precipitate of barium sulphate is formed	Sulphate is Present
4. Test for Ammonia a. 3 mL urine + 2 mL of NaOH (10%) boil (Hold red litmus paper near mouth of test tube).	Evolution of ammonia (Red litmus turns to blue)	Ammonia is present
b. 2 mL urine + drop of Phenolphthalein indicator + make it alkaline by few drops of NaOH. Hold a glass rod dipped in phenolphthalein at the mouth of the test tube and heat the content.	Phenolphthalein indicator in the glass rod changes to pink colour. (The appearance of pink colour is due to evolution of ammonia from decomposition of ammonia salt in urine.)	Ammonia is present
5. Test for Calcium and Phosphorus 10 mL Urine + 10 drops of Strong ammonia, Boil and cool. A white precipitate formed. Filter it and discard the filtrate. Add 5 mL dilute acetic acid through sides of filter paper placed over the test tube so that precipitate in the filter paper get dissolved in acetic acid. Collect this is test tube and divide it into 2 parts.		

Test	Observation	Inference
Test for Calcium First part + 1 mL of potassium oxalate solution	White precipitate is obtained	Calcium present
Test for Phosphorus Second part + 1 mL Conc. HNO_3 + 3mL ammonium molybdate	A canary yellow precipitate is obtained.	Phosphorous present.

E. Test for Normal Organic Constituents in Urine

Test	Observation	Inference
1. Test for Urea **a. Alkaline Hypobromite Test** 3 mL urine + few drops of freshly prepare alkaline sodium hypobromite solution.	Brisk effervescence of nitrogen gas is produced	Urea is present
b. Specific Urease Test 3 mL Urine + few drops of phenolphthalein indictor. *(If a pink colour appears, add a few drops of 1% acetic acid until the pink colour disappears)* Add spatula full urease powder. Mix and allow stand for few minutes.	A pink colour develops	Urea is Present
2. Test for uric acid **a. Benedict's uric acid test** 3 mL urine + 1 mL Benedict's Uric acid reagent (phosphotungstic acid) + 1 ml Sodium Carbonate (anhydrous) solution.	A deep blue colour develops	Uric acid Present
b. Schiff's Test A piece of filter paper is moistened with silver nitrate solution and then add few drops of urine on the same paper	A black or brown colour develops	Uric acid Present
3. Test for Ethereal Sulphate 5 mL urine + 2 mL Barium chloride + 2mL HCl. Mix and filter. Divide filtrate into two tubes. Boil the contents in one tube. Carefully look for the turbidity developing in the tubes.	A white precipitate of barium sulphate develops in the first tube.	Ethereal sulphate present
4. Test for Creatinine - Jaffe' test 5 mL Urine + 1 mL 1% picric acid + 10 drops of 10% NaOH.	Orange red colour develops	Creatinine present

Test	Observation	Inference
5. Test for urobilinogen – Ehrlich's Test 3 mL urine + 1 mL Ehrlich reagent (p-dimethylaminobenzaldehyde), mix and allow to stand for 5 min.	Red colour is observed when viewed through the mouth of the test tube.	Urobilinogen present

8. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

9. Result

Given sample of urine contains

a. Inorganic constituents: _____

b. Organic constituents: _____

10. Conclusion

The normal constituents in the given sample of urine were detected by qualitative tests.

11. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.

12. Related questions

a. What is the typical color range for normal urine? What factors can influence urine color?
b. Describe a simple test for the presence of chloride in a urine sample.
c. What are the normal constituents present in a urine sample, and what are their typical levels?
d. State the condition in which creatinine excretion is increased in urine.

(Space for Answers)

13. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No 14
Qualitative analysis of Urine for Normal Constituents (UN2)

1. Aim:

To detect normal constituents in a given sample of urine by qualitative tests (UN2).

2. Practical significance:

Urine is an excretory product of the body produced by the kidneys. Analysis of urine provides valuable information about the condition of the human body and its metabolic state. Examination of urine is one of the basic procedures in clinical biochemistry that significantly contributes to the diagnostic process, as well as monitoring the disease course and effects of therapy. In this experiment, students will learn to analyze the given sample of urine for normal constituents.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Differentiate between organic and inorganic constituents of urine.	CO 2	BTL3
2	Perform the qualitative analysis of urine for normal constituents.	CO 2	BTL5
3	Write analytical reports systematically.	CO 2	BTL2
4	Interpret the result and diagnose the various renal and systemic diseases	CO 2	BTL6

4. Relevant Theoretical Background

Urine is the ultrafiltrate of plasma formed when blood perfuses the two kidneys. The glomerulus filters plasma, producing about 180 liters of glomerular filtrate in 24 hours for an adult. The tubules of the kidney modify the glomerular filtrate by reabsorption and secretion of water and solutes, resulting in a final urine volume of 1-2 liters per day. The glomerular filtration rate is about 120 ml per minute. Thus, the kidneys retain essential substances and excrete waste products from the body. This process also helps maintain acid-base balance. Clinical laboratory analysis of urine can provide information about kidney dysfunction (e.g., nephrotic syndrome, glomerulonephritis) and certain systemic diseases (e.g., phenylketonuria, diabetes mellitus) in an individual.

The main inorganic constituents of urine are Na+, K+, Ca+, Mg++, NH4+, Cl–, phosphates and sulfates. Important organic constituents in urine are urea, uric acid, ethereal sulfates, creatinine, organic sulfates, urinary pigments.

5. Requirements

Glassware: Beakers, Test tubes, Test tube stands, Burner, Glass rod, Filter paper.

Chemicals: Dil. HCl, Dil. H_2SO_4, Conc. HNO_3, silver Nitrate solution, Ammonium molybdate (1N), Barium chloride solution, NaOH (40%), Phenolphthalein indicator, Acetic acid solution (1%), Ammonium oxalate solution (1N), Sodium hypobromite solution, Urease powder, Sodium carbonate (anhydrous), Sodium nitroprusside solution, Picric acid solution (saturated), Ehrlich reagent

6. Requirements Used

_____

_____

7. Procedure:

A. Collection of Urine Sample

For qualitative urine analysis, begin by voiding a small amount of urine first. Then, collect 15-20 ml of midstream urine in a clean glass or plastic container.

B. Preparation of Simulated Urine Sample (If a urine sample is not available, a simulated urine sample should be used.)

Dissolve a sufficient quantity of some normal constituents (Urea, Creatinine, Sodium Chloride, Calcium Chloride, Potassium Chloride, Magnesium Sulfate, Sodium Bicarbonate, Sodium Phosphate etc) in 1 liter of distilled water. Adjust the pH to around 6.0 using dilute hydrochloric acid (HCl) or sodium hydroxide (NaOH) if necessary.

C. Physical Properties of Normal Urine: Refer Experiment No. 13

D. Tests for Normal Inorganic constituents in Urine: Refer Experiment No. 13

E. Test for Normal Organic Constituents in Urine: Refer Experiment No. 13

8. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

9. Result

Given sample of urine contains

a. Inorganic constituents: _____

b. Organic constituents: _____

10. Conclusion

The normal constituents in the given sample of urine were detected by qualitative tests.

11. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.

12. Related questions

a. What precautions should be taken when handling and analyzing urine samples in the laboratory?
b. Write the physiological and pathological condition in which urine volume is increased and decreased than normal volume.
c. Write a procedure for the detection test of ammonia in urine sample?
d. Define Polyuria, Oliguria and Anuria.

(Space for Answers)

13. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No 15
Qualitative Analysis of Urine for Abnormal Constituents (UA1)

1. Aim:

To detect abnormal constituents in given sample of urine by qualitative tests (UA1).

2. Practical significance:

Urine is an excretory product of the body produced by the kidneys. Analyzing urine provides valuable information about the condition of the human body and its metabolic state. Urine examination is one of the basic procedures in clinical biochemistry, significantly contributing to the diagnostic process, as well as monitoring the disease course and effects of therapy. Some diseases are associated with the excretion of abnormal constituents in urine, such as glucose in diabetes mellitus, bilirubin in liver diseases, and protein in glomerulonephritis. The identification of such compounds in urine is of great diagnostic importance. In this experiment, students will learn to analyze a given urine sample, record their observations, and draw conclusions.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Differentiate between various pathological conditions caused due to presence of abnormal constituents in urine.	CO 2	BTL4
2	Perform the qualitative analysis of urine for abnormal constituents.	CO 2	BTL5
3	Write analytical reports systematically.	CO 2	BTL2
4	Interpret the result and diagnose the various renal and systemic diseases.	CO 2	BTL6

4. Relevant Theoretical Background

Some substances that are normally absent in urine may appear in it under pathological conditions. These pathological constituents are referred to as abnormal constituents of urine. Additionally, certain substances present in normal urine in trace amounts may be found in significantly higher levels under specific pathological or abnormal conditions. These substances may also be considered abnormal constituents. The presence of an abnormal constituent in urine can aid in the diagnosis of diseased conditions.

Abnormal Condition	Pathological Condition	Associated Ailments
Glucose	Glycosuria	Diabetes Mellitus
Bilirubin	Bilirubinuria	Liver Disease (e.g., Hepatitis, Cirrhosis)
Proteins (mainly albumin)	Proteinuria (Albuminuria)	Glomerulonephritis
Ketone bodies	Ketonuria	Diabetes Mellitus, Starvation
Red Blood Cells	Hematuria	Urinary Tract Infection (UTI), Kidney Stones
White Blood Cells	Pyuria	Pyelonephritis, Interstitial Cystitis
Hemoglobin	Hemoglobinuria	Hemolytic Anemia
Bacteria	Bacteriuria	Urinary Tract Infection

5. Requirements

Glassware: Beakers, Test tubes, Test tube stands, Burner, Glass rod, Filter paper.
Chemicals: Dilute HCl, Dilute H_2SO_4, Conc. HNO_3, Benedict's reagent, Fehling's reagent, Acetic acid, Sulphosalicylic acid, Ammonium sulphate powder, Sodium nitroprusside solution, Strong ammonia, Benzidine powder, Sulphur powder, Magnesium sulphate solution, Barium chloride solution, Fouchet's reagent.

6. Requirements Used

_____

_____

7. Procedure:

A. Collection of Urine Sample

For qualitative urine analysis, begin by voiding a small amount of urine first. Then, collect 15-20 ml of midstream urine in a clean glass or plastic container.

B. Preparation of Simulated Urine Sample (If a urine sample is not available, a simulated urine sample should be used.)

Dissolve a sufficient quantity of some abnormal constituents (glucose, albumin, bilirubin, acetone, microbial culture, blood, etc.) in 1 liter of distilled water. Adjust the pH to around 6.0 using dilute hydrochloric acid (HCl) or sodium hydroxide (NaOH) if necessary.

C. Physical Properties of Normal Urine

Test	Observation	Inference
Appearance	Clear and Transparent	Normal
	Turbid	Abnormal due to presence of pus cells or increased excretion of phosphates in alkaline urine
Colour	Straw or amber yellow colour	Normal
	Deep yellow	Abnormal due to presence of bile pigment (Jaundice).
	Reddish	Abnormal due to hematuria
	Reddish brown	Abnormal due to haemoglobinuria.
	Milky white	Abnormal due to chyluria.
	Black on standing	Abnormal due to presence of homogenic acid.
Odour	Aromatic Odour	Normal
	Putrid odour	Abnormal – Bacterial decomposition.
	Fruity odour	Abnormal – Diabetic ketoacidosis.
	Mousy odour	Abnormal – Phenylketonuria.
	Burnt sugar	Abnormal – maple syrup urine disease
Volume	800 – 2000 ml/ day	Normal
	More than 2000 mL/day	Abnormal – Polyuria – Increase volume due to more water intake, disease state like diabetes, high protein diet, diuretic drugs.

Test	Observation	Inference
	Less than 800 mL/day	Oliguria – Decreased volume due to excessive fluid loss due to vomiting, diarrhea, etc.
	No urine	Anuria – Total absence of urine due to acute tubular necrosis, shock, blood transfusion reaction.
pH	6 – 7.5	Normal
	Acidic (below 6)	Normally seen after high meat diet. Abnormally seen in acidosis.
	Alkaline (Above 8)	Alkalosis

D. Tests for Abnormal Constituents in Urine

Test	Observation	Inference
1. Test for Sugar (Glucose) **a. Benedict's Test** 5 mL Benedict's reagent + 2 mL Urine, boil for two minutes and cool.	Green / Yellow/ Red precipitate is obtained.	Presence of reducing sugar such as glucose.
b. Fehling's Test 2 mL of Fehling's A + 2 mL Fehling's B, boil for few minutes + 2-3 ml of urine, boil again.	Red / Yellow precipitate is obtained.	Presence of glucose
2. Test for Proteins **a. Heat coagulation test** Take 6-8 mL of urine in the test tube. Incline the test tube at an angle and heat the upper one-third of the test tube by a low flame. Turbidity develops in the heated portion of the urine. Add 1% acetic acid solution drop by drop.	Turbidity or precipitates Turbidity remains	Presence of albumin Presence of albumin
b. Sulphosalicylic acid test 2 mL urine + sulphosalicylic acid (if necessary, heat it).	White precipitate is formed	Presence of protein (albumin)
c. Heller's Test 3 mL urine + few drops of Conc. HNO_3.	White ring at the junction of two fluids.	Presence of protein (albumin)
3. Test for Ketone bodies – Rothera's Test 3 mL urine sample saturated with solid ammonium sulphate powder + 2 – 5 drops of sodium nitroprusside (freshly prepared) + 2 mL strong ammonia (added slowly along the sides of the tube) keep it for 10 min.	Permanganate colored ring is developed at the junction of two liquids.	Presence of ketone bodies.

Test	Observation	Inference
4. Test for Blood **Benzidine Test** Small quantity of benzidine powder + 1 ml glacial acetic acid + 1 mL hydrogen peroxide. Mix well and add 1 mL urine.	Blue / green colour develops (It changes to black colour within few minutes).	Presence of blood
5. Test for Bile salt **Hay's Test** Take 2 test tubes. Add 2 mL urine in the first test tube and 2 mL of distilled water in the second test tube. A small quantity of sulphur powder is sprinkled over the surface of each liquid in each tube.	Sulphur powder sinks in the test tube containing urine.	Presence of bile salts.
6. Test for Bile pigments **a. Modified Fouchet's Test** 10 mL urine + 1 mL magnesium sulphate solution. Boil it. While boiling add a few drops of barium chloride solution (10%). A white precipitate is formed. Filter it and discard the filtrate. The precipitate in filter paper is dried. Add 2 drops of Fouchet's reagent to precipitate.	A blue or green colour is produced on filter paper.	Presence of bile pigments.
b. Gmelin's Test 5 mL conc HNO_3 + few drops of urine (added slowly along the sides of the tube).	Green / blue/ violet / red / yellowish colour developed at the junction of two liquids.	Presence of bile pigments.

8. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

9. Result

The given sample of abnormal urine contains_____.

10. Conclusion

The abnormal constituents in the given sample of urine were detected by qualitative tests.

11. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.

12. Related questions

a. What are abnormal constituents in urine, and how do they differ from normal constituents?
b. Describe the appearance and characteristics of a urine sample containing abnormal constituents.
c. Why are glucose, albumin, and ketone bodies called abnormal constituents of urine?
d. What is proteinuria? In which disease protein will be present in urine?

(Space for Answers)

13. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No 16
Qualitative Analysis of Urine for Abnormal Constituents (UA2)

1. Aim:

To detect abnormal constituents in given sample of urine by qualitative tests (UA2).

2. Practical significance:

Urine is an excretory product of the body produced by the kidneys. Analyzing urine provides valuable information about the condition of the human body and its metabolic state. Urine examination is one of the basic procedures in clinical biochemistry, significantly contributing to the diagnostic process, as well as monitoring the disease course and effects of therapy. Some diseases are associated with the excretion of abnormal constituents in urine, such as glucose in diabetes mellitus, bilirubin in liver diseases, and protein in glomerulonephritis. The identification of such compounds in urine is of great diagnostic importance. In this experiment, students will learn to analyze a given urine sample, record their observations, and draw conclusions.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Differentiate between various pathological conditions caused due to presence of abnormal constituents in urine.	CO 2	BTL4
2	Perform the qualitative analysis of urine for abnormal constituents.	CO 2	BTL5
3	Write analytical reports systematically.	CO 2	BTL2
4	Interpret the result and diagnose the various renal and systemic diseases.	CO 2	BTL6

4. Relevant Theoretical Background

Refer Experiment No 15.

5. Requirements

Glassware: Beakers, Test tubes, Test tube stands, Burner, Glass rod, Filter paper.

Chemicals: Dilute HCl, Dilute H_2SO_4, Conc. HNO_3, Benedict's reagent, Fehling's reagent, Acetic acid, Sulphosalicylic acid, Ammonium sulphate powder, Sodium nitroprusside solution, Strong ammonia, Benzidine powder, Sulphur powder, Magnesium sulphate solution, Barium chloride solution, Fouchet's reagent.

6. Requirements Used

_____

_____

7. Procedure:

A. Collection of Urine Sample

For qualitative urine analysis, begin by voiding a small amount of urine first. Then, collect 15-20 ml of midstream urine in a clean glass or plastic container.

B. Preparation of Simulated Urine Sample (If a urine sample is not available, a simulated urine sample should be used.)

Dissolve a sufficient quantity of some abnormal constituents (glucose, albumin, bilirubin, acetone, microbial culture, blood, etc.) in 1 liter of distilled water. Adjust the pH to around 6.0 using dilute hydrochloric acid (HCl) or sodium hydroxide (NaOH) if necessary.

C. Physical Properties of Normal Urine: Refer Experiment No 15.

D. Tests for Abnormal Constituents in Urine: Refer Experiment No 15.

8. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

9. Result

The given sample of abnormal urine contains_____.

10. Conclusion

The abnormal constituents in the given sample of urine were detected by qualitative tests.

11. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.

12. Related questions

a. Name the bile pigments.
b. What is hemoglobinuria? In which disease will there be hemoglobinuria?
c. Name five abnormal constituents of urine along with their pathological conditions.
d. Name the test to identify reducing sugar in urine. Write its significance.

(Space for Answers)

13. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No 17
Qualitative Analysis of Urine for Abnormal Constituents (UA3)

1. Aim:

To detect abnormal constituents in given sample of urine by qualitative tests (UA3).

2. Practical significance:

Urine is an excretory product of the body produced by the kidneys. Analyzing urine provides valuable information about the condition of the human body and its metabolic state. Urine examination is one of the basic procedures in clinical biochemistry, significantly contributing to the diagnostic process, as well as monitoring the disease course and effects of therapy. Some diseases are associated with the excretion of abnormal constituents in urine, such as glucose in diabetes mellitus, bilirubin in liver diseases, and protein in glomerulonephritis. The identification of such compounds in urine is of great diagnostic importance. In this experiment, students will learn to analyze a given urine sample, record their observations, and draw conclusions.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Differentiate between various pathological conditions caused due to presence of abnormal constituents in urine.	CO 2	BTL4
2	Perform the qualitative analysis of urine for abnormal constituents.	CO 2	BTL5
3	Write analytical reports systematically.	CO 2	BTL2
4	Interpret the result and diagnose the various renal and systemic diseases.	CO 2	BTL6

4. Relevant Theoretical Background

Refer Experiment No 15.

5. Requirements

Glassware: Beakers, Test tubes, Test tube stands, Burner, Glass rod, Filter paper.

Chemicals: Dilute HCl, Dilute H_2SO_4, Conc. HNO_3, Benedict's reagent, Fehling's reagent, Acetic acid, Sulphosalicylic acid, Ammonium sulphate powder, Sodium nitroprusside solution, Strong ammonia, Benzidine powder, Sulphur powder, Magnesium sulphate solution, Barium chloride solution, Fouchet's reagent.

6. Requirements Used

_____

_____

7. Procedure:

A. Collection of Urine Sample

For qualitative urine analysis, begin by voiding a small amount of urine first. Then, collect 15-20 ml of midstream urine in a clean glass or plastic container.

B. Preparation of Simulated Urine Sample (If a urine sample is not available, a simulated urine sample should be used.)

Dissolve a sufficient quantity of some abnormal constituents (glucose, albumin, bilirubin, acetone, microbial culture, blood, etc.) in 1 liter of distilled water. Adjust the pH to around 6.0 using dilute hydrochloric acid (HCl) or sodium hydroxide (NaOH) if necessary.

C. Physical Properties of Normal Urine: Refer Experiment No 15.

D. Tests for Abnormal Constituents in Urine: Refer Experiment No 15.

8. Observations (Students to write test, observation, and inference)

Test	Observation	Inference

Test	Observation	Inference

9. Result

The given sample of abnormal urine contains_____.

10. Conclusion

The abnormal constituents in the given sample of urine were detected by qualitative test.

11. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (p) ltd, first edition.

12. Related questions

a. Define Pathological urine
b. What is glycosuria? In which disease will there be glycosuria?
c. Name the test to identify protein in urine. Write its significance.
d. Why is identifying abnormal constituents in urine important for medical diagnosis and treatment?

(Space for Answers)

13. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 18
Estimation of Glucose in Urine

1. Aim

To estimate the amount of glucose present in the given sample of urine by using Benedict's quantitative method.

2. Practical Significance

The presence of glucose in the urine is called glycosuria or glucosuria. The amount of sugar (glucose) in a urine sample can be measured using a glucose urine test. However, blood tests to measure glucose levels are easier and are often used instead of the glucose urine test. The glucose urine test may be ordered when the doctor suspects renal glycosuria, a rare condition in which glucose is released from the kidneys into the urine, even when the blood glucose level is normal. Determining sugar concentration in urine is of great value in managing diabetes mellitus. In this experiment, students will learn to estimate the amount of glucose in a given urine sample using Benedict's quantitative method.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind Benedict's quantitative method.	CO 2	BTL2
2	Estimate the amount of glucose in a urine sample quantitatively	CO 2	BTL6
3	Write the analytical reports systematically	CO 2	BTL2

4. Relevant Theoretical Background

Benedict's Quantitative reagents is a common method for measuring the amount of glucose in urine. It works based on the redox reaction between reducing sugar and copper (Cu^{2+}) ions. Benedict's solution has a characteristic blue color because of the presence of copper (II) ions. When a solution containing reducing sugar is added to Benedict's solution, the copper II (Cu^{2+}) ions are reduced to copper I (Cu^{1+}) ions by reducing sugar, causing a color change.

A reducing sugar is a sugar that contains a free aldehyde or α-hydroxyketone group capable of reducing iron (Fe^{3+}) or copper (Cu^{2+}) ions. In a reaction with iron (Fe^{3+}) or copper (Cu^{2+}) ions, an aldehyde is oxidized to carboxylic acid, while an α-hydroxyketone is oxidized to a diketone. Examples of reducing sugars include fructose, glucose, maltose, galactose, and lactose. Notably, sucrose is not a reducing sugar.

Benedict's Quantitative Solution contains copper sulfate, sodium citrate, sodium carbonate, potassium thiocyanate, and potassium ferrocyanide. Each component plays an important role in determining reducing sugars:

a. **Copper Sulfate**: This component provides the cupric (Cu^{2+}) ions that are reduced by reducing sugars.

b. **Sodium Carbonate**: This creates an alkaline environment essential for the redox reaction to occur.

c. **Sodium Citrate**: This prevents the formation of a copper carbonate precipitate. Copper (Cu^{2+}) ions can combine with carbonate ions (CO_3^{2-}) to form insoluble copper carbonate.

Citrate ions "chelate" (form a stable bond) with Cu^{2+} ions before they can react with CO_3^{2-} and precipitate.

d. **Reducing Sugar**: The reducing sugar acts as the titrant, reducing Cu^{2+} ions to cuprous (Cu^{+}) ions. These Cu^{+} ions then combine with thiocyanate ions (SCN^{-}) from potassium thiocyanate to form a white precipitate of copper thiocyanate (CuSCN), marking the endpoint of the titration.

e. **Potassium Ferrocyanide**: A small amount of this compound helps prevent the reoxidation of Cu^{+} ions back to Cu^{2+} ions.

5. Requirements

Glassware: Conical flask, burette, pipette, tripod stand, beaker, glass rod, test tubes.

Chemicals: Benedict's Quantitative reagent, Sodium carbonate, Urine sample, Distilled water

Preparation of Benedict's Quantitative reagent

Mix 100g of sodium carbonate, 125g of potassium thiocyanate, and 200g of sodium citrate. Add distilled water to make 600 mL of solution. Heat the mixture to dissolve the chemicals, then cool and filter it. Next, add 18g of copper sulfate and 5 ml of 5% w/v potassium ferrocyanide to the filtered solution. Mix well and add distilled water to make the volume up to 1000 mL.

6. Requirements used

_____

_____

7. Precautions to be taken

a. Use freshly prepared reagent solutions.
b. Do not ingest acids or other chemicals orally; use a pipette aid or suction bulb.
c. Perform titrations carefully, especially when nearing the endpoint.

8. Procedure

a. Add 8 drops of the given urine sample to 5 mL of Benedict’s quantitative reagent in a test tube. Boil the mixture and then cool it. Observe the precipitate or color obtained.
b. Dilute the given urine sample based on the color obtained in step (a).

Sr. No.	Colour obtained in Step (a)	Dilution factor	Procedure
1	Green	**2** (1 in 2)	10 mL urine sample + 10 mL distilled water to make total volume **20 mL**
2	Yellow	**5** (1 in 5)	10 mL urine sample + 40 mL distilled water to make total volume **50 mL**
3	Brick red	**10** (1 in 10)	10 mL urine sample + 90 mL distilled water to make total volume **100 mL**

c. Fill the burette with diluted urine.
d. Pipette out 10 mL of Benedict’s quantitative reagent into a 100 mL conical flask.
e. Add 20 mL of distilled water and 5g of anhydrous sodium carbonate.
f. Shake the mixture well, add a few pieces of porcelain, and heat the flask on a flame or hot plate.
g. Ensure that the mixture in the flask remains boiling throughout the titration.

h. Add the diluted urine rapidly from the burette into the flask until a white precipitate appears.
i. Then, add the diluted urine from the burette drop by drop with constant stirring until the blue color of the reagent disappears. Note the burette reading.

9. Observations

Sr. No.	Particulars	Observations
1	Colour obtained in step I	
2	Dilution factor of given urine sample (DF)	
3	End Point	White precipitate with complete disappearance of blue colour.
4	Burette Reading (Volume of diluted urine required to reduce 10 mL Benedict's quantitative reagent (X mL)	

10. Calculations:

20 mg / 0.020 g of glucose required to completely reduce 10 mL of Benedict's quantitative reagent.
∴ X mL of diluted urine sample contains 0.020 g of glucose.
_____ mL ≡ 0.020 g of glucose

100 mL diluted sample of urine contains = $\frac{100 \times 0.02}{\text{X mL of Urine}}$ g of glucose

= $\frac{2}{}$ g of glucose

= _____ g of glucose

∴ 100 mL diluted sample of urine contains = _____ g of glucose

Percentage of glucose in diluted urine sample = Y %

Percentage of glucose in diluted urine sample = _____ %

∴ Percentage of glucose in given urine sample = Y % × Dilution factor (DF)

= _____ × _____

Percentage of glucose in given urine sample = _____ g % or g/100 mL

11. Result:

a. The amount of glucose in given sample of urine was found to be _____ g/100 mL (_____ g %).
b. Concentration of glucose in given sample of urine was found to be _____ (less/more) than normal value. (*Normal value of glucose excretion in urine is 2 to 10 mg glucose/100 mL)*

12. Conclusion

The amount of glucose in the given sample of urine was estimated by Benedict's quantitative method.

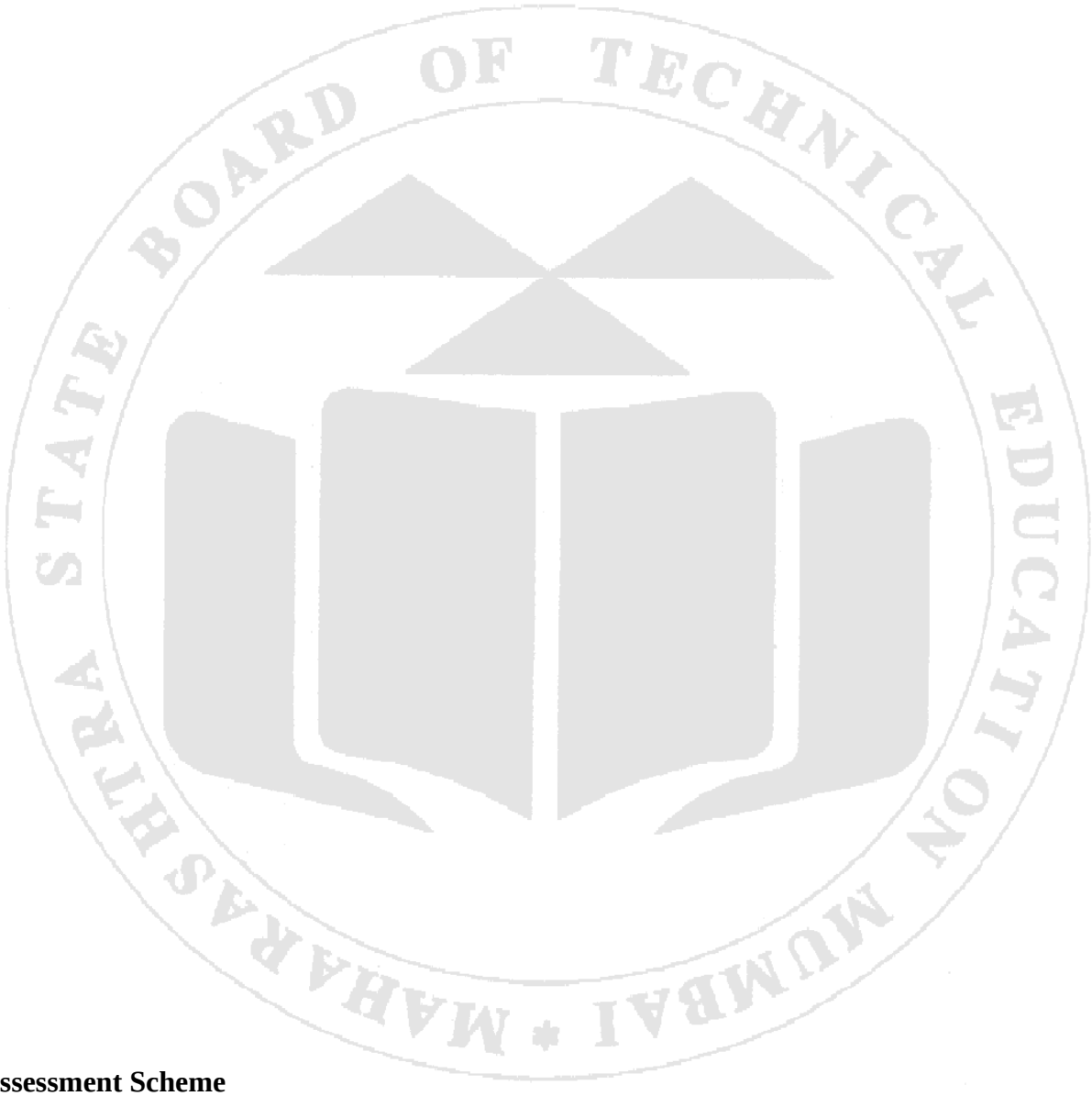
13. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (P) Ltd, First edition.
b. Frais, F. Practical Biochemistry: An Introductory Course; Butterworths: London, 1972; pp 30–32, 150–151.

14. Related questions

a. Explain the reaction and principle involved in the determination of the concentration of glucose in urine using Benedict's Quantitative Method.
b. Calculate the amount of glucose in urine when the volume of the urine sample required to reduce 10 mL of Benedict's quantitative reagent is 8.5 mL, and the dilution factor is 5.
c. How would you prepare Benedict's quantitative reagent?
d. If the glucose content in the urine is more than normal, then patient is suffering from which disease?

(Space for Answers)



15. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 19
Estimation of Creatinine in Urine

1. Aim
To estimate the amount of creatinine present in the given sample of urine by Jaffe's method.

2. Practical Significance
Creatinine is a waste product derived from the dehydration of creatinine phosphate. Creatine is synthesized within the body from amino acids through a series of two enzyme-mediated reactions. After synthesis, creatine is transported in the blood to other organs such as the muscles and brain, where it is phosphorylated to creatine phosphate, a high-energy compound. Some of the free creatine in the muscles is spontaneously converted to creatinine, which is the anhydride form of creatine. Both compounds are converted spontaneously to creatinine at a rate of 1–2%. Creatinine is entirely eliminated from the body by the kidneys. However, if kidney function is impaired, the level of creatinine in the urine increases. In this experiment, students will learn to estimate the amount of creatinine in the given sample of urine.

3. Practical Outcomes (PrO)
The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind Jaffe's reaction.	CO 2	BTL2
2	Estimate the amount of creatinine in a urine sample quantitatively.	CO 2	BTL6
3	Write the analytical reports systematically	CO 2	BTL2
4	Handle the colorimeter correctly	CO 2	BTL2

4. Relevant Theoretical Background
Methods for measuring creatinine often rely on the Jaffe reaction. In this reaction, creatinine reacts with picric acid in an alkaline solution. This forms a colored complex (red-orange) called creatinine picrate, which absorbs light at a specific wavelength (520 nm). The stronger the color, the higher the concentration of creatinine in the sample. The color intensity is compared to a standard creatinine solution treated the same way. Both the sample and standard are measured against a blank solution (containing no creatinine) using a colorimeter at a green filter (around 520 nm) or a spectrophotometer at precisely 520 nm.

Creatinine in Urine (Colourless) + Picric Acid $\xrightarrow{\text{Alkaline media}}$ Creatinine picrate (Red- orange coloured complex)

5. Requirements
Equipment: Photoelectric colorimeter / Spectrophotometer.

Glassware: Conical flask, pipette, test tubes, cuvettes, volumetric flask, beaker, glass rod.

Chemicals: Urine sample (collected before 24 hours), Picric acid (1%), Sodium hydroxide solution (10%), Distilled water, Creatinine, Hydrochloric acid (1N).

6. Requirements used

_____

_____

7. Procedure

a. Preparation of Standard Creatinine solution (stock solution)

Dissolve 1g of creatinine in 100 ml 1N HCl. Dilute it to 1000 mL with distilled water.

1g ≡ 1000 mg in 1000 mL

1 mL standard creatinine solution ≡ 1 mg of creatinine

b. Prepare creatinine working standard by diluting 1.0 mL of stock solution to 100 mL with distilled water. (Concentration of creatinine will be 0.01 mg/mL)

c. Dilute 1.0 mL of given urine sample to 100 mL in volumetric flask.

d. Prepare standard, test and blank solutions as mentioned in the following table:

Sr. No.	Content	Standard	Test	Blank
1	Creatinine working standard (0.01mg/ml)	5.0 mL	-	-
2	Diluted Urine	-	5.0 mL	-
3	Distilled Water	-	-	5.0 mL
4	Picric acid (1%)	1.0 mL	1.0 mL	1.0 mL
5	Sodium Hydroxide (10%)	1.0 mL	1.0 mL	1.0 mL

e. Allow to stand for 15 minutes.

f. Set photoelectric colorimeter at green filter / spectrophotometer at 510 nm.

g. Adjust 0% absorbance with distilled water

h. Record the intensity of colours obtained for blank, test and standard.

8. Precautions to be taken

Dry picric acid is explosive upon percussion. Discharging picric acid waste through copper pipes can cause the formation of copper picrate, which may lead to an explosion.

9. Observations

A. Concentration of creatinine in test solution (5 mL) CS = 0.05mg

B. Absorbance of blank solution (AB) = _____

C. Absorbance of standard solution (AS) = _____

D. Absorbance of test solution (AT) = _____

10. Calculations:

1 mL of urine is diluted to 100 mL and 5.0 mL is taken in test solution

Concentration of creatinine in test solution (0.05 mL undiluted urine) = CT

$$CT = [(AT - AB) / (AS-AB)] \times CS$$

$$CT = [(AT - AB) / (AS-AB)] \times 0.05$$

$$CT = [\quad / \quad] \times 0.05$$

CT =

Hence, concentration of creatinine in 100 mL urine (X) = (100 × CT) / 0.05

= 2000 × _____

Concentration of creatinine in 100 mL urine (X) = _____ mg/100mL

Concentration of creatinine in 1000 mL urine (Y) = X × 10 mg/lit

= ____ × 10 mg/lit

Concentration of creatinine in 1000 mL urine (Y) = _____ mg/lit

Concentration of creatinine in 1000 mL urine (Z) = Y/1000 g/lit

= _____/1000 g/lit

= _____ g/lit

Therefore, amount of creatinine in 24 hours urine = Volume of 24 hr urine sample in liters × Z

= 1.5 × _____ (Z)

= _____g / 24h

11. Result:

Amount of creatinine in a given sample of urine was found to be _____ g/24 Hrs. (Normal range is 1.5 to 3.0 g/24 Hrs).

12. Conclusion:

The amount of creatinine in the given sample of urine was estimated by Jaffe's method.

13. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (P) Ltd, First edition.

14. Related questions

a. State the condition in which creatinine excretion is increased in urine.
b. What is the basic principle behind Jaffe's reaction for creatinine measurement?
c. What is the significance of using a specific wavelength (520 nm) for measuring the color intensity?
d. Are there other methods available for measuring creatinine, and what are their potential advantages compared to Jaffe's reaction?

(Space for Answers)

15. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 20
Estimation of Creatinine in Human Blood

1. Aim

To estimate the amount of creatinine in the given sample of human blood by Jaffe's reaction using colorimeter.

2. Practical Significance

Normal creatinine levels in males are 0.7-1.5 mg%, while in females they are 0.4 – 1.2 mg%. Because males have more muscle mass, higher levels of creatinine are found. A high creatinine level in serum or urine is an indication of health risks such as kidney failure, kidney obstruction, protein catabolism, dehydration, diabetic nephropathy, etc. This experiment will help students learn to estimate creatinine levels by Jaffe's reaction using a colorimeter.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind Jaffe's reaction.	CO 2	BTL2
2	Estimate the amount of creatinine in a blood sample quantitatively.	CO 2	BTL6
3	Write the analytical reports systematically	CO 2	BTL2
4	Handle the colorimeter correctly	CO 2	BTL2

4. Relevant Theoretical Background

Creatinine is an anhydride of creatine phosphate that is present in serum, red blood cells (RBC), and all body secretions such as sweat, bile, and cerebrospinal fluid. Creatine is synthesized in the liver and kidneys and is then carried by the blood to muscular tissues, where it is converted to creatine phosphate, serving as a storage form of energy.

During muscle contraction, energy is required, which is provided by the breakdown of ATP to form ADP. ATP is then regenerated from ADP and creatine phosphate by the action of creatine kinase. Creatine phosphate donates its phosphate group to ADP to form ATP, and creatine is formed. Free creatine is subsequently converted to creatinine through spontaneous dehydration. About 2% of the total creatine is converted to creatinine per day. The daily production of creatinine remains constant to maintain its concentration in plasma and urine unless there is a change in muscle mass.

Jaffe's reaction is used to determine creatinine levels in serum. A whole blood sample should not be used as blood contains other components that can interfere with Jaffe's reaction; therefore, blood serum is used. In this reaction, serum creatinine reacts with an alkaline picrate solution to form a red-orange colored creatinine-picrate complex. The absorbance of the complex is measured with a green filter in a colorimeter or at 520 nm in a spectrophotometer.

$$\text{Creatinine in Urine (Colourless)} + \text{Picric Acid} \xrightarrow{\text{Alkaline media}} \text{Creatinine picrate (Red- orange coloured complex)}$$

5. Requirements

Equipment: Photoelectric colorimeter / Spectrophotometer.

Glassware: Conical flask, Pipette, Test tubes, Cuvettes, Centrifuge tube, Volumetric flask.

Chemicals:

a. Serum sample or simulated creatinine sample
b. **0.75 N Sodium hydroxide solution**: Dissolve 30 g AR NaOH in 200-300 mL water, make up volume to 1 L.
c. **Picric acid solution (0.04 mol/L or 9.16 g/L):** Dissolve 9.16 g of picric acid AR in 200-300 mL water, make up volume to 1 L.
d. **Sodium tungstate (5%):** Dissolve 5 g of sodium tungstate dihydrate in 20 mL of water and make up the volume to 100 mL.
e. **Stock creatinine standard (1 mg/mL)**: Dissolve 100 mg creatinine in 50 mL 0.1N HCl, and make up the volume to 100 mL with 0.1 N HCl in volumetric flask.
f. **0.1N HCl**: Dilute 8.2 mL of 37% concentrated HCl up to 1000 mL distilled water.
g. **Working creatinine standard solution (1 mg/dL or 0.01 mg/mL):** Dilute 1 mL of creatinine stock solution to 100 mL with 0.1 N HCl in volumetric flask.
h. **2/3 N Sulphuric acid solution**: Transfer 8.7 mL of sulphuric acid AR in volumetric flask containing 250 mL distilled water slowly. Make up the volume to 500 mL using distilled water, mix well.

6. Requirements used

_____

_____

7. Procedure

a. **Preparation of protein-free filtrate**: In a centrifuge tube add 1 mL of distilled water, 1 mL serum sample, 1 mL sodium tungstate, 1 mL 2/3N sulphuric acid, mix well and centrifuge for 10 min. Use the supernatant solution as protein-free filtrate (PFF). (This step can be omitted if a simulated creatinine sample is used).

b. Prepare the blank, standard, test solutions as shown in the table.

Sr. No.	Content	Blank	Standard	Test
1	PFF or Creatinine test solution	-	-	3.0 mL
2	Distilled Water	3.0 mL	2.5 mL	-
3	Woking creatinine standard solution (1mg/dL)	-	0.5 mL	-
4	Picric acid (1%)	1.0 mL	1.0 mL	1.0 mL
5	0.75 N Sodium Hydroxide	1.0 mL	1.0 mL	1.0 mL

c. Keep all the tubes at room temperature for 15 minutes.
d. Adjust absorbance or optical density (OD) of blank to zero, using a green filter in the colorimeter (520 nm).
e. Record the absorbance of standard and test solutions.

8. Precautions to be taken

Dry picric acid is explosive upon percussion. Discharging picric acid waste through copper pipes can cause the formation of copper picrate, which may lead to an explosion

9. Observations

A. Concentration of creatinine in standard solution CS = 0.005 mg

B. Absorbance or optical density of standard solution (AS) = _____

C. Absorbance of optical density test solution (AT) = _____

10. Calculations:

To find out concentration of creatinine in 100 mL blood (mg %), use following formula.

$$\text{Serum creatinine (mg \%)} = \frac{AT}{AS} \times \frac{\text{Concentration of standard (mg)}}{\text{Volume of sample (mL)}} \times 100$$

$$\text{Serum creatinine (mg \%)} = \frac{AT}{AS} \times \frac{0.005}{0.5} \times 100$$

$$\text{Serum creatinine (mg \%)} = \frac{\quad}{\quad} \times 1$$

Serum creatinine (mg %) = _____ mg%

Reference range: Males - 0.7 to 1.5 mg %, Females – 0.4-1.2 mg %

11. Result:

The amount in creatinine in the given sample of blood was found to be _____ mg %.

12. Conclusion:

The amount of creatinine in the given sample of human blood was estimated by Jaffe's reaction using colorimeter.

13. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (P) Ltd, First edition.
b. Indian Pharmacopoeia 2018.
c. Practical Biochemistry, G. Rajagopal, B.D. Torra, Ahuja's Publishing House, Fourth edition.

14. Related questions

a. Explain the principle behind Jaffe's reaction for estimating creatinine in human serum.
b. Discuss the significance of measuring creatinine levels in human serum and its relevance in clinical diagnosis.
c. How is creatinine formed in the body? Give its normal range.
d. Give the reason for lower creatinine concentration in females?

(Space for Answers)

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15. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 21
Estimation of Glucose in Blood

1. Aim

To estimate the amount of glucose in the given sample of human blood using colorimeter.

2. Practical Significance

Blood glucose measurement is crucial for diagnosing diabetes mellitus. High glucose levels signify diabetes, whereas low levels may suggest hypoglycemia, necessitating proper care and treatment. Irregularities in glucose levels can signal metabolic issues like insulin resistance, metabolic syndrome, or pancreatic disorders. In essence, serum glucose estimation is essential for guiding clinical decisions, managing patients, and diagnosing diseases. While working in the pathology laboratory, the student needs to adapt expertise in blood sugar estimation. In this experiment, the student will learn to estimate the levels of glucose by various methods using a colorimeter

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind various methods used for estimation of blood glucose.	CO 2	BTL2
2	Estimate the amount of glucose in a blood sample quantitatively.	CO 2	BTL6
3	Handle the colorimeter correctly	CO 2	BTL2

4. Relevant Theoretical Background

Glucose is a primary source of energy for cells and is essential for various metabolic processes in the body. It is derived from dietary carbohydrates and glycogen stores in the liver and muscles. Glucose metabolism involves glycolysis, where glucose is converted into pyruvate, followed by aerobic or anaerobic pathways to produce ATP (adenosine triphosphate).

Blood glucose levels are tightly regulated to maintain homeostasis and provide energy to tissues. Abnormalities in blood glucose levels, such as hyperglycemia (high glucose) or hypoglycemia (low glucose), can indicate underlying health conditions. Glucose monitoring is crucial in critical care settings, diabetes management, preoperative assessments, and research studies focused on metabolic disorders

The estimation of glucose levels employs several methods:

a) The Folin-Wu method
b) The Ortho-toluidine method
c) The Glucose oxidase-peroxidase method (GOD/POD method)

Among these, the Ortho-toluidine and GOD/POD methods are commonly used for estimating glucose levels in plasma.

Ortho-toluidine method

In the Ortho-toluidine method, glucose reacts with ortho-toluidine in the presence of glacial acetic acid at 100°C, forming N-glucosylamine, which imparts a blue-green colour. Glucose concentration is proportional to the intensity of the colour. The colour intensity is then measured using a colorimeter or spectrophotometer.

5. Requirements

Equipment: Photoelectric colorimeter / Spectrophotometer, Centrifuge.

Glassware: Conical flask, Pipette, Test tubes, Centrifuge tube.

Specimen solution:

a. **Simulated sample, plasma, or serum**. The container for collecting blood plasma should contain a potassium oxalate-sodium fluoride mixture (3 parts potassium oxalate and one part sodium fluoride is added to 3 mg/ml plasma) to prevent clotting and glycolysis. If serum is to be used, it should be separated immediately after clotting.

Reagents for o-Toluidine method:

a. **Saturated benzoic acid solution**: Dissolve 2.5 g benzoic acid in hot distilled water and makeup volume to 1000 mL with distilled water. This solution acts as a preservative for glucose.

b. **Ortho-toluidine reagent**: Dissolve 1.5 g of thiourea in 940 mL of glacial acetic acid. Add 60 mL of ortho toluidine. Mix well and store in an amber-coloured bottle. Keep for 24 hours before using it.

c. **Glucose stock solution (1 g%)**: Weigh 1 g dry anhydrous glucose (dextrose) and dissolve it in 80 mL of saturated benzoic acid solution. Make the volume to 100 mL with a saturated benzoic acid solution.

d. **Working glucose standard solution (100 mg%):** Dilute 1 mL of glucose stock solution to 10 mL with a saturated benzoic acid solution. This solution is stable for 3-4 days.

6. Requirements used

_____

_____

7. Procedure

Ortho-toluidine method:

a. If using blood samples, take 0.5 mL of blood, add 3 mL distilled water and 1.5 mL of 10% trichloroacetic acid, mix and wait for 10 min. If using plasma or serum or simulated samples skip this step.

b. Prepare the Blank, standard, test solution in test tubes as shown in the table.

Sr. No.	Content	Blank	Standard	Test
1	Ortho-toluidine reagent	5.0 mL	5.0 mL	5.0 mL
2	Distilled Water	1.0 mL	-	-
3	Woking glucose standard solution	-	1.0 mL	-
4	Specimen solution	-	-	1.0 mL

c. Place all the test tubes in a boiling water bath for 10-12 minutes. Then cool the test tubes for 5 minutes in cold water.

d. Select an orange or red filter in a colorimeter or 600-630 nm wavelength in a spectrophotometer.

e. Adjust absorbance or optical density (OD) of blank to zero, then record the absorbance of standard and test solutions.

8. Observations

Ortho-toluidine method:

a. Concentration of glucose in standard solution CS = 1 mg

b. Absorbance or optical density of standard solution (AS) = _____

c. Absorbance of optical density test solution (AT) = _____

9. Calculations:

To find out the concentration of glucose (mg/dL or mg%) use the following formula.

$$\text{Blood glucose (mg \%)} = \frac{AT}{AS} \times \frac{\text{Concentration of standard (mg)}}{\text{Volume of sample (mL)}} \times 100$$

$$\text{Blood glucose (mg \%)} = \frac{AT}{AS} \times \frac{1}{1} \times 100$$

$$\text{Blood glucose (mg \%)} = \frac{\quad}{\quad} \times 1$$

Blood glucose (mg %) = _____ mg%

Reference ranges

Normal Blood Sugar Level	: 60-100 mg%
Random Blood Sugar Level	: <140 mg%
Fasting Blood Sugar Level	: 60-100 mg% (12-hour fasting sample)
Postprandial Blood Sugar Level	: 100-140 mg% (sample is taken after 2 hours of meal or intake of 75 g of oral glucose intake)

10. Result:

The amount of glucose in the given sample of blood was found to be _____ mg %.

11. Conclusion:

The amount of glucose in the given sample of human blood was estimated using colorimeter.

12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (P) Ltd, First edition.

b. Practical Biochemistry, G. Rajagopal, B.D. Torra, Ahuja's Publishing House, Fourth edition.

13. Related questions

a. Enlist the methods for estimating blood glucose levels.

b. Discuss the importance of patient education and self-monitoring of blood glucose levels in diabetes care.
c. Explain the principle of the ortho-toluidine method.
d. What are the WHO criteria for the diagnosis of diabetes mellitus?
e. What are the different types of diabetes mellitus?

(Space for Answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 22
Estimation of Cholesterol in Human Blood

1. Aim

To estimate the amount of cholesterol in the given sample of human blood.

2. Practical Significance

Cholesterol plays a crucial role in the human body by facilitating the synthesis of hormones such as testosterone, estrogen, and adrenocorticoids. Additionally, cholesterol is essential for the synthesis of vitamin D and contributes to maintaining the fluidity of cell membranes. Elevated cholesterol levels can lead to the deposition of cholesterol on artery walls, resulting in arteriosclerosis or atherosclerosis, which can further lead to conditions like stroke, chest pain, cardiac arrest, gallstones, and numbness in the legs. Conversely, low cholesterol levels may contribute to conditions such as anxiety, depression, and certain types of cancer. In this experiment, students will estimate cholesterol levels using Zak's method with a colorimeter.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind Zak's method for estimation of blood cholesterol.	CO 2	BTL2
2	Estimate the amount of cholesterol in a blood sample quantitatively.	CO 2	BTL6
3	Handle the colorimeter correctly.	CO 2	BTL2

4. Relevant Theoretical Background

Zak's method:

This is a procedure used to estimate cholesterol levels in an unknown serum sample. The method relies on the formation of a red-violet color through the reaction of cholesterol with acetic acid, ferric chloride, and sulfuric acid. The intensity of this color is directly proportional to the amount of cholesterol present in the sample. It is recorded at 540 nm (using a green filter). The basic principle of the method is to precipitate proteins in serum with a ferric chloride-acetic acid reagent. The protein-free filtrate is then treated with sulfuric acid and acetic acid. The cholesterol present in the protein-free filtrate is oxidized and dehydrated by ferric chloride, acetic acid, and sulfuric acid to form a red-colored compound.

5. Requirements

Equipment: Photoelectric colorimeter / Spectrophotometer, Centrifuge.

Glassware: Conical flask, Pipette, Test tubes, Centrifuge tube.

Specimen: Simulated sample or serum.

Reagents

a. **Ferric chloride-acetic acid reagent:** Dissolve 0.05 g ferric chloride in 100 mL glacial acetic acid AR.

b. **Cholesterol standard stock (1 mg/mL):** Dissolve 10 mg cholesterol in 10 mL glacial acetic acid AR.

c. **Working cholesterol standard (0.04 mg/mL):** Dilute 1 mL of cholesterol standard stock solution to make up a volume of 25 mL with a ferric chloride-acetic acid solution

6. Requirements used

_____

_____

7. Procedure

a. Preparation of PFF (Protein free filtrate) from serum: Place 0.2 mL of serum in a 15 mL centrifuge tube, then add 9.8 mL of working ferric chloride- acetic acid reagent, stopper the tube, and shake well. Keep aside for 15 min. Centrifuge the mixture.

b. Prepare the blank, standard, test solution in test tubes as shown in the following table:

Sr. No.	Content	Blank	Standard	Test
1	Protein-free filtrate	-	-	5.0 mL
2	Conc Sulphuric acid	3.0 mL	3.0 mL	3.0 mL
3	Working cholesterol standard	-	5.0 mL	-
4	Ferric chloride-acetic acid reagent	5.0 mL	-	-

c. Mix well, set aside for 30 min at room temperature.

d. Adjust absorbance or optical density (OD) of blank to zero, using green filter (540 nm), then record the absorbance of standard and test solutions.

8. Observations

A. Absorbance or optical density of standard solution (AS) = _____

B. Absorbance of optical density test solution (AT) = _____

9. Calculations:

Concentration of standard in 5 mL solution = 5×0.04 mg/mL = 0.2 mg/mL

Volume of serum in 5 mL of solution = 0.1 mL (0.2 mL in 10 mL)

To find out concentration of cholesterol (mg/dL or mg%), use following formula.

$$\text{Cholesterol creatinine (mg \%)} = \frac{AT}{AS} \times \frac{\text{Concentration of standard (mg)}}{\text{Volume of serum (mL)}} \times 100$$

$$\text{Cholesterol creatinine (mg \%)} = \frac{AT}{AS} \times \frac{0.2}{0.1} \times 100$$

$$\text{Cholesterol creatinine (mg \%)} = \frac{\quad}{\quad} \times 200$$

Cholesterol creatinine (mg %) = _____ mg%

Reference range: 150-250 mg%

10. Result:

The amount in cholesterol in the given sample of blood was found to be _____ mg %.

11. Conclusion:

The amount of cholesterol in the given sample of human blood was estimated using colorimeter.

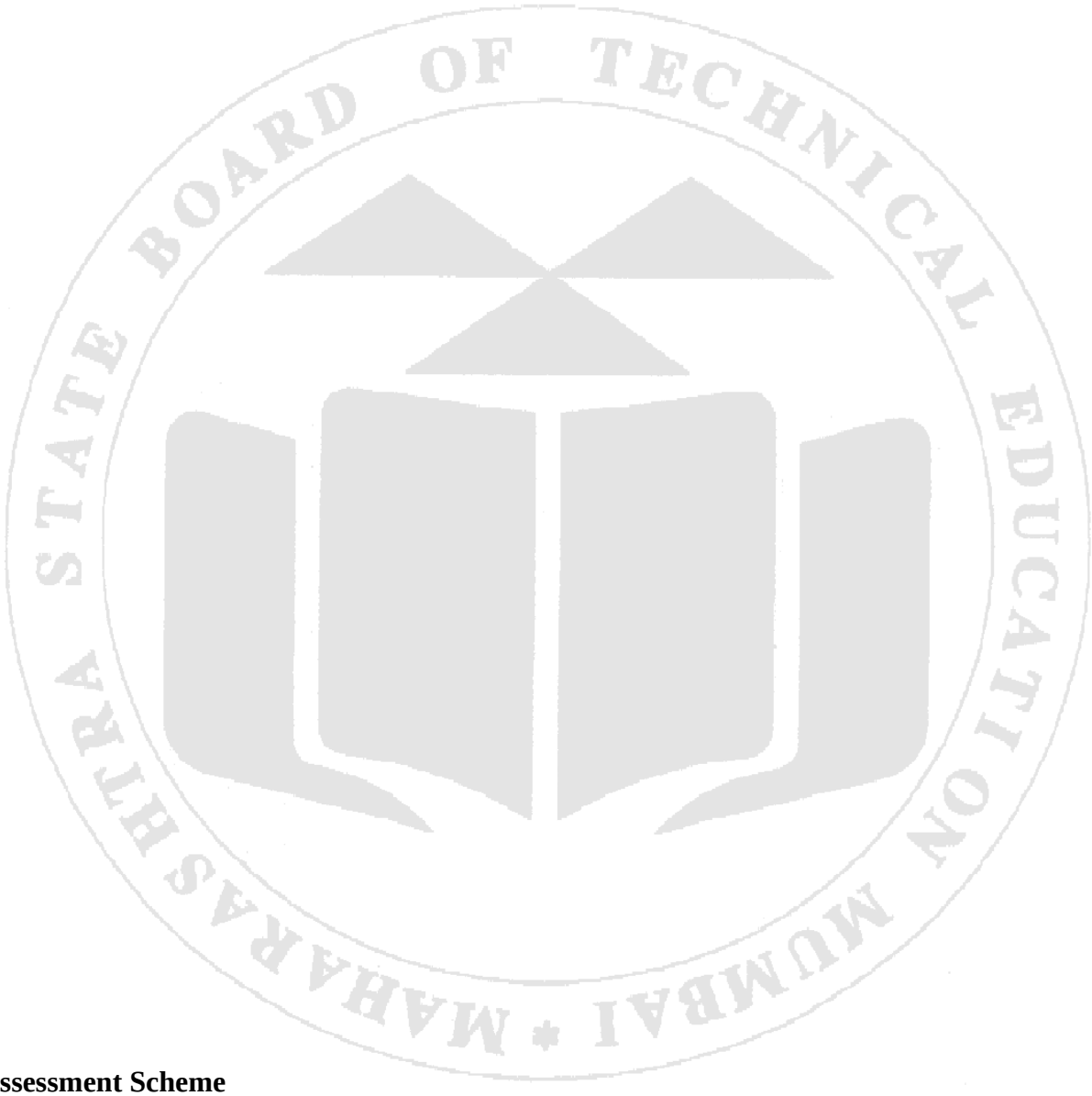
12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (P) Ltd, First edition.
b. Indian Pharmacopoeia 2018.
c. Practical Biochemistry, G. Rajagopal, B.D. Torra, Ahuja's Publishing House, Fourth edition.

13. Related questions

a. Give the principle of Zak’s method using ferric chloride.
b. What are the specific reagents and chemicals used in Zak's method, and what role does each play in the estimation process?
c. What are the primary functions of cholesterol in the human body?
d. Name the two methods by which serum total cholesterol can be estimated.
e. Enlist rich sources of cholesterol.
f. What are the major causes of hypercholesterolemia?

(Space for Answers)



14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 23
Estimation of Calcium in Human Blood

1. Aim

To estimate the amount of calcium in the given sample of human blood by titration method.

2. Practical Significance

The skeleton and teeth contribute to 99% of the total body calcium storage. Plasma and other bodily fluids contain the remaining 1% of calcium. Calcium in the blood is 50% free, 40% protein-bound, and 10% complexed with diffusible ions such as bicarbonate, lactate, phosphate, and citrate. Intracellular calcium aids in muscle contraction, hormone secretion, hormone action, metabolic activities, enzyme actions, exocytosis, and cell division. Increased neuromuscular excitability and tetany can result from a drop in serum-free calcium (either due to an actual decrease or a relative decrease caused by alkalosis). Most biochemical functions depend on the free form of calcium, i.e., ionized form, but in practice, total calcium in serum is estimated. In this experiment, students will learn to estimate the levels of calcium in blood using the titration method.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle behind titration method for estimation of blood calcium	CO 2	BTL2
2	Perform the titrimetric analysis for estimation of level of calcium in blood.	CO 2	BTL5
3	Estimate the amount of calcium in the given sample of blood.	CO 2	BTL6

4. Relevant Theoretical Background

Calcium estimation is primarily conducted using:

A. Photometric Method: O-Cresolphthalein Method

B. Titration Method: Method of Clark and Collip

O-Cresolphthalein method necessitates an ethylamine reagent containing dissolved potassium cyanide. Therefore, the titration method is preferred in the student's laboratory.

Titration Method:

This method involves the precipitation of total calcium in serum as calcium oxalate by introducing ammonium oxalate solution. Any excess ammonium oxalate is removed by rinsing the precipitated calcium oxalate with an ammonia solution. The calcium oxalate precipitate is then dissolved in 1N sulfuric acid to generate oxalic acid, which is subsequently titrated against a 0.01N potassium permanganate solution. The endpoint of this method is indicated by the appearance of a pink color.

5. Requirements

Specimen: Simulated sample or serum.

Glassware: Conical flask, Pipette, Test tubes, Glass rod, Micro burette (calibrated to 0.02 mL), Centrifuge tube, and apparatus.

Reagents

a. **1N Sulphuric acid**: Add 28 mL concentrated sulfuric acid to 950 mL distilled water in a volumetric flask slowly, cool, makeup the volume to 1 L with distilled water.

b. **2% (v/v) Ammonia solution**: Dilute 2 mL ammonia solution to 100 mL with water.

c. **4% Ammonium oxalate**: 100 g of oxalic acid can be dissolved in 800 mL of water and then neutralized with approximately 83 grams of ammonium carbonate.

d. **0.1N $KMnO_4$ stock solution**: Dissolve 3.162 g potassium permanganate in 1000 mL of distilled water. Heat and allow to stand for 2 days and filter through glass wool. Standardize against 0.1 N oxalic acid solution.

e. **0.01N $KMnO_4$ solution**: Dilute 10 mL of 0.1 N $KMnO_4$ stock solution to 100 mL with distilled water.

6. Requirements used

_____

_____

7. Procedure

a. Add 2 mL of serum to a centrifuge tube.
b. Add 2 mL of distilled water and 1 mL of 4% ammonium oxalate to it. Mix thoroughly to ensure complete precipitation within half an hour.
c. Centrifuge the mixture at 2000 rpm for 30 minutes.
d. Discard the supernatant fluid without disturbing the precipitate. Invert the tubes over filter paper to drain off any remaining supernatant for 5 minutes.
e. Add 3 mL of 2% ammonia down the sides of the tube and mix the precipitate thoroughly with it.
f. Centrifuge the mixture again and pour off the supernatant.
g. Add 2 mL of 1N sulfuric acid and dissolve the precipitate using a glass rod. Leave the glass rod in the solution.
h. Dissolve the calcium oxalate precipitate by warming it in a water bath and stirring with the same glass rod.
i. Without removing the glass rod, titrate the solution against 0.01N potassium permanganate using a micro burette graduated to 0.02 mL until a pink colour persists for a minute.
j. Perform a blank titration of 0.01N permanganate taken in a micro burette against 2 mL of 1N sulfuric acid.
k. The difference between these titrations indicates the volume of 0.01N potassium permanganate required to titrate the calcium oxalate precipitate.

8. Observations

A. Burette reading (test) = _____

B. Burette reading (blank) = _____

C. Final Burette reading (test-blank) = _____

9. Calculations:

Factor - Each mL of 0.01 N $KMnO_4$ equivalent to 0.2 mg of calcium.

$$\text{Concentration of calcium (mg \%)} = \frac{\text{Final burette reading X Factor}}{\text{Volume of serum (mL)}} \text{ X } 100$$

$$\text{Concentration of calcium (mg \%)} = \frac{\text{Final burette reading X 0.2}}{2} \text{ X } 100$$

Concentration of calcium (mg %) = Final burette reading X 10

Concentration of calcium (mg %) = _____ X 10

Concentration of calcium (mg %) = _____ mg %

Reference Range in Adults: Total calcium – 8.6 – 10.3 mg%
Free calcium – 4.6 – 5.3 mg%

10. Result:

The amount in calcium in the given sample was found to be _____ mg %.

11. Conclusion:

The amount of calcium in the given sample of human blood was estimated by titration method.

12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (P) Ltd, First edition.
b. Indian Pharmacopoeia 2018.
c. Practical Biochemistry, G. Rajagopal, B.D. Torra, Ahuja's Publishing House, Fourth edition.

13. Related questions

a. Describe the principle of calcium estimation by the titration method.
b. Enlist calcium deficiency disorders.
c. What are the different functions offered by calcium in the body?
d. What is normal serum calcium level?
e. What role does calcium play in muscle contraction and nerve function?
f. What are the dietary sources of calcium, and why is adequate calcium intake important?

(Space for Answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 24
Estimation of Urea in Human Blood

1. Aim

To estimate the amount of urea in the given sample of human blood.

2. Practical Significance

Urea levels are pivotal in assessing vital organ functioning. Abnormal urea concentrations can indicate kidney damage or dysfunction, as well as liver dysfunction, providing crucial insights into renal health. Elevated urea levels can be associated with diabetic nephropathy and increased cardiovascular risk factors such as hypertension and heart disease. Monitoring urea levels helps evaluate protein breakdown and utilization, aiding cardiovascular health assessments and nutritional assessment, managing kidney-related issues in diabetic patients, and dietary management for treating kidney-related conditions like kidney stones or renal infections.

Urea measurements, along with other renal function tests, assist healthcare providers in making informed clinical decisions regarding patient care, medication dosages, and treatment plans. Learning the colorimetric method for urea estimation enhances students' understanding of biochemical analyses and their practical applications in healthcare and laboratory settings. In this experiment, the students will learn to estimate the levels of urea in blood by colorimetric method.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the principle of estimation of blood urea.	CO 2	BTL2
2	Perform the colorimetric analysis for estimation of level of urea in blood	CO 2	BTL5
3	Estimate the amount of urea in the given sample of blood.	CO 2	BTL6
4	Handle the colorimeter correctly.	CO 2	BTL2

4. Relevant Theoretical Background

Protein catabolism results in the production of amino acids. Subsequently, amino acid deamination generates ammonia, which undergoes detoxification in the liver to form urea, making urea the end product of protein catabolism. The majority of the urea produced is excreted in urine, while the remainder is eliminated through the gastrointestinal tract and skin.

Urea levels in blood or plasma can be measured using the diacetyl monoxime thiosemicarbazide method. Initially, a protein-free filtrate containing urea is obtained by adding trichloroacetic acid. When diacetyl monoxime is heated in an acidic environment, it decomposes into hydroxylamine and diacetyl. Diacetyl then reacts with urea, resulting in a pink-red coloration. The presence of thiosemicarbazide and ferric ions enhances and stabilizes this reaction.

5. Requirements

Equipment: Photoelectric colorimeter / Spectrophotometer, Centrifuge.

Glassware: Conical flask, Pipette, Test tubes, Centrifuge tube.

Specimen: Simulated sample or Serum or anticoagulated whole blood or plasma.

Reagents

a. **Urea stock standard (1 g%):** Dissolve 1 g of dry urea in 100 mL distilled water.

b. **Urea Working standard (50 mg%):** Dilute 5 mL of urea stock solution to 100 mL with distilled water.

c. **Trichloroacetic acid (10 %):** Dissolve 10 g of trichloroacetic acid in 50 mL of distilled water and then make up the volume to 100 ml with distilled water.

d. **Ferric chloride (5 %):** Dissolve 5 g of ferric chloride in 80 mL of distilled water, add 1 mL conc sulphuric acid, make up the volume to 100 mL with distilled water.

e. **Thiosemicarbazide (0.25 %):** Dissolve 0.25 g of thiosemicarbazide in 100 ml distilled water.

f. **Acid reagent:** Take 50 ml of distilled water in a 100 mL volumetric flask and add with caution 8 mL of conc sulfuric acid and 2 mL of orthophosphoric acid and 1 mL of ferric chloride (5%) solution. Make up the volume to 100 mL with distilled water. It is stable for 24 hours only.

g. **Colour reagent:** Mix 75 mL acid reagent, 50 mL distilled water, 2.5 mL diacetyl monoxime (2.5 %) and 0.6 mL of thiosemicarbazide (0.25 %). This solution shall be prepared freshly as it is stable for 3-4 hours only.

6. Requirements used

_____

_____

7. Procedure

a. Add 0.2 mL blood, serum or plasma to 1.8 mL 3% trichloroacetic acid (0.2 mL blood diluted to 2 mL; Dilution =2/0.2 = 10). Use this protein-free filtrate as a test solution in the procedure. This step is avoided if a simulated urea sample is used.

b. Prepare the blank, standard, test solution in test tubes as shown in the following table:

Sr. No.	Content	Blank	Standard	Test (Serum)
1	Protein-free filtrate or simulated sample	-	-	0.2 mL
2	Working urea standard	-	0.2 mL	-
3	Colour reagent	5.2 mL	5.2 mL	5.2 mL

c. Keep these three tubes in a boiling water bath for 20 minutes.

d. Cool the tubes to room temperature.

e. Adjust absorbance or optical density (OD) of blank to zero, using green filter (540 nm), then record the absorbance of standard and test solutions.

8. Observations

A. Absorbance or optical density of standard solution (AS) = _____

B. Absorbance of optical density test solution (AT) = _____

9. Calculations:

To find out concentration of urea (mg/dL or mg%) use following formula

Concentration of urea working standard solution = 50 mg/ 100 mL

0.2 mL of urea working standard solution ≡ 0.1 mg

$$\text{Urea concentration (mg \%)} = \frac{AT}{AS} \times \frac{\text{Concentration of standard (mg)}}{\text{Volume of serum (mL)}} \times 100$$

$$\text{Urea concentration (mg \%)} = \frac{AT}{AS} \times \frac{0.1}{0.2} \times 100$$

$$\text{Urea concentration (mg \%)} = \_\_\_\_\_ \times 50$$

Urea concentration (mg %) = _____ mg%

Reference range: 20-40 mg %

10. Result:

The amount in urea in the given sample was found to be _____ mg %.

11. Conclusion:

The amount of urea in the given sample of human blood was estimated.

12. References

a. Practical Biochemistry, Geetha Damodaran K, Jaypee Brothers Medical Publishers (P) Ltd, First edition.
b. Indian Pharmacopoeia 2018.
c. Practical Biochemistry, G. Rajagopal, B.D. Torra, Ahuja's Publishing House, Fourth edition.

13. Related questions

a. What principle underlies the process of urea estimation?
b. What are the factors that can lead to elevated urea levels in the blood of an otherwise healthy individual?
c. Can you explain uremia and list the various causes of this condition?
d. How is urea formed in the body?

(Space for Answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 25
Hydrolysis of Starch

1. Aim

To study the hydrolysis of starch by salivary amylase enzyme.

2. Practical Significance

The metabolism of starch and other carbohydrates begins in the mouth through the action of the salivary amylase enzyme. Amylase is a bio-catalyst that breaks down polysaccharides such as starch, dextrin, and amylose by randomly hydrolyzing α-(1,4) glycosidic linkages, resulting in the formation of maltose and glucose. Abnormalities in salivary amylase activity can be indicative of certain medical conditions. For example, changes in enzyme levels might be observed in diseases affecting the salivary glands or pancreas, aiding in diagnostic processes. In this experiment, students will learn to identify the hydrolysis of starch by salivary amylase and determine the optimum conditions for this hydrolysis process.

3. Practical Outcomes (PrO)

The students will be able to

PrO	Practical Outcomes	Mapped CO	BTL
1	Explain the hydrolysis process of starch in saliva.	CO 2	BTL2
2	Evaluate amylase activity by a visual test	CO 2	BTL6
3	Observe color changes and estimate the enzyme activity level.	CO 2	BTL3
4	Determine optimum temperature and effect of substrate concentration on hydrolysis reaction.	CO 2	BTL6

4. Relevant Theoretical Background

Amylase in humans is α-amylase. Its activity is observed under optimal conditions such as a pH range of 5.6-6.9, a body temperature of 37-38°C, and the presence of chlorides and bromides, which enhance its effectiveness. Amylase activity can be observed by monitoring the stages of starch hydrolysis:

Product	Iodine test colour change	Amount of starch remaining	Test (Serum)
Starch	Dark Blue-Black	All	None
Amylodextrin	Purple-Blue	Most	Moderate
Erythrodextrin	Brown-Red	Some	Low
Acrodextrin	Yellow-Colorless	Almost None	High
Maltose	Yellow-Colorless	None	High

The resulting color indicates the amount of starch remaining and the level of enzyme activity. When the enzyme is inactive, a blue-black color persists for a longer duration. Therefore, students can determine the enzyme activity level and the remaining amount of starch to be hydrolyzed by observing these color changes.

5. Requirements

Specimen: Saliva.

Glassware: Beaker, Dropper, and Test tubes.

Reagents

a. **Saliva solution or Amylase solution**: Rinse your mouth with distilled water and discard the water. Again take 20-30 mL distilled water in the mouth and churn it in the mouth for 3-5 min. Pour into the clean beaker and filter.

b. **Starch solution (1%):** Dissolve 5 g of soluble starch in 300 mL of distilled water. Warm to solubilize and makeup the volume to 500 mL with distilled water.

c. **Phosphate buffer solution (pH 6.8):** Dissolve 28.80 g of disodium hydrogen phosphate and 11.45 g of potassium dihydrogen Phosphate in sufficient water to produce 1000 mL.

d. **Iodine solution:** Dissolve 2.0 g of iodine and 3 g of potassium iodide in water to produce 100 mL.

e. **NaCl solution (5%):** Dissolve 5 g of sodium chloride in sufficient distilled water and make up a volume of 100 mL with water.

6. Requirements used

_____

_____

7. Procedure

Part A: Effect of temperature on hydrolysis of starch by salivary amylase

a. Add 1 mL starch solution, 1 mL phosphate buffer (pH 6.8) into 4 test tubes labelled differently.
b. Place each test tube in a different water bath with temperatures adjusted approximately between 0-10^{0}C, 35-38^{0}C, 70-75^{0}C and 90-100^{0}C respectively.
c. Wait for 1-3 min and add 1 mL of saliva solution into each test tube and add 1 mL NaCl solution.
d. Then, place 1 drop of iodine solution on a watch glass and one drop solution from the test tube and observe the colour change from dark blue to yellow-colourless (Repeat this procedure per minute for 10 times). Record the time for hydrolysis or colour change.

Part B: Effect of substrate concentration on hydrolysis of starch by salivary amylase

a. Take 4 test tubes and label them differently.
b. Add solution to test tubes as shown in the table and maintain optimum temperature i.e. 35-38^{0}C in a water bath.

Reagent	Test Tube 1 Control	Test Tube 2	Test Tube 3	Test Tube 4
Starch solution	1 mL	1 mL	2 mL	3 mL
Distilled water	4 mL	4 mL	3 mL	2 mL
NaCl solution	1 mL	1 mL	1 mL	1 mL
Saliva solution	-	1 mL	1 mL	1 mL

c. Place 1 drop of iodine solution on the watch glass and one drop of solution from the test tube and observe the colour change from dark blue to yellow-colourless (Repeat this procedure per minute for 10-15 times).
d. Record the time for hydrolysis or color change.

8. Observations

Part A

Test Tube Time (min)	A Record colour	B Record colour	C Record colour	D Record colour

Part B

Test Tube Time (min)	A Record colour	B Record colour	C Record colour	D Record colour

9. Result:

a. Optimum temperature for the amylase activity was found to be _____as per part A.

b. As the substrate concentration increases time required for amylase activity_____ (increases/ decreases) as per part B.

10. Conclusion:

The hydrolysis of starch by salivary amylase enzyme was studied and optimum temperature for amylase activity was estimated.

11. References

a. Practical Biochemistry, Ritu Mahajan, Vayu Education of India. First edition.

b. Indian Pharmacopoeia 2018.

12. Related questions

a. What is the role of salivary amylase in carbohydrate digestion?

b. How does salivary amylase break down starch molecules?

c. What are the products formed during the hydrolysis of starch by salivary amylase?

d. What are the optimum conditions (e.g., pH, temperature) for salivary amylase activity?

e. What is the effect of temperature on amylase activity?

(Space for Answers)

13. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Guidelines for Conducting the Sessional Practical Examination

Course Name: Biochemistry & Clinical Pathology – Practical (BCP)
Course Code: 20058 Year: Second Year (PH2J)
Max Time: 3 Hrs Max. Marks: 80

Q. 1. Synopsis **(10 M)**

Q. 2. Experiments **(50 M)**

a. Major experiment (30 M)

b. Minor experiment (20 M)

Q. 3. Viva-voce **(10 M)**

Q. 4. Practical Record Maintenance **(10 M)**

Internal assessment: The marks secured by the students out of the total of 80 shall be reduced to 10 in each sessional, and then the internal assessment shall be calculated based on the best two averages for 10 marks from the sessional. An additional 10 marks shall be awarded based on the average of all three assignments.

Guidelines for Conducting the Annual Practical Examination

Course Name: Biochemistry & Clinical Pathology – Practical (BCP)
Course Code: 20058 Year: Second Year (PH2J)
Max Time: 3 Hrs Max. Marks: 80

Q. 1. Synopsis **(10 M)**

5 questions of 2 marks each or 10 questions of 1 mark each based on qualitative analysis of carbohydrates, proteins, amino acids, lipids, urine for normal and abnormal constituents; quantitative analysis of biomolecules in the given samples of blood or urine can be asked.

Q. 2. Experiments **(60 M)**

a. Major experiment **(40 M)**

Qualitative analysis of carbohydrates **OR**

Quantitative estimation of biomolecules in the given biological samples of urine or blood.

b. Minor experiment **(20 M)**

Qualitative analysis of Proteins, Amino acids, Lipids, Urine for normal or abnormal constituents.

Q. 3. Viva-voce **(10 M)**

(Viva should be conducted on practical and theory-based questions)