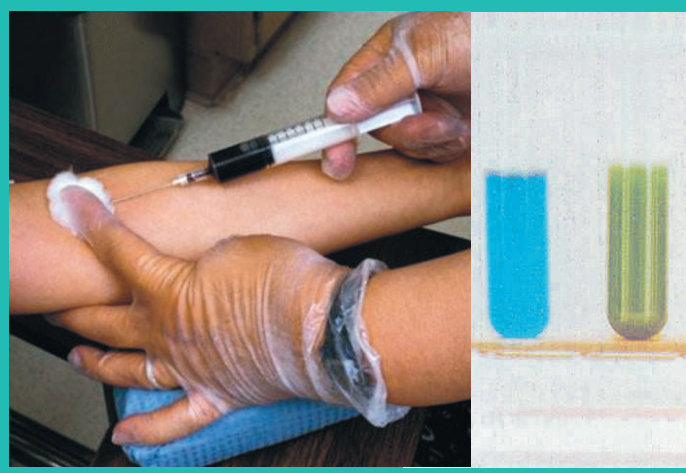
FIRST YEAR DIPLOMA IN PHARMACY

PH



A LABORATORY MANUAL FOR BIO-CHEMISTRY AND CLINICAL PATHOLOGY

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 changing technological and 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 followings:

- Education industry produces live products.
- Market requirements do not wait for curriculum changes.
- Question paper is the reflector of academic standards of educational organization.
- Well designed curriculum needs effective implementation too.
- Competency based curriculum is the backbone of need based program.
- Technical skills do need support of life skills.
- Best teachers are the national assets.
- Effective teaching learning process is impossible without learning resources.

A Laboratory Manual for

BIOCHEMISTRY AND CLINICAL PATHOLOGY

(0808)

First year Diploma in
Pharmacy (PH)

Maharashtra State

Board of Technical Education, Mumbai

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

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 June, 2014)

MAHARASHTRA STATE BOARD OF TECHNICAL EDUCATION

Certificate

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

Roll No. _____ of First Year Diploma in pharmacy _____ of

_____ (Institute) has Completed the term work satisfactorily in **Biochemistry and Clinical Pathology PR. (0808)** for the academic year 200 _____ to 200 _____ as prescribed in the curriculum.

Place : _____ Enrolment No.: _____

Date : _____ Exam. Seat No.: _____

( ) ( )

Subject Teacher **Principal**

Seal of Institution

LEARNING OVERVIEW

Importance of subject :

Biochemistry is a science concerned with chemical constituents of living cells, reactions and processes undergoing, in living cells. From biochemical point of view health may be considered as a situation in which all of the cellular reactions that occur in the body proceed at rates proportional with its maximum survival in physiologic state.

While pathology is a branch of biological science which deals with nature of disease through study of its causes, processes and effects with associated structural and functional alternations in body.

Clinical pathology is a application of laboratory techniques to find out cause of disease. It provides clinical data for diagnosis, prognosis and treatment of disease. Determination of chemical constituents of various body fluids such as blood, serum, urine, cerebrospinal fluid are useful in diagnosing various clinical conditions like diabetes mellitus, jaundice, gout etc. It is also useful in determining severity of diseases of organs, disorders of endocrine glands, disturbances in acid-base balance of body.

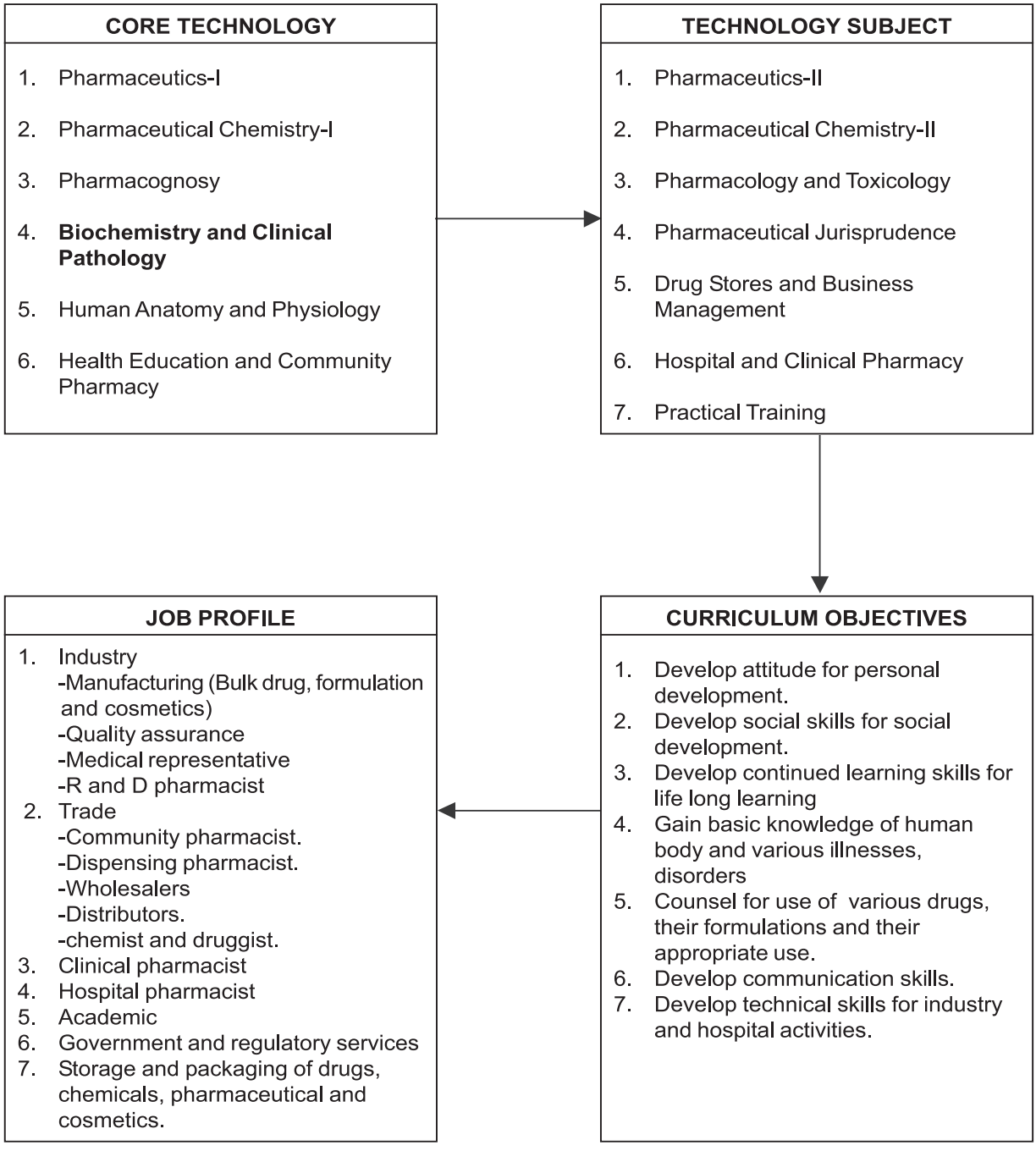
All the diseases are manifestation of abnormalities of molecules, chemical reactions or processes. All of these are caused by a different factors, which affect biochemical mechanisms in the cell or in the body. Knowledge of biochemistry helps the physicians in diagnosis and prognosis of diseases. Clinicians must have knowledge of biochemical changes of various foodstuff's, harmones, vitamins, minerals, etc. to diagnose a disease properly and for its cure because certain diseases are caused due to alterations of these constituents in the body.

Thus knowledge of biochemistry is essential to determine cause of disease, to assist in monitoring process of particular treatment and for assessing response to therapy.

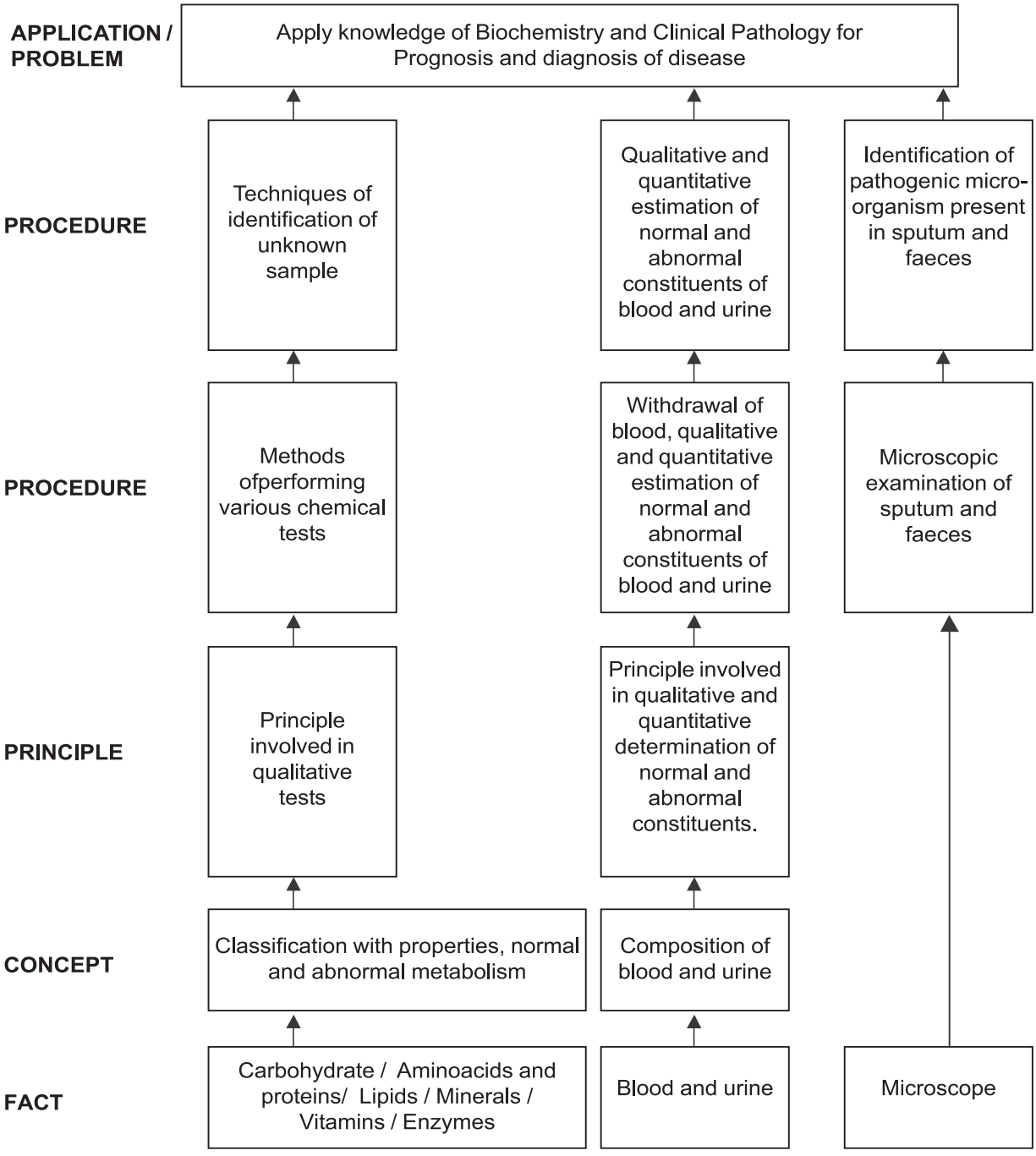
Biochemistry is a subject linking chemistry and biology with medicine and pharmacy. It has provided a major break through in the development of drugs. The pharmacist, is a link between physician and patient.

It is also a basis for understanding other branches of medicine. For example Genetics is based on biochemistry of nucleic acids. Immunology employs various biochemical techniques. Pharmacology and pharmacy are based on the biochemical role of various drugs in the body.

LINK / BLOCK DIAGRAM
SHOWING INTER RELATIONSHIP OF SUBJECT AREAS, CURRICULUM OBJECTIVES AND JOB PROFILE



GRAPHICAL STRUCTURE OF SUBJECT AREA
FIRST YEAR DIPLOMA IN PHARMACY
BIOCHEMISTRY AND CLINICAL PATHOLOGY (0808)



DEVELOPMENT OF SKILLS

Following is the broad perspective of acquisition of intellectual and motor skills. Due care is to be taken, that a student systematically studying the subject will acquire the skills enlisted below.

A) Intellectual skills

1. Understanding the concept of experiment. (I_1)
2. Identification of colours obtained in test. (I_2)
3. Interpreting the test results. (I_3)
4. Calculations as per formula. (I_4)

B) Motor skills :

1. Measuring and withdrawing the solutions accurately with the help of pipette. (M_1)
2. Handling and using correctly the instrument / equipment. (M_2)
3. Observing colour precipitate etc. produce in the test. (M_3)

GRID TABLE

Following table gives grid of the experiments and related intellectual and motor skills.

- Teacher shall ensure for development of generic skills during the practicals.
- Students are expected to focus on acquiring specific skills mentioned therein

No.	Experiment No. & Title	Intellectual skills				Motor skills		
		I_1	I_2	I_3	I_4	M_1	M_2	M_3
1.	Introduction to Biochemistry and Clinical Pathology Laboratory	√	√			√	√	
	Carbohydrates							
2.	To identify given sample of carbohydrate by qualitative tests.	√	√			√		√
3.	To identify given sample of carbohydrate by qualitative tests.	√	√			√		√
4.	To identify given sample of carbohydrate by qualitative tests.	√	√			√		√
5.	To identify given sample of carbohydrate by qualitative tests.	√	√			√		√
6.	To identify given sample of carbohydrate by qualitative tests.	√	√			√		√
7.	To identify given sample of carbohydrate by qualitative tests.	√	√			√		√
	Amino Acids							
8.	To identify given sample of amino acid by qualitative tests.	√	√	√		√		√
9.	To identify given sample of amino acid by qualitative tests.	√	√	√		√		√
10.	To identify given sample of amino acid by qualitative tests.	√	√	√		√		√
11.	To identify given sample of amino acid by qualitative tests.	√	√	√		√		√
12.	To identify given sample of amino acid by qualitative tests.	√	√	√		√		√
	Proteins							
13.	To isolate casein from milk and its confirmation by chemical tests.	√		√		√		√
14.	To identify given sample of protein by qualitative tests.		√	√		√		√
15.	To identify given sample of protein by qualitative tests.		√	√		√		√

No.	Experiment No. & Title	Intellectual skills				Motor skills		
		I_1	I_2	I_3	I_4	M_1	M_2	M_3
16.	To identify given sample of protein by qualitative tests.		√	√		√		√
	Lipids							
17.	To study physical and chemical properties of fats and oils.	√		√				
18.	To conduct test on cholesterol to verify physical properties and chemical tests.	√	√			√		√
	Urine							
19.	To detect normal constituents in given sample of urine by qualitative tests.	√		√		√		√
20.	To detect abnormal constituents in given sample of urine by qualitative test.	√		√		√		√
21.	To detect abnormal constituents in given sample of urine by qualitative test.	√		√		√		√
22.	To detect abnormal constituents in given sample of urine by qualitative test.	√		√		√		√
23.	To estimate quantity of glucose in given sample of urine.	√		√	√	√		√
24.	To estimate quantity of creatitine in given sample of urine	√			√	√	√	
25.	To estimate quantity of urea nitrogen in given sample of urine.	√			√	√	√	
	Blood							
26.	To estimate quantity of total cholesterol in given sample of blood plasma.	√			√	√	√	
27.	To estimate quantity of serum alkaline phophatase in given sample of serum.	√			√	√	√	
28.	To estimate quantity of calcium in given sample of serum.	√			√	√	√	
29.	To estimate quantity of glucose in given sample of serum	√			√	√	√	
	Sputum and Faeces							
30.	To study microscopic examination of sputum	√	√				√	
31.	To study microscopic examination of faeces.	√	√	√			√	
32.	To visit a hospital to study methods of injecting drugs.	√						√
33.	To visit to a pathology laboratory to study methods of withdrawal of blood.	√						√

NOTE : √- Identified Skills

STRATEGY FOR IMPLEMENTATION

It is suggested that 40 to 50 % experiments shall be completed in first term and remaining experiments in the second term.

GUIDELINES FOR TEACHERS

Teacher shall discuss the following points with students before start of practicals of the subject.

1) **Learning Overview :** To develop better understanding of importance of the subject. To know related skills to be developed such as intellectual skills and motor skills.
2) **Link / Block Diagram :** Context of the subject in the form of link diagram showing inter relationship of various subject areas, curriculum objectives and job profile.
3) **Graphical structure :** In this topics and sub topics are organized in systematic way so that ultimate purpose of learning the subject is achieved. This is arranged in the form of fact, concept, principle, procedure, application and problem.
4) **Know your laboratory work :** To understand the layout of laboratory, specifications of equipment / instruments / chemicals, procedure, working in groups, planning time etc. Also to know total amount of work to be done in the laboratory.
5) Teacher shall ensure that required equipment are in working condition before start of experiment, also keep operating instruction manual available.
6) Explain prior concepts to the students before starting of each experiment.
7) Involve students' activity at the time of conduct of each experiment.
8) While taking reading / observation each student (from batch of 20 students) shall be given a chance to perform / observe the experiment.
9) List of questions is given at the end of each experiment. Teacher shall instruct the students to attempt all questions given at the end each experiment / exercise. Teacher shall ensure that each student writes the answers to the allotted questions in the laboratory manual after performance is over.
10) If the experimental setup have variations in the specifications of the equipment, the teachers are advised to make the necessary changes, wherever needed.
11) Teacher shall assess the performance of students continuously as per norms prescribed by MSBTE.
12) Teacher should ensure that the respective skills and competencies are developed in the students after the completion of the practical exercise.
13) Teacher is expected to share the skills and competencies to be developed in the students.
14) Teacher may provide additional knowledge and skills to the students even though not covered in the manual but are expected from the students by the industries.
15) Teacher shall ensure that visits recommended in the manual are covered.
16) Teacher may suggest the students to refer additional related literature of the technical papers / reference books / seminar proceedings, etc.
17) During assessment teacher is expected to ask questions to the students to tap their achievements regarding related knowledge and skills so that students can prepare while submitting record of the practicals. Focus should be given on development of enlisted skills rather than theoretical / codified knowledge.
18) Teacher should enlist the skills to be developed in the students that are expected by the industry.
19) Teacher should organize group discussions/ brain storming sessions / seminars to facilitate the exchange of knowledge amongst the students.
20) Teacher should ensure that revised CIAAN-2004 norms are followed simultaneously and progressively.
21) Teacher should give more focus on hands on skills and should actually share the same.
22) Few experiments may be combined and conducted in single turn to accommodate in given time schedule.
23) Artificial samples of urine, serum, blood may be used wherever required. Prepared / permanent slides be obtained from pathology laboratory for microscopic examinations of sputum and faeces.
24) New experiments have been included as per present day requirement, even though these are not stated in the curriculum. A thought to accommodate all the experiments in the given limit (total No. of available periods) is also given.
25) Teacher shall also refer to the circular No. MSBTE/D-50/Pharm Lab Manual/2006/3160 dated 04/05/2006 for additional guidelines.

INSTRUCTIONS FOR STUDENTS

Students shall read the points given below for understanding the theoretical concepts and practical applications.

1) Listen carefully to the lecture given by teacher about importance of subject, curriculum philosophy, graphical structure, skills to be developed, information about equipment, instruments, procedure, method of continuous assessment, tentative plan of work in laboratory and total amount of work to be done in a year.
2) Students shall undergo study visit of the laboratory for types of equipment, instruments, chemicals be used before performing experiments.
3) Read the write up of each experiment to be performed, a day in advance.
4) Organize the work in the group and make a record of all observations.
5) Understand the work in the group and make a record of all observations.
6) Write the answers of the questions allotted by the teacher during the same practical hours if possible or afterwards, but immediately.
7) Student should not hesitate to ask any difficulty faced during conduct of practical / exercise.
8) The students shall study all the questions given in the laboratory manual and practice to write the answers to these questions.
9) Student shall visit the pharmaceutical industries and pathology laboratories / hospitals / medical stores and should make a project report on it as directed by the teacher.
10) Student shall learn GMP / CGMP as expected by the industries.
11) Student should develop the habit of pocket discussion / group discussion related to the experiments / exercises so that exchanges of knowledge / skills could take place.
12) Students shall attempt to develop related hands-on-skill and gain confidence.
13) Student shall focus on development of skills rather than theoretical or codified knowledge.
14) Student shall visit the nearby drug stores, medicinal gardens, technical exhibitions, trade fair, etc. even not included in the lab manual. In short, students should have exposure to the area of work right in the student hood.
15) Student shall insist for the completion of recommended laboratory work, pharmaceutical industrial visits, answers to the given questions, etc.
16) Student shall develop the habit of evolving more ideas, innovations, skills etc. than included in the scope of the manual.
17) Student shall refer periodicals / journals / pharmacopoeias, magazines, proceedings of the seminars, refer websites related to the scope of the subjects and update their knowledge and skills.
18) Student should develop the habit of not to depend totally on teachers but to develop self learning techniques.
19) Student should develop the habit to react with the teacher without hesitation with respect to the academics involved.
20) Student should develop habit to submit the practical exercises continuously and progressively on the scheduled dates and should get the assessment done.
21) Student should be well prepared while submitting the write up of the exercise. This will develop the continuity of the studies and he will not be over loaded at the end of the term.

Special instructions while working in the laboratory.

1) Student should wear apron, caps, face mask and footware.
2) Student should not taste any chemicals, crude drug, etc. in the laboratory.
3) Student should not suck harmful irritants like strong acids, alkalis, organic solvent by mouth.
4) Student should handle instrument / equipment carefully in the laboratory.

List of Experiments and Record of Progressive Assessment

Sr. No.	Name of the Experiments	Page No.	Date of Perfor-mance	Date of submi - ssion	Assess-ment Max. Marks 10	Teacher's Signa-ture
1	Introduction to Biochemistry and Clinical Pathology Laboratory	1				
	Carbohydrates					
2	To identify given sample of carbohydrate by qualitative tests.	6				
3	To identify given sample of carbohydrate by qualitative tests.	15				
4	To identify given sample of carbohydrate by qualitative tests.	24				
5	To identify given sample of carbohydrate by qualitative tests.	33				
6	To identify given sample of carbohydrate by qualitative tests.	42				
7	To identify given sample of carbohydrate by qualitative tests.	51				
	Amino Acids					
8	To identify given sample of amino acid by qualitative tests.	60				
9	To identify given sample of amino acid by qualitative tests.	67				
10	To identify given sample of amino acid by qualitative tests.	74				
11	To identify given sample of amino acid by qualitative tests.	81				
12	To identify given sample of amino acid by qualitative tests.	88				
	Proteins					
13	To isolate casein from milk and its confirmation by chemical tests.	95				
14	To identify given sample of protein by qualitative tests.	100				
15	To identify given sample of protein by qualitative tests.	109				

Sr. No.	Name of the Experiments	Page No.	Date of Perfor-mance	Date of submi - ssion	Assess-ment Max. Marks 10	Teacher's Signa-ture
	Lipids					
16	To identify given sample of protein by qualitative tests.	118				
17	To study physical and chemical properties of fats and oils.	127				
18	To conduct test on cholesterol to verify physical properties and chemical tests.	133				
	Urine					
19	To detect normal constituents in given sample of urine by qualitative tests.	139				
20	To detect abnormal constituents in given sample of urine by qualitative test.	146				
21	To detect abnormal constituents in given sample of urine by qualitative test.	153				
22	To detect abnormal constituents in given sample of urine by qualitative test.	160				
23	To estimate quantity of glucose in given sample of urine.	167				
24	To estimate quantity of creatitine in given sample of urine.	173				
25	To estimate quantity of urea nitrogen in given sample of urine.	178				
	Blood					
26	To estimate quantity of total cholesterol in given sample of blood plasma.	182				
27	To estimate quantity of serum alkaline phophatase in given sample of serum.	188				
28	To estimate quantity of calcium in given sample of serum.	193				
29	To estimate quantity of glucose in given sample of serum	197				
	Sputum and Faeces					
30	To study microscopic examination of sputum	201				

Sr. No.	Name of the Experiments	Page No.	Date of Perfor-mance	Date of submi - ssion	Assess-ment Max. Marks 10	Teacher's Signa-ture
31	To study microscopic examination of faeces.	208				
32	To visit a hospital to study methods of injecting drugs.	214				
33	To visit to a pathology laboratory to study methods of withdrawal of blood.	220				

Total marks obtained for the number of experiments considered for	Average marks obtained for the experiments out of 10.
First sessional = (1 to 12) Second sessional = (13 to 24) Third sessional = (25 to 33)	First sessional = Second sessional = Third sessional =

* To be transferred to proforma of CIAAN-2004 (Proforma I-1)

Note :- The guidelines for the conduct of annual practical examination are enclosed in the end at page number 229.

Experiment No. 1

1.0 TITLE : Introduction to Biochemistry and Clinical Pathology laboratory.

2.0 PRIOR CONCEPTS:

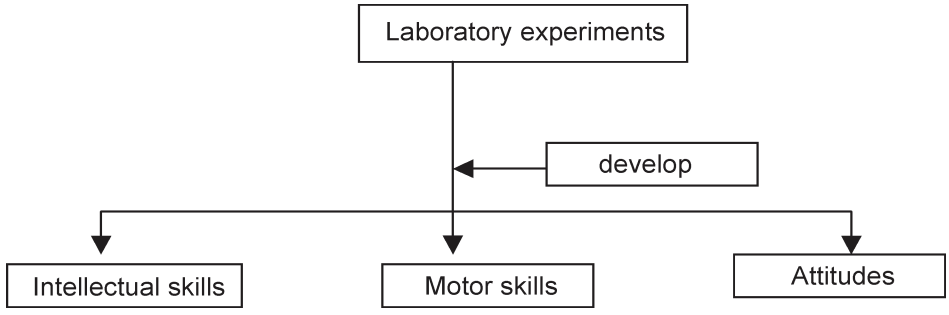
Curriculum contents, scope of work, planning, assessment

3.0 NEW CONCEPTS:

Proposition1 : Laboratory experiment

Laboratory experiments are expected to develop intellectual skills motor skills and attitudes in students.

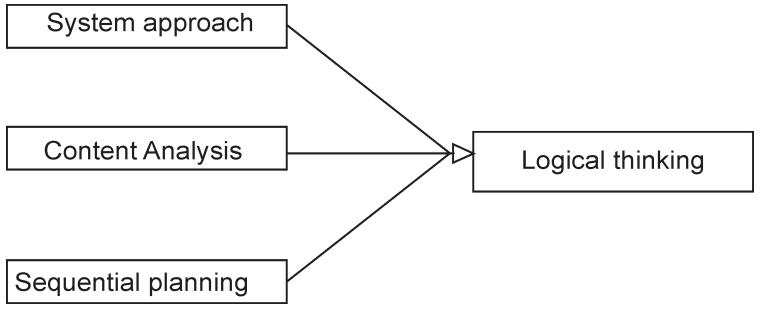
Concept Structure :



***Proposition 2 :* Logical Thinking**

Logical thinking is developed in students through systems approach, content analysis and sequential planning of laboratory work.

Concept Structure :



4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand the concept of working of each laboratory equipment.
2. To identify corrosive, irritant chemicals and to take care during its handling.

Motor skills

1. Ability to proper withdrawal and addition of proper reagents.
2. Ability to operate colorimeter, centrifuge, microscope.

5.0 APPARATUS :

Centrifuge, Photoelectric colorimeter / spectrophotometer, microscope, charts.

6.0 DIAGRAM :

Photoelectric Colorimeter

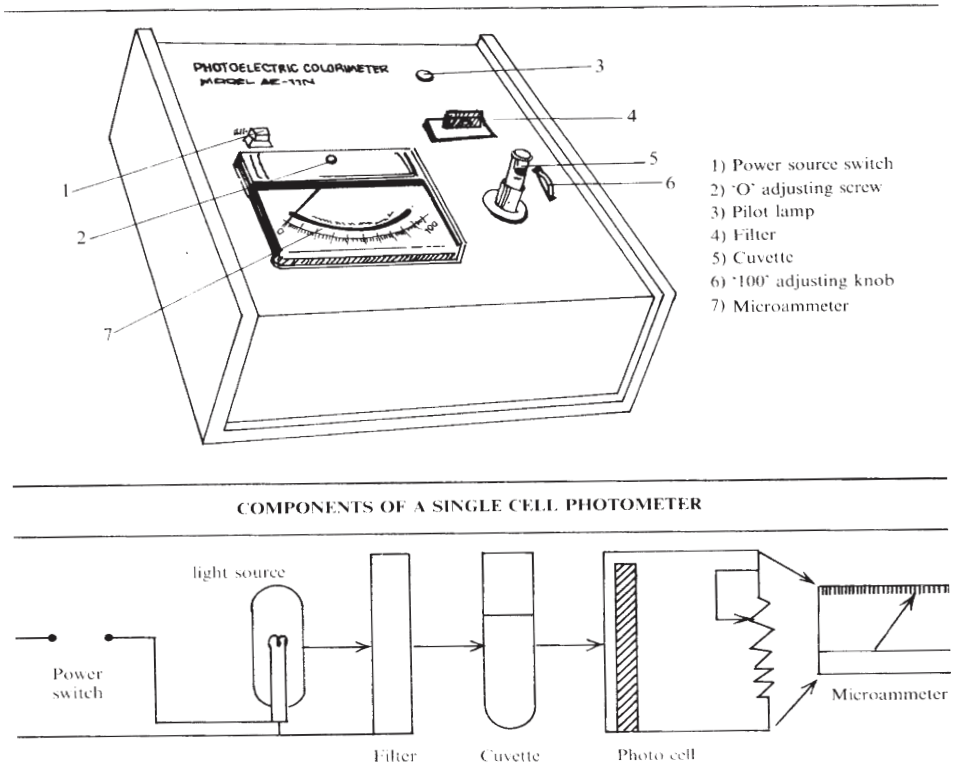


Fig. 1.1

Laboratory centrifuge

Fig. 1.2

Spectrophotometer

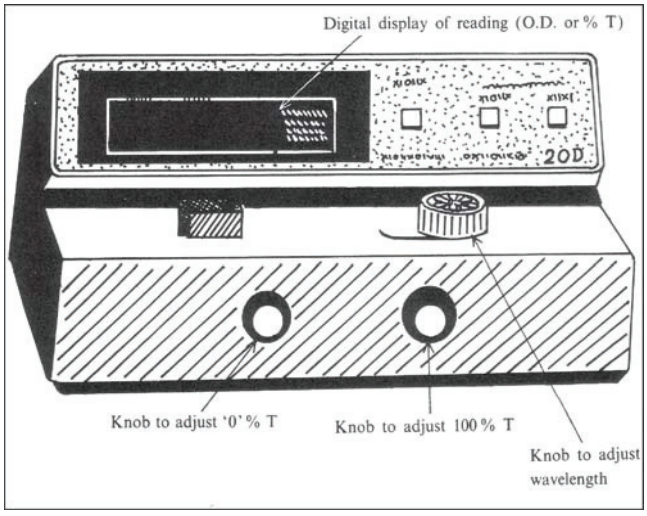


Fig. 1.3

7.0 STEPWISE PROCEDURE :

1. Read the learning overview carefully.
2. Listen to the lecture given by teacher about importance of subject, curriculum philosophy, graphical structure, skills to be developed, information about equipment, instrument, procedure method of continuous assessment and tentative plan of work in laboratory.
3. Visit the laboratory for types of equipment, instrument, materials, reagents, charts to be used, while performing experiments.
4. Receive instructions about working in the laboratory, dress code, precautions, etc.

8.0 OBSERVATION :

Student to write the observation / information as directed Parts of Photoelectric colorimeter.

1. _____ 2. _____ 3. _____

4. _____ 5. _____

Parts of centrifuge.

1. _____ 2. _____ 3. _____

Names of charts displayed in laboratory.

1. _____ 2. _____ 3. _____

4. _____ 5. _____

Ten reagents on reagent rack in the laboratory (Give names and chemical formula)

1. _____ 2. _____ 3. _____

4. _____ 5. _____ 6. _____

7. _____ 8. _____ 9. _____

10. _____

9.0 QUESTIONS :

Student to answer one question from each category, category A Q....., category B Q....., category CQ..... and the question numbers shall be allotted by the teacher.

Category A

1. What is the importance of link diagram of the curriculum of the subject.
2. How graphical structure of a subject is useful in understanding the scope of subject.
3. Classify the curriculum of diploma in different groups of subjects.
4. List two roles of pharmacists given in the job profile in block diagram.
5. State the fact and principle of Biochemistry and clinical pathology given in graphical structure.

Category B

6. State two applications of centrifuge in Biochemistry and Clinical Pathology laboratory.
7. State one application of colorimeter in Biochemistry and Clinical Pathology laboratory.
8. State one application of microscope in Biochemistry and Clinical Pathology laboratory.
9. State the type of balances available in laboratory for weighing.
10. State the precautions to be taken while operating centrifuge.

Category C

11. State the safety precautions to be taken while working in the laboratory.
12. What is importance of using aprons in laboratory in view of safety ?
13. State precautions to be taken during pipetting the solution.
14. State the first aid in each case of burn, splashing of strong acids, alkalis, bleeding.
15. Draw a layout of Biochemistry and Clinical Pathology Laboratory.

Space for writing answers

Space for writing answers

Signature of Teacher

Experiment No. 2

1.0 TITLE : To identify given sample of carbohydrate (sample No. 1)

2.0 PRIOR CONCEPTS:

Carbohydrates are present in natural products such as maize, rice, potato, cane sugar, honey, etc.

3.0 NEW CONCEPTS:

Proposition 1 :
Carbohydrate is detected by Molisch's test.

Proposition 2 :
Polysaccharides are detected by iodine test.

Proposition 3 :
Reducing sugars are detected by Fehling's and Benedict's test.

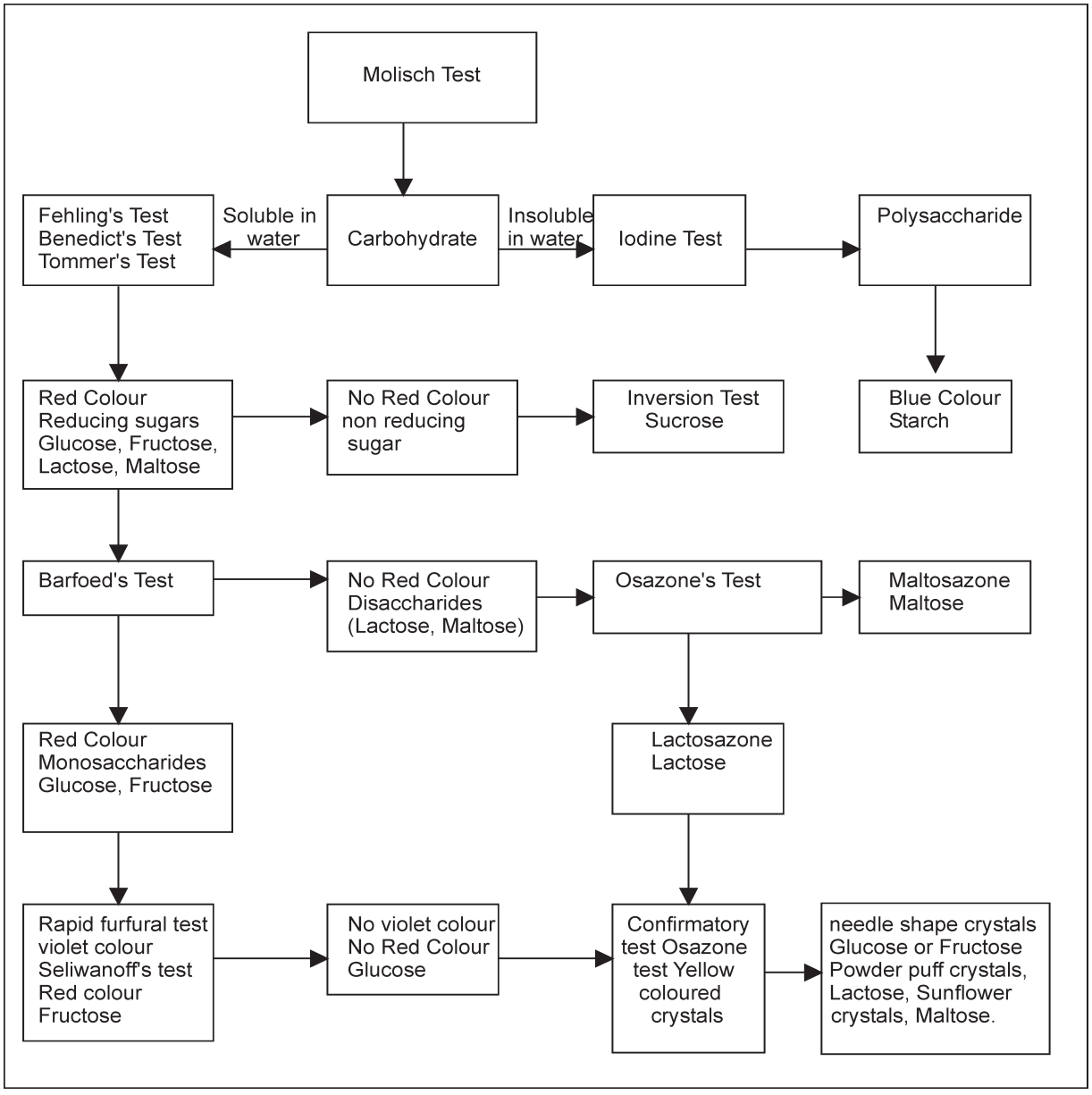
Proposition 4 :
Aldoses and Ketoses are distinguished by Seliwanoff's test.

Proposition 5 :
Lactose & Maltose distinguished by Tollen's reagent test.

Proposition 6 :
Sucrose is confirmed by inversion test.

Proposition 7 :
Glucose, Fructose, Maltose, Lactose are confirmed by osazone test.

Concept Structure :



4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand formation of precipitate.
2. Application of chemical test of carbohydrates to identify given sample of carbohydrate according to concept structure.

Motor skills

1. Ability to distinguish the colours while performing various chemical tests.
2. Ability to add required amount of test solution and chemical reagent while performing the chemical test.
3. Ability to provide required quantity of heat safely while performing the chemical test whenever required.
4. Ability to observe and differentiate respective osazone crystals while observing them under microscope.

5.0 APPARATUS :

Glassware

Beaker, water bath, test tubes, graduated pipettes, funnel, filter paper, watchglass, slide, microscope.

Chemicals

Molisch reagent (1% ∝naphthol in alcohol), concentrated sulphuric acid, N/50 Iodine solution, distilled water, Fehling's solution A (7.93% copper sulphate in water), Fehling's solution B (250g sodium hydroxide and 320g sodium potassium tartarate in 500 ml water), Benedict's reagent (dissolve 173g sodium citrate and 100g anhydrous sodium carbonate in about 800 ml water, separately dissolve 17.3g copper sulphate in 100ml water, mix both the solution and make volume to 1000 ml with water), Tommer's reagent (prepared fresh- 5 % copper sulphate in water and equal volume of sodium hydroxide solution), Barfoed's reagent (13.3g copper acetate in 200 ml water filter, to it add 1.8 ml glacial acetic acid), Seliwanoff's reagent (dissolve 50 mg of resorcinol in 33 ml concentrated hydrochloric acid diluted to 1000 ml with water)

Concentrated nitric acid, phenylhydrazine hydrochloride,

Acetate buffer - PH 5.0 Dissolve 40.8 g sodium acetate in 70 ml water. To it add 8.2 ml glacial acetc acid. Make the volume to 100ml with water.

Fougler's reagent (Dissolve 40 g urea in 80 ml of 40% w/w sulphuric acid to it add 2 g stannous chloride and boil till clear solution is obtained. Cool make volume to 40 ml with 40% w/w sulphuric acid.

6.0 STEPWISE PROCEDURE :

1. Prepare a solution of given carbohydrate sample by dissolving 1 gm sample in 20 ml of water. And use same solution for analysis.
2. Perform the chemical test of carbohydrates according to general concept, structure, to identify the given sample.
3. Adjust the microscope at low power properly before observing osazone crystals.
4. Confirm Glucose, Fructose, Maltose, Lactose by Osazone test, Sucrose by inversion test and starch by iodine test.
5. Report your analysis for identification of given sample of carbohydrate in given proforma.

Table to perform tests for identification of carbohydrate sample (with reagents)

Test	Observation	Inference
1. Molisch's test : Mix 2ml of carbohydrate sample with 5 drops of Molisch's Reagent in a test tube. Add gently through the side by tilting the tube, about 2 ml of concentrated H_2SO_4 so as to form a bottom layer.	Violet / purple ring at the junction of two liquids	Carbohydrate present.
2. Solubility Compound + water	Soluble Insoluble	Mono and disaccharides present Polysaccharides present
3. Fehling's test 2 ml of Fehling's solution A + 2ml of Fehling's solution B+ 2 ml of Sugar solution Boil.	Yellow or brick red ppt is observed.	Reducing sugar present
4. Benedict's test Take 5ml of Benedict's qualitative reagent, add 8 drops of sugar solution. Boil over a flame for 2 minutes or place in boiling water bath for 3 minutes. Allow to cool.	Green, yellow, orange or brick red ppt is observed	Reducing sugar present

Test	Observation	Inference
5. Tommer's test To 2 ml of Tommer's reagent add 3 ml of sugar solution boil for 2 minutes cool.	Yellow or red ppt is observed	Reducing sugar present
6. Barfoed's test 2 ml of Barfoed's reagent add 2ml of sugar solution and keep in boiling water bath for 2 minutes cool.	Brick red precipitate is observed at the bottom of test tube.	Reducing sugarpresent.
7. Seliwanoff's test To 3 ml of Seliwanoff's reagent add 1 ml of sugar solution and heat the mixture to boil for 2 minutes. cool.	Red colour or red precipitate is observed.	Ketoses like fructose, sucrose present.
8. Rapid furfural test To 2 ml of sugar solution add 1 ml of A-napthol solution (1% in alcohol) and 5 ml concentrated HCL boil.	Deep purple colour is observed.	Ketoses like fructose, sucrose present
9. Osazone test Set up a boiling water bath. Take test tube, add 1 g of Phenyl hydrazine hydrochloride 2 ml of acetate buffer, P^H 5.0. add 5ml of water mixwell and warm gently. Filter it. To the filterate add 5 ml sugar solution and keep in boiling water bath and cool. Mount the crystals under microscope and examine under microscope.	a) Greenish yellow needle shaped crystals. b) Badminton ball, powder puff shaped crystals. c) Sunflower or its petals shaped crystals.	Glucosazone i.e. glucose present. Lactosazone i.e. lactose present. Maltasazone i.e. maltose present.
10. Iodine test a) To about 2 ml Suspension or solution of polysaccharides add 1-2 drops of N/50 iodine solution.	Blue violet colour developsBrown wine colour develops.	Starch is present Glycogen is present.
b) Warm above solution c) Cool the above solution d) To about 2 ml suspension of starch add 1ml 5 % sodium hydroxide and 2 drops of N/50 iodine.	Blue colour disappears. Blue colour reappears No blue colour	Starch is present. Starch is present. Starch is present.
C.T. for Glucose a) Osazone test	Greenish yellow needle shaped crystal	Glucose confirmed
b) To 2ml of test solution add 5 % NaOH	Brown resinous precipitate is observed.	Glucose confirmed
c) Take 3 ml water to it add drop of Methylene blue solution and 1 ml of 5% sodium hydroxide and 2ml of sugar solution and boil.	Solution is decolourised	Glucose confirmed
d) To 3 ml of sugar solution add 1 ml of picric acid solution and 1ml 5% NaOH and heat.	Red colour develops	Glucose confirmed
C. T. for Lactose 1. Mucic acid test To 1 ml sugar solution add 1 ml concentrated HNO_3 boil and cool.	Broken glass like crystals obtained which can be identified under microscope.	Lactose confirmed

Test	Observation	Inference
2. Silver mirror test 1 ml sugar solution add 1 ml Ammonical silver nitrate solution add excess NaOH Warm.	Silver mirror is observed.	Lactose confirmed
C.T. for sucrose a) Seliwanoff's test b) Rapid furfural test c) Inversion test. To 25 ml of test solution add 5 ml of concentrated HCl boil for 3 minutes and cool under tap water. Neutralize with sodium hydroxide solution. Now perform following test for this solution. i) Fehling's test ii) Tommer's test iii) Benedict's test iv) Barfoed's test	 Red precipitate is observed. Red precipitate is observed. Red / yellow / green precipitate is observed.	 Sucrose confirmed. Sucrose confirmed. Sucrose confirmed.
C.T. for fructose Fougler's test To 3 ml fougler's reagent add 0.5 ml of sugar solution. Boil for one minute.	 Blue colour develops	 Fructose confirmed.

7.0 OBSERVATION :

Sample 1 :

Student to write test, observation and inference

Test	Observation	Inference

Student to write test, observation and inference

Test	Observation	Inference

8.0 RESULT :

1. Given sample of carbohydrate is _____
2. It is _____ (Monosaccharide /Disaccharide/ Polysaccharide)

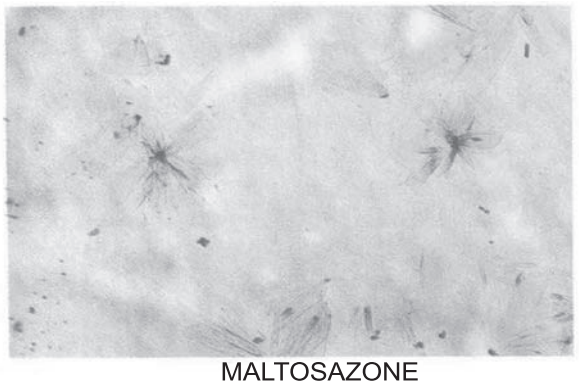
9.0 QUESTIONS :

Student to answer question Q....., Q....., Q.....,Q.....
and the question numbers shall be allotted by the teacher.

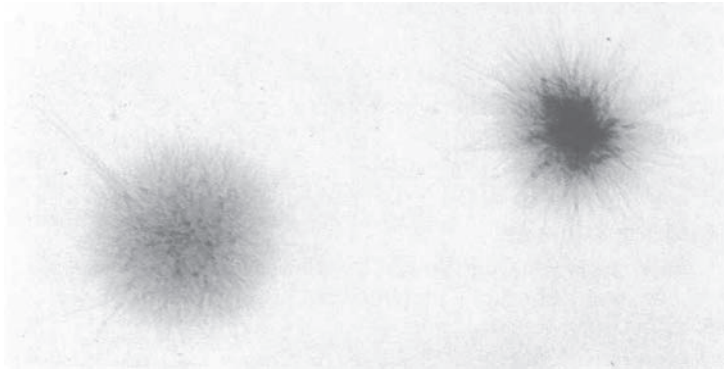
1. State the principle of Molisch test.
2. State the component of starch.
3. State the name of two sources of starch.
4. State the principle of Benedict test.
5. State the name of most sensitive test of reducing sugars.
6. State the composition of Tollen's reagent.
7. Write the reactions involved in Osazone test.
8. Draw the shape of Maltosazone crystals.
9. State the names of carbohydrates distinguished by iodine test.
10. State the names of epimers of glucose.
11. What is purpose of inversion test.
12. State the name of acid obtained by oxidation of glucose.
13. State the name of chemical test required to distinguish monosaccharide and disaccharide.
14. State the colours produced by iodine with starch, dextrine and glycogen.
15. State the name of non-reducing diassacharides.
16. Draw the structures of Glucose and Fructose.
17. Draw the structures of Sucrose, Lactose and Maltose.
18. State the hydrolysis products of each : Sucrose.
19. State two disease related to abnormal metabolism of glucose.
20. Name two reducing diassacharides and give its hydrolysis products.
21. State two examples of monosaccharides, diasaccharide and polysaccharides each.
22. Name two chemical test to discriminate between glucose and fructose sample.
23. State name of sugar sample which gives needle shape crystals of osazone.
24. State name of sugar sample which gives powder puff shape crystals of osazone.
25. State the two sources of fructose.
26. State rich source of lactose and sucrose each.

10.0 REFERENCE:

GLUCOSAZONE



MALTOSAZONE



LACTOSAZONE

PLATE (COLOURS OF THE TESTS)
REACTIONS OF CARBOHYDRATES

TEST	OBSERVATION	COLOR	CONFIRMS
Molisch's Test	Violet Ring		Carbohydrate
	Blue Negative		Absence of reducing sugar 0%
	Green Color (+)		Presence of reducing sugar 0.1 – 0.5 g %
Benedict's Test	Yellow (++)		Presence of reducing sugar 0.5 – 1.0 g %
	Orange (+++)		Presence of reducing sugar 1.0 – 2.0 g %
	Brick red (++++)		Presence of reducing sugar > 2.0 g %
Barfoed's Test	Deep Blue Color		Monosaccharides
Foulger's Test	Blue Color		Keto Sugar
Selivanoff's Test	Red Color		Keto Sugar
Iodine Test	Purple		Dextrine

Fig. 2.1

Space for writing answers

Signature of Teacher

Experiment No. 3

1.0 TITLE : To identify given sample of carbohydrate (sample No. 2)

2.0 PRIOR CONCEPTS:

Carbohydrates are present in natural products such as maize, rice, potato, cane sugar, honey, etc.

3.0 NEW CONCEPTS:

Proposition 1 :

Carbohydrate is detected by Molisch's test.

Proposition 2 :

Polysaccharides are detected by iodine test.

Proposition 3 :

Reducing sugars are detected by Fehling's and Benedict's test.

Proposition 4 :

Aldoses and Ketoses are distinguished by Seliwanoff's test.

Proposition 5 :

Lactose & Maltose distinguished by Tollen's reagent test.

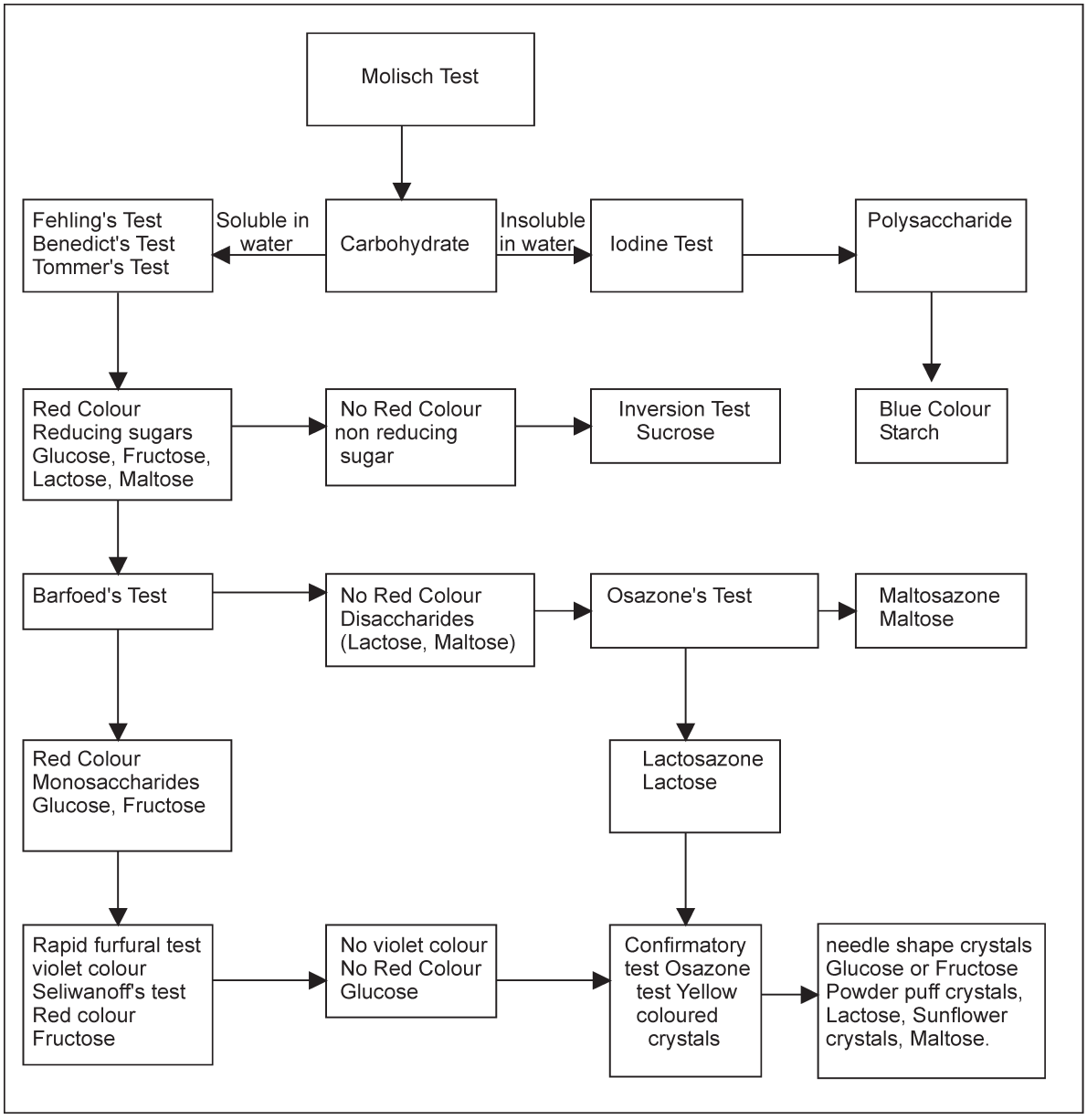
Proposition 6 :

Sucrose is confirmed by inversion test.

Proposition 7 :

Glucose, Fructose, Maltose, Lactose are confirmed by osazone test.

Concept Structure :



4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand formation of precipitate.
2. Application of chemical test of carbohydrates to identify given sample of carbohydrate according to concept structure.

Motor skills

1. Ability to distinguish the colours while performing various chemical tests.
2. Ability to add required amount of test solution and chemical reagent while performing the chemical test.
3. Ability to provide required quantity of heat safely while performing the chemical test whenever required.
4. Ability to observe and differentiate respective osazone crystals while observing them under microscope.

5.0 APPARATUS :

Glassware

Beaker, water bath, test tubes, graduated pipettes, funnel, filter paper, watchglass, slide, microscope.

Chemicals

Molisch reagent (1% ∝naphthol in alcohol), concentrated sulphuric acid, N/50 Iodine solution, distilled water, Fehling's solution A (7.93% copper sulphate in water), Fehling's solution B (250g sodium hydroxide and 320g sodium potassium tartarate in 500 ml water), Benedict's reagent (dissolve 173g sodium citrate and 100g anhydrous sodium carbonate in about 800 ml water, separately dissolve 17.3g copper sulphate in 100ml water, mix both the solution and make volume to 1000 ml with water), Tommer's reagent (prepared fresh- 5 % copper sulphate in water and equal volume of sodium hydroxide solution), Barfoed's reagent (13.3g copper acetate in 200 ml water filter, to it add 1.8 ml glacial acetic acid), Seliwanoff's reagent (dissolve 50 mg of resorcinol in 33 ml concentrated hydrochloric acid diluted to 1000 ml with water)

Concentrated nitric acid, phenylhydrazine hydrochloride,

Acetate buffer - PH 5.0 Dissolve 40.8 g sodium acetate in 70 ml water. To it add 8.2 ml glacial acetc acid. Make the volume to 100ml with water.

Fougler's reagent (Dissolve 40 g urea in 80 ml of 40% w/w sulphuric acid to it add 2 g stannous chloride and boil till clear solution is obtained. Cool make volume to 40 ml with 40% w/w sulphuric acid.

6.0 STEPWISE PROCEDURE :

1. Prepare a solution of given carbohydrate sample by dissolving 1 gm sample in 20 ml of water. And use same solution for analysis.
2. Perform the chemical test of carbohydrates according to general concept, structure, to identify the given sample.
3. Adjust the microscope at low power properly before observing osazone crystals.
4. Confirm Glucose, Fructose, Maltose, Lactose by Osazone test, Sucrose by inversion test and starch by iodine test.
5. Report your analysis for identification of given sample of carbohydrate in given proforma.

Table to perform tests for identification of carbohydrate sample (with reagents)

Test	Observation	Inference
1. Molisch's test : Mix 2ml of carbohydrate sample with 5 drops of Molisch's Reagent in a test tube. Add gently through the side by tilting the tube, about 2 ml of concentrated H_2SO_4 so as to form a bottom layer.	Violet / purple ring at the junction of two liquids	Carbohydrate present.
2. Solubility Compound + water	Soluble Insoluble	Mono and disaccharides present Polysaccharides present
3. Fehling's test 2 ml of Fehling's solution A + 2ml of Fehling's solution B+ 2 ml of Sugar solution Boil.	Yellow or brick red ppt is observed.	Reducing sugar present
4. Benedict's test Take 5ml of Benedict's qualitative reagent, add 8 drops of sugar solution. Boil over a flame for 2 minutes or place in boiling water bath for 3 minutes. Allow to cool.	Green, yellow, orange or brick red ppt is observed	Reducing sugar present

Test	Observation	Inference
5. Tommer's test To 2 ml of Tommer's reagent add 3 ml of sugar solution boil for 2 minutes cool.	Yellow or red ppt is observed	Reducing sugar present
6. Barfoed's test 2 ml of Barfoed's reagent add 2ml of sugar solution and keep in boiling water bath for 2 minutes cool.	Brick red precipitate is observed at the bottom of test tube.	Reducing sugarpresent.
7. Seliwanoff's test To 3 ml of Seliwanoff's reagent add 1 ml of sugar solution and heat the mixture to boil for 2 minutes. cool.	Red colour or red precipitate is observed.	Ketoses like fructose, sucrose present.
8. Rapid furfural test To 2 ml of sugar solution add 1 ml of A-napthol solution (1% in alcohol) and 5 ml concentrated HCl boil.	Deep purple colour is observed.	Ketoses like fructose, sucrose present
9. Osazone test Set up a boiling water bath. Take test tube, add 1 g of Phenyl hydrazine hydrochloride 2 ml of acetate buffer, P^H 5.0. add 5ml of water mixwell and warm gently. Filter it. To the filterate add 5 ml sugar solution and keep in boiling water bath and cool. Mount the crystals under microscope and examine under microscope.	a) Greenish yellow needle shaped crystals. b) Badminton ball, powder puff shaped crystals. c) Sunflower or its petals shaped crystals.	Glucosazone i.e. glucose present. Lactosazone i.e. lactose present. Maltasazone i.e. maltose present.
10. Iodine test a) To about 2 ml Suspension or solution of polysaccharides add 1-2 drops of N/50 iodine solution.	Blue violet colour developsBrown wine colour develops.	Starch is present Glycogen is present.
b) Warm above solution c) Cool the above solution d) To about 2 ml suspension of starch add 1ml 5 % sodium hydroxide and 2 drops of N/50 iodine.	Blue colour disappears. Blue colour reappears No blue colour	Starch is present. Starch is present. Starch is present.
C.T. for Glucose a) Osazone test	Greenish yellow needle shaped crystal	Glucose confirmed
b) To 2ml of test solution add 5 % NaOH	Brown resinous precipitate is observed.	Glucose confirmed
c) Take 3 ml water to it add drop of Methylene blue solution and 1 ml of 5% sodium hydroxide and 2ml of sugar solution and boil.	Solution is decolourised	Glucose confirmed
d) To 3 ml of sugar solution add 1 ml of picric acid solution and 1ml 5% NaOH and heat.	Red colour develops	Glucose confirmed
C. T. for Lactose 1. Mucic acid test To 1 ml sugar solutionadd 1 ml concentrated HNO_3 boil and cool.	Broken glass like crystals obtained which can be identified under microscope.	Lactose confirmed

Test	Observation	Inference
2. Silver mirror test 1 ml sugar solution add 1 ml Ammonical silver nitrate solution add excess NaOH Warm.	Silver mirror is observed.	Lactose confirmed
C.T. for sucrose a) Seliwanoff's test b) Rapid furfural test c) Inversion test. To 25 ml of test solution add 5 ml of concentrated HCL boil for 3 minutes and cool under tap water. Neutralize with sodium hydroxide solution. Now perform following test for this solution. i) Fehling's test ii) Tommer's test iii) Benedict's test iv) Barfoed's test	 Red precipitate is observed. Red precipitate is observed. Red / yellow / green precipitate is observed.	 Sucrose confirmed. Sucrose confirmed. Sucrose confirmed.
C.T. for fructose Fougler's test To 3 ml fougler's reagent add 0.5 ml of sugar solution. Boil for one minute.	 Blue colour develops	 Fructose confirmed.

7.0 OBSERVATION :

Sample 2 :

Student to write test, observation and inference

Test	Observation	Inference

Student to write test, observation and inference

Test	Observation	Inference

8.0 RESULT :

1. Given sample of carbohydrate is _____
2. It is _____ (Monosaccharide /Disaccharide/ Polysaccharide)

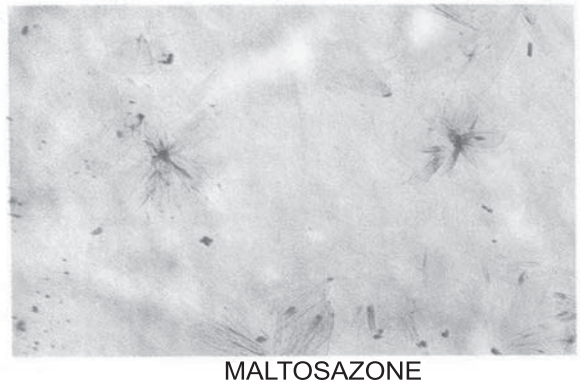
9.0 QUESTIONS :

Student to answer question Q....., Q....., Q.....,Q.....
and the question numbers shall be allotted by the teacher.

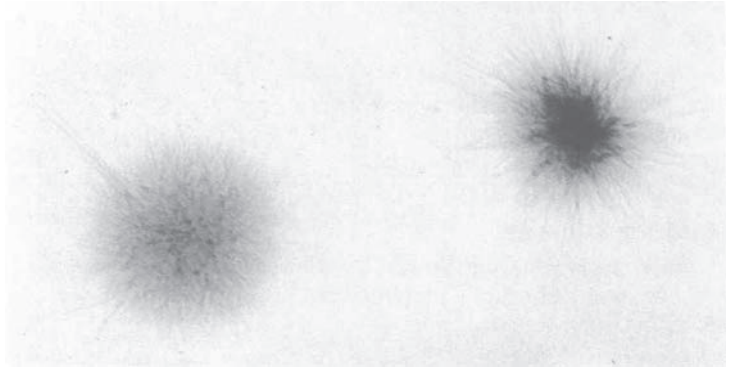
1. State the principle of Molisch test.
2. State the component of starch.
3. State the name of two sources of starch.
4. State the principle of Benedict test.
5. State the name of most sensitive test of reducing sugars.
6. State the composition of Tollen's reagent.
7. Write the reactions involved in Osazone test.
8. Draw the shape of Maltosazone crystals.
9. State the names of carbohydrates distinguished by iodine test.
10. State the names of epimers of glucose.
11. What is purpose of inversion test.
12. State the name of acid obtained by oxidation of glucose.
13. State the name of chemical test required to distinguish monosaccharide and disaccharide.
14. State the colours produced by iodine with starch, dextrine and glycogen.
15. State the name of non-reducing diassacharides.
16. Draw the structures of Glucose and Fructose.
17. Draw the structures of Sucrose, Lactose and Maltose.
18. State the hydrolysis products of each : Sucrose.
19. State two disease related to abnormal metabolism of glucose.
20. Name two reducing diassacharides and give its hydrolysis products.
21. State two examples of monosaccharides, diasaccharide and polysaccharides each.
22. Name two chemical test to discriminate between glucose and fructose sample.
23. State name of sugar sample which gives needle shape crystals of osazone.
24. State name of sugar sample which gives powder puff shape crystals of osazone.
25. State the two sources of fructose.
26. State rich source of lactose and sucrose each.

10.0 REFERENCE:

GLUCOSAZONE



MALTOSAZONE



LACTOSAZONE

PLATE (COLOURS OF THE TESTS)
REACTIONS OF CARBOHYDRATES

TEST	OBSERVATION	COLOR	CONFIRMS
Molisch's Test	Violet Ring		Carbohydrate
	Blue Negative		Absence of reducing sugar 0%
	Green Color (+)		Presence of reducing sugar 0.1 – 0.5 g %
Benedict's Test	Yellow (++)		Presence of reducing sugar 0.5 – 1.0 g %
	Orange (+++)		Presence of reducing sugar 1.0 – 2.0 g %
	Brick red (++++)		Presence of reducing sugar > 2.0 g %
Barfoed's Test	Deep Blue Color		Monosaccharides
Foulger's Test	Blue Color		Keto Sugar
Selivanoff's Test	Red Color		Keto Sugar
Iodine Test	Purple		Dextrine

Fig. 2.1

Space for writing answers

Signature of Teacher

Experiment No. 4

1.0 TITLE : To identify given sample of carbohydrate (sample No. 3)

2.0 PRIOR CONCEPTS:

Carbohydrates are present in natural products such as maize, rice, potato, cane sugar, honey, etc.

3.0 NEW CONCEPTS:

Proposition 1 :

Carbohydrate is detected by Molisch's test.

Proposition 2 :

Polysaccharides are detected by iodine test.

Proposition 3 :

Reducing sugars are detected by Fehling's and Benedict's test.

Proposition 4 :

Aldoses and Ketoses are distinguished by Seliwanoff's test.

Proposition 5 :

Lactose & Maltose distinguished by Tollen's reagent test.

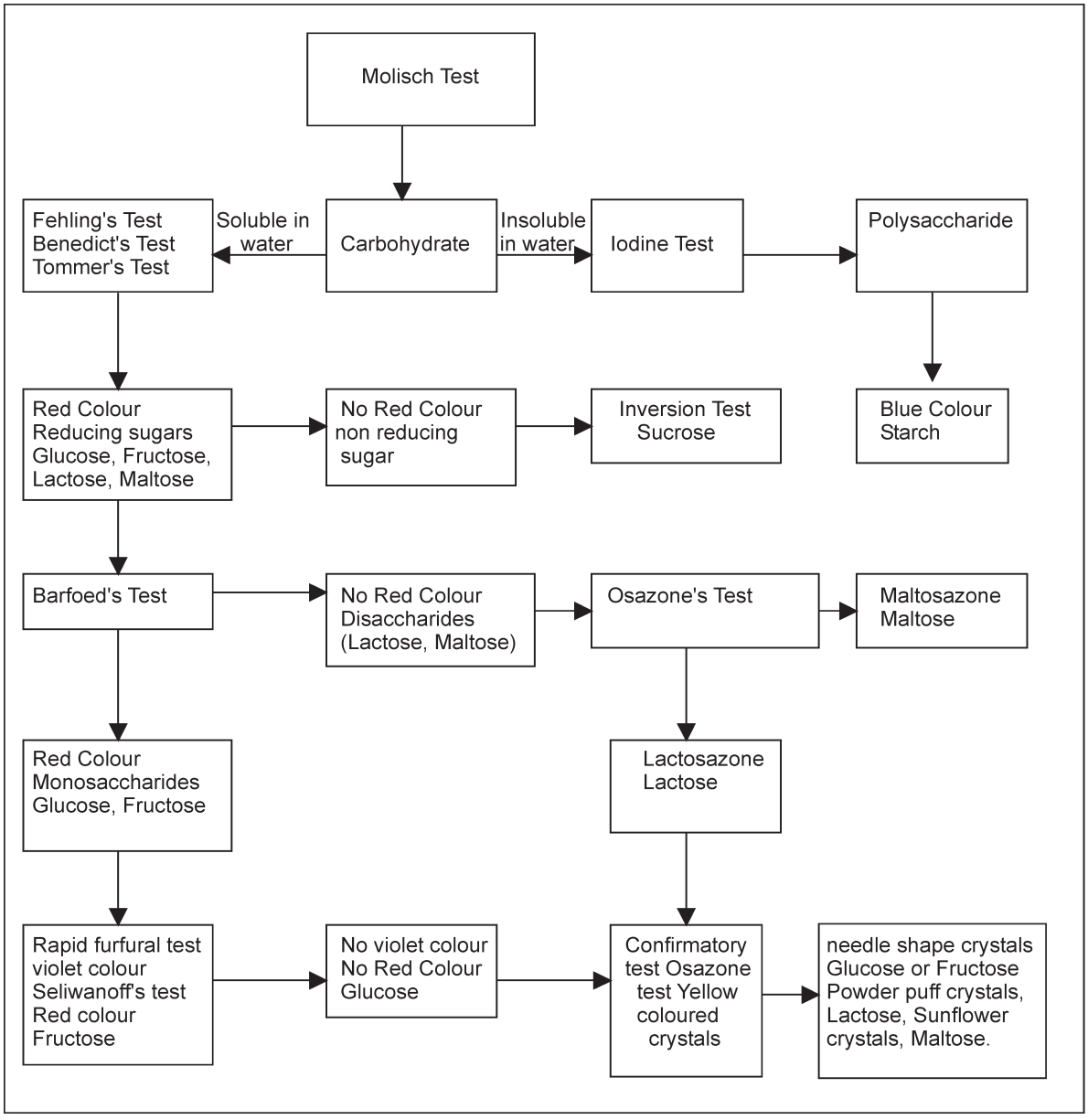
Proposition 6 :

Sucrose is confirmed by inversion test.

Proposition 7 :

Glucose, Fructose, Maltose, Lactose are confirmed by osazone test.

Concept Structure :



4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand formation of precipitate.
2. Application of chemical test of carbohydrates to identify given sample of carbohydrate according to concept structure.

Motor skills

1. Ability to distinguish the colours while performing various chemical tests.
2. Ability to add required amount of test solution and chemical reagent while performing the chemical test.
3. Ability to provide required quantity of heat safely while performing the chemical test whenever required.
4. Ability to observe and differentiate respective osazone crystals while observing them under microscope.

5.0 APPARATUS :

Glassware

Beaker, water bath, test tubes, graduated pipettes, funnel, filter paper, watchglass, slide, microscope.

Chemicals

Molisch reagent (1% ∝naphthol in alcohol), concentrated sulphuric acid, N/50 Iodine solution, distilled water, Fehling's solution A (7.93% copper sulphate in water), Fehling's solution B (250g sodium hydroxide and 320g sodium potassium tartarate in 500 ml water), Benedict's reagent (dissolve 173g sodium citrate and 100g anhydrous sodium carbonate in about 800 ml water, separately dissolve 17.3g copper sulphate in 100ml water, mix both the solution and make volume to 1000 ml with water), Tommer's reagent (prepared fresh- 5 % copper sulphate in water and equal volume of sodium hydroxide solution), Barfoed's reagent (13.3g copper acetate in 200 ml water filter, to it add 1.8 ml glacial acetic acid), Seliwanoff's reagent (dissolve 50 mg of resorcinol in 33 ml concentrated hydrochloric acid diluted to 1000 ml with water)

Concentrated nitric acid, phenylhydrazine hydrochloride,

Acetate buffer - PH 5.0 Dissolve 40.8 g sodium acetate in 70 ml water. To it add 8.2 ml glacial acetc acid. Make the volume to 100ml with water.

Fougler's reagent (Dissolve 40 g urea in 80 ml of 40% w/w sulphuric acid to it add 2 g stannous chloride and boil till clear solution is obtained. Cool make volume to 40 ml with 40% w/w sulphuric acid.

6.0 STEPWISE PROCEDURE :

1. Prepare a solution of given carbohydrate sample by dissolving 1 gm sample in 20 ml of water. And use same solution for analysis.
2. Perform the chemical test of carbohydrates according to general concept, structure, to identify the given sample.
3. Adjust the microscope at low power properly before observing osazone crystals.
4. Confirm Glucose, Fructose, Maltose, Lactose by Osazone test, Sucrose by inversion test and starch by iodine test.
5. Report your analysis for identification of given sample of carbohydrate in given proforma.

Table to perform tests for identification of carbohydrate sample (with reagents)

Test	Observation	Inference
1. Molisch's test : Mix 2ml of carbohydrate sample with 5 drops of Molisch's Reagent in a test tube. Add gently through the side by tilting the tube, about 2 ml of concentrated H_2SO_4 so as to form a bottom layer.	Violet / purple ring at the junction of two liquids	Carbohydrate present.
2. Solubility Compound + water	Soluble Insoluble	Mono and disaccharides present Polysaccharides present
3. Fehling's test 2 ml of Fehling's solution A + 2ml of Fehling's solution B+ 2 ml of Sugar solution Boil.	Yellow or brick red ppt is observed.	Reducing sugar present
4. Benedict's test Take 5ml of Benedict's qualitative reagent, add 8 drops of sugar solution. Boil over a flame for 2 minutes or place in boiling water bath for 3 minutes. Allow to cool.	Green, yellow, orange or brick red ppt is observed	Reducing sugar present

Test	Observation	Inference
5. Tommer's test To 2 ml of Tommer's reagent add 3 ml of sugar solution boil for 2 minutes cool.	Yellow or red ppt is observed	Reducing sugar present
6. Barfoed's test 2 ml of Barfoed's reagent add 2ml of sugar solution and keep in boiling water bath for 2 minutes cool.	Brick red precipitate is observed at the bottom of test tube.	Reducing sugarpresent.
7. Seliwanoff's test To 3 ml of Seliwanoff's reagent add 1 ml of sugar solution and heat the mixture to boil for 2 minutes. cool.	Red colour or red precipitate is observed.	Ketoses like fructose, sucrose present.
8. Rapid furfural test To 2 ml of sugar solution add 1 ml of A-napthol solution (1% in alcohol) and 5 ml concentrated HCl boil.	Deep purple colour is observed.	Ketoses like fructose, sucrose present
9. Osazone test Set up a boiling water bath. Take test tube, add 1 g of Phenyl hydrazine hydrochloride 2 ml of acetate buffer, P^H 5.0. add 5ml of water mixwell and warm gently. Filter it. To the filterate add 5 ml sugar solution and keep in boiling water bath and cool. Mount the crystals under microscope and examine under microscope.	a) Greenish yellow needle shaped crystals. b) Badminton ball, powder puff shaped crystals. c) Sunflower or its petals shaped crystals.	Glucosazone i.e. glucose present. Lactosazone i.e. lactose present. Maltasazone i.e. maltose present.
10. Iodine test a) To about 2 ml Suspension or solution of polysaccharides add 1-2 drops of N/50 iodine solution.	Blue violet colour developsBrown wine colour develops.	Starch is present Glycogen is present.
b) Warm above solution c) Cool the above solution d) To about 2 ml suspension of starch add 1ml 5 % sodium hydroxide and 2 drops of N/50 iodine.	Blue colour disappears. Blue colour reappears No blue colour	Starch is present. Starch is present. Starch is present.
C.T. for Glucose a) Osazone test	Greenish yellow needle shaped crystal	Glucose confirmed
b) To 2ml of test solution add 5 % NaOH	Brown resinous precipitate is observed.	Glucose confirmed
c) Take 3 ml water to it add drop of Methylene blue solution and 1 ml of 5% sodium hydroxide and 2ml of sugar solution and boil.	Solution is decolourised	Glucose confirmed
d) To 3 ml of sugar solution add 1 ml of picric acid solution and 1ml 5% NaOH and heat.	Red colour develops	Glucose confirmed
C. T. for Lactose 1. Mucic acid test To 1 ml sugar solutionadd 1 ml concentrated HNO_3 boil and cool.	Broken glass like crystals obtained which can be identified under microscope.	Lactose confirmed

Test	Observation	Inference
2. Silver mirror test 1 ml sugar solution add 1 ml Ammonical silver nitrate solution add excess NaOH Warm.	Silver mirror is observed.	Lactose confirmed
C.T. for sucrose a) Seliwanoff's test b) Rapid furfural test c) Inversion test. To 25 ml of test solution add 5 ml of concentrated HCL boil for 3 minutes and cool under tap water. Neutralize with sodium hydroxide solution. Now perform following test for this solution. i) Fehling's test ii) Tommer's test iii) Benedict's test iv) Barfoed's test	 Red precipitate is observed. Red precipitate is observed. Red / yellow / green precipitate is observed.	 Sucrose confirmed. Sucrose confirmed. Sucrose confirmed.
C.T. for fructose Fougler's test To 3 ml fougler's reagent add 0.5 ml of sugar solution. Boil for one minute.	Blue colour develops	Fructose confirmed.

7.0 OBSERVATION :

Sample 3 :

Student to write test, observation and inference

Test	Observation	Inference

Student to write test, observation and inference

Test	Observation	Inference

8.0 RESULT :

1. Given sample of carbohydrate is _____
2. It is _____ (Monosaccharide /Disaccharide/ Polysaccharide)

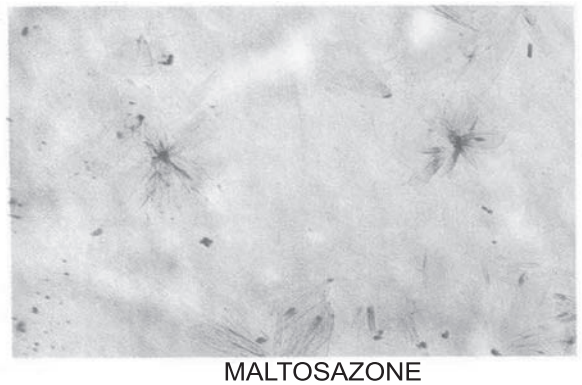
9.0 QUESTIONS :

Student to answer question Q....., Q....., Q.....,Q.....
and the question numbers shall be allotted by the teacher.

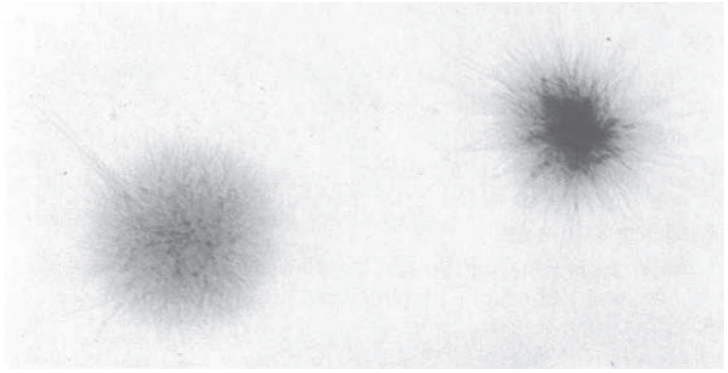
1. State the principle of Molisch test.
2. State the component of starch.
3. State the name of two sources of starch.
4. State the principle of Benedict test.
5. State the name of most sensitive test of reducing sugars.
6. State the composition of Tollen's reagent.
7. Write the reactions involved in Osazone test.
8. Draw the shape of Maltosazone crystals.
9. State the names of carbohydrates distinguished by iodine test.
10. State the names of epimers of glucose.
11. What is purpose of inversion test.
12. State the name of acid obtained by oxidation of glucose.
13. State the name of chemical test required to distinguish monosaccharide and disaccharide.
14. State the colours produced by iodine with starch, dextrine and glycogen.
15. State the name of non-reducing diassacharides.
16. Draw the structures of Glucose and Fructose.
17. Draw the structures of Sucrose, Lactose and Maltose.
18. State the hydrolysis products of each : Sucrose.
19. State two disease related to abnormal metabolism of glucose.
20. Name two reducing diassacharides and give its hydrolysis products.
21. State two examples of monosaccharides, diasaccharide and polysaccharides each.
22. Name two chemical test to discriminate between glucose and fructose sample.
23. State name of sugar sample which gives needle shape crystals of osazone.
24. State name of sugar sample which gives powder puff shape crystals of osazone.
25. State the two sources of fructose.
26. State rich source of lactose and sucrose each.

10.0 REFERENCE:

GLUCOSAZONE



MALTOSAZONE



LACTOSAZONE

PLATE (COLOURS OF THE TESTS)
REACTIONS OF CARBOHYDRATES

TEST	OBSERVATION	COLOR	CONFIRMS
Molisch's Test	Violet Ring		Carbohydrate
	Blue Negative		Absence of reducing sugar 0%
	Green Color (+)		Presence of reducing sugar 0.1 – 0.5 g %
Benedict's Test	Yellow (++)		Presence of reducing sugar 0.5 – 1.0 g %
	Orange (+++)		Presence of reducing sugar 1.0 – 2.0 g %
	Brick red (++++)		Presence of reducing sugar > 2.0 g %
Barfoed's Test	Deep Blue Color		Monosaccharides
Foulger's Test	Blue Color		Keto Sugar
Selivanoff's Test	Red Color		Keto Sugar
Iodine Test	Purple		Dextrine

Fig. 2.1

Space for writing answers

Signature of Teacher

Experiment No. 5

1.0 TITLE : To identify given sample of carbohydrate (sample No. 4)

2.0 PRIOR CONCEPTS:

Carbohydrates are present in natural products such as maize, rice, potato, cane sugar, honey, etc.

3.0 NEW CONCEPTS:

Proposition 1 :

Carbohydrate is detected by Molisch's test.

Proposition 2 :

Polysaccharides are detected by iodine test.

Proposition 3 :

Reducing sugars are detected by Fehling's and Benedict's test.

Proposition 4 :

Aldoses and Ketoses are distinguished by Seliwanoff's test.

Proposition 5 :

Lactose & Maltose distinguished by Tollen's reagent test.

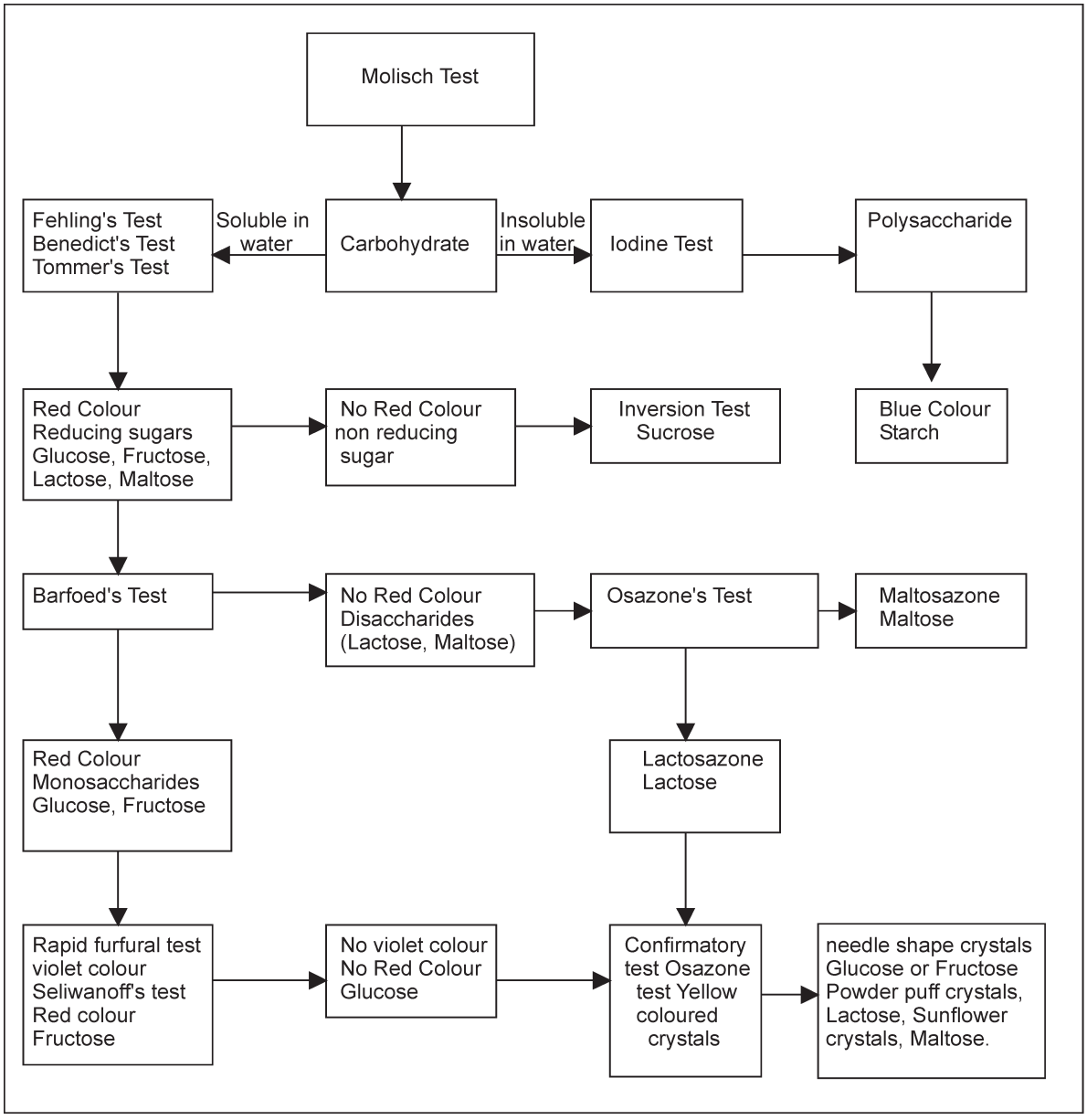
Proposition 6 :

Sucrose is confirmed by inversion test.

Proposition 7 :

Glucose, Fructose, Maltose, Lactose are confirmed by osazone test.

Concept Structure :



4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand formation of precipitate.
2. Application of chemical test of carbohydrates to identify given sample of carbohydrate according to concept structure.

Motor skills

1. Ability to distinguish the colours while performing various chemical tests.
2. Ability to add required amount of test solution and chemical reagent while performing the chemical test.
3. Ability to provide required quantity of heat safely while performing the chemical test whenever required.
4. Ability to observe and differentiate respective osazone crystals while observing them under microscope.

5.0 APPARATUS :

Glassware

Beaker, water bath, test tubes, graduated pipettes, funnel, filter paper, watchglass, slide, microscope.

Chemicals

Molisch reagent (1% ∝naphthol in alcohol), concentrated sulphuric acid, N/50 Iodine solution, distilled water, Fehling's solution A (7.93% copper sulphate in water), Fehling's solution B (250g sodium hydroxide and 320g sodium potassium tartarate in 500 ml water), Benedict's reagent (dissolve 173g sodium citrate and 100g anhydrous sodium carbonate in about 800 ml water, separately dissolve 17.3g copper sulphate in 100ml water, mix both the solution and make volume to 1000 ml with water), Tommer's reagent (prepared fresh- 5 % copper sulphate in water and equal volume of sodium hydroxide solution), Barfoed's reagent (13.3g copper acetate in 200 ml water filter, to it add 1.8 ml glacial acetic acid), Seliwanoff's reagent (dissolve 50 mg of resorcinol in 33 ml concentrated hydrochloric acid diluted to 1000 ml with water)

Concentrated nitric acid, phenylhydrazine hydrochloride,

Acetate buffer - PH 5.0 Dissolve 40.8 g sodium acetate in 70 ml water. To it add 8.2 ml glacial acetc acid. Make the volume to 100ml with water.

Fougler's reagent (Dissolve 40 g urea in 80 ml of 40% w/w sulphuric acid to it add 2 g stannous chloride and boil till clear solution is obtained. Cool make volume to 40 ml with 40% w/w sulphuric acid.

6.0 STEPWISE PROCEDURE :

1. Prepare a solution of given carbohydrate sample by dissolving 1 gm sample in 20 ml of water. And use same solution for analysis.
2. Perform the chemical test of carbohydrates according to general concept, structure, to identify the given sample.
3. Adjust the microscope at low power properly before observing osazone crystals.
4. Confirm Glucose, Fructose, Maltose, Lactose by Osazone test, Sucrose by inversion test and starch by iodine test.
5. Report your analysis for identification of given sample of carbohydrate in given proforma.

Table to perform tests for identification of carbohydrate sample (with reagents)

Test	Observation	Inference
1. Molisch's test : Mix 2ml of carbohydrate sample with 5 drops of Molisch's Reagent in a test tube. Add gently through the side by tilting the tube, about 2 ml of concentrated H_2SO_4 so as to form a bottom layer.	Violet / purple ring at the junction of two liquids	Carbohydrate present.
2. Solubility Compound + water	Soluble Insoluble	Mono and disaccharides present Polysaccharides present
3. Fehling's test 2 ml of Fehling's solution A + 2ml of Fehling's solution B+ 2 ml of Sugar solution Boil.	Yellow or brick red ppt is observed.	Reducing sugar present
4. Benedict's test Take 5ml of Benedict's qualitative reagent, add 8 drops of sugar solution. Boil over a flame for 2 minutes or place in boiling water bath for 3 minutes. Allow to cool.	Green, yellow, orange or brick red ppt is observed	Reducing sugar present

Test	Observation	Inference
5. Tommer's test To 2 ml of Tommer's reagent add 3 ml of sugar solution boil for 2 minutes cool.	Yellow or red ppt is observed	Reducing sugarpresent
6. Barfoed's test 2 ml of Barfoed's reagent add 2ml of sugar solution and keep in boiling water bath for 2 minutes cool.	Brick red precipitate is observed at the bottom of test tube.	Reducing sugar present.
7. Seliwanoff's test To 3 ml of Seliwanoff's reagent add 1 ml of sugar solution and heat the mixture to boil for 2 minutes. cool.	Red colour or red precipitate is observed.	Ketoses like fructose, sucrose present.
8. Rapid furfural test To 2 ml of sugar solution add 1 ml of A-napthol solution (1% in alcohol) and 5 ml concentrated HCL boil.	Deep purple colour is observed.	Ketoses like fructose, sucrose present
9. Osazone test Set up a boiling water bath. Take test tube, add 1 g of Phenyl hydrazine hydrochloride 2 ml of acetate buffer, P^H 5.0. add 5ml of water mixwell and warm gently. Filter it. To the filterate add 5 ml sugar solution and keep in boiling water bath and cool. Mount the crystals under microscope and examine under microscope.	a) Greenish yellow needle shaped crystals. b) Badminton ball, powder puff shaped crystals. c) Sunflower or its petals shaped crystals.	Glucosazone i.e. glucose present. Lactosazone i.e. lactose present. Maltasazone i.e. maltose present.
10. Iodine test a) To about 2 ml Suspension or solution of polysaccharides add 1-2 drops of N/50 iodine solution.	Blue violet colour developsBrown wine colour develops.	Starch is present Glycogen is present.
b) Warm above solution c) Cool the above solution d) To about 2 ml suspension of starch add 1ml 5 % sodium hydroxide and 2 drops of N/50 iodine.	Blue colour disappears. Blue colour reappears No blue colour	Starch is present. Starch is present. Starch is present.
C.T. for Glucose a) Osazone test	Greenish yellow needle shaped crystal	Glucose confirmed
b) To 2ml of test solution add 5 % NaOH	Brown resinous precipitate is observed.	Glucose confirmed
c) Take 3 ml water to it add drop of Methylene blue solution and 1 ml of 5% sodium hydroxide and 2ml of sugar solution and boil.	Solution is decolourised	Glucose confirmed
d) To 3 ml of sugar solution add 1 ml of picric acid solution and 1ml 5% NaOH and heat.	Red colour develops	Glucose confirmed
C. T. for Lactose 1. Mucic acid test To 1 ml sugar solutionadd 1 ml concentrated HNO_3 boil and cool.	Broken glass like crystals obtained which can be identified under microscope.	Lactose confirmed

Test	Observation	Inference
2. Silver mirror test 1 ml sugar solution add 1 ml Ammonical silver nitrate solution add excess NaOH Warm.	Silver mirror is observed.	Lactose confirmed
C.T. for sucrose a) Seliwanoff's test b) Rapid furfural test c) Inversion test. To 25 ml of test solution add 5 ml of concentrated HCL boil for 3 minutes and cool under tap water. Neutralize with sodium hydroxide solution. Now perform following test for this solution. i) Fehling's test ii) Tommer's test iii) Benedict's test iv) Barfoed's test	 Red precipitate is observed. Red precipitate is observed. Red / yellow / green precipitate is observed.	 Sucrose confirmed. Sucrose confirmed. Sucrose confirmed.
C.T. for fructose Fougler's test To 3 ml fougler's reagent add 0.5 ml of sugar solution. Boil for one minute.	Blue colour develops	Fructose confirmed.

7.0 OBSERVATION :

Sample 4 :

Student to write test, observation and inference

Test	Observation	Inference

Student to write test, observation and inference

Test	Observation	Inference

8.0 RESULT :

1. Given sample of carbohydrate is _____
2. It is _____ (Monosaccharide /Disaccharide/ Polysaccharide)

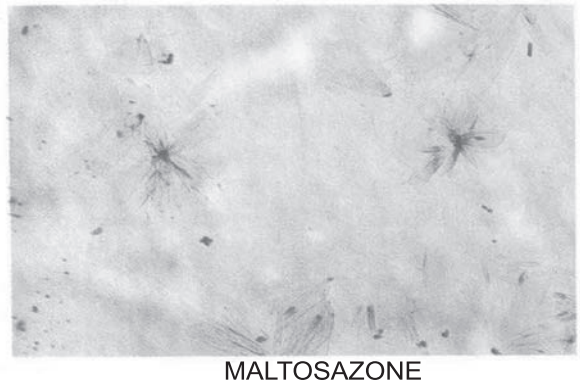
9.0 QUESTIONS :

Student to answer question Q....., Q....., Q.....,Q.....
and the question numbers shall be allotted by the teacher.

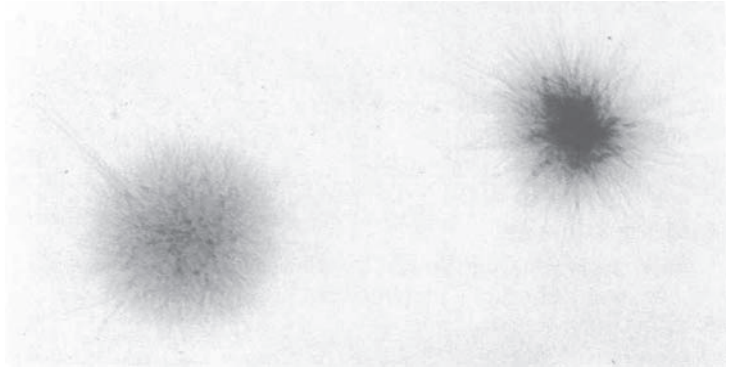
1. State the principle of Molisch test.
2. State the component of starch.
3. State the name of two sources of starch.
4. State the principle of Benedict test.
5. State the name of most sensitive test of reducing sugars.
6. State the composition of Tollen's reagent.
7. Write the reactions involved in Osazone test.
8. Draw the shape of Maltosazone crystals.
9. State the names of carbohydrates distinguished by iodine test.
10. State the names of epimers of glucose.
11. What is purpose of inversion test.
12. State the name of acid obtained by oxidation of glucose.
13. State the name of chemical test required to distinguish monosaccharide and disaccharide.
14. State the colours produced by iodine with starch, dextrine and glycogen.
15. State the name of non-reducing diassacharides.
16. Draw the structures of Glucose and Fructose.
17. Draw the structures of Sucrose, Lactose and Maltose.
18. State the hydrolysis products of each : Sucrose.
19. State two disease related to abnormal metabolism of glucose.
20. Name two reducing diassacharides and give its hydrolysis products.
21. State two examples of monosaccharides, diasaccharide and polysaccharides each.
22. Name two chemical test to discriminate between glucose and fructose sample.
23. State name of sugar sample which gives needle shape crystals of osazone.
24. State name of sugar sample which gives powder puff shape crystals of osazone.
25. State the two sources of fructose.
26. State rich source of lactose and sucrose each.

10.0 REFERENCE:

GLUCOSAZONE



MALTOSAZONE



LACTOSAZONE

PLATE (COLOURS OF THE TESTS)
REACTIONS OF CARBOHYDRATES

TEST	OBSERVATION	COLOR	CONFIRMS
Molisch's Test	Violet Ring		Carbohydrate
	Blue Negative		Absence of reducing sugar 0%
	Green Color (+)		Presence of reducing sugar 0.1 – 0.5 g %
Benedict's Test	Yellow (++)		Presence of reducing sugar 0.5 – 1.0 g %
	Orange (+++)		Presence of reducing sugar 1.0 – 2.0 g %
	Brick red (++++)		Presence of reducing sugar > 2.0 g %
Barfoed's Test	Deep Blue Color		Monosaccharides
Foulger's Test	Blue Color		Keto Sugar
Selivanoff's Test	Red Color		Keto Sugar
Iodine Test	Purple		Dextrine

Fig. 2.1

Space for writing answers

Signature of Teacher

Experiment No. 6

1.0 TITLE : To identify given sample of carbohydrate (sample No. 5)

2.0 PRIOR CONCEPTS:

Carbohydrates are present in natural products such as maize, rice, potato, cane sugar, honey, etc.

3.0 NEW CONCEPTS:

Proposition 1 :

Carbohydrate is detected by Molisch's test.

Proposition 2 :

Polysaccharides are detected by iodine test.

Proposition 3 :

Reducing sugars are detected by Fehling's and Benedict's test.

Proposition 4 :

Aldoses and Ketoses are distinguished by Seliwanoff's test.

Proposition 5 :

Lactose & Maltose distinguished by Tollen's reagent test.

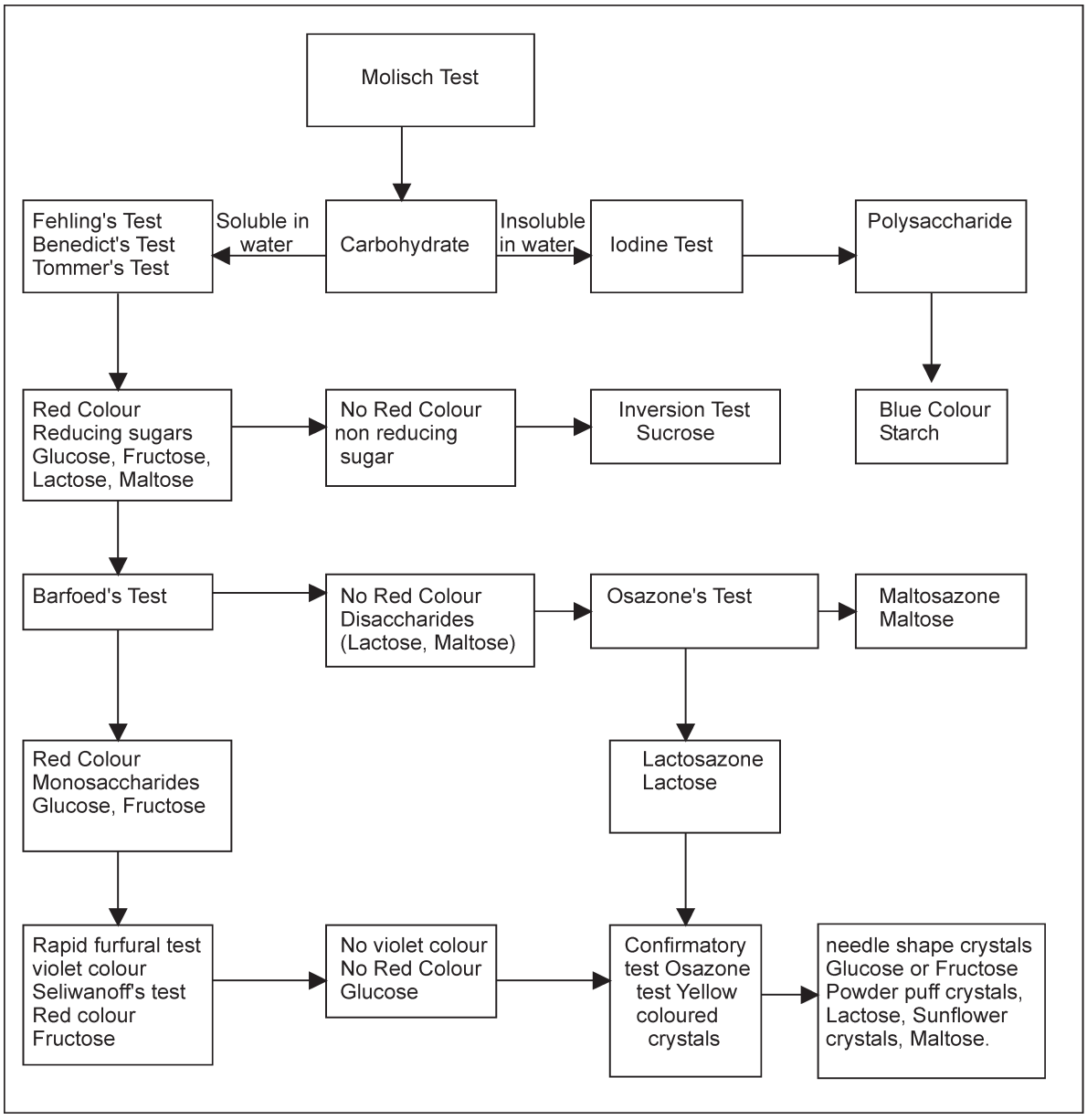
Proposition 6 :

Sucrose is confirmed by inversion test.

Proposition 7 :

Glucose, Fructose, Maltose, Lactose are confirmed by osazone test.

Concept Structure :



4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand formation of precipitate.
2. Application of chemical test of carbohydrates to identify given sample of carbohydrate according to concept structure.

Motor skills

1. Ability to distinguish the colours while performing various chemical tests.
2. Ability to add required amount of test solution and chemical reagent while performing the chemical test.
3. Ability to provide required quantity of heat safely while performing the chemical test whenever required.
4. Ability to observe and differentiate respective osazone crystals while observing them under microscope.

5.0 APPARATUS :

Glassware

Beaker, water bath, test tubes, graduated pipettes, funnel, filter paper, watchglass, slide, microscope.

Chemicals

Molisch reagent (1% ∝naphthol in alcohol), concentrated sulphuric acid, N/50 Iodine solution, distilled water, Fehling's solution A (7.93% copper sulphate in water), Fehling's solution B (250g sodium hydroxide and 320g sodium potassium tartarate in 500 ml water), Benedict's reagent (dissolve 173g sodium citrate and 100g anhydrous sodium carbonate in about 800 ml water, separately dissolve 17.3g copper sulphate in 100ml water, mix both the solution and make volume to 1000 ml with water), Tommer's reagent (prepared fresh- 5 % copper sulphate in water and equal volume of sodium hydroxide solution), Barfoed's reagent (13.3g copper acetate in 200 ml water filter, to it add 1.8 ml glacial acetic acid), Seliwanoff's reagent (dissolve 50 mg of resorcinol in 33 ml concentrated hydrochloric acid diluted to 1000 ml with water)

Concentrated nitric acid, phenylhydrazine hydrochloride,

Acetate buffer - PH 5.0 Dissolve 40.8 g sodium acetate in 70 ml water. To it add 8.2 ml glacial acetc acid. Make the volume to 100ml with water.

Fougler's reagent (Dissolve 40 g urea in 80 ml of 40% w/w sulphuric acid to it add 2 g stannous chloride and boil till clear solution is obtained. Cool make volume to 40 ml with 40% w/w sulphuric acid.

6.0 STEPWISE PROCEDURE :

1. Prepare a solution of given carbohydrate sample by dissolving 1 gm sample in 20 ml of water. And use same solution for analysis.
2. Perform the chemical test of carbohydrates according to general concept, structure, to identify the given sample.
3. Adjust the microscope at low power properly before observing osazone crystals.
4. Confirm Glucose, Fructose, Maltose, Lactose by Osazone test, Sucrose by inversion test and starch by iodine test.
5. Report your analysis for identification of given sample of carbohydrate in given proforma.

Table to perform tests for identification of carbohydrate sample (with reagents)

Test	Observation	Inference
1. Molisch's test : Mix 2ml of carbohydrate sample with 5 drops of Molisch's Reagent in a test tube. Add gently through the side by tilting the tube, about 2 ml of concentrated H_2SO_4 so as to form a bottom layer.	Violet / purple ring at the junction of two liquids	Carbohydrate present.
2. Solubility Compound + water	Soluble Insoluble	Mono and disaccharides present Polysaccharides present
3. Fehling's test 2 ml of Fehling's solution A + 2ml of Fehling's solution B+ 2 ml of Sugar solution Boil.	Yellow or brick red ppt is observed.	Reducing sugar present
4. Benedict's test Take 5ml of Benedict's qualitative reagent, add 8 drops of sugar solution. Boil over a flame for 2 minutes or place in boiling water bath for 3 minutes. Allow to cool.	Green, yellow, orange or brick red ppt is observed	Reducing sugar present

Test	Observation	Inference
5. Tommer's test To 2 ml of Tommer's reagent add 3 ml of sugar solution boil for 2 minutes cool.	Yellow or red ppt is observed	Reducing sugarpresent
6. Barfoed's test 2 ml of Barfoed's reagent add 2ml of sugar solution and keep in boiling water bath for 2 minutes cool.	Brick red precipitate is observed at the bottom of test tube.	Reducing sugar present.
7. Seliwanoff's test To 3 ml of Seliwanoff's reagent add 1 ml of sugar solution and heat the mixture to boil for 2 minutes. cool.	Red colour or red precipitate is observed.	Ketoses like fructose, sucrose present.
8. Rapid furfural test To 2 ml of sugar solution add 1 ml of A-napthol solution (1% in alcohol) and 5 ml concentrated HCl boil.	Deep purple colour is observed.	Ketoses like fructose, sucrose present
9. Osazone test Set up a boiling water bath. Take test tube, add 1 g of Phenyl hydrazine hydrochloride 2 ml of acetate buffer, P^H 5.0. add 5ml of water mixwell and warm gently. Filter it. To the filterate add 5 ml sugar solution and keep in boiling water bath and cool. Mount the crystals under microscope and examine under microscope.	a) Greenish yellow needle shaped crystals. b) Badminton ball, powder puff shaped crystals. c) Sunflower or its petals shaped crystals.	Glucosazone i.e. glucose present. Lactosazone i.e. lactose present. Maltasazone i.e. maltose present.
10. Iodine test a) To about 2 ml Suspension or solution of polysaccharides add 1-2 drops of N/50 iodine solution.	Blue violet colour developsBrown wine colour develops.	Starch is present Glycogen is present.
b) Warm above solution c) Cool the above solution d) To about 2 ml suspension of starch add 1ml 5 % sodium hydroxide and 2 drops of N/50 iodine.	Blue colour disappears. Blue colour reappears No blue colour	Starch is present. Starch is present. Starch is present.
C.T. for Glucose a) Osazone test	Greenish yellow needle shaped crystal	Glucose confirmed
b) To 2ml of test solution add 5 % NaOH	Brown resinous precipitate is observed.	Glucose confirmed
c) Take 3 ml water to it add drop of Methylene blue solution and 1 ml of 5% sodium hydroxide and 2ml of sugar solution and boil.	Solution is decolourised	Glucose confirmed
d) To 3 ml of sugar solution add 1 ml of picric acid solution and 1ml 5% NaOH and heat.	Red colour develops	Glucose confirmed
C. T. for Lactose 1. Mucic acid test To 1 ml sugar solutionadd 1 ml concentrated HNO_3 boil and cool.	Broken glass like crystals obtained which can be identified under microscope.	Lactose confirmed

Test	Observation	Inference
2. Silver mirror test 1 ml sugar solution add 1 ml Ammonical silver nitrate solution add excess NaOH Warm.	Silver mirror is observed.	Lactose confirmed
C.T. for sucrose a) Seliwanoff's test b) Rapid furfural test c) Inversion test. To 25 ml of test solution add 5 ml of concentrated HCl boil for 3 minutes and cool under tap water. Neutralize with sodium hydroxide solution. Now perform following test for this solution. i) Fehling's test ii) Tommer's test iii) Benedict's test iv) Barfoed's test	 Red precipitate is observed. Red precipitate is observed. Red / yellow / green precipitate is observed.	 Sucrose confirmed. Sucrose confirmed. Sucrose confirmed.
C.T. for fructose Fougler's test To 3 ml fougler's reagent add 0.5 ml of sugar solution. Boil for one minute.	Blue colour develops	Fructose confirmed.

7.0 OBSERVATION :

Sample 5 :

Student to write test, observation and inference

Test	Observation	Inference

Student to write test, observation and inference

Test	Observation	Inference

8.0 RESULT :

1. Given sample of carbohydrate is _____
2. It is _____ (Monosaccharide /Disaccharide/ Polysaccharide)

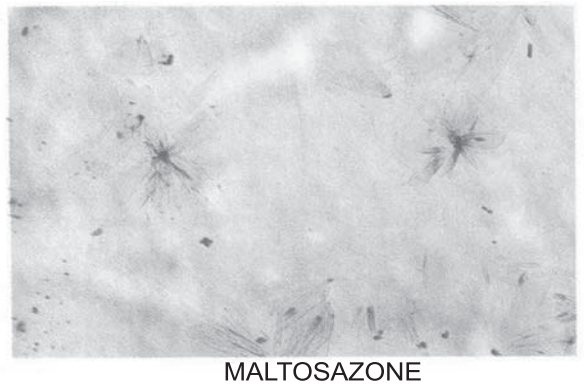
9.0 QUESTIONS :

Student to answer question Q....., Q....., Q.....,Q.....
and the question numbers shall be allotted by the teacher.

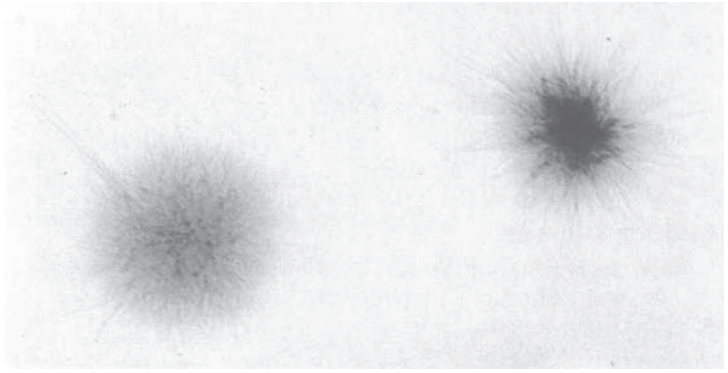
1. State the principle of Molisch test.
2. State the component of starch.
3. State the name of two sources of starch.
4. State the principle of Benedict test.
5. State the name of most sensitive test of reducing sugars.
6. State the composition of Tollen's reagent.
7. Write the reactions involved in Osazone test.
8. Draw the shape of Maltosazone crystals.
9. State the names of carbohydrates distinguished by iodine test.
10. State the names of epimers of glucose.
11. What is purpose of inversion test.
12. State the name of acid obtained by oxidation of glucose.
13. State the name of chemical test required to distinguish monosaccharide and disaccharide.
14. State the colours produced by iodine with starch, dextrine and glycogen.
15. State the name of non-reducing diassacharides.
16. Draw the structures of Glucose and Fructose.
17. Draw the structures of Sucrose, Lactose and Maltose.
18. State the hydrolysis products of each : Sucrose.
19. State two disease related to abnormal metabolism of glucose.
20. Name two reducing diassacharides and give its hydrolysis products.
21. State two examples of monosaccharides, diasaccharide and polysaccharides each.
22. Name two chemical test to discriminate between glucose and fructose sample.
23. State name of sugar sample which gives needle shape crystals of osazone.
24. State name of sugar sample which gives powder puff shape crystals of osazone.
25. State the two sources of fructose.
26. State rich source of lactose and sucrose each.

10.0 REFERENCE:

GLUCOSAZONE



MALTOSAZONE



LACTOSAZONE

PLATE (COLOURS OF THE TESTS)
REACTIONS OF CARBOHYDRATES

TEST	OBSERVATION	COLOR	CONFIRMS
Molisch's Test	Violet Ring		Carbohydrate
	Blue Negative		Absence of reducing sugar 0%
	Green Color (+)		Presence of reducing sugar 0.1 – 0.5 g %
Benedict's Test	Yellow (++)		Presence of reducing sugar 0.5 – 1.0 g %
	Orange (+++)		Presence of reducing sugar 1.0 – 2.0 g %
	Brick red (++++)		Presence of reducing sugar > 2.0 g %
Barfoed's Test	Deep Blue Color		Monosaccharides
Foulger's Test	Blue Color		Keto Sugar
Selivanoff's Test	Red Color		Keto Sugar
Iodine Test	Purple		Dextrine

Fig. 2.1

Space for writing answers

Signature of Teacher

Experiment No. 7

1.0 TITLE : To identify given sample of carbohydrate (sample No. 6)

2.0 PRIOR CONCEPTS:

Carbohydrates are present in natural products such as maize, rice, potato, cane sugar, honey, etc.

3.0 NEW CONCEPTS:

Proposition 1 :

Carbohydrate is detected by Molisch's test.

Proposition 2 :

Polysaccharides are detected by iodine test.

Proposition 3 :

Reducing sugars are detected by Fehling's and Benedict's test.

Proposition 4 :

Aldoses and Ketoses are distinguished by Seliwanoff's test.

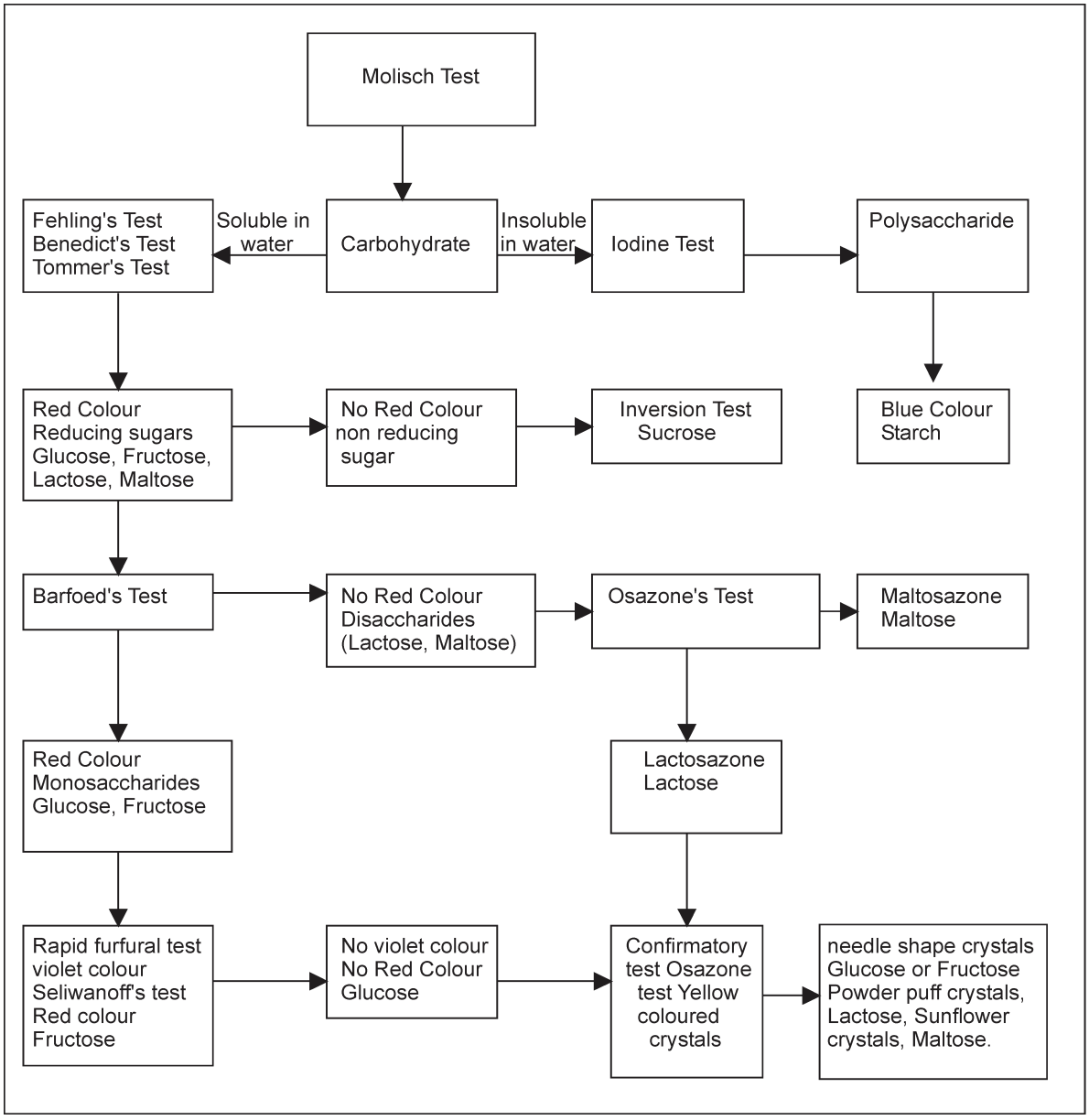
Proposition 5 :
Lactose & Maltose distinguished by Tollen's reagent test.

Proposition 6 :
Sucrose is confirmed by inversion test.

Proposition 7 :

Glucose, Fructose, Maltose, Lactose are confirmed by osazone test.

Concept Structure :



4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand formation of precipitate.
2. Application of chemical test of carbohydrates to identify given sample of carbohydrate according to concept structure.

Motor skills

1. Ability to distinguish the colours while performing various chemical tests.
2. Ability to add required amount of test solution and chemical reagent while performing the chemical test.
3. Ability to provide required quantity of heat safely while performing the chemical test whenever required.
4. Ability to observe and differentiate respective osazone crystals while observing them under microscope.

5.0 APPARATUS :

Glassware

Beaker, water bath, test tubes, graduated pipettes, funnel, filter paper, watchglass, slide, microscope.

Chemicals

Molisch reagent (1% ∝naphthol in alcohol), concentrated sulphuric acid, N/50 Iodine solution, distilled water, Fehling's solution A (7.93% copper sulphate in water), Fehling's solution B (250g sodium hydroxide and 320g sodium potassium tartarate in 500 ml water), Benedict's reagent (dissolve 173g sodium citrate and 100g anhydrous sodium carbonate in about 800 ml water, separately dissolve 17.3g copper sulphate in 100ml water, mix both the solution and make volume to 1000 ml with water), Tommer's reagent (prepared fresh- 5 % copper sulphate in water and equal volume of sodium hydroxide solution), Barfoed's reagent (13.3g copper acetate in 200 ml water filter, to it add 1.8 ml glacial acetic acid), Seliwanoff's reagent (dissolve 50 mg of resorcinol in 33 ml concentrated hydrochloric acid diluted to 1000 ml with water)

Concentrated nitric acid, phenylhydrazine hydrochloride,

Acetate buffer - PH 5.0 Dissolve 40.8 g sodium acetate in 70 ml water. To it add 8.2 ml glacial acetc acid. Make the volume to 100ml with water.

Fougler's reagent (Dissolve 40 g urea in 80 ml of 40% w/w sulphuric acid to it add 2 g stannous chloride and boil till clear solution is obtained. Cool make volume to 40 ml with 40% w/w sulphuric acid.

6.0 STEPWISE PROCEDURE :

1. Prepare a solution of given carbohydrate sample by dissolving 1 gm sample in 20 ml of water. And use same solution for analysis.
2. Perform the chemical test of carbohydrates according to general concept, structure, to identify the given sample.
3. Adjust the microscope at low power properly before observing osazone crystals.
4. Confirm Glucose, Fructose, Maltose, Lactose by Osazone test, Sucrose by inversion test and starch by iodine test.
5. Report your analysis for identification of given sample of carbohydrate in given proforma.

Table to perform tests for identification of carbohydrate sample (with reagents)

Test	Observation	Inference
1. Molisch's test : Mix 2ml of carbohydrate sample with 5 drops of Molisch's Reagent in a test tube. Add gently through the side by tilting the tube, about 2 ml of concentrated H_2SO_4 so as to form a bottom layer.	Violet / purple ring at the junction of two liquids	Carbohydrate present.
2. Solubility Compound + water	Soluble Insoluble	Mono and disaccharides present Polysaccharides present
3. Fehling's test 2 ml of Fehling's solution A + 2ml of Fehling's solution B+ 2 ml of Sugar solution Boil.	Yellow or brick red ppt is observed.	Reducing sugar present
4. Benedict's test Take 5ml of Benedict's qualitative reagent, add 8 drops of sugar solution. Boil over a flame for 2 minutes or place in boiling water bath for 3 minutes. Allow to cool.	Green, yellow, orange or brick red ppt is observed	Reducing sugar present

Test	Observation	Inference
5. Tommer's test To 2 ml of Tommer's reagent add 3 ml of sugar solution boil for 2 minutes cool.	Yellow or red ppt is observed	Reducing sugarpresent
6. Barfoed's test 2 ml of Barfoed's reagent add 2ml of sugar solution and keep in boiling water bath for 2 minutes cool.	Brick red precipitate is observed at the bottom of test tube.	Reducing sugar present.
7. Seliwanoff's test To 3 ml of Seliwanoff's reagent add 1 ml of sugar solution and heat the mixture to boil for 2 minutes. cool.	Red colour or red precipitate is observed.	Ketoses like fructose, sucrose present.
8. Rapid furfural test To 2 ml of sugar solution add 1 ml of A-napthol solution (1% in alcohol) and 5 ml concentrated HCl boil.	Deep purple colour is observed.	Ketoses like fructose, sucrose present
9. Osazone test Set up a boiling water bath. Take test tube, add 1 g of Phenyl hydrazine hydrochloride 2 ml of acetate buffer, P^H 5.0. add 5ml of water mixwell and warm gently. Filter it. To the filterate add 5 ml sugar solution and keep in boiling water bath and cool. Mount the crystals under microscope and examine under microscope.	a) Greenish yellow needle shaped crystals. b) Badminton ball, powder puff shaped crystals. c) Sunflower or its petals shaped crystals.	Glucosazone i.e. glucose present. Lactosazone i.e. lactose present. Maltasazone i.e. maltose present.
10. Iodine test a) To about 2 ml Suspension or solution of polysaccharides add 1-2 drops of N/50 iodine solution.	Blue violet colour developsBrown wine colour develops.	Starch is present Glycogen is present.
b) Warm above solution c) Cool the above solution d) To about 2 ml suspension of starch add 1ml 5 % sodium hydroxide and 2 drops of N/50 iodine.	Blue colour disappears. Blue colour reappears No blue colour	Starch is present. Starch is present. Starch is present.
C.T. for Glucose a) Osazone test	Greenish yellow needle shaped crystal	Glucose confirmed
b) To 2ml of test solution add 5 % NaOH	Brown resinous precipitate is observed.	Glucose confirmed
c) Take 3 ml water to it add drop of Methylene blue solution and 1 ml of 5% sodium hydroxide and 2ml of sugar solution and boil.	Solution is decolourised	Glucose confirmed
d) To 3 ml of sugar solution add 1 ml of picric acid solution and 1ml 5% NaOH and heat.	Red colour develops	Glucose confirmed
C. T. for Lactose 1. Mucic acid test To 1 ml sugar solutionadd 1 ml concentrated HNO_3 boil and cool.	Broken glass like crystals obtained which can be identified under microscope.	Lactose confirmed

Test	Observation	Inference
2. Silver mirror test 1 ml sugar solution add 1 ml Ammonical silver nitrate solution add excess NaOH Warm.	Silver mirror is observed.	Lactose confirmed
C.T. for sucrose a) Seliwanoff's test b) Rapid furfural test c) Inversion test. To 25 ml of test solution add 5 ml of concentrated HCL boil for 3 minutes and cool under tap water. Neutralize with sodium hydroxide solution. Now perform following test for this solution. i) Fehling's test ii) Tommer's test iii) Benedict's test iv) Barfoed's test	 Red precipitate is observed. Red precipitate is observed. Red / yellow / green precipitate is observed.	 Sucrose confirmed. Sucrose confirmed. Sucrose confirmed.
C.T. for fructose Fougler's test To 3 ml fougler's reagent add 0.5 ml of sugar solution. Boil for one minute.	 Blue colour develops	 Fructose confirmed.

7.0 OBSERVATION :

Sample 6 :

Student to write test, observation and inference

Test	Observation	Inference

Student to write test, observation and inference

Test	Observation	Inference

8.0 RESULT :

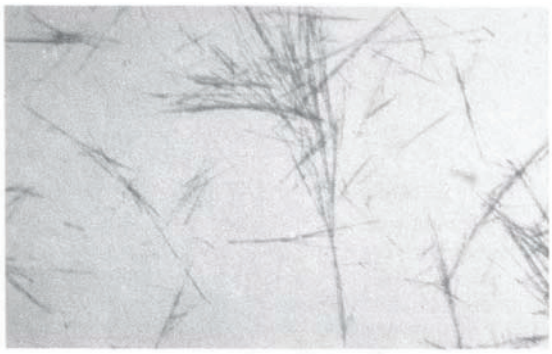
1. Given sample of carbohydrate is _____
2. It is _____ (Monosaccharide /Disaccharide/ Polysaccharide)

9.0 QUESTIONS :

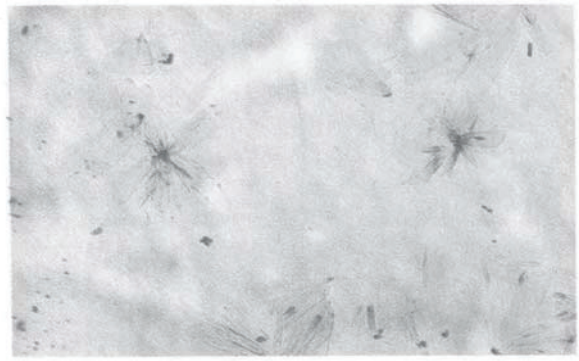
Student to answer question Q....., Q....., Q.....,Q.....
and the question numbers shall be allotted by the teacher.

1. State the principle of Molisch test.
2. State the component of starch.
3. State the name of two sources of starch.
4. State the principle of Benedict test.
5. State the name of most sensitive test of reducing sugars.
6. State the composition of Tollen's reagent.
7. Write the reactions involved in Osazone test.
8. Draw the shape of Maltosazone crystals.
9. State the names of carbohydrates distinguished by iodine test.
10. State the names of epimers of glucose.
11. What is purpose of inversion test.
12. State the name of acid obtained by oxidation of glucose.
13. State the name of chemical test required to distinguish monosaccharide and disaccharide.
14. State the colours produced by iodine with starch, dextrine and glycogen.
15. State the name of non-reducing diassacharides.
16. Draw the structures of Glucose and Fructose.
17. Draw the structures of Sucrose, Lactose and Maltose.
18. State the hydrolysis products of each : Sucrose.
19. State two disease related to abnormal metabolism of glucose.
20. Name two reducing diassacharides and give its hydrolysis products.
21. State two examples of monosaccharides, diasaccharide and polysaccharides each.
22. Name two chemical test to discriminate between glucose and fructose sample.
23. State name of sugar sample which gives needle shape crystals of osazone.
24. State name of sugar sample which gives powder puff shape crystals of osazone.
25. State the two sources of fructose.
26. State rich source of lactose and sucrose each.

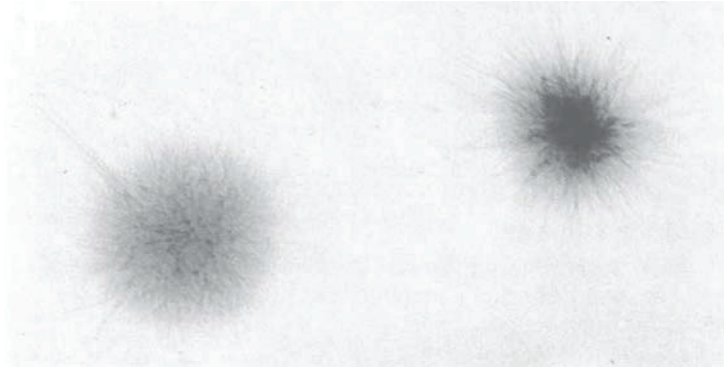
10.0 REFERENCE:



GLUCOSAZONE



MALTOSAZONE



LACTOSAZONE

PLATE (COLOURS OF THE TESTS)
REACTIONS OF CARBOHYDRATES

TEST	OBSERVATION	COLOR	CONFIRMS
Molisch's Test	Violet Ring		Carbohydrate
	Blue Negative		Absence of reducing sugar 0%
	Green Color (+)		Presence of reducing sugar 0.1 – 0.5 g %
Benedict's Test	Yellow (++)		Presence of reducing sugar 0.5 – 1.0 g %
	Orange (+++)		Presence of reducing sugar 1.0 – 2.0 g %
	Brick red (++++)		Presence of reducing sugar > 2.0 g %
Barfoed's Test	Deep Blue Color		Monosaccharides
Foulger's Test	Blue Color		Keto Sugar
Selivanoff's Test	Red Color		Keto Sugar
Iodine Test	Purple		Dextrine

Fig. 2.1

Space for writing answers

Signature of Teacher

Experiment No. 8

Experiment No. 8 and Experiment No. 9 may be conducted in single turn.

1.0 TITLE : To identify given sample of amino acid by qualitative tests. (Sample No. 1)

2.0 PRIOR CONCEPTS:

Basic unit of protein.

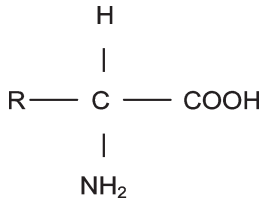
3.0 NEW CONCEPTS:

Proposition 1 : Amino acids

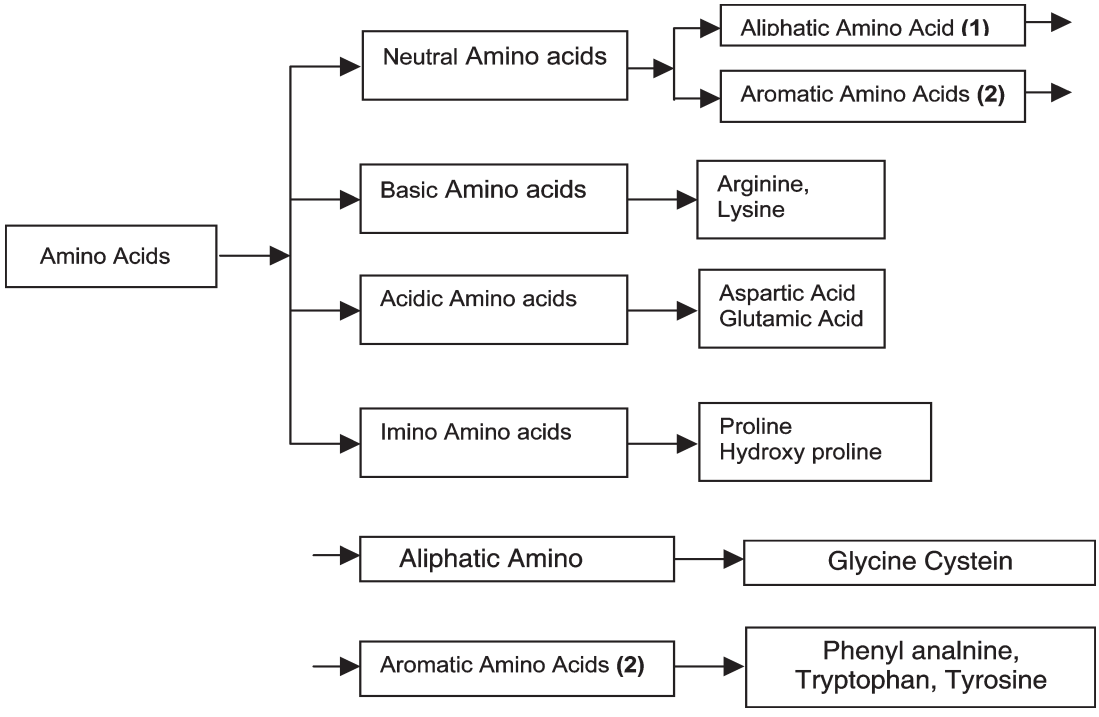
Amino acids are organic acids which carry at least one amino group in it. Generally at alpha Carbon atom.

Concept Structure :

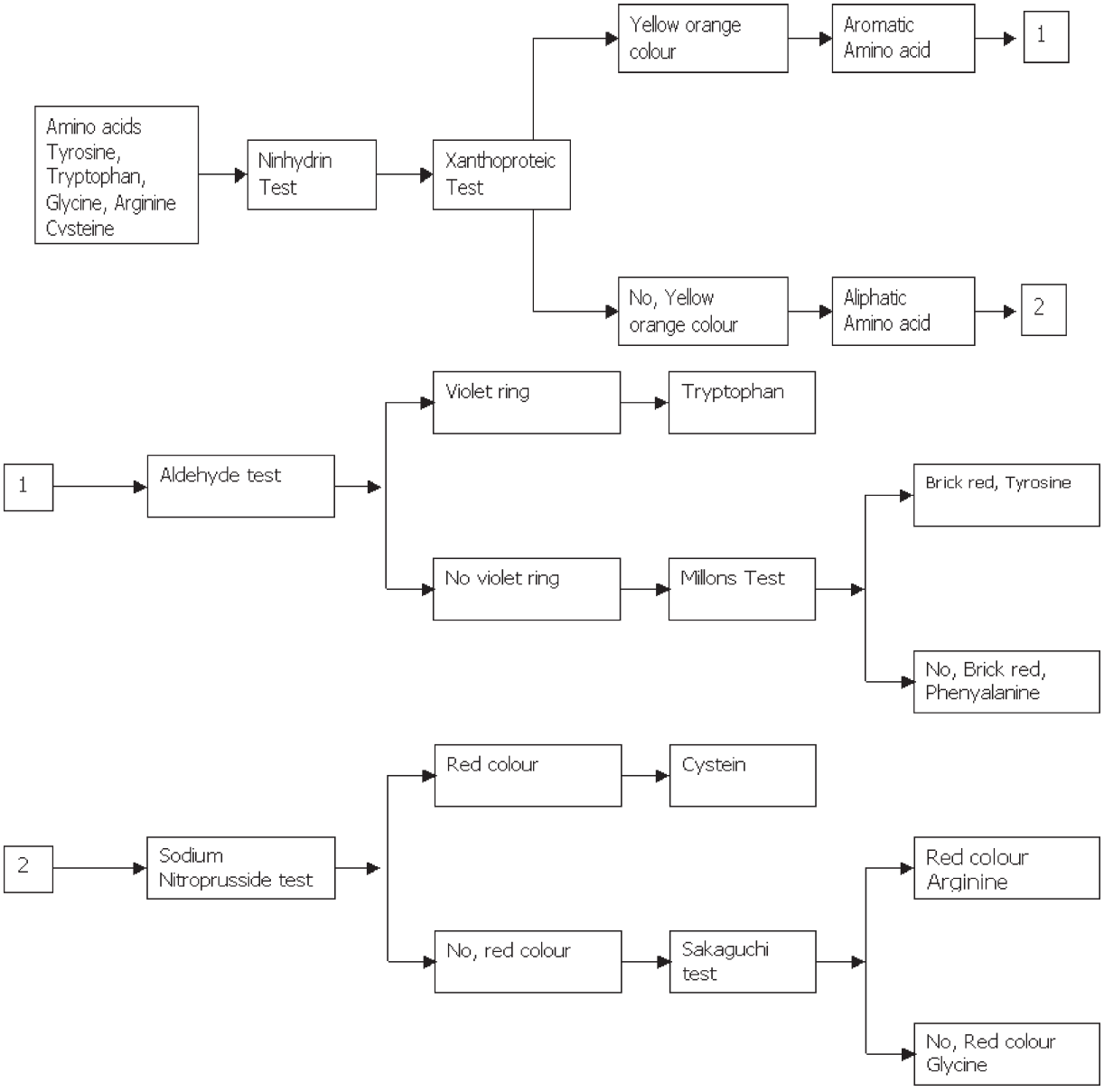
General structure of amino acid



Proposition 2 : Types of amino acids.



General concept structure for identification of amino acids.



4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand concept of amino acids and their types.
2. To identify colour obtained in a identification of amino acid.
3. To distinguish between aromatic and aliphatic amino acids by interpreting test results.
4. To select proper reagent for the test.

Motor skills

1. Ability to add proper reagent in a proper quantity in a original solution.
2. Ability to observe color, turbidity, and precipitate produced in test.

5.0 APPARATUS :

Glassware

Test tubes, Beakers, Test tube stand, Brush, Gas burner, Glass rod etc.

Chemicals

Ninhydrin reagent (1% in acetone), Concentrated HNO_3, 40% w/v NaOH solution, Millon's reagent, Concentrated H_2SO_4, Dilute Sodium Nitroprusside Solution, 2% Lead acetate solution, 2% Alpha Napthol, 10 % w/v NaOH, Ferric chloride solution, Dilute HCl, Strong ammonia solution

Preparation of Reagent

1)	Ninhydrin Reagent	–	0.1 % in a Acetone
2)	Millon's Reagent	–	1 part of mercury (by weight) + 2 parts of HNO_3 (by weight) . Dissolve with help of Heat. Dilute resulting Solution by 2 volumes of water.
3)	Sodium Nitroprusside	–	Dissolve 5 g of Sodium Nitroprusside in 100 ml of water.
4)	Alpha Napthol reagent	–	2g of alpha napthol in a 100 ml of Alcohol
5)	Sodium hypo bromite	–	10 ml of bromine to 100 ml of 40 % NaOH with a constant stirring while adding bromine.
6)	Sodium Nitrite	–	5 g. Of $NaNO_2$ in a 100 ml of water.
7)	Glyoxalic acid reagent	–	Expose acetic acid to sunlight.
8)	Mormer reagent	–	1 ml. Formaline + 45 ml. Distilled water + 55 ml. Concentrated H_2SO_4.

6.0 STEPWISE PROCEDURE :

1. Prepare 5% amino acid samples in distilled water. Use this solution as original solution. (O.S.)
2. Perform the following tests on amino acid sample, observe the colour and interpret as given in following table.

Table for performing tests on amino acid sample with reagents.

Test	Observation	Inference
Ninhydrin Test 2 ml. O. S. + 0.5 ml Ninhydrin reagent (0.1 % in Acetone) boil for 2 min.	Blue / Violet colour	Amino Acids arepresent
Xanthoproteic Test 2 ml. O. S. + 1 ml. Con. HNO_3 boil + 40% NaOH drop by drop.	Yellow orange colour	Aromatic Amino acids are present
Aldehyde Test 2 ml. O.S. + 5 drops of Millon's reagent + 5 drops of Formalin mix + 2 ml Con. H_2SO_4 from side of Test tube.	Violet ring at junction	Tryptophan present
Millon's Test 2 ml. O. S. + 2 ml. Millon's reagent boil cool, add few drops of $NaNO_2$ Solution.	Brick red colour	Tyrosine present
Sodium Nitroprusside Test 1 ml. O. S. + 1 ml. Dilute NaOH + 1 ml. Sodium Nitroprusside solution	Red colour	Cystein present
Sakaguchi's Test (Arginine Test) 1 ml. O. S. + 1 ml. 10 % NaOH + 5 drops of alpha Napthol + 1 ml. Dilute Sodium Nitroprusside Solution	Red colour	Arginine present

Confirmatory Test (C.T.)

Test	Observation	Inference
C. T. for Cystein 1 ml. O. S. + 1 ml. 40 % NaOH solution boil cool add 1 ml. Lead acetate solution	Dark gray colour	Cystein present
C. T. for Tryptophan **(Hopkin cole Test)** 1 ml. O. S. + 1 ml.Glyoxalic acid shake add Con. H_2SO_4 from side of test tube	Violet ring at junction	Tryptophan present
C. T. for Tyrosine 1 ml. O. S. + 1 ml. Morner reagent heat to boil	Green colour appears	Tyrosine present
C. T. for Arginine (Sakaguchi's Test) 1 ml. O. S. + 1 ml. 10 % NaOH solution + 5 drops of alpha Napthol + 1 ml. Sodium Nitroprusside Solution	Red colour	Arginine present
C. T. for Glycine 1. To 5 ml. of a 1 . in 10 ml of solution, add 5 drops of a 1in 2 ml. solution of Sodium Nitrite 2. To 2 ml. of 1 in 10 ml. Solution add 1 ml. of Ferric Chloride solution To above solution add excess dilute HCl To above solution add excess of Strong Ammonia Solution.	Vigorous evolution of colourless gas Red colour produced Red colour disappears Red colour reappears	Glycine present Glycine present Glycine present Glycine present.

7.0 OBSERVATION TABLE :

Student to write test observation for chemical test Performed on given amino acid sample.

Test	Observation	Inference

Test	Observation	Inference

8.0 RESULT :

1. Given sample of amino acid is _____
2. It is _____ (aromatic/aliphatic) amino acid.

9.0 QUESTIONS :

(Note - Student to answer question Q....., Q....., Q..... and question number shall be allotted by teacher.)

1. Which colour is developed in Ninhydrin Test.
2. State the use of Sakaguchi's Test.
3. Write general structure of amino acid.
4. List two sulphur containing amino acids.
5. Name the optically inactive amino acid.
6. Draw the structure's of aromatic Amino acid.
7. Name and draw structure of the aromatic amino acids which gives aldehyde test positive.
8. Which colour is developed in Millon's test for tyrosine.
9. State the use of Xanthoproteic test.
10. Write confirmatory test of tryptophan.
11. Which chemical test should be performed to differentiate between Glycine and Arginine.
12. Name the four essential Amino acids.
13. Define iso electric point of Amino acids.
14. Define essential and non essential amino acids.
15. Write acid base properties of Amino acids.

Space for writing answers

Space for writing answers

Signature of Teacher

Experiment No. 9

1.0 TITLE : To identify given sample of amino acid by qualitative tests. (Sample No. 2)

2.0 PRIOR CONCEPTS:

Basic unit of protein.

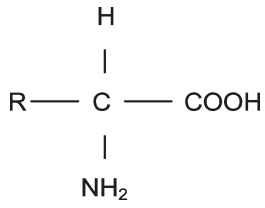
3.0 NEW CONCEPTS:

Proposition 1 : Amino acids

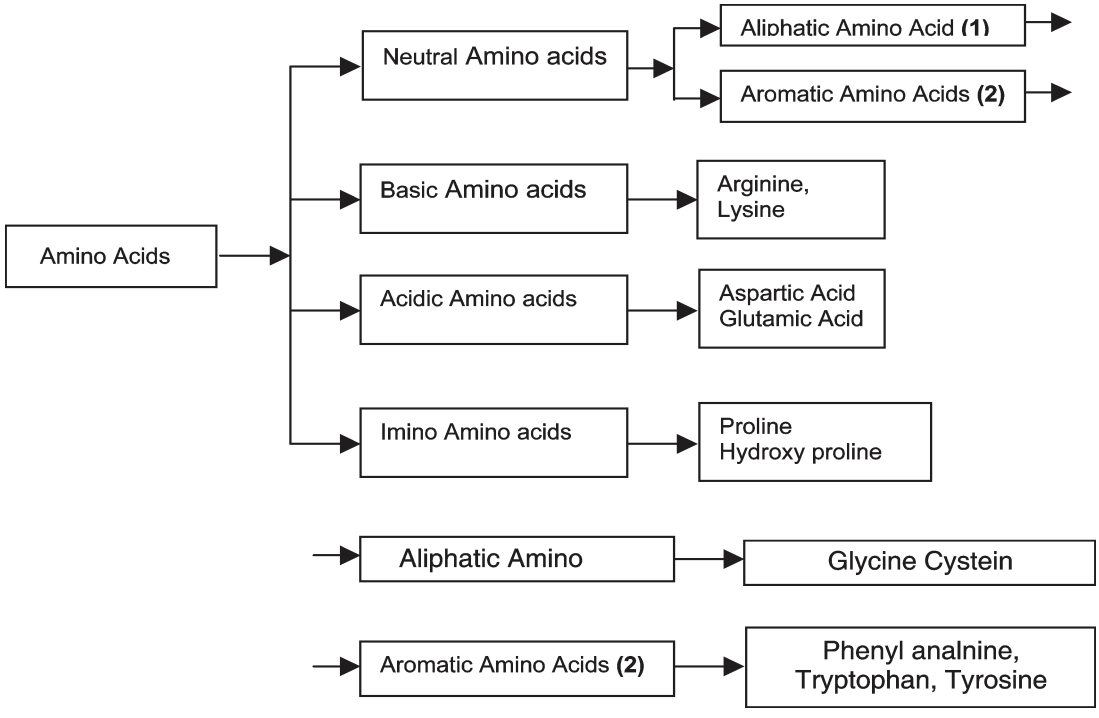
Amino acids are organic acids which carry at least one amino group in it. Generally at alpha Carbon atom.

Concept Structure :

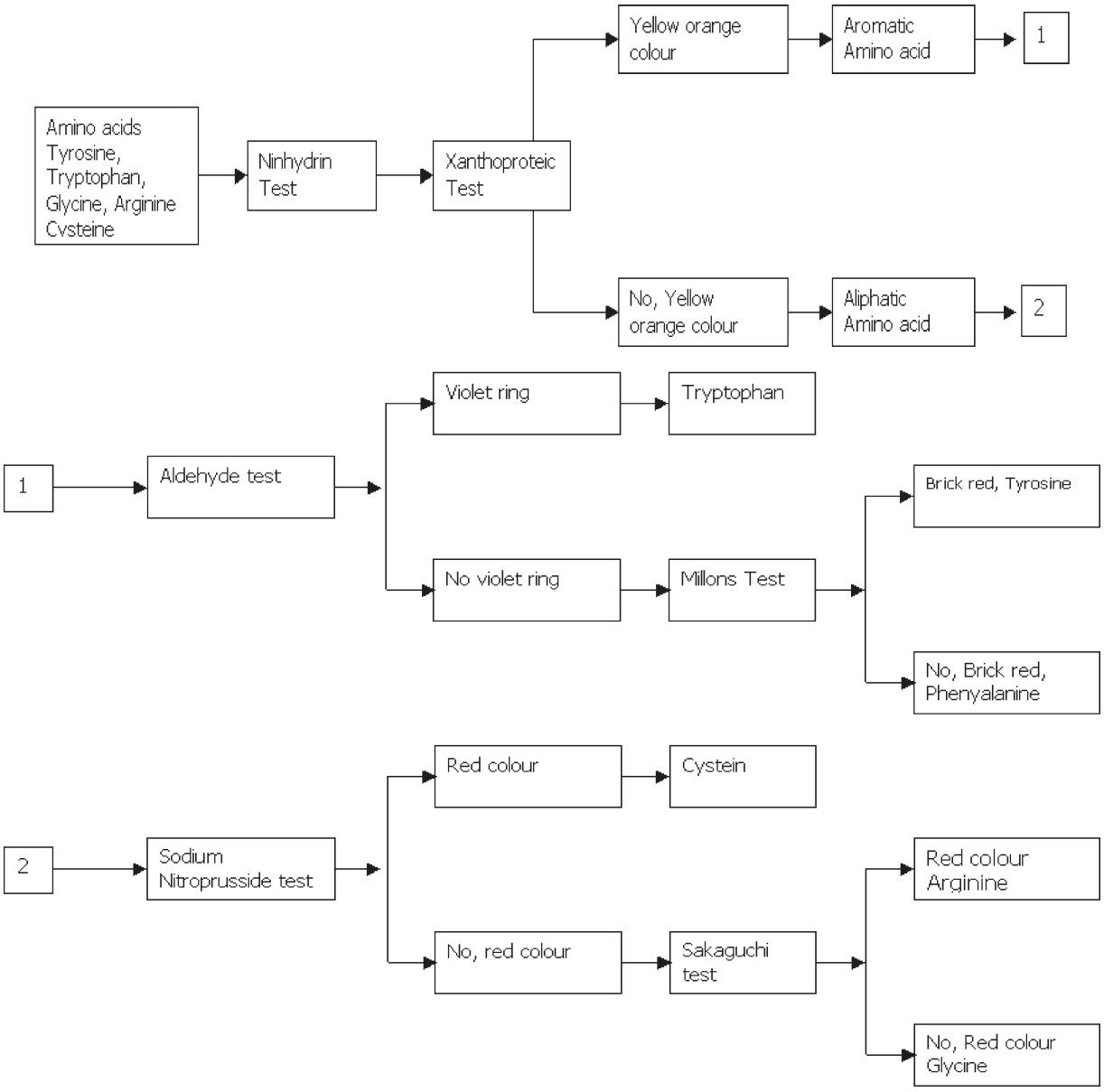
General structure of amino acid



Proposition 2 : Types of amino acids.



General concept structure for identification of amino acids.



4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand concept of amino acids and their types.
2. To identify colour obtained in a identification of amino acid.
5. To distinguish between aromatic and aliphatic amino acids by interpreting test results.
6. To select proper reagent for the test.

Motor skills

1. Ability to add proper reagent in a proper quantity in a original solution.
2. Ability to observe color, turbidity, and precipitate produced in test.

5.0 APPARATUS :

Glassware

Test tubes, Beakers, Test tube stand, Brush, Gas burner, Glass rod etc.

Chemicals

Ninhydrin reagent (1% in acetone), Concentrated HNO_3, 40% w/v NaOH solution, Millon's reagent, Concentrated H_2SO_4, Dilute Sodium Nitroprusside Solution, 2% Lead acetate solution, 2% Alpha Napthol, 10 % w/v NaOH, Ferric chloride solution, Dilute HCl, Strong ammonia solution

Preparation of Reagent

1)	Ninhydrin Reagent	–	0.1 % in a Acetone
2)	Millon's Reagent	–	1 part of mercury (by weight) + 2 parts of HNO_3 (by weight) . Dissolve with help of Heat. Dilute resulting Solution by 2 volumes of water.
3)	Sodium Nitroprusside	–	Dissolve 5 g of Sodium Nitroprusside in 100 ml of water.
4)	Alpha Napthol reagent	–	2 g of alpha napthol in a 100 ml of Alcohol
5)	Sodium hypo bromite	–	10 ml of bromine to 100 ml of 40 % NaOH with a constant stirring while adding bromine.
6)	Sodium Nitrite	–	5 g. Of $NaNO_2$ in a 100 ml of water.
7)	Glyoxalic acid reagent	–	Expose acetic acid to sunlight.
8)	Mormer reagent	–	1 ml. Formaline + 45 ml. Distilled water + 55 ml. Concentrated H_2SO_4.

6.0 STEP WISE PROCEDURE:

1. Prepare 5% amino acid samples in distilled water. Use this solution as original solution. (O.S.)
2. Perform the following tests on amino acid sample, observe the colour and interpret as given in following table.

Table for performing tests on amino acid sample with reagents.

Test	Observation	Inference
Ninhydrin Test 2 ml. O. S. + 0.5 ml Ninhydrin reagent (0.1 % in Acetone) boil for 2 min.	Blue / Violet colour	Amino Acids arepresent
Xanthoproteic Test 2 ml. O. S. + 1 ml. Con. HNO_3 boil + 40% NaOH drop by drop.	Yellow orange colour	Aromatic Amino acids are present
Aldehyde Test 2 ml. O.S. + 5 drops of Millon's reagent + 5 drops of Formalin mix + 2 ml Con. H_2SO_4 from side of Test tube.	Violet ring at junction	Tryptophan present
Millon's Test 2 ml. O. S. + 2 ml. Millon's reagent boil cool, add few drops of $NaNO_2$ Solution.	Brick red colour	Tyrosine present
Sodium Nitroprusside Test 1 ml. O. S. + 1 ml. Dilute NaOH + 1 ml. Sodium Nitroprusside solution	Red colour	Cystein present
Sakaguchi's Test (Arginine Test) 1 ml. O. S. + 1 ml. 10 % NaOH + 5 drops of alpha Napthol + 1 ml. Dilute Sodium Nitroprusside Solution	Red colour	Arginine present

Confirmatory Test (C.T.)

Test	Observation	Inference
C. T. for Cystein 1 ml. O. S. + 1 ml. 40 % NaOH solution boil cool add 1 ml. Lead acetate solution	Dark gray colour	Cystein present
C. T. for Tryptophan **(Hopkin cole Test)** 1 ml. O. S. + 1 ml.Glyoxalic acid shake add Con. H_2SO_4 from side of test tube	Violet ring at junction	Tryptophan present
C. T. for Tyrosine 1 ml. O. S. + 1 ml. Morner reagent heat to boil	Green colour appears	Tyrosine present
C. T. for Arginine (Sakaguchi's Test) 1 ml. O. S. + 1 ml. 10 % NaOH solution + 5 drops of alpha Napthol + 1 ml. Sodium Nitroprusside Solution	Red colour	Arginine present
C. T. for Glycine 1. To 5 ml. of a 1. in 10 ml of solution, add 5 drops of a 1in 2 ml. solution of Sodium Nitrite 2. To 2 ml. of 1 in 10 ml. Solution add 1 ml. of Ferric Chloride solution To above solution add excess dilute HCl To above solution add excess of Strong Ammonia Solution.	Vigorous evolution of colourless gas Red colour produced Red colour disappears Red colour reappears	Glycine present Glycine present Glycine present Glycine present.

7.0 OBSERVATION TABLE :

Student to write test observation for chemical test Performed on given amino acid sample.

Test	Observation	Inference

Test	Observation	Inference

8.0 RESULT :

1. Given sample of amino acid is _____
2. It is _____ (aromatic/aliphatic) amino acid.

9.0 QUESTIONS :

(Note - Student to answer question Q....., Q....., Q..... and question number shall be allotted by teacher.)

1. Which colour is developed in Ninhydrin Test.
2. State the use of Sakaguchi's Test.
3. Write general structure of amino acid.
4. List two sulphur containing amino acids.
5. Name the optically inactive amino acid.
6. Draw the structure's of aromatic Amino acid.
7. Name and draw structure of the aromatic amino acids which gives aldehyde test positive.
8. Which colour is developed in Millon's test for tyrosine.
9. State the use of Xanthoproteic test.
10. Write confirmatory test of tryptophan.
11. Which chemical test should be performed to differentiate between Glycine and Arginine.
12. Name the four essential Amino acids.
13. Define iso electric point of Amino acids.
14. Define essential and non essential amino acids.
15. Write acid base properties of Amino acids.

Space for writing answers

Space for writing answers

Signature of Teacher

Experiment No. 10

Experiment No. 10, Experiment No. 11 and Experiment No. 12 may be conducted in single turn.

1.0 TITLE : To identify given sample of amino acid by qualitative tests. (Sample No. 3)

2.0 PRIOR CONCEPTS:

Basic unit of protein.

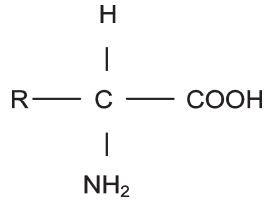
3.0 NEW CONCEPTS:

Proposition 1 : Amino acids

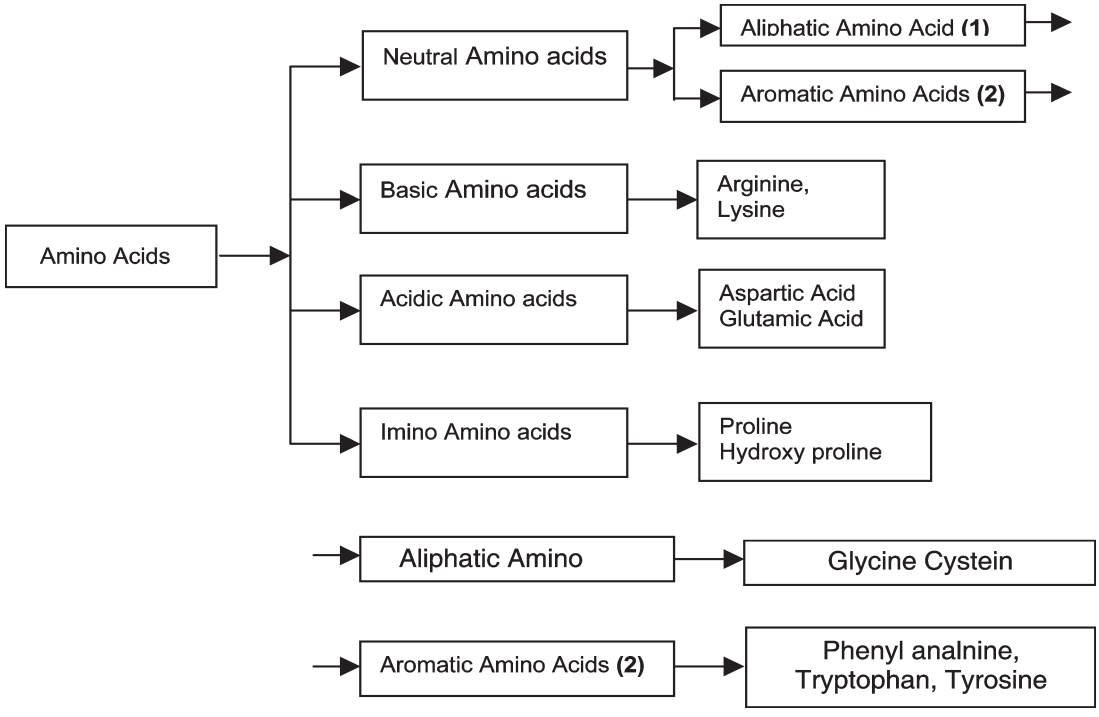
Amino acids are organic acids, which carry at least one amino group in it. Generally at alpha Carbon atom.

Concept Structure :

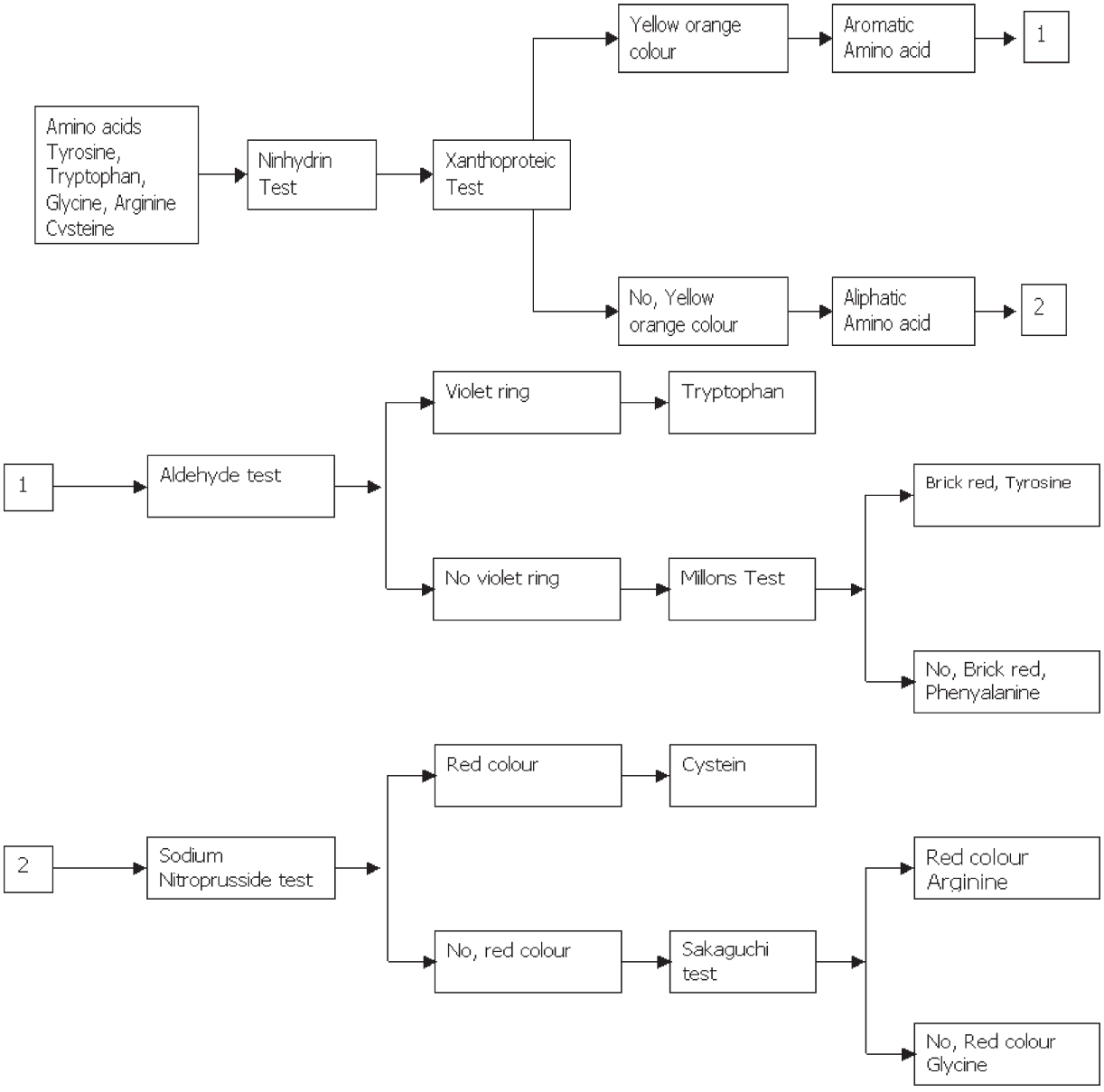
General structure of amino acid



Proposition 2 : Types of amino acids.



General concept structure for identification of amino acids.



4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand concept of amino acids and their types.
2. To identify colour obtained in a identification of amino acid.
3. To distinguish between aromatic and aliphatic amino acids by interpreting test results.
4. To select proper reagent for the test.

Motor skills

1. Ability to add proper reagent in a proper quantity in a original solution.
2. Ability to observe color, turbidity, and precipitate produced in test.

5.0 APPARATUS :

Glassware

Test tubes, Beakers, Test tube stand, Brush, Gas burner, Glass rod etc.

Chemicals

Ninhydrin reagent (1% in acetone), Concentrated HNO_3, 40% w/v NaOH solution, Millon's reagent, Concentrated H_2SO_4, Dilute Sodium Nitroprusside Solution, 2% Lead acetate solution, 2% Alpha Napthol, 10 % w/v NaOH, Ferric chloride solution, Dilute HCl, Strong ammonia solution

Preparation of Reagent

1)	Ninhydrin Reagent	–	0.1 % in a Acetone
2)	Millon's Reagent	–	1 part of mercury (by weight) + 2 parts of HNO_3 (by weight) . Dissolve with help of Heat. Dilute resulting Solution by 2 volumes of water.
3)	Sodium Nitroprusside	–	Dissolve 5 g of Sodium Nitroprusside in 100 ml of water.
4)	Alpha Napthol reagent	–	2 g of alpha napthol in a 100 ml of Alcohol
5)	Sodium hypo bromite	–	10 ml of bromine to 100 ml of 40 % NaOH with a constant stirring while adding bromine.
6)	Sodium Nitrite	–	5 g. Of $NaNO_2$ in a 100 ml of water.
7)	Glyoxalic acid reagent	–	Expose acetic acid to sunlight.
8)	Mormer reagent	–	1 ml. Formaline + 45 ml. Distilled water + 55 ml. Concentrated H_2SO_4.

6.0 STEP WISE PROCEDURE:

1. Prepare 5% amino acid samples in distilled water. Use this solution as original solution. (O.S.)
2. Perform the following tests on amino acid sample, observe the colour and interpret as given in following table.

Table for performing tests on amino acid sample with reagents.

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Xanthoproteic Test 2 ml. O. S. + 1 ml. Con. HNO_3 boil + 40% NaOH drop by drop.	Yellow orange colour	Aromatic Amino acids are present
Aldehyde Test 2 ml. O.S. + 5 drops of Millon's reagent + 5 drops of Formalin mix + 2 ml Con. H_2SO_4 from side of Test tube.	Violet ring at junction	Tryptophan present
Millon's Test 2 ml. O. S. + 2 ml. Millon's reagent boil cool, add few drops of $NaNO_2$ Solution.	Brick red colour	Tyrosine present
Sodium Nitroprusside Test 1 ml. O. S. + 1 ml. Dilute NaOH + 1 ml. Sodium Nitroprusside solution	Red colour	Cystein present
Sakaguchi's Test (Arginine Test) 1 ml. O. S. + 1 ml. 10 % NaOH + 5 drops of alpha Napthol + 1 ml. Dilute Sodium Nitroprusside Solution	Red colour	Arginine present

Confirmatory Test (C.T.)

Test	Observation	Inference
C. T. for Cystein 1 ml. O. S. + 1 ml. 40 % NaOH solution boil cool add 1 ml. Lead acetate solution	Dark gray colour	Cystein present
C. T. for Tryptophan **(Hopkin cole Test)** 1 ml. O. S. + 1 ml.Glyoxalic acid shake add Con. H_2SO_4 from side of test tube	Violet ring at junction	Tryptophan present
C. T. for Tyrosine 1 ml. O. S. + 1 ml. Morner reagent heat to boil	Green colour appears	Tyrosine present
C. T. for Arginine (Sakaguchi's Test) 1 ml. O. S. + 1 ml. 10 % NaOH solution + 5 drops of alpha Napthol + 1 ml. Sodium Nitroprusside Solution	Red colour	Arginine present
C. T. for Glycine 1. To 5 ml. of a 1. in 10 ml of solution, add 5 drops of a 1in 2 ml. solution of Sodium Nitrite 2. To 2 ml. of 1 in 10 ml. Solution add 1 ml. of Ferric Chloride solution To above solution add excess dilute HCl To above solution add excess of Strong Ammonia Solution.	 Vigorous evolution of colourless gas Red colour produced Red colour disappears Red colour reappears	 Glycine present Glycine present Glycine present Glycine present.

7.0 OBSERVATION TABLE :

Student to write test observation for chemical test Performed on given amino acid sample.

Test	Observation	Inference

Test	Observation	Inference

8.0 RESULT :

1. Given sample of amino acid is _____
2. It is _____ (aromatic/aliphatic) amino acid.

9.0 QUESTIONS :

(Note - Student to answer question Q....., Q....., Q..... and question number shall be allotted by teacher.)

1. Which colour is developed in Ninhydrin Test.
2. State the use of Sakaguchi's Test.
3. Write general structure of amino acid.
4. List two sulphur containing amino acids.
5. Name the optically inactive amino acid.
6. Draw the structure's of aromatic Amino acid.
7. Name and draw structure of the aromatic amino acids which gives aldehyde test positive.
8. Which colour is developed in Millon's test for tyrosine.
9. State the use of Xanthoproteic test.
10. Write confirmatory test of tryptophan.
11. Which chemical test should be performed to differentiate between Glycine and Arginine.
12. Name the four essential Amino acids.
13. Define iso electric point of Amino acids.
14. Define essential and non essential amino acids.
15. Write acid base properties of Amino acids.

Space for writing answers

Space for writing answers

Signature of Teacher

Experiment No. 11

1.0 TITLE : To identify given sample of amino acid by qualitative tests. (Sample No. 4)

2.0 PRIOR CONCEPTS:

Basic unit of protein.

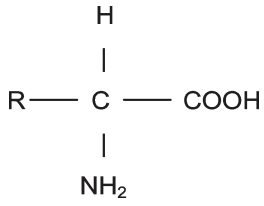
3.0 NEW CONCEPTS:

Proposition 1 : Amino acids

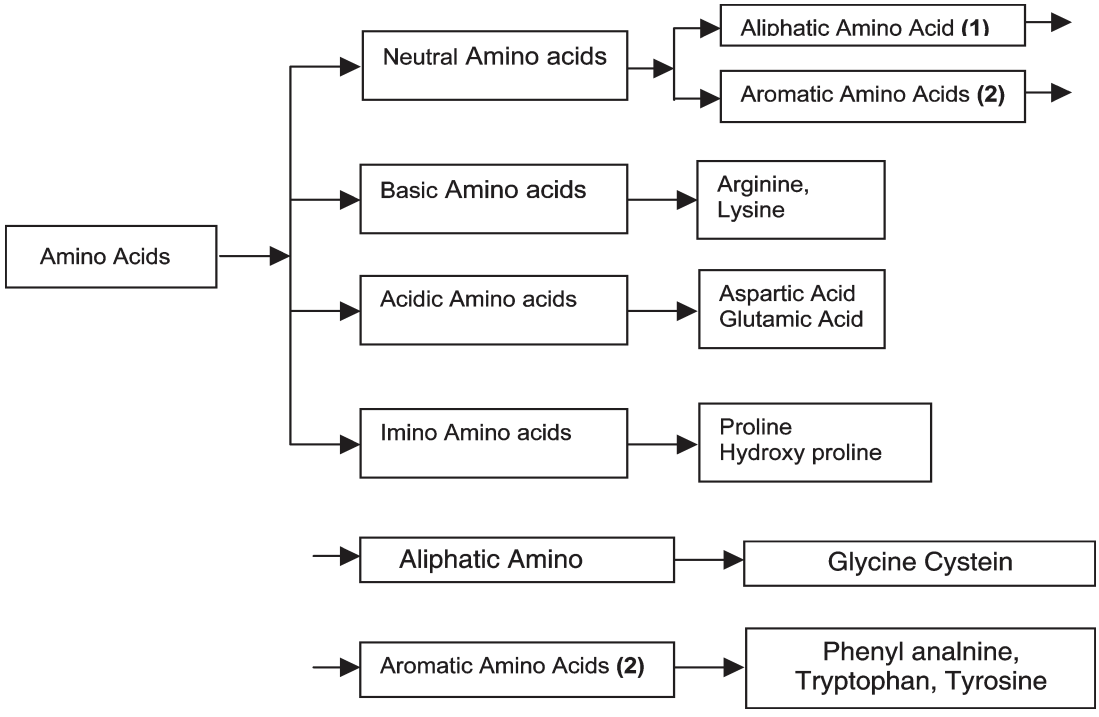
Amino acids are organic acids, which carry at least one amino group in it. Generally at alpha Carbon atom.

Concept Structure :

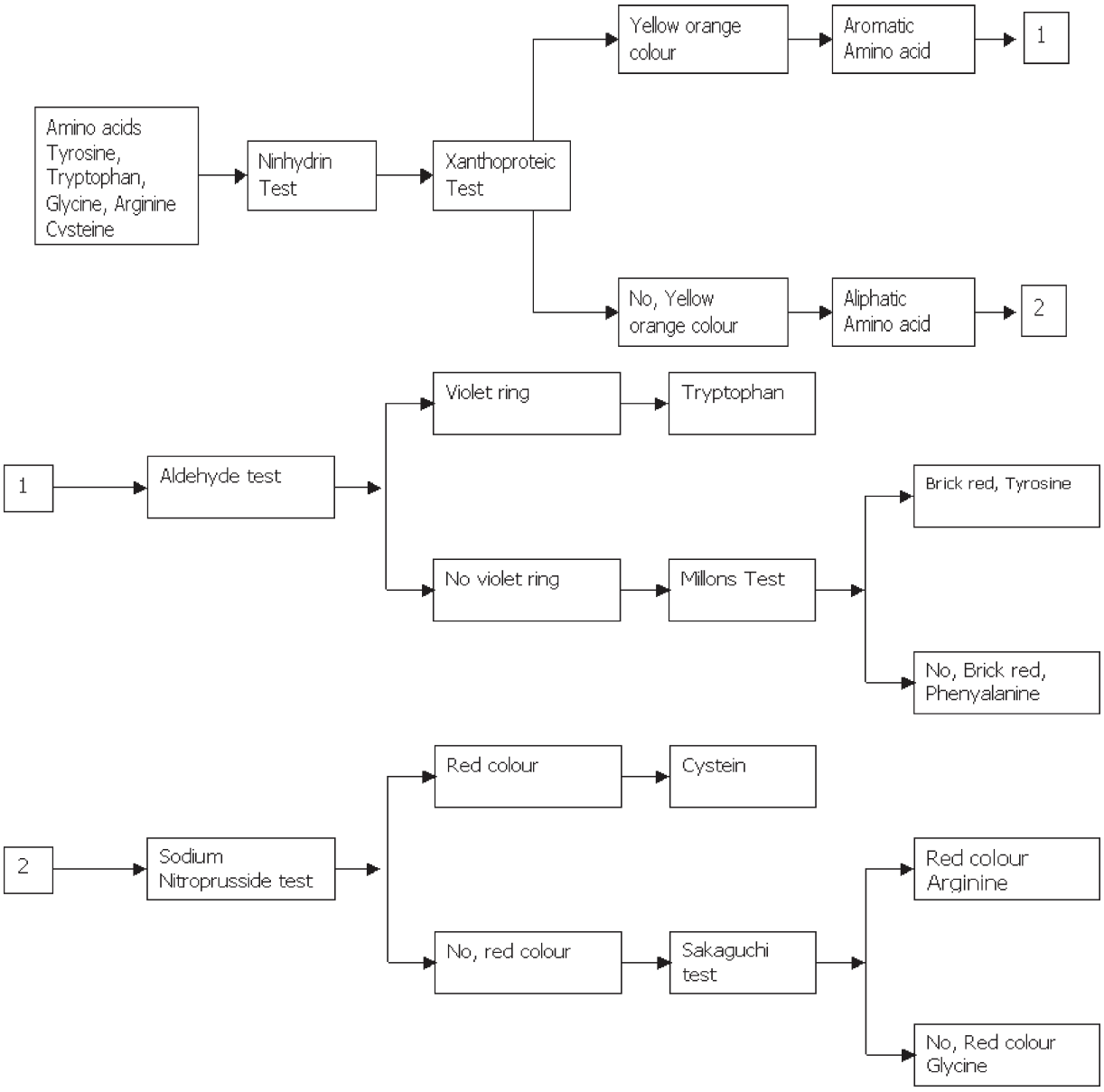
General structure of amino acid



Proposition 2 : Types of amino acids.



General concept structure for identification of amino acids.



4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand concept of amino acids and their types.
2. To identify colour obtained in a identification of amino acid.
3. To distinguish between aromatic and aliphatic amino acids by interpreting test results.
4. To select proper reagent for the test.

Motor skills

1. Ability to add proper reagent in a proper quantity in a original solution.
2. Ability to observe color, turbidity, and precipitate produced in test.

5.0 APPARATUS :

Glassware

Test tubes, Beakers, Test tube stand, Brush, Gas burner, Glass rod etc.

Chemicals

Ninhydrin reagent (1% in acetone), Concentrated HNO_3, 40% w/v NaOH solution, Millon's reagent, Concentrated H_2SO_4, Dilute Sodium Nitroprusside Solution, 2% Lead acetate solution, 2% Alpha Napthol, 10 % w/v NaOH, Ferric chloride solution, Dilute HCl, Strong ammonia solution

Preparation of Reagent

1)	Ninhydrin Reagent	–	0.1 % in a Acetone
2)	Millon's Reagent	–	1 part of mercury (by weight) + 2 parts of HNO_3 (by weight) . Dissolve with help of Heat. Dilute resulting Solution by 2 volumes of water.
3)	Sodium Nitroprusside	–	Dissolve 5 g of Sodium Nitroprusside in 100 ml of water.
4)	Alpha Napthol reagent	–	2 g of alpha napthol in a 100 ml of Alcohol
5)	Sodium hypo bromite	–	10 ml of bromine to 100 ml of 40 % NaOH with a constant stirring while adding bromine.
6)	Sodium Nitrite	–	5 g. Of $NaNO_2$ in a 100 ml of water.
7)	Glyoxalic acid reagent	–	Expose acetic acid to sunlight.
8)	Mormer reagent	–	1 ml. Formaline + 45 ml. Distilled water + 55 ml. Concentrated H_2SO_4.

6.0 STEP WISE PROCEDURE:

1. Prepare 5% amino acid samples in distilled water. Use this solution as original solution. (O.S.)
2. Perform the following tests on amino acid sample, observe the colour and interpret as given in following table.

Table for performing tests on amino acid sample with reagents.

Test	Observation	Inference
Ninhydrin Test 2 ml. O. S. + 0.5 ml Ninhydrin reagent (0.1 % in Acetone) boil for 2 min.	Blue / Violet colour	Amino Acids arepresent
Xanthoproteic Test 2 ml. O. S. + 1 ml. Con. HNO_3 boil + 40% NaOH drop by drop.	Yellow orange colour	Aromatic Amino acids are present
Aldehyde Test 2 ml. O.S. + 5 drops of Millon's reagent + 5 drops of Formalin mix + 2 ml Con. H_2SO_4 from side of Test tube.	Violet ring at junction	Tryptophan present
Millon's Test 2 ml. O. S. + 2 ml. Millon's reagent boil cool, add few drops of $NaNO_2$ Solution.	Brick red colour	Tyrosine present
Sodium Nitroprusside Test 1 ml. O. S. + 1 ml. Dilute NaOH + 1 ml. Sodium Nitroprusside solution	Red colour	Cystein present
Sakaguchi's Test (Arginine Test) 1 ml. O. S. + 1 ml. 10 % NaOH + 5 drops of alpha Napthol + 1 ml. Dilute Sodium Nitroprusside Solution	Red colour	Arginine present

Confirmatory Test (C.T.)

Test	Observation	Inference
C. T. for Cystein 1 ml. O. S. + 1 ml. 40 % NaOH solution boil cool add 1 ml. Lead acetate solution	Dark gray colour	Cystein present
C. T. for Tryptophan **(Hopkin cole Test)** 1 ml. O. S. + 1 ml.Glyoxalic acid shake add Con. H_2SO_4 from side of test tube	Violet ring at junction	Tryptophan present
C. T. for Tyrosine 1 ml. O. S. + 1 ml. Morner reagent heat to boil	Green colour appears	Tyrosine present
C. T. for Arginine (Sakaguchi's Test) 1 ml. O. S. + 1 ml. 10 % NaOH solution + 5 drops of alpha Napthol + 1 ml. Sodium Nitroprusside Solution	Red colour	Arginine present
C. T. for Glycine 1. To 5 ml. of a 1. in 10 ml of solution, add 5 drops of a 1in 2 ml. solution of Sodium Nitrite 2. To 2 ml. of 1 in 10 ml. Solution add 1 ml. of Ferric Chloride solution To above solution add excess dilute HCl To above solution add excess of Strong Ammonia Solution.	Vigorous evolution of colourless gas Red colour produced Red colour disappears Red colour reappears	Glycine present Glycine present Glycine present Glycine present.

7.0 OBSERVATION TABLE :

Student to write test observation for chemical test Performed on given amino acid sample.

Test	Observation	Inference

Test	Observation	Inference

8.0 RESULT :

1. Given sample of amino acid is _____
2. It is _____ (aromatic/aliphatic) amino acid.

9.0 QUESTIONS :

(Note - Student to answer question Q....., Q....., Q..... and question number shall be allotted by teacher.)

1. Which colour is developed in Ninhydrin Test.
2. State the use of Sakaguchi's Test.
3. Write general structure of amino acid.
4. List two sulphur containing amino acids.
3. Name the optically inactive amino acid.
4. Draw the structure's of aromatic Amino acid.
5. Name and draw structure of the aromatic amino acids which gives aldehyde test positive.
6. Which colour is developed in Millon's test for tyrosine.
7. State the use of Xanthoproteic test.
8. Write confirmatory test of tryptophan.
9. Which chemical test should be performed to differentiate between Glycine and Arginine.
10. Name the four essential Amino acids.
11. Define iso electric point of Amino acids.
12. Define essential and non essential amino acids.
13. Write acid base properties of Amino acids.

Space for writing answers

Space for writing answers

Signature of Teacher

Experiment No. 12

1.0 TITLE : To identify given sample of amino acid by qualitative tests. (Sample No. 5)

2.0 PRIOR CONCEPTS:

Basic unit of protein.

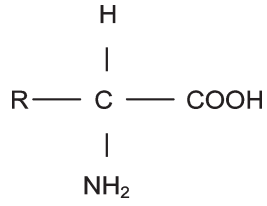
3.0 NEW CONCEPTS:

Proposition 1 : Amino acids

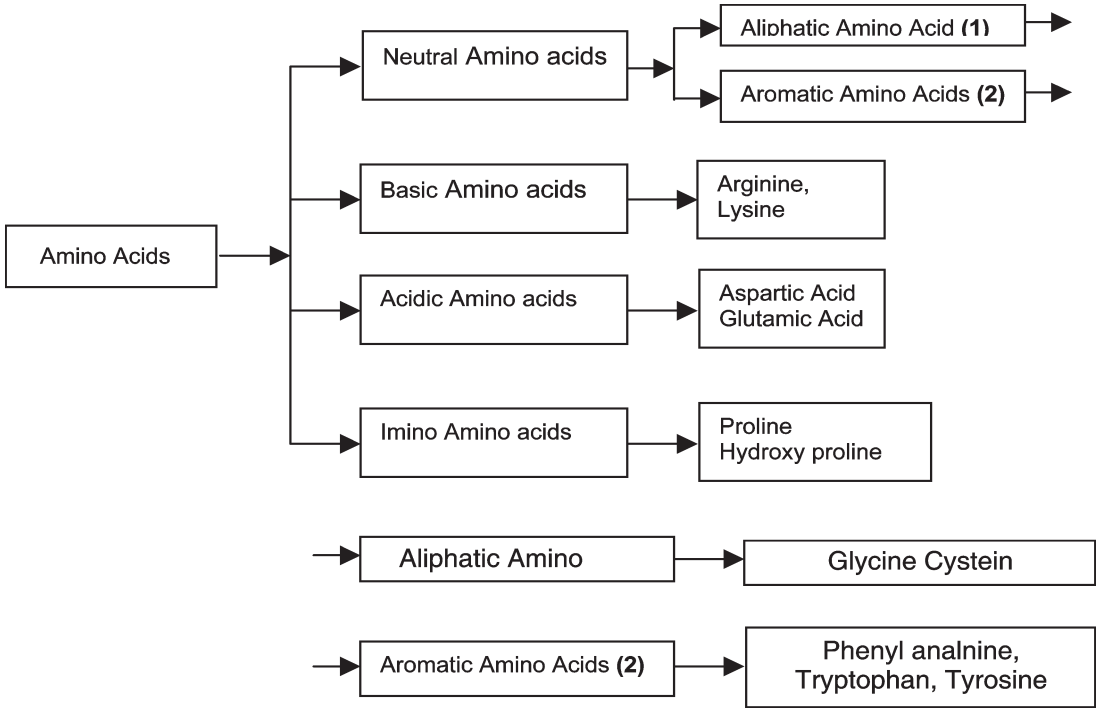
Amino acids are organic acids, which carry at least one amino group in it. Generally at alpha Carbon atom.

Concept Structure :

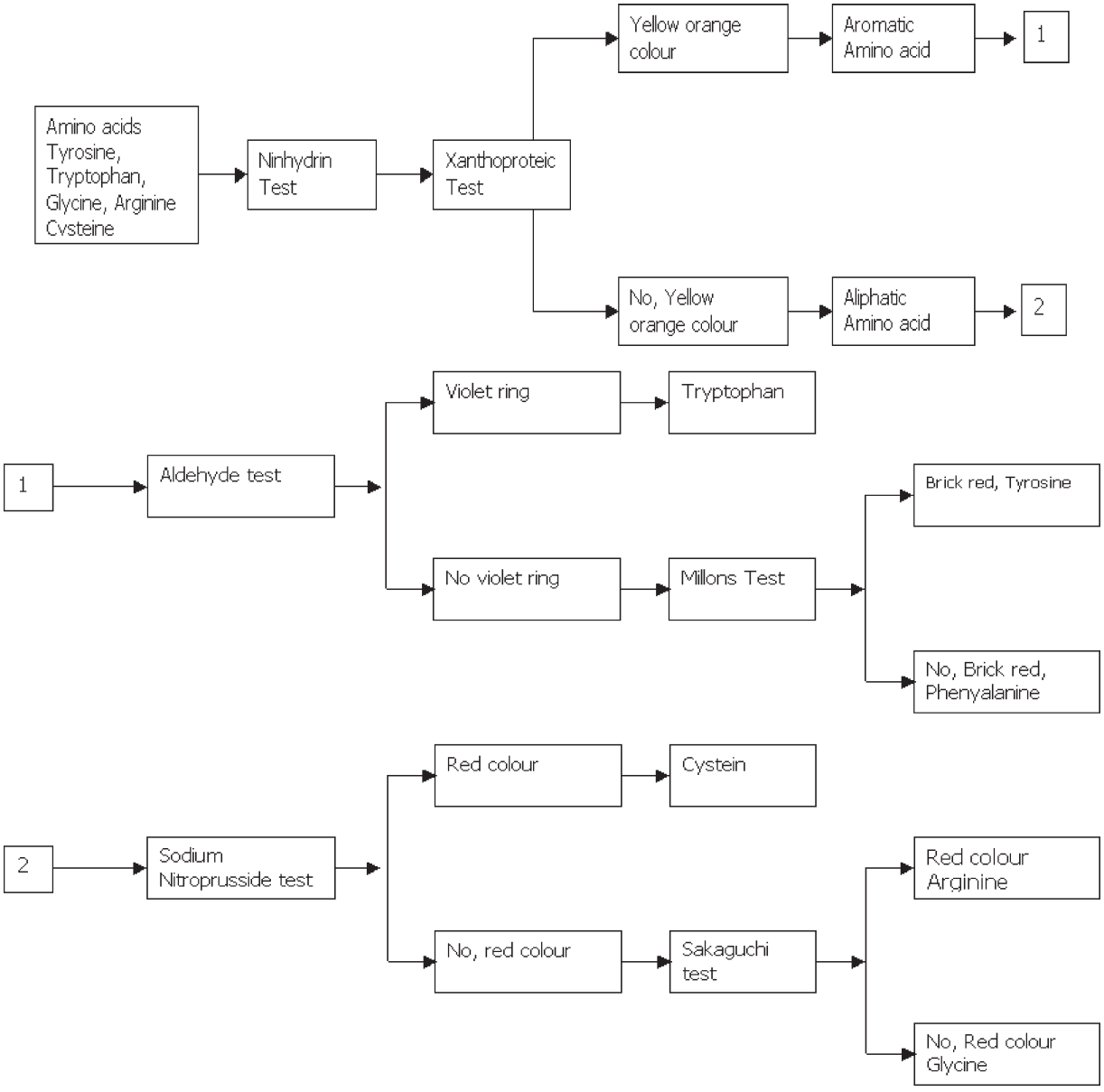
General structure of amino acid



Proposition 2 : Types of amino acids.



General concept structure for identification of amino acids.



4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand concept of amino acids and their types.
2. To identify colour obtained in a identification of amino acid.
3. To distinguish between aromatic and aliphatic amino acids by interpreting test results.
4. To select proper reagent for the test.

Motor skills

1. Ability to add proper reagent in a proper quantity in a original solution.
2. Ability to observe color, turbidity, and precipitate produced in test.

5.0 APPARATUS :

Glassware

Test tubes, Beakers, Test tube stand, Brush, Gas burner, Glass rod etc.

Chemicals

Ninhydrin reagent (1% in acetone), Concentrated HNO_3, 40% w/v NaOH solution, Millon's reagent, Concentrated H_2SO_4, Dilute Sodium Nitroprusside Solution, 2% Lead acetate solution, 2% Alpha Napthol, 10 % w/v NaOH, Ferric chloride solution, Dilute HCl, Strong ammonia solution

Preparation of Reagent

1)	Ninhydrin Reagent	–	0.1 % in a Acetone
2)	Millon's Reagent	–	1 part of mercury (by weight) + 2 parts of HNO_3 (by weight) . Dissolve with help of Heat. Dilute resulting Solution by 2 volumes of water.
3)	Sodium Nitroprusside	–	Dissolve 5 g of Sodium Nitroprusside in 100 ml of water.
4)	Alpha Napthol reagent	–	2 g of alpha napthol in a 100 ml of Alcohol
5)	Sodium hypo bromite	–	10 ml of bromine to 100 ml of 40 % NaOH with a constant stirring while adding bromine.
6)	Sodium Nitrite	–	5 g. Of $NaNO_2$ in a 100 ml of water.
7)	Glyoxalic acid reagent	–	Expose acetic acid to sunlight.
8)	Mormer reagent	–	1 ml. Formaline + 45 ml. Distilled water + 55 ml. Concentrated H_2SO_4.

6.0 STEP WISE PROCEDURE:

1. Prepare 5% amino acid samples in distilled water. Use this solution as original solution. (O.S.)
2. Perform the following tests on amino acid sample, observe the colour and interpret as given in following table.

Table for performing tests on amino acid sample with reagents.

Test	Observation	Inference
Ninhydrin Test 2 ml. O. S. + 0.5 ml Ninhydrin reagent (0.1 % in Acetone) boil for 2 min.	Blue / Violet colour	Amino Acids arepresent
Xanthoproteic Test 2 ml. O. S. + 1 ml. Con. HNO_3 boil + 40% NaOH drop by drop.	Yellow orange colour	Aromatic Amino acids are present
Aldehyde Test 2 ml. O.S. + 5 drops of Millon's reagent + 5 drops of Formalin mix + 2 ml Con. H_2SO_4 from side of Test tube.	Violet ring at junction	Tryptophan present
Millon's Test 2 ml. O. S. + 2 ml. Millon's reagent boil cool, add few drops of $NaNO_2$ Solution.	Brick red colour	Tyrosine present
Sodium Nitroprusside Test 1 ml. O. S. + 1 ml. Dilute NaOH + 1 ml. Sodium Nitroprusside solution	Red colour	Cystein present
Sakaguchi's Test (Arginine Test) 1 ml. O. S. + 1 ml. 10 % NaOH + 5 drops of alpha Napthol + 1 ml. Dilute Sodium Nitroprusside Solution	Red colour	Arginine present

Confirmatory Test (C.T.)

Test	Observation	Inference
C. T. for Cystein 1 ml. O. S. + 1 ml. 40 % NaOH solution boil cool add 1 ml. Lead acetate solution	Dark gray colour	Cystein present
C. T. for Tryptophan **(Hopkin cole Test)** 1 ml. O. S. + 1 ml.Glyoxalic acid shake add Con. H_2SO_4 from side of test tube	Violet ring at junction	Tryptophan present
C. T. for Tyrosine 1 ml. O. S. + 1 ml. Morner reagent heat to boil	Green colour appears	Tyrosine present
C. T. for Arginine (Sakaguchi's Test) 1 ml. O. S. + 1 ml. 10 % NaOH solution + 5 drops of alpha Napthol + 1 ml. Sodium Nitroprusside Solution	Red colour	Arginine present
C. T. for Glycine 1. To 5 ml. of a 1. in 10 ml of solution, add 5 drops of a 1in 2 ml. solution of Sodium Nitrite 2. To 2 ml. of 1 in 10 ml. Solution add 1 ml. of Ferric Chloride solution To above solution add excess dilute HCl To above solution add excess of Strong Ammonia Solution.	 Vigorous evolution of colourless gas Red colour produced Red colour disappears Red colour reappears	 Glycine present Glycine present Glycine present Glycine present.

7.0 OBSERVATION TABLE :

Student to write test observation for chemical test Performed on given amino acid sample.

Test	Observation	Inference

Test	Observation	Inference

8.0 RESULT :

1. Given sample of amino acid is _____
2. It is _____ (aromatic/aliphatic) amino acid.

9.0 QUESTIONS :

(Note - Student to answer question Q....., Q....., Q..... and question number shall be allotted by teacher.)

1. Which colour is developed in Ninhydrin Test.
2. State the use of Sakaguchi's Test.
3. Write general structure of amino acid.
4. List two sulphur containing amino acids.
5. Name the optically inactive amino acid.
6. Draw the structure's of aromatic Amino acid.
7. Name and draw structure of the aromatic amino acids which gives aldehyde test positive.
8. Which colour is developed in Millon's test for tyrosine.
9. State the use of Xanthoproteic test.
10. Write confirmatory test of tryptophan.
11. Which chemical test should be performed to differentiate between Glycine and Arginine.
12. Name the four essential Amino acids.
13. Define iso electric point of Amino acids.
14. Define essential and non essential amino acids.
15. Write acid base properties of Amino acids.

Space for writing answers

Space for writing answers

Signature of Teacher

Experiment No. 13

1.0 TITLE : To isolate casein from milk and its confirmation by chemical tests.

2.0 PRIOR CONCEPTS:

Protein

Casein is a conjugate protein (phosphoprotein) present in milk.

3.0 NEW CONCEPTS:

Proposition 1 :

Proteins are precipitated at isoelectric pH

Proposition 2 :

At isoelectric pH proteins exists as zwitter ions or dipolar ions.
They are electrically neutral with minimum solubility maximum precipitability and least buffering capacity. Casein is precipitated at its isoelectric pH4.6.

4.0 LEARNING OBJECTIVES :

Intellectual skill

1. To understand precipitation
2. To confirm sample by interpreting test results.

Motor skills

1. Ability to add sufficient quantity of 2% HCl and precipitate casein.
2. Ability to observe color, turbidity obtained in each test.

5.0 APPARATUS :

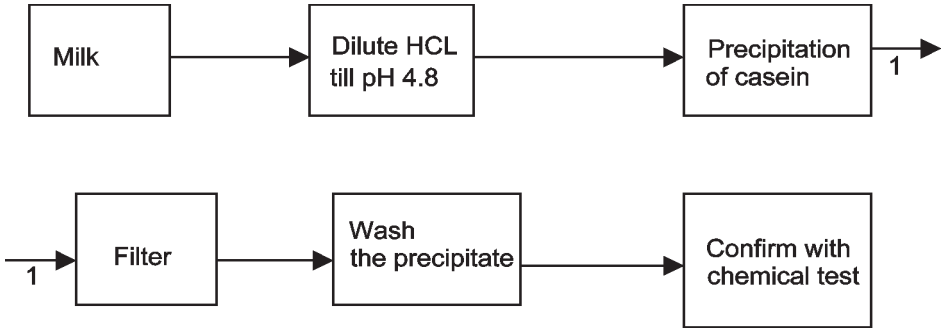
Glassware
Conical flask, centrifuge, funnel, pipettes, test tubes, glass rod.

Chemicals

Milk, 2 % HCL, Bromocresol green indicator, 0.1N NaOH, 0.1N HCl, 10% NaOH, 1%$CuSO_4$, Ninhydrin solution (1%in acetone), 2% lead acetate 20% sulphosalicyclic acid, 1% acetic acid.

6.0 DIAGRAM :

Block diagram for isolation of casein from milk.



7.0 STEPWISE PROCEDURE :

1) Dilute 100 ml of milk with 100 ml of water. Centrifuge for 10 min.
2) Remove the fat layer which floats on top with spatula.
3) Add 2% HCL drop by drop with constant stirring.
4) Check the pH with Bromocresol green indicator.
5) Addition of HCL is continued until bromocresol green gives green color. (Color changes from blue to green, if acid is added in excess it changes to yellow)
6) Observe the visible precipitate of casein.
7) Allow the precipitate to settle and filter.
8) Wash the precipitate in funnel with water and finally with ethanol.
9) Collect, dry the precipitate and report yield.
10) Confirmation by chemical tests.
    - ❖ Solubility test :-
      Check solubility of casein by adding 0.5g of casein in 2 ml 0.1N NaOH, 2ml 0.1N HCL and 2ml of distilled water separately. Keep the tubes in water bath for 10 min and observe, casein is completely soluble in 0.1N NaOH.
    - ❖ Preparation of sample solution :-

      Dissolve 1g of casein in 100 ml 0.1 N NaOH to prepare solution
11) Perform Biuret test, Nihydrin test, precipitation by heavy metals, precipitation by alkaloidal agents, heat coagulation test and Neumann's test.
    - ❖ **Biuret test :-** (General test for proteins)

      To 2ml protein solution add 2ml 10% NaOH solution and then 3 to 4 drops of 1% copper sulphate solution and mix. Purple or pinkish purple color is produced which confirm presence of peptide bond and solution under analysis is protein.
    - ❖ **Ninhydrin test :-**

      To 3ml of protein solution add 3 drops of Ninydrin solution (0.1% in acetone) heat to boil and cool. A bluish purple color is produced. The test is positive for all alpha amino acids.
    - ❖ **Precipitation by heavy metals :-**

      To 2ml solution of protein add 2 to 3 drops of 2% lead acetate solution. Casein is precipitated as white precipitate.
    - ❖ **Precipitation by alkaloidal reagents :-**

      To 3ml solution of protein, add 2 drops of 20% solution of sulphosalicyclic acid, Casein is precipitated as white precipitate or turbidity.
    - ❖ **Heat coagulation test :-**

      Fill the protein solution upto 2/3rd of the test tube. Boil the upper portion of test tube by slightly tilting the test tube. Add 2 to 3 drops of 1% acetic acid and again boil vigorously for 2 to 3 min. No appearance of turbidity or ppt in test tube confirm the casein.
    - ❖ **Neumann's test :-**

      To 5ml protein solution add 0.5ml of 40% NaOH. Heat for 1 minute and cool spontaneously. Add 1.5ml of concentrated nitric acid (till pH is acidic). To this solution add 1ml of ammonium molybdate solution (saturated) and heat. A cannary yellow precipitate is formed which confirms casein.

8.0 OBSERVATION :

Student to write test observation and inference.

Test	Observation	Inference
Solubility test		
Biuret test		
Ninhydrin test		
Precipitation by heavy metal		
Precipitation by alkoloidal reagents		
Heat coagulation test		
Neumann's test		

9.0 RESULT :

Casein is separated from milk and confirmed by chemical tests _____

10.0 QUESTIONS :

Student to answer Q....., Q....., Q....., Q..... and the question numbers shall be allotted by the teacher.

1. State chemical nature of casein.
2. State isoelectric pH of casein.
3. What is the color of Bromocresol green indicator at isoelectric pH of casein ?

4. What is principle involved in Neumann's test ?
5. How to differentiate between casein and albumin by performing heat coagulation test ?
6. What is principle behind Biuret test and Ninhydrin test ?
7. Name the test for identification of peptide bond.
8. How peptide bond is formed between two aminoacids?
9. Write the primary structure of protein.
10. Name protein present in milk.
11. State three examples of conjugated protien.

Space for writing answers

Signature of Teacher

Experiment No. 14

1.0 TITLE : To identify given sample of protein by qualitative tests. (sample No. 1)

2.0 PRIOR CONCEPTS:

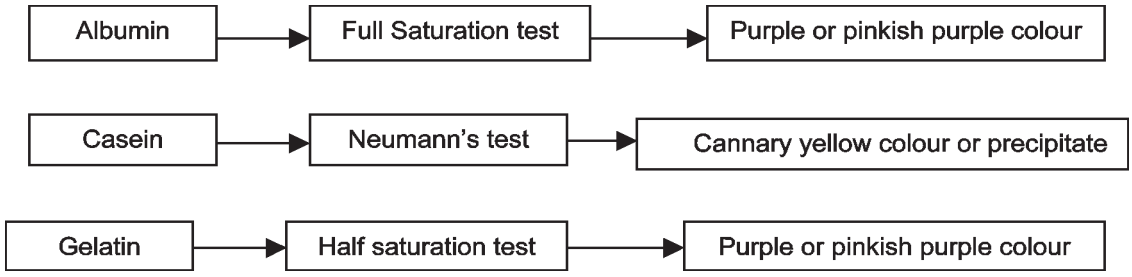
Protein, amino acid, precipitation by heavy metals and alkaloidal reagents, precipitation of protein at isoelectric pH

3.0 NEW CONCEPTS:

Proposition 1 : Confirmatory test for albumin, casein and gelatin

Albumin is confirmed by full saturation test, casein is confirmed by Neumann's test and gelatin is confirmed by half saturation test.

General concept structure:



4.0 LEARNING OBJECTIVES:

Intellectual skill

1. To discriminate between coagulation and no coagulation
2. To interpret by observation of different colours obtained in the test
3. To select tests for identification of protein sample.

Motor skills

1. Ability to add proper amount of reagent
2. Ability to observe coagulation, turbidity, colour obtained in each test.

5.0 APPARATUS :

Glassware

Beaker, test tubes, pipettes, glass rod, litmus red/blue, funnel, filter paper.

Chemicals

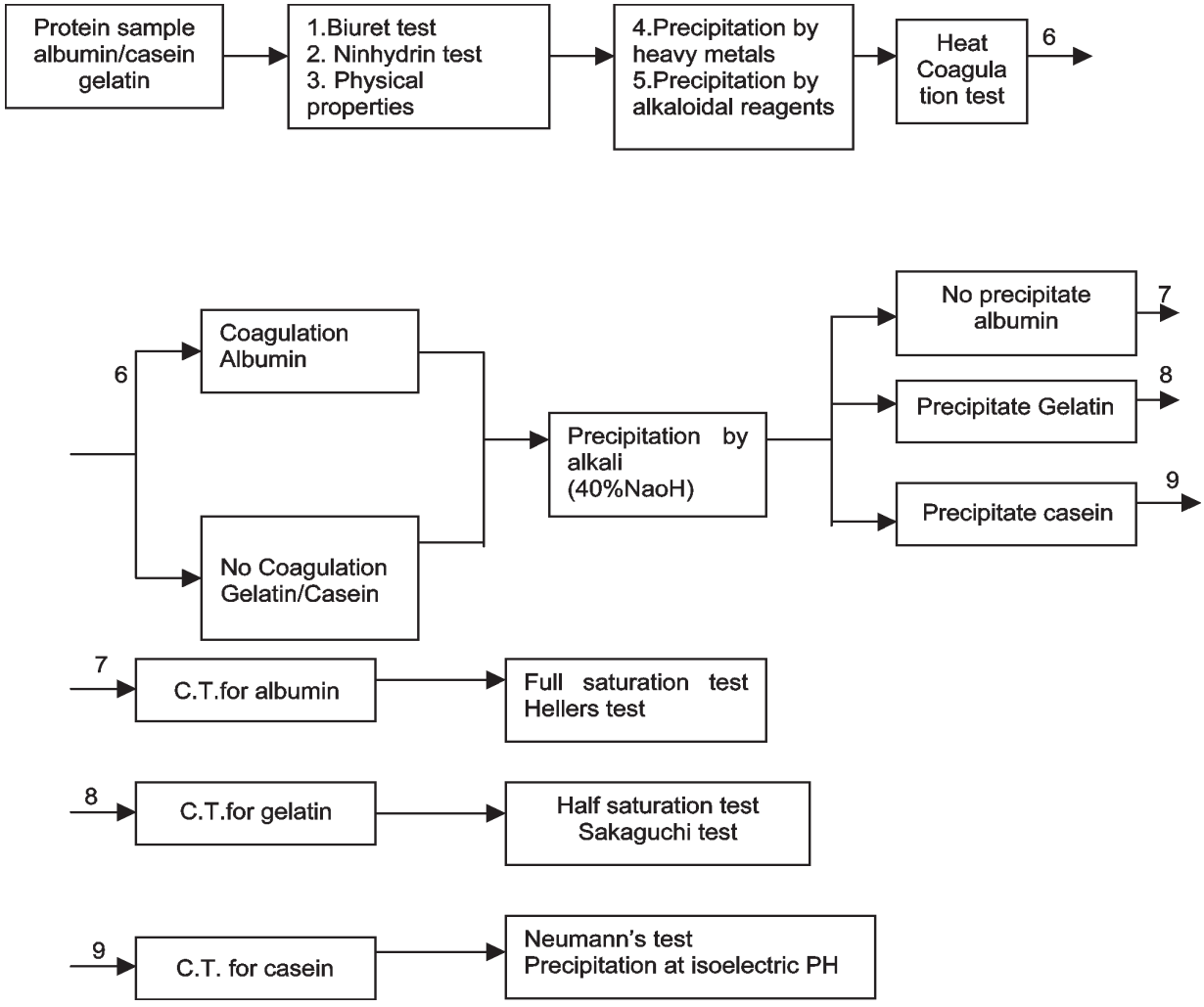
Albumin, Casein, Gelatin, distilled water,

0.1N sodium hydroxide, 5% sodium hydroxide, 1% copper sulphate, ninhydrin reagent, 5% mercuric nitrate, 1% lead acetate,5% ferric chloride, 20% sulphosalicyclic acid. dilute acetic acid, 40% sodium hydroxide, ammonium sulphate powder, concentrated nitric acid, bromocresol green indicator, 2% acetic acid, saturated solution of ammonium sulphate, 10% sodium hydroxide, 1% alpha napthol in alcohol, bromine water or freshly prepared sodium hypobromite solution, soda lime.

Esbach's reagent (1g picric acid+2g citric acid in 100ml of water),

6.0 DIAGRAM :

Block diagram for isolation of casein from milk.



7.0 STEPWISE PROCEDURE :

1. Prepare protein sample as

   Albumin 5g in 100ml water.

   Casein 1 g in 100ml 0.1 N Sodium hydroxide.

   Gelatin 1g in 100ml hot water and cool

2. Perform following tests on protein sample, observe the colour and interpret as given in following table

Table for identification of protein sample (with reagents)

Test	Observation	Inference
Biuret test		
General test for proteins		
3ml of protein solution+3ml of 5% sodium hydroxide + 3 to 4 drops of 1% copper sulphate	Purple or pinkish purple colour is developed.	Proteins are present (i.e presence of peptide bond)
Control test		Serves as control for differentiation between positive test and negative test
3ml water + 3ml of 5% sodium hydroxide + 3 to 4 drops of 1% $CuSO_4$	Blue colour	
Ninhydrin test		Amino acids present
3ml protein solution + 3 drops of Ninhydrin reagent (0.1% in acetone) Heat to boil and cool	Bluish purple colour is produced	
Physical Properties		
Appearance of solution	Turbid	Albumin/Casein may be present
	Clear solution	Gelatin/Peptone may be present
Colour of solution	Milky Faint yellow Faint brown	Albumin/Casein may be present Peptone may be present Gelatin may be present
Smell	Egg like Milk like Meat like	Albumin, may be present Casein may be present Gelatin, peptone may be present
Litmus test	Neutral Acidic Alkaline	Albumin, gelatin may be present Peptone may be present Casein may be present
Precipitation by		
Heavy metals	White precipitate	Protein(Albumin,gelatin, casein peptone may be present)
3ml protein solution + 5% mercuric nitrate solution drop by drop		
Heat above solution	White precipitate changes to brick red	Gelatin may be present
3ml protein solution + 2 to 3 drops of 2% lead acetate solution	White precipitate No precipitate	Albumin, casein may be present Gelatin may be present
3ml protein solution + 2 to 3 drops of 5% ferric chloride solution	White precipitate No precipitate	Albumin, casein may be present Gelatin may be present
Precipitation by alkaloidal reagents		
3ml protein solution + 2 to 3 drops 20% solution of sulphosalicyclic acid	White precipitate	Proteins(Albumin, Gelatin, casein, peptone) may be present

Test	Observation	Inference
3ml protein solution + 2ml Esbach's reagent	Yellow precipitate	Proteins (Albumin, casein, gelatin, peptone) may be present
Heat Coagulation test		
Fill 2/3rd of the test tube with protein solution. Heat upper portion of test tube by slightly tilting the test tube so that lower portion serves as control. To it add 2 to 3 drops of dilute acetic acid and again heat vigorously	Coagulation of protein	Albumin present
	No coagulation of protein but formation of gel on vigorous boiling and cooling	Gelatin present
	No coagulation of protein	Casein present
Precipitation by 40% NaoH		
3ml protein solution + 2ml 40% NaoH	No precipitate Precipitate	Albumin present Gelatin, casein present
Confirmatory test for Albumin		
Full saturation test		
5ml of protein solution + Ammonium sulphate powder till the solution is saturated. A precipitate is formed. Filter the precipitate.		
Dissolve the precipitate in water and perform Biuret test using 40% NaoH	Purple or pinkish purple colour is developed.	Albumin present
Perform Biuret test with filtrate	No purple or pinkish purple colour	Albumin present
Hellers test		
To 3ml concentrated nitric acid in a test tube add gently through side 3ml protein solution	A white precipitate at the interface of acid and protein	Albumin confirmed
C.T. for Casein		
Precipitation at isoelectric PH To 5ml of protein solution add 3 drops of bromocresol green as indicator. Mix and note the colour. If it is blue the solution is alkaline add 2% acetic acid till blue colour changes to light green i.e pH 4.6	Precipitate	Casein present
Neumann's test		
To 5ml of protein solution add 0.5 ml of 40% NaoH. Heat for one minute and cool. Add 1.5 ml of concentrated nitric acid + 1ml ammonium molybdate solution (Saturated) and heat	Cannery yellow colour or cannery yellow precipitate	Casein confirmed

Test	Observation	Inference
C.T. for gelatin		
Gel test		
Heat about 5ml protein solution till volume is reduced to about 1 ml cool	Gel is formed	Gelatin present
Half saturation test		
5ml protein solution + 5ml ammonium sulphate solution (saturated)	Gelatinous precipitate	Gelatin present
Filter the precipitate 3ml filtrate + 3ml 40% NaoH + 3 to 4 drops of 1% Cuso4 solution	No Purple colour	Gelatin present
Sakaguchi test		
2ml of protein solution + 1ml 1% NaoH + 5 drops 1% a napthol in alcohol + 10 drops fresh bromine water or freshly prepared sodium hypobromite solution	Intense red colour	Presence amino acid arginine Gelatin confirmed
5ml protein solution + soda lime + Heat	Ammonia gas evolved	Gelatin confirmed

8.0 OBSERVATION :

Sample 1

Student to write test, observation and inference

Test	Observation	Inference

Student to write test, observation and inference

Test	Observation	Inference

Student to write test, observation and inference

Test	Observation	Inference

9.0 RESULT:

Given protein sample is ……

It is ……..…… (Simple protein/ Conjugated protein/ Derived protein)

10.0 Questions:

Note : Students to answer Q....., Q....., Q....., Q....., and the question numbers shall be allotted by teacher.

1. Name the protein sample which will give no precipitate in heat coagulation test and yellow precipitate in Neumann's test
2. Name the protein sample which gives precipitate in heat coagulation test and purple colour in full saturation test.
3. What is composition of Biuret reagent.
4. State principle involved in Biuret test and Ninhydrin test.
5. State principle involved in Full saturation test and half saturation test.
6. State principle involved in Neumann's test
7. State major source of Albumin, gelatin and casein.
8. State isoelectric pH of casein
9. How to differentiate albumin and gelatin sample.
10. How to differentiate between albumin and casein by performing test precipitation by 40.0% NaoH.
11. State one example of simple protein and derived protein.
12. Name two diseases related to deficiency of proteins in body.
13. State three biological functions of protein.
14. State oxygen carrier protein in blood.

Space for answers

(Space for answers)

Signature of Teacher

Experiment No. 15

1.0 TITLE : To identify given sample of protein by qualitative tests. (sample No. 2)

2.0 PRIOR CONCEPTS:

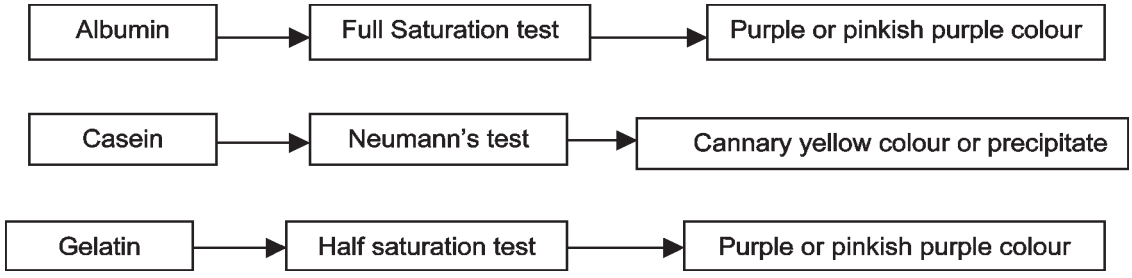
Protein, amino acid, precipitation by heavy metals and alkaloidal reagents, precipitation of protein at isoelectric pH

3.0 NEW CONCEPTS:

Proposition 1 : Confirmatory test for albumin, casein and gelatin

Albumin is confirmed by full saturation test, casein is confirmed by Neumann's test and gelatin is confirmed by half saturation test.

General concept structure:



4.0 LEARNING OBJECTIVES:

Intellectual skill

1. To discriminate between coagulation and no coagulation
2. To interpret by observation of different colours obtained in the test
3. To select tests for identification of protein sample.

Motor skills

1. Ability to add proper amount of reagent
2. Ability to observe coagulation, turbidity, colour obtained in each test.

5.0 APPARATUS :

Glassware

Beaker, test tubes, pipettes, glass rod, litmus red/blue, funnel, filter paper.

Chemicals

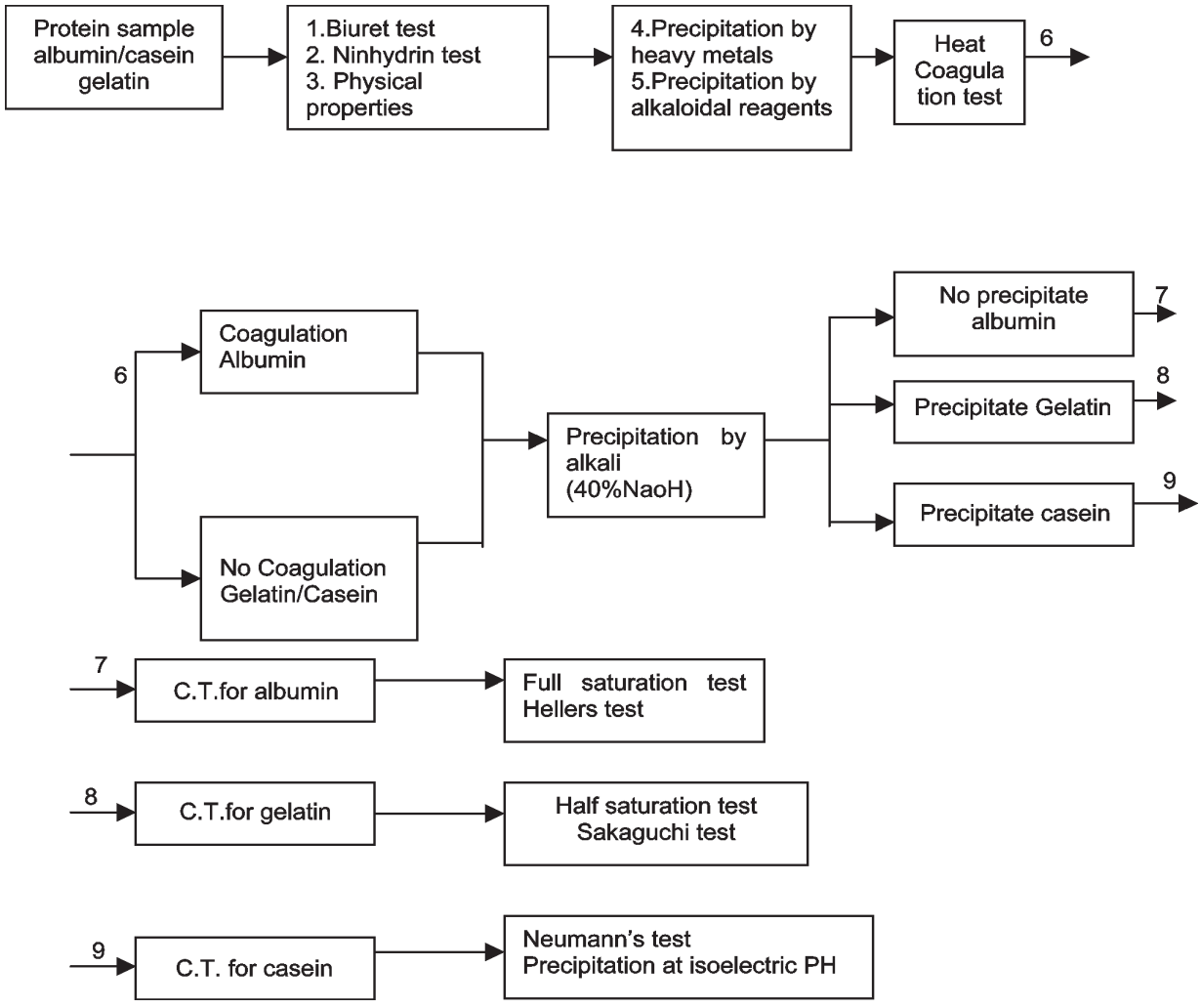
Albumin, Casein, Gelatin, distilled water,

0.1N sodium hydroxide, 5% sodium hydroxide, 1% copper sulphate, ninhydrin reagent, 5% mercuric nitrate, 1% lead acetate,5% ferric chloride, 20% sulphosalicyclic acid. dilute acetic acid, 40% sodium hydroxide, ammonium sulphate powder, concentrated nitric acid, bromocresol green indicator, 2% acetic acid, saturated solution of ammonium sulphate, 10% sodium hydroxide, 1% alpha napthol in alcohol, bromine water or freshly prepared sodium hypobromite solution, soda lime.

Esbach's reagent (1g picric acid+2g citric acid in 100ml of water),

6.0 DIAGRAM :

Block diagram for isolation of casein from milk.



7.0 STEPWISE PROCEDURE :

1. Prepare protein sample as

   Albumin 5g in 100ml water.

   Casein 1 g in 100ml 0.1 N Sodium hydroxide.

   Gelatin 1g in 100ml hot water and cool

2. Perform following tests on protein sample, observe the colour and interpret as given in following table

Table for identification of protein sample (with reagents)

Test	Observation	Inference
Biuret test		
General test for proteins		
3ml of protein solution+3ml of 5% sodium hydroxide + 3 to 4 drops of 1% copper sulphate	Purple or pinkish purple colour is developed.	Proteins are present (i.e presence of peptide bond)
Control test		Serves as control for differentiation between positive test and negative
3ml water + 3ml of 5% sodium hydroxide + 3 to 4 drops of 1% $CuSO_4$	Blue colour	test
Ninhydrin test		Amino acids present
3ml protein solution + 3 drops of Ninhydrin reagent (0.1% in acetone) Heat to boil and cool	Bluish purple colour is produced	
Physical Properties		
Appearance of solution	Turbid	Albumin/Casein may be present
	Clear solution	Gelatin/Peptone may be present
Colour of solution	Milky Faint yellow Faint brown	Albumin/Casein may be present Peptone may be present Gelatin may be present
Smell	Egg like Milk like Meat like	Albumin, may be present Casein may be present Gelatin, peptone may be present
Litmus test	Neutral Acidic Alkaline	Albumin, gelatin may be present Peptone may be present Casein may be present
Precipitation by		
Heavy metals	White precipitate	Protein(Albumin,gelatin, casein
3ml protein solution + 5% mercuric nitrate solution drop by drop		peptone may be present)
Heat above solution	White precipitate changes to brick red	Gelatin may be present
3ml protein solution + 2 to 3 drops of 2% lead acetate solution	White precipitate No precipitate	Albumin, casein may be present Gelatin may be present
3ml protein solution + 2 to 3 drops of 5% ferric chloride solution	White precipitate No precipitate	Albumin, casein may be present Gelatin may be present
Precipitation by alkaloidal reagents		
3ml protein solution + 2 to 3 drops 20% solution of sulphosalicyclic acid	White precipitate	Proteins(Albumin, Gelatin, casein, peptone) may be present

Test	Observation	Inference
3ml protein solution + 2ml Esbach's reagent	Yellow precipitate	Proteins (Albumin, casein, gelatin, peptone) may be present
Heat Coagulation test		
Fill 2/3rd of the test tube with protein solution. Heat upper portion of test tube by slightly tilting the test tube so that lower portion serves as control. To it add 2 to 3 drops of dilute acetic acid and again heat vigorously	Coagulation of protein	Albumin present
	No coagulation of protein but formation of gel on vigorous boiling and cooling	Gelatin present
	No coagulation of protein	Casein present
Precipitation by 40% NaoH		
3ml protein solution + 2ml 40% NaoH	No precipitate Precipitate	Albumin present Gelatin, casein present
Confirmatory test for Albumin		
Full saturation test		
5ml of protein solution + Ammonium sulphate powder till the solution is saturated. A precipitate is formed. Filter the precipitate.		
Dissolve the precipitate in water and perform Biuret test using 40% NaoH	Purple or pinkish purple colour is developed.	Albumin present
Perform Biuret test with filtrate	No purple or pinkish purple colour	Albumin present
Hellers test		
To 3ml concentrated nitric acid in a test tube add gently through side 3ml protein solution	A white precipitate at the interface of acid and protein	Albumin confirmed
C.T. for Casein		
Precipitation at isoelectric PH To 5ml of protein solution add 3 drops of bromocresol green as indicator. Mix and note the colour. If it is blue the solution is alkaline add 2% acetic acid till blue colour changes to light green i.e pH 4.6	Precipitate	Casein present
Neumann's test		
To 5ml of protein solution add 0.5 ml of 40% NaoH. Heat for one minute and cool. Add 1.5 ml of concentrated nitric acid + 1ml ammonium molybdate solution (Saturated) and heat	Cannery yellow colour or cannery yellow precipitate	Casein confirmed

Test	Observation	Inference
C.T. for gelatin		
Gel test		
Heat about 5ml protein solution till volume is reduced to about 1 ml cool	Gel is formed	Gelatin present
Half saturation test		
5ml protein solution + 5ml ammonium sulphate solution (saturated)	Gelatinous precipitate	Gelatin present
Filter the precipitate 3ml filtrate + 3ml 40% NaoH + 3 to 4 drops of 1% Cuso4 solution	No Purple colour	Gelatin present
Sakaguchi test		
2ml of protein solution + 1ml 1% NaoH + 5 drops 1% a napthol in alcohol + 10 drops fresh bromine water or freshly prepared sodium hypobromite solution	Intense red colour	Presence amino acid arginine Gelatin confirmed
5ml protein solution + soda lime + Heat	Ammonia gas evolved	Gelatin confirmed

8.0 OBSERVATION :

Sample 2

Student to write test, observation and inference

Test	Observation	Inference

Student to write test, observation and inference

Test	Observation	Inference

Student to write test, observation and inference

Test	Observation	Inference

9.0 RESULT:

Given protein sample is ……

It is ……..…… (Simple protein/ Conjugated protein/ Derived protein)

10.0 Questions:

Note : Students to answer Q....., Q....., Q....., Q....., and the question numbers shall be allotted by teacher.

1. Name the protein sample which will give no precipitate in heat coagulation test and yellow precipitate in Neumann's test
2. Name the protein sample which gives precipitate in heat coagulation test and purple colour in full saturation test.
3. What is composition of Biuret reagent.
4. State principle involved in Biuret test and Ninhydrin test.
5. State principle involved in Full saturation test and half saturation test.
6. State principle involved in Neumann's test
7. State major source of Albumin, gelatin and casein.
8. State isoelectric pH of casein
9. How to differentiate albumin and gelatin sample.
10. How to differentiate between albumin and casein by performing test precipitation by 40.0% NaoH.
11. State one example of simple protein and derived protein.
12. Name two diseases related to deficiency of proteins in body.
13. State three biological functions of protein.
14. State oxygen carrier protein in blood.

Space for answers

(Space for answers)

Signature of Teacher

Experiment No. 16

1.0 TITLE:

To identify given sample of protein by qualitative tests. (sample No. 3)

2.0 PRIOR CONCEPTS:

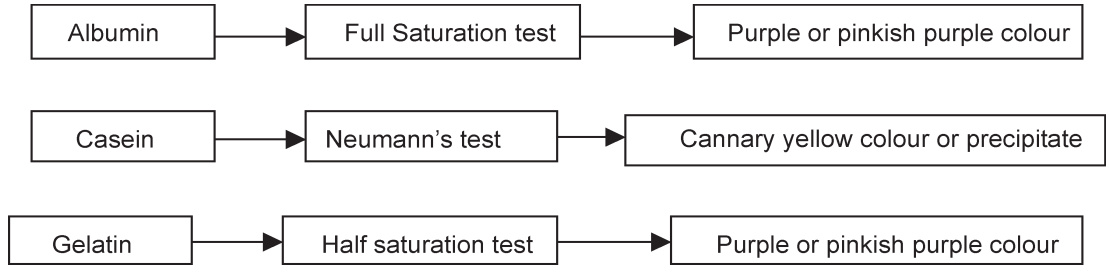
Protein, amino acid, precipitation by heavy metals and alkaloidal reagents, precipitation of protein at isoelectric pH

3.0 NEW CONCEPTS:

Proposition 1 : Confirmatory test for albumin, casein and gelatin

Albumin is confirmed by full saturation test, casein is confirmed by Neumann's test and gelatin is confirmed by half saturation test.

General concept structure:



4.0 LEARNING OBJECTIVES:

Intellectual skill:

1. To discriminate between coagulation and no coagulation
2. To interpret by observation of different colours obtained in the test
3. To select tests for identification of protein sample.

Motor skills:

1. Ability to add proper amount of reagent
2. Ability to observe coagulation, turbidity, colour obtained in each test.

5.0 APPARATUS:

Glassware

Beaker, test tubes, pipettes, glass rod, litmus red/blue, funnel, filter paper.

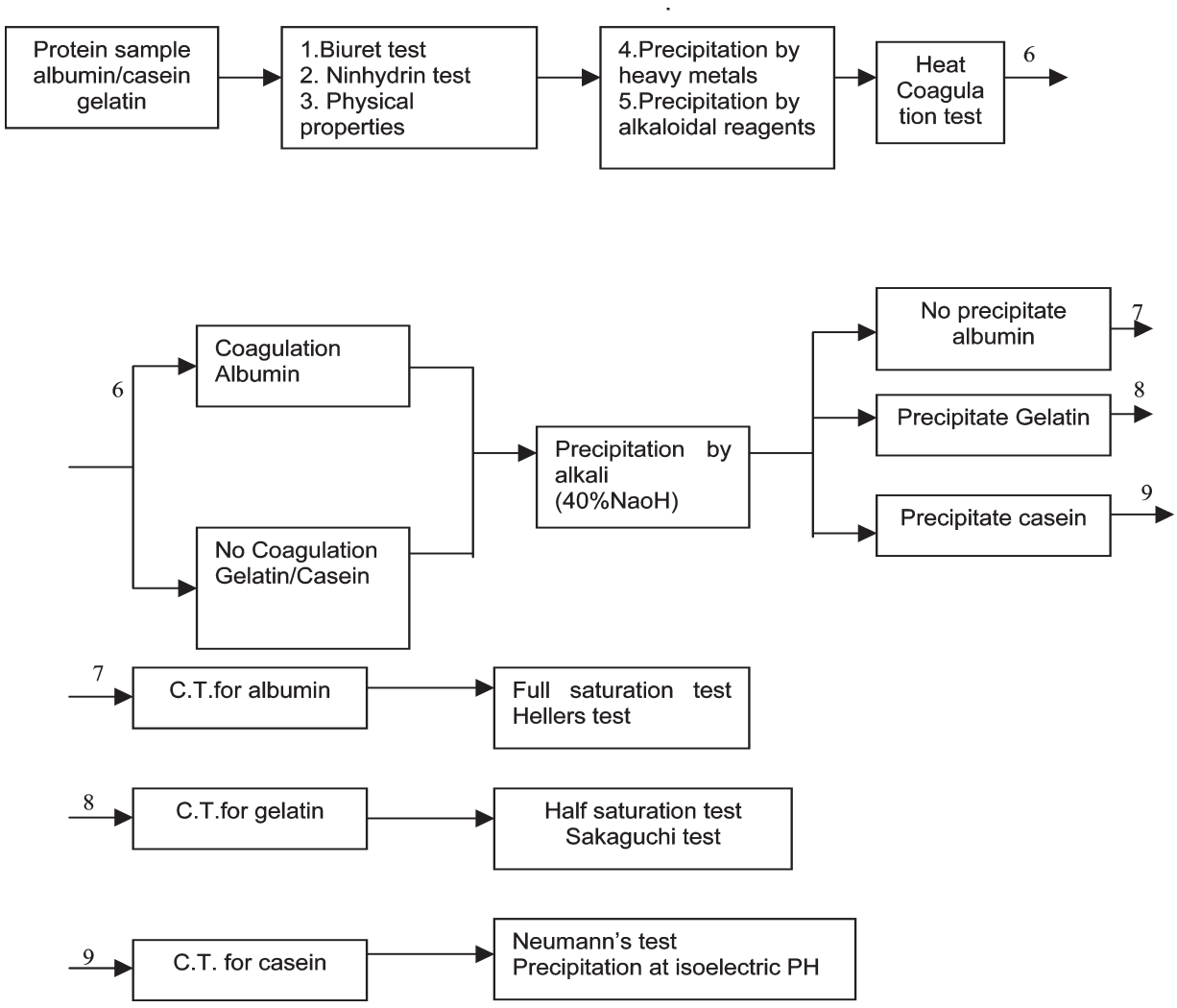
Chemicals

Albumin, Casein, Gelatin, distilled water,

0.1N sodium hydroxide, 5% sodium hydroxide, 1% copper sulphate, ninhydrin reagent, 5% mercuric nitrate, 1% lead acetate,5% ferric chloride, 20% sulphosalicyclic acid. dilute acetic acid, 40% sodium hydroxide, ammonium sulphate powder, concentrated nitric acid, bromocresol green indicator, 2% acetic acid, saturated solution of ammonium sulphate, 10% sodium hydroxide, 1% alpha napthol in alcohol, bromine water or freshly prepared sodium hypobromite solution, soda lime. Esbach's reagent (1g picric acid+2g citric acid in 100ml of water),

6.0 DIAGRAM:

Block diagram showing scheme for identification of protein.



7.0 STEPWISE PROCEDURE:

1. Prepare protein sample as

   Albumin 5g in 100ml water.

   Casein 1 g in 100ml 0.1 N Sodium hydroxide.

   Gelatin 1g in 100ml hot water and cool

2. Perform following tests on protein sample, observe the colour and interpret as given in following table

Table for identification of protein sample (with reagents)

Test	Observation	Inference
Biuret test		
General test for proteins		
3ml of protein solution+3ml of 5% sodium hydroxide + 3 to 4 drops of 1% copper sulphate	Purple or pinkish purple colour is developed.	Proteins are present (i.e presence of peptide bond)
Control test		Serves as control for differentiation between positive test and negative
3ml water + 3ml of 5% sodium hydroxide + 3 to 4 drops of 1% CuSO4	Blue colour	test
Ninhydrin test		Amino acids present
3ml protein solution + 3 drops of Ninhydrin reagent (0.1% in acetone) Heat to boil and cool	Bluish purple colour is produced	
Physical Properties		
Appearance of solution	Turbid	Albumin/Casein may be present
	Clear solution	Gelatin/Peptone may be present
Colour of solution	Milky Faint yellow Faint brown	Albumin/Casein may be present Peptone may be present Gelatin may be present
Smell	Egg like Milk like Meat like	Albumin, may be present Casein may be present Gelatin, peptone may be present
Litmus test	Neutral Acidic Alkaline	Albumin, gelatin may be present Peptone may be present Casein may be present
Precipitation by		
Heavy metals	White precipitate	Protein(Albumin,gelatin, casein
3ml protein solution + 5% mercuric nitrate solution drop by drop		peptone may be present)
Heat above solution	White precipitate changes to brick red	Gelatin may be present
3ml protein solution + 2 to 3 drops of 2% lead acetate solution	White precipitate No precipitate	Albumin, casein may be present Gelatin may be present
3ml protein solution + 2 to 3 drops of 5% ferric chloride solution	White precipitate No precipitate	Albumin, casein may be present Gelatin may be present
Precipitation by alkaloidal reagents		
3ml protein solution + 2 to 3 drops 20% solution of sulphosalicyclic acid	White precipitate	Proteins(Albumin, Gelatin, casein, peptone) may be present

Test	Observation	Inference
3ml protein solution + 2ml Esbach's reagent	Yellow precipitate	Proteins (Albumin, casein, gelatin, peptone) may be present
Heat Coagulation test		
Fill 2/3rd of the test tube with protein solution. Heat upper portion of test tube by slightly tilting the test tube so that lower portion serves as control. To it add 2 to 3 drops of dilute acetic acid and again heat vigorously	Coagulation of protein	Albumin present
	No coagulation of protein but formation of gel on vigorous boiling and cooling	Gelatin present
	No coagulation of protein	Casein present
Precipitation by 40% NaoH		
3ml protein solution + 2ml 40% NaoH	No precipitate Precipitate	Albumin present Gelatin, casein present
Confirmatory test for Albumin		
Full saturation test		
5ml of protein solution + Ammonium sulphate powder till the solution is saturated. A precipitate is formed. Filter the precipitate.		
Dissolve the precipitate in water and perform Biuret test using 40% NaoH	Purple or pinkish purple colour is developed.	Albumin present
Perform Biuret test with filtrate	No purple or pinkish purple colour	Albumin present
Hellers test		
To 3ml concentrated nitric acid in a test tube add gently through side 3ml protein solution	A white precipitate at the interface of acid and protein	Albumin confirmed
C.T. for Casein		
Precipitation at isoelectric PH To 5ml of protein solution add 3 drops of bromocresol green as indicator. Mix and note the colour. If it is blue the solution is alkaline add 2% acetic acid till blue colour changes to light green i.e pH 4.6	Precipitate	Casein present
Neumann's test		
To 5ml of protein solution add 0.5 ml of 40% NaoH. Heat for one minute and cool. Add 1.5 ml of concentrated nitric acid + 1ml ammonium molybdate solution (Saturated) and heat	Cannery yellow colour or cannery yellow precipitate	Casein confirmed

Test	Observation	Inference
C.T. for gelatin		
Gel test		
Heat about 5ml protein solution till volume is reduced to about 1 ml cool	Gel is formed	Gelatin present
Half saturation test		
5ml protein solution + 5ml ammonium sulphate solution (saturated)	Gelatinous precipitate	Gelatin present
Filter the precipitate 3ml filtrate + 3ml 40% NaoH + 3 to 4 drops of 1% Cuso4 solution	No Purple colour	Gelatin present
Sakaguchi test		
2ml of protein solution + 1ml 1% NaoH + 5 drops 1% a napthol in alcohol + 10 drops fresh bromine water or freshly prepared sodium hypobromite solution	Intense red colour	Presence amino acid arginine Gelatin confirmed
5ml protein solution + soda lime + Heat	Ammonia gas evolved	Gelatin confirmed

8.0 OBSERVATION :

Sample 3

Student to write test, observation and inference

Test	Observation	Inference

Student to write test, observation and inference

Test	Observation	Inference

Student to write test, observation and inference

Test	Observation	Inference

9.0 RESULT:

Given protein sample is ……

It is ……..…… (Simple protein/ Conjugated protein/ Derived protein)

10.0 Questions:

Note : Students to answer Q....., Q....., Q....., Q....., and the question numbers shall be allotted by teacher.

1. Name the protein sample which will give no precipitate in heat coagulation test and yellow precipitate in Neumann's test
2. Name the protein sample which gives precipitate in heat coagulation test and purple colour in full saturation test.
3. What is composition of Biuret reagent.
4. State principle involved in Biuret test and Ninhydrin test.
5. State principle involved in Full saturation test and half saturation test.
6. State principle involved in Neumann's test
7. State major source of Albumin, gelatin and casein.
8. State isoelectric pH of casein
9. How to differentiate albumin and gelatin sample.
10. How to differentiate between albumin and casein by performing test precipitation by 40.0% NaoH.
11. State one example of simple protein and derived protein.
12. Name two diseases related to deficiency of proteins in body.
13. State three biological functions of protein.
14. State oxygen carrier protein in blood.

Space for answers

(Space for answers)

Signature of Teacher

Experiment No. 17

Experiment No. 17 and Experiment No. 18 may be conducted in single turn.

1.0 TITLE:

To study physical and chemical properties of fats and oils.

2.0 PRIOR CONCEPTS:

Basic unit of lipid.

3.0 NEW CONCEPTS:

Proposition 1 :

Fats are esters of long chain fatty acids with glycerol's.

Concept structure:

General structure of Triglyceride.

CH_2 — O — CO — R
|
CH — O — CO — R (R = Long chain fatty acid)
|
CH_2 — O — CO — R

Proposition 2 :

Fats are esters of saturated fatty acids and solid at room temp.

Proposition 3:

Oils are esters of unsaturated fatty acids and liquid at room temp.

4.0 LEARNING OBJECTIVES:

Intellectual skill:

1. To understand concepts of fats, fatty acid and triglycerides.
2. To distinguish fats and oils by interpreting test results.

Motor skills:

1. Ability to add proper amount of reagent.
2. Ability to observe the colours produced in test.

5.0 APPARATUS:

Glassware

Test tubes, Beaker, Test tube stand, Gas burner etc.

Chemicals

1% bile salt, solid NaCl, 20 % NaOH, Alcohol, 2 % CaCl2 Solution, Distilled water, Choloroform etc.

Fat-cocoa -butter, oil-castor oil. Red/blue litmus, concentrated HCL. H2SO4, bromine water.

6.0 STEPWISE PROCEDURE :

Perform following tests on given fat and oil sample observe and interpret as mentioned in the following table.

Table for performing test on fat and oil sample with reagent.

Test	Observation	Inference
Touch	They are greasy and oily to touch	Fats and oils are present
Greasy spot formation Drop of fat /oil is added on paper	Paper becomes semitransparent	Fat, oil are present
Solubility 1) 1 ml. Fat / oil + 5 ml. Water 2) 1 ml. Fat or oil + 5 ml. Organic solvent (ether ,alcohol, chloroform)	 Immiscible Soluble	 Fat or oil present Fat or oil present
Action on moist litmus paper	Blue (litmus) turns red Red litmus turns to blue No action on litmus paper	Acidic in nature Basic in nature. Neutral in nature.
Specific gravity Add small quantity of fat/oil to the test tube of fully filled with water	Fat or oil float on water	Specific gravity of fat / oil is less than one.

Test	Observation	Inference
Emulsification 1) 1 ml. alcoholic solution of fat or oil + 3 ml of water shake vigorously.	A White homogeneous emulsion is formed which break on standing by separating fatty phase in form of oily droplets	Fat or oil form emulsion in water.
2) 1 ml. alcoholic solution of fat / oil + 5 ml. 1 % bilesalt solution + 5 ml. distilled water, shake vigorously.	A White homogenous emulsion is formed which remains stable on standing.	Fats/ oil form emulsion in water, which can be stabilized by emulsifying agent like bile salt.
Saponification 10 ml of 20 % NaOH + 10 ml. alcoholic solution of fat / oil. Heat on water bath until drop of this mixture do not separate oil drop when added to distilled water. Add mixture with equal quantities of distilled water and divide in three equal parts.		
A) 2 ml. of above mixture + solid 0.5g NaCl shake vigorously.	Cake of soap floats on a top. Separate this cake and make 2 parts.	Fat / oil is saponified.
i) One part of above test + 10 ml. water	Frothing takes place.	Formation of soap confirmed.
ii) Second part of soap cake + 5 ml. sater	Precipitate dissolves	Sodium soap of fat / oil is dissolved in water.

Test	Observation	Inference
B) 2 ml. of above mixture + 3 ml 2% $CaCl_2$ solution shake	White flocculant precipitate is formed insoluble in water.	Calcium soap is insoluble in water.
C) above mixture 3 ml. + 2-3 drops of con. HCl or H_2SO_4 boil	Fatty acid seprated and float on water	Soap salt are formed with (H_2SO_4 / HCL)
Test for Unsaturation 1 g fat / oil + bromine water	Decolourisation of bromine water	Unsaturated fatty acids present in oil.

7.0 OBSERVATION :

Student to write observations of tests performed on a fat/oil.

Test	Observation	Inference

Student to write observations of tests performed on a fat/oil.

Test	Observation	Inference

8.0 RESULT:

Given sample of fat / oil .....(complies/Not complies) all physical and chemical test.

9.0 Questions:

(Note - Student to answer question Q....., Q....., Q..... and question number shall be allotted by teacher.)

1. Draw the general structure of triglycerides.
2. State two examples of oils.
3. Which emulsifying agent is added in emulsion test?
4. State use of bromine water test.
5. State two differences between fat and oil.
6. State two examples of fats.
7. Define saponification of fat and oil.
8. Which test should be performed to detect specific gravity of fat and oil?
9. Draw the structures of two saturated fatty acid.
10. Draw the structures of three unsaturated fatty acid.

Space for answers

(Space for answers)

Signature of Teacher

Experiment No. 18

1.0 TITLE:

To conduct test on cholesterol to verify physical and chemical tests.

2.0 PRIOR CONCEPTS:

It is constituents of tissue of brain, nervous system and spinal cord.

3.0 NEW CONCEPTS:

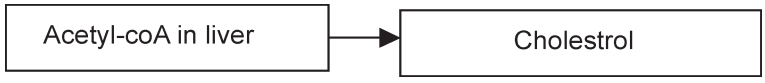
Proposition 1 : Source of Cholestrol

Cholesterol is present in all mammalian tissue mainly in brain, spinal cord, nervous system. It is also present in a fish liver oils and egg yolk. Gall stone is chief source of Cholesterol.

Proposition 2 : Formation of Cholestrol

It is formed in liver from acetyl-coA by number of steps.

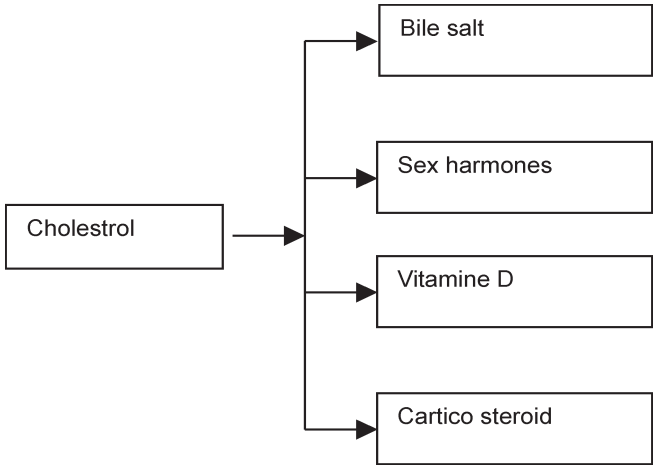
Concept structure:



Proposition 3 : Biosynthesis product of Cholestrol

Cholesterol is precursor for synthesis of bile salt, sex harmones, Vitamin D and Cortico steroid in body.

Concept structure:



Proposition 4 : Athrosclerosis

Cholesterol is deposited on a wall of arteries and destroy their normal elasticity is know as athrosclerosis

4.0 LEARNING OBJECTIVES:

Intellectual skill:

1. To understand concept of cholesterol and its property.
2. To identify colours obtained in its identification test.

Motor skills:

1. Ability to add proper amount of reagent for test.
2. Ability to observe the colours produced in test.

5.0 APPARATUS:

Glassware

Test tubes, Beaker, Test tube stand, Burner, Microscope, slides etc.

Chemicals

Cholesterol sample, Chloroform, Acetic anhydride, Concentrate H_2SO_4 , Chlorobenzene, ether

6.0 STEPWISE PROCEDURE :

Perform following tests on given cholesterol sample, observe and interpret as mentioned in following table.

Microscopic Examination

Examine the crystals of cholesterol under the microscope. Cholesterol crystals are colourless and rhombic in shape and characteristically have a notch at one of the edges.

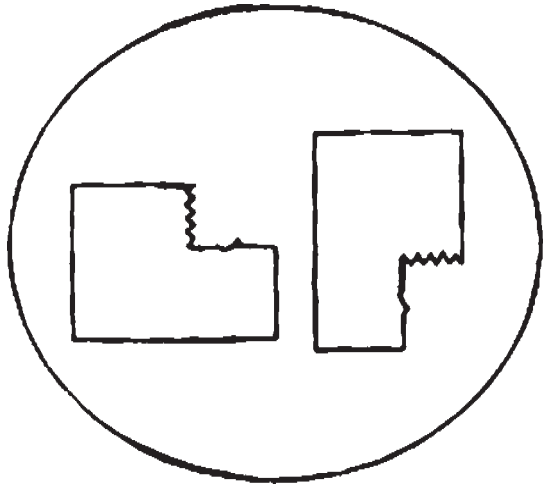


Fig. 18.1 Shape of Cholesterol Crystal under Microscope

Table for performing test on Cholesterol sample with reagents.

Test	Observation	Inference
Physical Properties 0.5 g Cholesterol + 2 ml. Water	Insoluble	Being Lipid it is insoluble in water
0.5 g Cholesterol + 2 ml. organic solvent like Chlorobenzen / Ether.	Soluble	Being lipid it is soluble.
Microscopic appearance	White shining rhombic crystal	Cholesterol may be present
Chemical Tests **Salkowski test** 2 ml Cholesterol solution in chloroform + Slowly add 2 ml. Con H2SO4 wait for 3 min.	Upper Chloroform layer shows red colour while lower H2SO4 layer shows green fluorescence.	Cholesterol is confirmed.
Liberman - Burchrdt's Test 2 ml. Of Cholesterol solution in Chloroform + 10 drops of acetic anhydride + 2 drops Con. H2SO4	Solution becomes deep red which rapidly changes to deep blue to green colour.	Cholesterol confirmed.

7.0 OBSERVATION :

Student to write observation and inference of the tests performed.

Test	Observation	Inference
Physical properties and solubility		

Student to write observation and inference of the tests performed.

Test	Observation	Inference

8.0 RESULT:

Hence the given sample of Cholestrol ..... (comply / does not comply) physical properties. ..... (comply / does not comply) chemical tests.

9.0 Questions:

(Note - Student to answer question Q....., Q....., Q..... and question number shall be allotted by teacher.)

1. Write two sources of Cholesterol.
2. Draw the structure of Cholesterol.
3. Write name of chemical reagents which are used in Liberman burchardt test.
4. State colour observed in upper and bottom layer of salkowski test.
5. Write chemical reagent, which are used in salkowski test.
6. Name the two tests by which Cholesterol is confirmed.
7. State the two uses of Cholesterol.
8. Draw the microscopic appearance of confirmation Cholesterol.
9. Describe solubility test of Cholesterol in water and organic solvent.
10. Which colour is developed in Liberman burchardt test?

Space for answers

(Space for answers)

Signature of Teacher

Experiment No. 19

1.0 TITLE:

To detect normal constituents in given sample of urine by qualitative test.

2.0 PRIOR CONCEPTS:

Urine is excretory fluid eliminated through kidney. The waste products (urea,creatinine,uric acid etc) are eliminated through Urine.

3.0 NEW CONCEPTS:

Proposition 1 :

Urine shows presence of Organic and Inorganic substance.

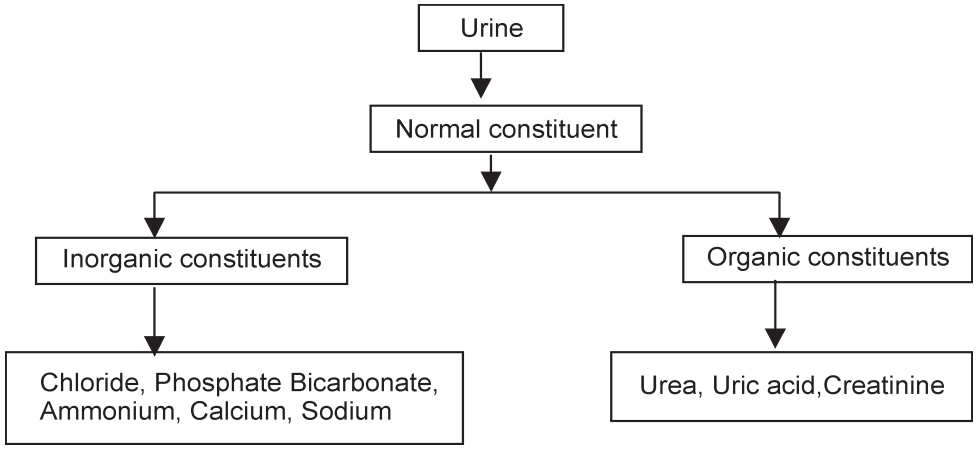
Proposition 2 :

Organic constituents are Urea, Uric Acid and creatinine.

Proposition 3 :

Inorganic ions are Chloride, Phosphate, Bicarbonate, Ammonium, Sulphate , Calcium and Sodium.

General Concept Structure:



4.0 LEARNING OBJECTIVES:

Intellectual skill:

1. To understand concept of detection of normal contituent of Urine.
2. To discreminate between organic and inorganic constituent of Urine.
3. To understand identification test of Inorganic ions and Organic constituesnt of Urine.

Motor skills:

1. Ability to add proper volume of reagent.
2. Ability to observe colour produced in test.

5.0 APPARATUS:

Glassware

Test tubes, Beakers, Test tube stand, Burner, Glass rod etc.

Chemicals

Dilute HCl solution, Dilute H_2SO_4 solution, Con.HNO_3, $AgNO_3$,solution 1 N Ammonium Molybdate Solution., 1 N Barium Chloridesolution,40 % NaOH solution, Phenolphthalein Indicator, 1 % Acetic Acid,solution,1NAmmonium Oxalate Solution, Sodium Hypobromite Solution, Urease Powder Anhydrous Sodium Carbonate 1N Sodium Nitroprusside Solution., Saturated Picric acid solution

6.0 STEPWISE PROCEDURE :

Perform following tests on given urine sample and interpret as mentioned in following table,

Physical properties

Table for performing test on urine sample with reagents.

Test	Observation	Inference
Volume	(a) 1000 ml-1500 ml per day	Normal
	(b) More than 1500 ml per day	(1) Polyuria may be due to more water intake, less perspiration, high protein diet diuretic substance like alcohol, coffee, tea, diseased state like diabetes insipidius
	(c) Less than 1000 ml/ day	may be due to 1. Hard physical work 2. Fever 3. Dehydration 4. Vomitting, diarrhoea 5. Acute nephrittis.
	(d) No urine	Anuria due to acute renal insufficiency.

INORGANIC CONSTITUENTS

TEST FOR NORMAL INORGANIC CONSTITUENTS OF URINE :

Test	Observation	Inference
Test for bi-carbonates : 3 ml urine + dilute HCl or dilute H_2So_4	Effervescence of CO_2 gas	Bi-carbonate present
Test for chlorides: 5 ml urine + 1 ml. concentrated HNO_3 (to prevent precipitation of other ions like phosphate) + 1 ml $AgNO_3$ solution	White curdy ppt. of Agcl soluble in NH_4OH solution.	Chlorides present.
Test for phosphates : 3 ml. Urine + 3 ml concentrate HNO_3 + 3 ml of 1 N. ammonium molybdate solution. Heat to boil.	Cannary yellow ppt.	Phosphate present
Test for sulphates : 5 ml. Urine + 1 ml concentrated HCl (to prevent phosphate ppt.) + 2 ml 1 N. $Bacl_2$ solution.	An opaque milkiness or a thick white ppt. of $BaSO_4$ insoluble in concentrated HCl.	Sulphate present.

Test	Observation	Inference
Test for ammonia : 1. 5 ml urine + 2 ml of 40 % NaOH, boil, hold a red litmus paper near mouth of test tube 2. Deep the glass rod in phenolphthalein indicator and hold near mouth of test tube	The red litmus paper turns blue. The colour becomes pink	Ammonia present Ammonia present
Test for calcium : 5 ml urine + 5 drops of 40 % NaOH + 1 % acetic acid + 2 to 3 ml of ammonium oxalate solution.	White ppt of calcium oxalate	Calcium present

ORGANIC CONSTITUENTS

TEST FOR NORMAL ORGANIC CONSTITUENTS OF URINE :

Test	Observation	Inference
Test for urea 1. 3 ml of urine + 5 drops of alkaline sodium hyprobromate solution (NaOBr) 2. 5 ml urine + 4 drops of phenolphthalein indicator + pinchfull of ureas powder and mix. Allow it to stand for five minutes.	Effervesence of Nitrogen gas Solution becomes pink (if solution is already pink before adding urease add 10 % acetic acid to decolourise it).	Urea present. Urea present.
Test for uric acid 1. (Schiffs test) Moisten a strip of filter paper with $AgNO_3$ solution to it add a drop of urine. 2. 5 ml urine + 5 drops of benedicts uric acid reagent + 3 g of anhydrous Na_2CO_3 and mix by shaking.	Black or yellow brown stain formed. A deep blue colour develops.	Uric acid present. Uric acid present.
Test for creatinine (Weyl's test) 1. 5 ml of urine + 5 drops of sodium nitropruside + 2 ml of 10 % NaOH 2. Jaffes test. 5 ml of urine + 1 ml of saturated solution of picric acid + 3 g of anhydrous Na_2CO_3 mix well by shaking.	Rubby red colour is formed and soon changes to yellow. A deep orange colour is formed.	Creatinine present Creatinine present.

7.0 OBSERVATION TABLE :

Student to write test observation of the tests performed on given urine sample.

Test	Observation	Inference

Test	Observation	Inference

8.0 RESULT:

Given urine sample contains normal constituents.

a. Inorganic ----------

b. Organic ----------

9.0 Questions:

(Note - Student to answer question Q....., Q....., Q..... and question number shall be allotted by teacher.)

1. Name organic constituents of urine.
2. Write the test for identification of creatinine in urine sample.
3. How bicarbonate's ions are detected in urine sample ?
4. Name inorganic constituents present in urine.
5. Write the name of apparatus by which specific gravity of Urine is measured
6. Write quantity of urea, uric acid and creatinine excreated per 24 hours.
7. Write the quantity of chloride, phosphate, sulphate, ammonium excreted per 24 hours.
8. Write the test for detection of ammonium in urine sample.
10. Write pH of normal urine.
11. Write the Physiological and Pathological condition in which urine volume is increased.
12. Name the harmones involved in urine formation.
13. Write the Physiological and Pathological condition in which urine volume is decreasesd.

Space for answers

(Space for answers)

Signature of Teacher

Experiment No. 20

1.0 TITLE:

To detect abnormal constituents in given sample of urine by qualitative tests. (Sample No.1)

2.0 PRIOR CONCEPTS:

The constituents which are not present in normal urine are excreted in urine such constituent are abnormal constituents.

3.0 NEW CONCEPTS:

Proposition 1 : Pathological urine

The urine which contains essential of body like glucose, proteins, ketone bodies, bile salt, bile pigment, blood , etc.

Proposition 2 :

Abnormal constituent	Significante disease
Sugar (Glucose)	Glycosuria
Protein	Protinuria
Ketone bodies	Ketonuria
Bile salt	Jaundice
Bile pigment	Hepatitis
Blood	Haematuria

4.0 LEARNING OBJECTIVES:

Intellectual skill:

1. To understand concept of detection of abnormal constituents of urine.
2. To understand identification test of abnormal constituents of urine.

Motor skills:

1. Ability to add proper volume of reagent.
2. Ability to observe colour produced in test.

5.0 APPARATUS:

Glassware

Test tubes, Beakers, Test tube stand, Gas, Burner, Glass rod etc.

Chemicals

Concentrated HNO3, 0.1 N.Chlorophenol red solution, Ammonium sulphate powder 1 N Sulphosalicylic acid solution, Fehling A solution, Glacial Acetic acid solution, Benzidine powder Sulphur powder Strong Ammonia.,Solution, Fehling B solution, 0.1 N. HCl solution, Hybrogen Peroxide Solution

6.0 STEPWISE PROCEDURE :

Table for performing test on urine sample with reagents.

1. Use urine sample as a original solution
2. Perform following tests on given urine sample and interpret as mentioned in following table.

Physical properties

Table for performing test on urine sample with reagents.

Test	Observation	Inference
Volume	(a) 1000 ml-1500 ml per day	Normal
	(b) More than 1500 ml per day	(1) Polyuria may be due to more water intake, less perspiration, high protein diet diuretic substance like alcohol, coffee, tea, diseased state like diabetes inspidius
	(c) Less than 1000 ml/ day	may be due to 1. Hard physical work 2. Fever 3. Dehydration 4. Vomitting, diarrhoea 5. Acute nephrittis.
	(d) No urine	Anuria due to acute renal insufficiency.
Colour (observe within 60 minutes)	(a) Pale yellow	(a) Normal due to presence of urochrome pigment.
	(b) Marked yellow	(b) Normal due to decreased urine output.
	(c) Light yellow (Slightly yellow than pale yellow)	(c) Seen after heavy meals.
	(d) Yellowish green brown	(d) Abnormal indicates condition like jaundice.
	(e) Reddish	(e) Abnormal due to haematuria.
	(f) Dirty bluish	(f) Abnormal like in cholera and typhus.
	(g) Milky	(g) Abnormal like in pyuria or chyluria (Pus and fat respectively)
	(h) Brown colour	(h) Abnormalprecipitates of urate, phosphates etc.
Odour :	(a) Peculier, aromatic	(a) Normal.
	(b) Unpleasant aromatic	(b) Due to various drug metabolites and microbial decomposition.
pH	(a) 6 - 7.5	(a) Normal
	(b) Acidic (below 6)	(b) Normally seen after high meat diet. Abnormally seen in acidosis.
	(c) Alkaline (above 8)	(c) Abnormally seen in alkalosis.

ABNORMAL CONSTITUENTS

ANALYSIS OF URINE FOR ABNORMAL CONSTITUENTS

Test	Observation	Inference
Test for proteins (Albumin and globulin)		
(i) Sulphosalicylic acid test : 3 ml clear urine + sulphosalicyclic acid drop by drop.	White ppt appears	Albumin present
(ii) Hellers nitric acid ring test : 3 ml conc. HNO_3 + add from side of test tube dropwise urine	White ring at the junction of two fluids.	Albumin confirmed

Test	Observation	Inference
(iii) Heat co-agulation test : 3ml urine + 2 drops of chlorophenol red, adjust the pH faint pink colour by adding 1 % Na_2CO_3 boil for two minutes.	Turbidity or precipitates	Albumin confirmed
Add 5 drops acetic acid.	Turbidity remains	Albumin confirmed
Test for sugar (Glucose)		
(i) Benedicts test : 5ml urine + 5ml benedicts reagent boil for two minutes and cool	(i) Green ppt. (ii) Yellow ppt. (iii) Red ppt.	Glucose present –1% Glucose present –2% Glucose present more than 2%
(ii) Fehlings test : 2 ml fehlings A + 2 ml fehlings B boil for few minutes, add 2-3 ml urine boil again.	Red/Yellow ppt appears	Glucose confirmed.
Test for Ketones : (Rothera's test) 5 ml urine + solid $(NH_4)_2 SO_4$ to saturate it completely + 2 drops of sodium nitropruside solution + 2 ml strong ammonia solution from sides of test tube wait for 10 minutes.	Permanganate colour develops	Ketones like acetone present.
Test for bile salts 5 ml urine in beaker sprinkle sublimed sulphur powder on surface.	(i) Powder sinks to bottom (ii) Powder floats on the surface	Bile salts present. Bile salts present.
Test for bile pigments		
(a) Gmelins test (modified) 10 ml urine + 2-3 drops of dilute. HCl filter it through paper, allow it to dry put a drop of conc. HNO_3 at the apex of paper.	Coloration on paper in following order green, blue, violet, red and yellowish red seen. Fine play of colours.	Bile pigments present.
(b) Nitric acid test : 3 ml conc. Nitric acid, add urine slowly from side of test tube.		Bile pigments present.
Test for blood (Benzidine test) Pinch of benzidine powder + 1 ml of glacial acetic acid shake for 1 minute + 2 ml urine + few drops of H_2O_2	Green/blue colour due to iron benzidine formation.	Blood present.

7.0 OBSERVATION TABLE :

Student to write observations of physical and chemical tests performed on given urine sample.

Test	Observation	Inference

Test	Observation	Inference

8.0 RESULT:

Given urine sample contains Abnormal constituents ----------

9.0 Questions:

(Note - Student to answer question Q……,Q……..,Q…….. and Question number shall be allotted by teacher.)

1. Name five abnormal constituents of urine ?
2. Define glycosuriya
3. Define Haematouria
4. Define Ketouria
5. Define Pentosuria
6. Why glucose albumin, ketone bodies are called abnormal constituents of urine
7. Define Albuminuria
8. Write Rothra's the test for detection of Ketone bodies in a urine
9. Write the test for detection of glucose in urine
10. Write the test for detection of blood in urine
11. Define Jaundice
12. Write the test for detection of bile salt in urine
13. Name two tests by which albumin can be detected in urine

Space for answers

(Space for answers)

Signature of Teacher

Experiment No. 21

1.0 TITLE:

To detect abnormal constituents in given sample of urine by qualitative tests. (Sample No.2)

2.0 PRIOR CONCEPTS:

The constituents which are not present in normal urine are excreted in urine such constituent are abnormal constituents .

3.0 NEW CONCEPTS: Abnormal constituents

Proposition 1: Pathological urine

The urine which contains essential of body like glucose, proteins, ketone bodies, bile salt, bile pigment, blood , etc.

Proposition 2 :

Abnormal constituent	Significante disease
Sugar (Glucose)	Glycosuria
Protein	Protinuria
Ketone bodies	Ketonuria
Bile salt	Jaundice
Bile pigment	Hepatitis
Blood	Haematuria

4.0 LEARNING OBJECTIVES:

Intellectual skill:

1. To understand concept of detection of abnormal constituents of urine.
2. To understand identification test of abnormal constituents of urine.

Motor skills:

1. Ability to add proper volume of reagent.
2. Ability to observe colour produced in test.

5.0 APPARATUS:

Glassware

Test tubes, Beakers, Test tube stand, Gas, Burner, Glass rod etc.

Chemicals

Concentrated HNO_3, 0.1 N.Chlorophenol red solution, Ammonium sulphate powder 1 N Sulphosalicylic acid solution, Fehling A solution, Glacial Acetic acid solution, Benzidine powder Sulphur powder Strong Ammonia.,Solution, Fehling B solution, 0.1 N. HCl solution, Hybrogen Peroxide Solution

6.0 STEPWISE PROCEDURE :

Table for performing test on urine sample with reagents.

1. Use urine sample as a original solution
2. Perform following tests on given urine sample and interpret as mentioned in following table

Physical properties

Table for performing test on urine sample with reagents.

Test	Observation	Inference
Volume	(a) 1000 ml-1500 ml per day	Normal
	(b) More than 1500 ml per day	(1) Polyuria may be due to more water intake, less perspiration, high protein diet diuretic substance like alcohol, coffee, tea, diseased state like diabetes inspidius
	(c) Less than 1000 ml/ day	may be due to 1. Hard physical work 2. Fever 3. Dehydration 4. Vomitting, diarrhoea 5. Acute nephrittis.
	(d) No urine	Anuria due to acute renal insufficiency.
Colour (observe within 60 minutes)	(a) Pale yellow	(a) Normal due to presence of urochrome pigment.
	(b) Marked yellow	(b) Normal due to decreased urine output.
	(c) Light yellow (Slightly yellow than pale yellow)	(c) Seen after heavy meals.
	(d) Yellowish green brown	(d) Abnormal indicates condition like jaundice.
	(e) Reddish	(e) Abnormal due to haematuria.
	(f) Dirty bluish	(f) Abnormal like in cholera and typhus.
	(g) Milky	(g) Abnormal like in pyuria or chyluria (Pus and fat respectively)
	(h) Brown colour	(h) Abnormalprecipitates of urate, phosphates etc.
Odour :	(a) Peculier, aromatic	(a) Normal.
	(b) Unpleasant aromatic	(b) Due to various drug metabolites and microbial decomposition.
pH	(a) 6 - 7.5	(a) Normal
	(b) Acidic (below 6)	(b) Normally seen after high meat diet. Abnormally seen in acidosis.
	(c) Alkaline (above 8)	(c) Abnormally seen in alkalosis.

ABNORMAL CONSTITUENTS

ANALYSIS OF URINE FOR ABNORMAL CONSTITUENTS

Test	Observation	Inference
Test for proteins (Albumin and globulin)		
(i) Salphosalicylic acid test : 3 ml clear urine + sulphosalicyclic acid drop by drop.	White ppt appears	Albumin present
(ii) Hellers nitric acid ring test : 3 ml conc. HNO_3 + add from side of test tube dropwise urine	White ring at the junction of two fluids.	Albumin confirmed

Test	Observation	Inference
(iii) Heat co-agulation test : 3ml urine + 2 drops of chlorophenol red, adjust the pH faint pink colour by adding 1 % Na_2CO_3 boil for two minutes. Add 5 drops acetic acid.	Turbidity or precipitates Turbidity remains	Albumin confirmed Albumin confirmed
Test for sugar (Glucose) (i) Benedicts test : 5ml urine + 5ml benedicts reagent boil for two minutes and cool (ii) Fehlings test : 2 ml fehlings A + 2 ml fehlings B boil for few minutes, add 2-3 ml urine boil again.	(i) Green ppt. (ii) Yellow ppt. (iii) Red ppt. Red/Yellow ppt appears	Glucose present –1% Glucose present –2% Glucose present more than 2% Glucose confirmed.
Test for Ketones : (Rothera's test) 5 ml urine + solid $(NH_4)_2\ SO_4$ to saturate it completely + 2 drops of sodium nitropruside solution + 2 ml strong ammonia solution from sides of test tube wait for 10 minutes.	Permanganate colour develops	Ketones like acetone present.
Test for bile salts 5 ml urine in beaker sprinkle sublimed sulphur powder on surface.	(i) Powder sinks to bottom (ii) Powder floats on the surface	Bile salts present. Bile salts present.
Test for bile pigments (a) Gmelins test (modified) 10 ml urine + 2-3 drops of dilute. HCl filter it through paper, allow it to dry put a drop of conc. HNO_3 at the apex of paper. (b) Nitric acid test : 3 ml conc. Nitric acid, add urine slowly from side of test tube.	Coloration on paper in following order green, blue, violet, red and yellowish red seen. Fine play of colours.	Bile pigments present. Bile pigments present.
Test for blood (Benzidine test) Pinch of benzidine powder + 1 ml of glacial acetic acid shake for 1 minute + 2 ml urine + few drops of H_2O_2	Green/blue colour due to iron benzidine formation.	Blood present.

7.0 OBSERVATION TABLE :

Student to write observations of physical and chemical tests performed on given urine sample.

Test	Observation	Inference

Test	Observation	Inference

8.0 RESULT:

Given urine sample contains Abnormal constituents ----------

9.0 Questions:

(Note - Student to answer question Q……,Q……,Q…….. and Question number shall be allotted by teacher.)

1. Name five abnormal constituents of urine ?
2. Define glycosuriya
3. Define Haematouria
4. Define Ketouria
5. Define Pentosuria
6. Why glucose albumin, ketone bodies are called abnormal constituents of urine
7. Define Albuminuria
8. Write Rothra's the test for detection of Ketone bodies in a urine
9. Write the test for detection of glucose in urine
10. Write the test for detection of blood in urine
11. Define Jaundice
12. Write the test for detection of bile salt in urine
13. Name two tests by which albumin can be detected in urine

Space for answers

(Space for answers)

Signature of Teacher

Experiment No. 22

1.0 TITLE:

To detect abnormal constituents in given sample of urine by qualitative tests. (Sample No.3)

2.0 PRIOR CONCEPTS:

The constituents which are not present in normal urine are excreted in urine such constituent are abnormal constituents.

3.0 NEW CONCEPTS: Abnormal constituents

Proposition 1: Pathological urine

The urine which contains essential of body like glucose, proteins, ketone bodies, bile salt, bile pigment, blood , etc.

Proposition 2 :

Abnormal constituent	Significante disease
Sugar (Glucose)	Glycosuria
Protein	Protinuria
Ketone bodies	Ketonuria
Bile salt	Jaundice
Bile pigment	Hepatitis
Blood	Haematuria

4.0 LEARNING OBJECTIVES:

Intellectual skill:

1. To understand concept of detection of abnormal constituents of urine.
2. To understand identification test of abnormal constituents of urine.

Motor skills:

1. Ability to add proper volume of reagent.
2. Ability to observe colour produced in test.

5.0 APPARATUS:

Glassware

Test tubes, Beakers, Test tube stand, Gas, Burner, Glass rod etc.

Chemicals

Concentrated HNO_3, 0.1 N.Chlorophenol red solution, Ammonium sulphate powder 1 N Sulphosalicylic acid solution, Fehling A solution, Glacial Acetic acid solution, Benzidine powder Sulphur powder Strong Ammonia.,Solution, Fehling B solution, 0.1 N. HCl solution, Hybrogen Peroxide Solution

6.0 STEPWISE PROCEDURE :

Table for performing test on urine sample with reagents.

1. Use urine sample as a original solution
2. Perform following tests on given urine sample and interpret as mentioned in following table

Physical properties

Table for performing test on urine sample with reagents.

Test	Observation	Inference
Volume	(a) 1000 ml-1500 ml per day	Normal
	(b) More than 1500 ml per day	(1) Polyuria may be due to more water intake, less perspiration, high protein diet diuretic substance like alcohol, coffee, tea, diseased state like diabetes inspidius
	(c) Less than 1000 ml/ day	may be due to 1. Hard physical work 2. Fever 3. Dehydration 4. Vomitting, diarrhoea 5. Acute nephrittis.
	(d) No urine	Anuria due to acute renal insufficiency.
Colour (observe within 60 minutes)	(a) Pale yellow	(a) Normal due to presence of urochrome pigment.
	(b) Marked yellow	(b) Normal due to decreased urine output.
	(c) Light yellow (Slightly yellow than pale yellow)	(c) Seen after heavy meals.
	(d) Yellowish green brown	(d) Abnormal indicates condition like jaundice.
	(e) Reddish	(e) Abnormal due to haematuria.
	(f) Dirty bluish	(f) Abnormal like in cholera and typhus.
	(g) Milky	(g) Abnormal like in pyuria or chyluria (Pus and fat respectively)
	(h) Brown colour	(h) Abnormalprecipitates of urate, phosphates etc.
Odour :	(a) Peculier, aromatic	(a) Normal.
	(b) Unpleasant aromatic	(b) Due to various drug metabolites and microbial decomposition.
pH	(a) 6 - 7.5	(a) Normal
	(b) Acidic (below 6)	(b) Normally seen after high meat diet. Abnormally seen in acidosis.
	(c) Alkaline (above 8)	(c) Abnormally seen in alkalosis.

ABNORMAL CONSTITUENTS

ANALYSIS OF URINE FOR ABNORMAL CONSTITUENTS

Test	Observation	Inference
Test for proteins (Albumin and globulin)		
(i) Salphosalicylic acid test : 3 ml clear urine + sulphosalicyclic acid drop by drop.	White ppt appears	Albumin present
(ii) Hellers nitric acid ring test : 3 ml conc. HNO_3 + add from side of test tube dropwise urine	White ring at the junction of two fluids.	Albumin confirmed

Test	Observation	Inference
(iii) Heat co-agulation test : 3ml urine + 2 drops of chlorophenol red, adjust the pH faint pink colour by adding 1 % Na_2CO_3 boil for two minutes. Add 5 drops acetic acid.	Turbidity or precipitates Turbidity remains	Albumin confirmed Albumin confirmed
Test for sugar (Glucose) (i) Benedicts test : 5ml urine + 5ml benedicts reagent boil for two minutes and cool (ii) Fehlings test : 2 ml fehlings A + 2 ml fehlings B boil for few minutes, add 2-3 ml urine boil again.	(i) Green ppt. (ii) Yellow ppt. (iii) Red ppt. Red/Yellow ppt appears	Glucose present –1% Glucose present –2% Glucose present more than 2% Glucose confirmed.
Test for Ketones : (Rothera's test) 5 ml urine + solid $(NH_4)_2\ SO_4$ to saturate it completely + 2 drops of sodium nitropruside solution + 2 ml strong ammonia solution from sides of test tube wait for 10 minutes.	Permanganate colour develops	Ketones like acetone present.
Test for bile salts 5 ml urine in beaker sprinkle sublimed sulphur powder on surface.	(i) Powder sinks to bottom (ii) Powder floats on the surface	Bile salts present. Bile salts present.
Test for bile pigments (a) Gmelins test (modified) 10 ml urine + 2-3 drops of dilute. HCl filter it through paper, allow it to dry put a drop of conc. HNO_3 at the apex of paper. (b) Nitric acid test : 3 ml conc. Nitric acid, add urine slowly from side of test tube.	Coloration on paper in following order green, blue, violet, red and yellowish red seen. Fine play of colours.	Bile pigments present. Bile pigments present.
Test for blood (Benzidine test) Pinch of benzidine powder + 1 ml of glacial acetic acid shake for 1 minute + 2 ml urine + few drops of H_2O_2	Green/blue colour due to iron benzidine formation.	Blood present.

7.0 OBSERVATION TABLE :

Student to write observations of physical and chemical tests performed on given urine sample.

Test	Observation	Inference

Test	Observation	Inference

8.0 RESULT:

Given urine sample contains Abnormal constituents ----------

9.0 Questions:

(Note - Student to answer question Q…….,Q……..,Q…….. and Question number shall be allotted by teacher.)

1. Name five abnormal constituents of urine ?
2. Define glycosuriya
3. Define Haematouria
4. Define Ketouria
5. Define Pentosuria
6. Why glucose albumin, ketone bodies are called abnormal constituents of urine
7. Define Albuminuria
8. Write Rothra's the test for detection of Ketone bodies in a urine
9. Write the test for detection of glucose in urine
10. Write the test for detection of blood in urine
11. Define Jaundice
12. Write the test for detection of bile salt in urine
13. Name two tests by which albumin can be detected in urine

Space for answers

(Space for answers)

Signature of Teacher

Experiment No. 23

1.0 TITLE:

To estimate quantity of glucose in given sample of urine (by Benedict's quantitative method)

2.0 PRIOR CONCEPTS: Glycosuria

It is abnormal quantity of glucose excreted in urine.

Diabetes mellitus

3.0 NEW CONCEPTS:

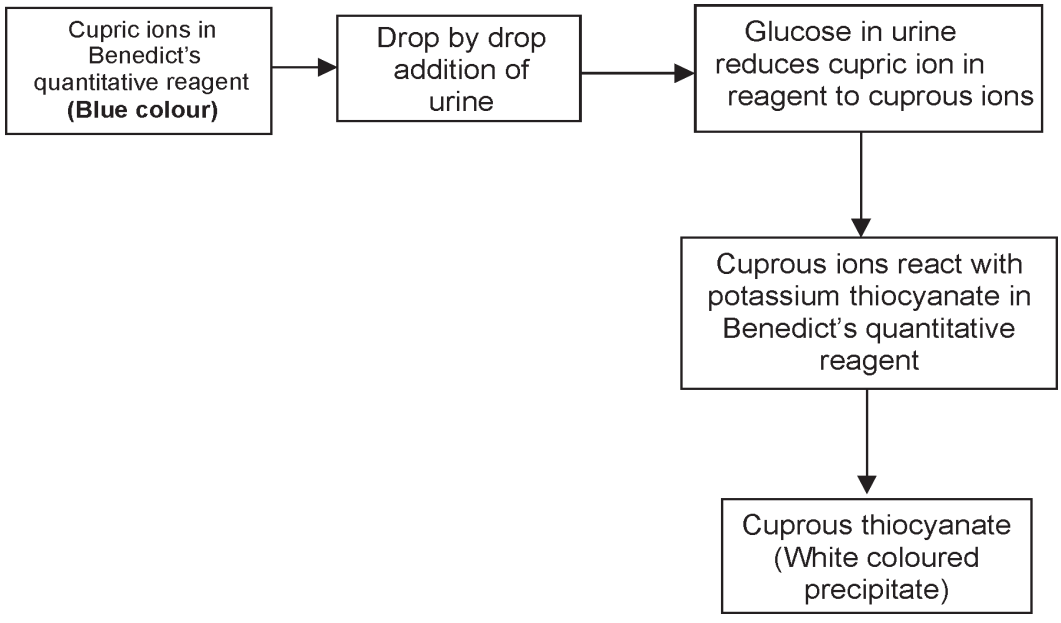
Proposition 1:

Amount of glucose in urine can be measured by Benedict's quantitative method.

Proposition 2 : Benedict's quantitative test

Benedict's quantitative reagent contains potassium thiocyanate, potassium ferrocyanide, sodium citrate, sodium carbonate and copper sulphate. Glucose reduces cupric ions in the reagent to cuprous ions, which react with potassium thiocyanate, to form white coloured precipitate of cuprous thiocyanate.

Concept structure:



Proposition 3 : Benedict's quantitative test

Determination of glucose concentration in urine is important for management of Diabetes mellitus.

4.0 LEARNING OBJECTIVES:

Intellectual skill:

1. To understand concept of Benedict's quantitative method.
2. To calculate amount of glucose in given urine sample.

Motor skills:

1. Ability to observe colour obtained in urine dilution test
2. Ability to add urine sample drop by drop into boiling solution of Benedict's quantitative regent.

5.0 APPARATUS:

Glassware

Conical flask, burette, pipette,

Chemicals

Benedict's qualitative reagent,

Benedict's quantitative reagent:- 100g of anhydrous sodium carbonate + 200g of sodium citrate + 125 g of potassium thiocyanate, add distilled water to make 600ml Heat to dissolve the chemicals, cool and filter(if necessary)

To it add copper sulphate 18g and 5ml 5%w/v potassium ferrocyanide mix and make volume upto 1000ml with distilled water.

Anhydrous sodium carbonate, urine sample.

6.0 DIAGRAM:

To estimate quantity of glucose in given sample of

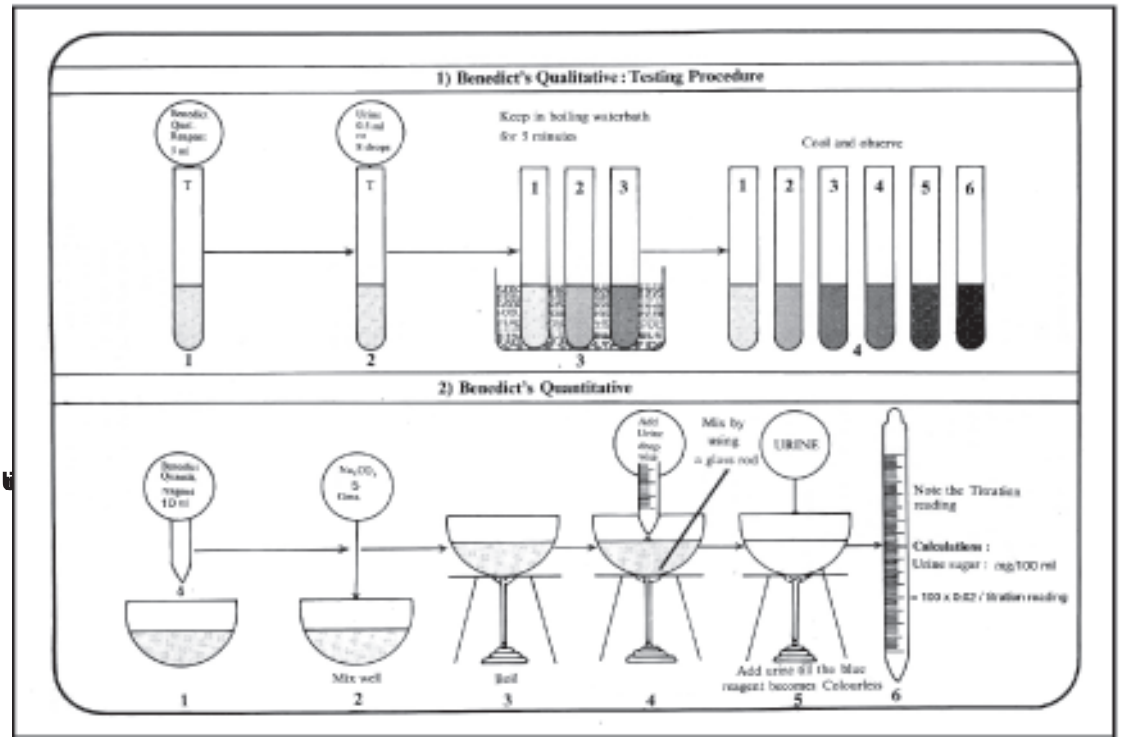


Fig. 23.1

7.0 STEPWISE PROCEDURE:

1. Take 5ml of benedict's qualitative reagent in a test tube and add 8 drops of given urine sample.Boil the mixture and cool. Observe the colour obtained.
2. If colour of solution/ precipitate is green, dilute given urine sample1: 2 i.e 10ml urine sample + 10ml distilled water to make the total volume 20ml.

3. If colour of solution/ precipitate is yellow, dilute given urine sample 1:5 i.e 10ml urine sample + 40ml distilled water to make the total volume 50ml.
4. If colour of solution/ precipitate is brick red, dilute given urine sample 1:10 i.e. 10ml urine sample + 90ml distilled water to make the total volume 100ml.
5. Pipette out 10 ml benedict's quantitative reagent in 100ml conical flask ; add 20 ml water and 5g anhydrous sodium carbonate mix well, add few pieces of porcelin.
6. Heat the flask on flame such that mixture is kept boiling throughout titration period.
7. Fill the burette with diluted urine.
8. Add urine sample rapidly in the flask until white precipitate appears.
9. After this add urine sample from burette drop by drop with constant stirring till blue colour of reagent disappears and white precipitate is formed. (Blue colour changes to greenish yellow then white)
10. Note the titration reading.

8.0 OBSERVATION :

1. Colour obtained in step 7.1 ……..
2. Dilution of urine sample
   10ml urine sample + ……. ml distilled water to make total volume ……..ml
3. Volume of urine sample required for 10ml Benedict's quantitative reagent = X ml
   (Burette reading)
   = ……ml

9.0 CALCULATIONS:

10ml of Benedict's quantitative reagent = 20mg of glucose = 0.020g of glucose

therefore X ml diluted urine sample contains = 0.020g of glucose

i.e ….. ml diluted urine sample contains = 0.020g of glucose.

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

$$= \frac{100 \times 0.02}{.....} \text{ g of glucose}$$

$$= \text{Y g}$$

therefore 100ml diluted sample of urine contains = ……g of glucose.

Percentage of glucose in diluted urine = Y %

Therefore Percentage of glucose in diluted urine = …… %

Percentage of glucose in given urine sample = Y % x dilution factor.

= .....x.....

= …. g %

Note : Dilution factor is 2 when 10ml urine sample is diluted with 10ml water to make total volume 20ml

Dilution factor is 5 when 10 ml urine is diluted with 40ml water to make total volume 50ml

Dilution factor is 10, when 10ml urine is diluted with 90ml distilled water to make total volume 100ml.

10.0 RESULT:

1. Quantity of glucose in given sample of urine is …….. g/100ml i.e ……g%
2. As glucose content of given sample of urine is …….. (less/more) than normal value.
   Patient is suffering from ……
   Normal value of glucose excretion in urine is 2 to 10mg glucose/100ml or 78.5 mg/day

11.0 QUESTIONS:

Note : Students to answer Q....., Q....., Q....., and the question numbers shall be allotted by teacher.

1. State principle involved in estimation of glucose in urine by Benedict's method.
2. Why there is colour change from blue to white after addition of urine sample to Benedict's quantitative reagent. Give specific reason.
3. What is composition of Benedict's quantitative reagent.
4. If glucose content of urine is 0.072g/100ml patient is suffering from which disorder/disease?
5. Calculate glucose content of urine when volume of urine sample required for 10ml Benedict's quantitative reagent is 12ml and dilution factor is 5.
6. State other method available for estimation of glucose in urine.

12.0 REFERENCE :

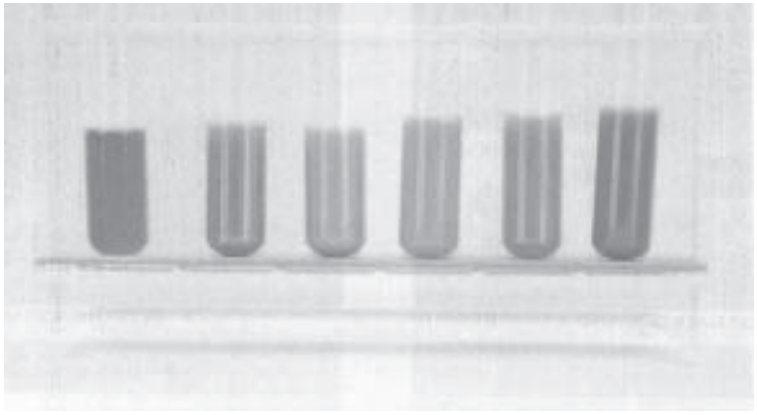


Fig. 23.2

(Space for answers)

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Signature of Teacher

Experiment No. 24

1.0 TITLE:

To estimate quantity of creatinine in given sample of urine by Alkaline picrate method (Jaffe's reaction)

2.0 PRIOR CONCEPTS: Urine

It is chief excretory fluid eliminated through kidney. Waste products are eliminated through urine.

Normal Constituents of urine

Urine Contains organic constituents urea, uric acid, creatinine and inorganic constituents chlorides, phosphates, sulphates, bicarbonates, ammonia and calcium etc.

3.0 NEW CONCEPTS :

Proposition 1: Creatinine

Creatinine is end product of creatine metabolism. It is an anhydride of creatine.

Proposition 2 : Creatine

Creatine is present in muscles, brain and blood in free form as well as in the form of creatine phosphate. Creatine phosphate gives supply of readily available energy to muscles.

Concept structure:

Creatine phosphate + ADP $\rightleftharpoons$ ATP + Creatine

(in muscles)

During severe exercise creatine phosphate stored in muscles is converted to ATP

Proposition 3 : Excretion of Creatinine

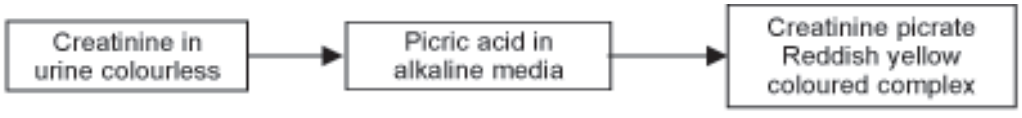
Creatinine is formed in body from spontaneous breakdown of creatine phosphate. It is a nonthreshold substance. It is filtered by glomerulli. It's excretion is not related with food protein. It is remarkably constant. It's variation in excretion indicates metabolic disorder. It's excretion in urine increases in fever, starvation, on carbohydrate free diet and in diabetes mellitus.

Proposition 4 : Alkaline Picrate Method

Creatinine reacts with picric acid in alkaline medium to form reddish yellow coloured complex creatinine picrate.The intensity of colour developed is directly proportional to the amount of creatinine present in urine and is compared with that of standard creatinine solution similarly treated against reagent blank at green filter / 520nm using colourimeter or spectrophotometer respectively.

Estimation of quantity of creatinine in urine is useful in calculation of creatinine clearance.

Concept Structure:



4.0 LEARNING OBJECTIVES:

Intellectual skill:

1. To understand concept of alkaline picrate method.
2. To calculate amount of creatinine in urine.

Motor skills:

1. Ability to add proper amount of urine, creatinine working standard, picric acid, sodium hydroxide solution and distilled water in blank, standard and test solution respectively.
2. Ability to operate colourimeter.
3. Ability to measure % Transmittance and Absorbance.

5.0 APPARATUS:

Glassware

Conical flask, pipette, test tubes, volumetric flask, cuvettes, photo electric colorimeter / spectrophotometer.

Chemicals

24 hours urine sample, picric acid (0.9%w/v),Sodium hydroxide (3%w/v), Distilled water Creatinine stock (1mg/ml) 100mg creatinine in 100ml of 0.1N HCl, Creatinine working standard (0.01mg/ml) Dilute 1.0 ml of stock creatinine solution to 100ml with distilled water in volumetric flask.

6.0 DIAGRAM:

To estimate quantity of creatitine in given sample of urine

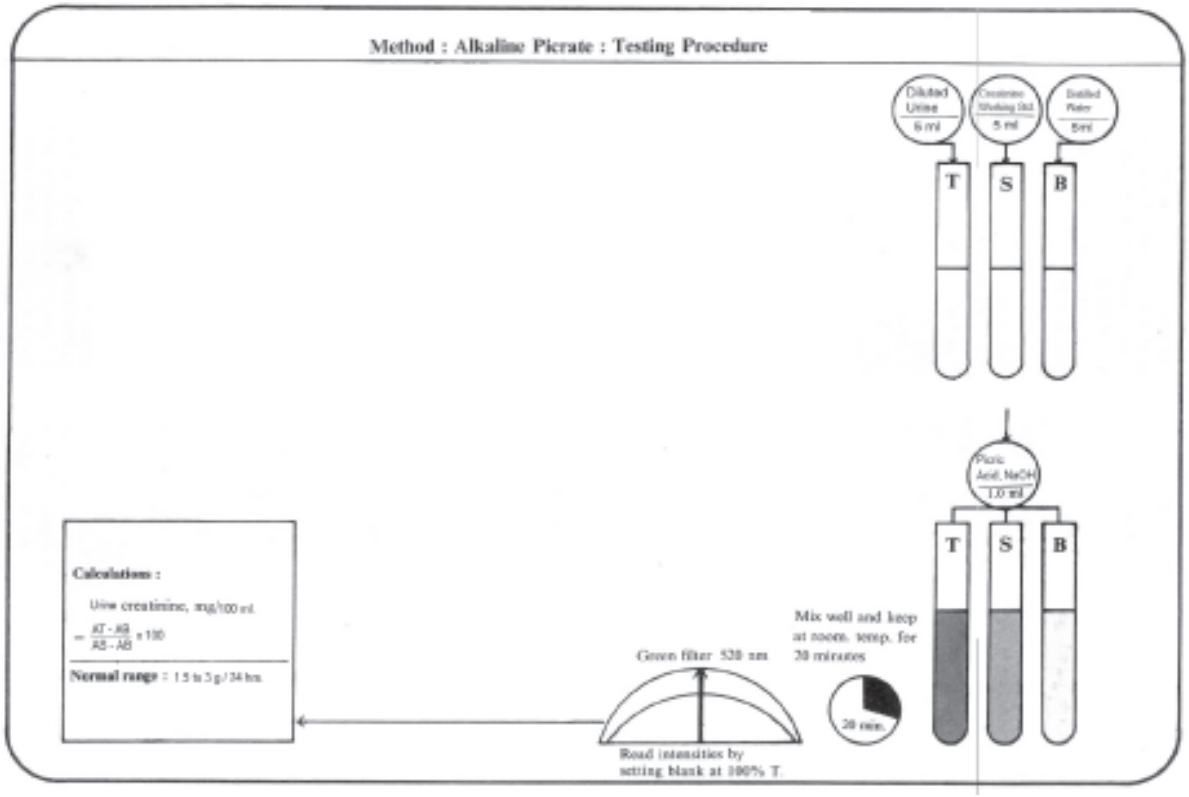


Fig. 24.1

7.0 STEPWISE PROCEDURE:

1. Prepare creatinine working standard by diluting 1.0ml of stock creatinine solution to 100ml with distilled water in volumetric flask. So the concentration of creatinine will be 0.01mg/ml.
2. Dilute 1.0ml of given urine sample to 100ml in volumetric flask.
3. Prepare blank standard, test solution as mentioned in following table

	Blank	Standard	Test
Distilled water	5.0 ml	--	--
Creatinine working standard (0.01mg/ml)	--	5.0 ml	--
Diluted urine	--	--	5.0ml
Picric acid (0.9%w/v)	1.0ml	1.0ml	1.0ml
Sodium hydroxide (3%w/v)	1.0ml	1.0ml	1.0ml

4. Allow it to stand for 15 minutes.
5. Set photoelectric colorimeter at green filter / spectrophotometer at 520 nm
6. Adjust 0% T (transmission)
7. Adjust 100% T (transmission) with distilled water.
8. Measure Absorbance or calculate Absorbance for blank standard and test

8.0 OBSERVATION :

Absorbance of blank A_B =

Absorbance of standard A_S=

Absorbance of Test A_T =

9.0 CALCULATIONS :

Concentration of creatinine in test solution C_T = $\frac{A_T - A_B}{A_S - A_B} \times C_S$

Where C_S = 0.05mg of creatinine

Concentration of creatinine in test solution C_T = $\frac{A_T - A_B}{A_S - A_B} \times 0.05$

Now as 1.0ml of urine is diluted to 100ml and 5.0 ml is taken in test solution concentration of creatinine in 0.05ml undiluted urine = C_T

Hence concentration of creatinine in 100ml urine = $\frac{C_T \times 100}{0.05}$

$= \frac{\frac{A_T - A_B}{A_S - A_B} \times 0.05 \times 100}{0.05}$

$= \frac{A_T - A_B}{A_S - A_B} \times 100$

Hence mg of creatinine in 100ml sample of urine = $\frac{\text{.....} - \text{.....}}{\text{.....} - \text{.....}} \times 100$ mg/100ml

= xmg/100ml

= mg/100ml

G of creatinine in 1000 ml sample of urine $= \frac{X}{1000} \times 10 \text{ g / lit}$

$= \frac{\ldots\ldots\ldots\ldots}{1000} \times 10 \text{ g / lit}$

$= Y \text{ g / lit}$

$= \ldots\ldots\ldots \text{ g/lit}$

hence amount of creatinine in 24 hours urine sample $= \frac{\text{Volume of 24 hours urine sample in liters x Y}}{1}$

$= \frac{\ldots\ldots\ldots \times \ldots\ldots\ldots}{1}$

$= \ldots\ldots\ldots \text{ g/lit}$

10.0 RESULT:

Amount of creatinine in given sample of urine …….. g / 24 hrs

Normal range is 1.5 to 3.0 g / 24 hrs.

11.0 QUESTIONS:

Note : Students to answer Q....., Q....., Q....., and the question numbers shall be allotted by teacher.

1. Name the method used for estimation of creatinine in urine?
2. Which colour developing agent is used for estimation of creatinine in urine by colorimeter?
3. What is colour of creatinine picrate?
4. State the wavelength and filter at which estimation of creatinine in urine is done by colorimeter
5. If patient's urine report 3.5g creatinine in 24 hours, Name the diseases he is suffering from?
6. State the condition in which creatinine excretion is increased in urine
7. Name the standard used for estimation of creatinine in urine by colorimeter
8. How creatinine is formed in body ?
9. State the factors by which creatinine excretion in urine is affected.
10. What is normal value of excretion of creatinine in urine?

(Space for answers)

(Space for answers)

Signature of Teacher

Experiment No. 25

1.0 TITLE:

To estimate quantity of urea nitrogen in given sample of urine by Diacetyl monoxime method.

2.0 PRIOR CONCEPTS : Urea cycle / Kreb cycle

Urea formation takes place in liver. Two molecules of ammonia and one molecule of carbon dioxide are converted to urea in each turn of cycle which is known as urea cycle or kreb cycle.

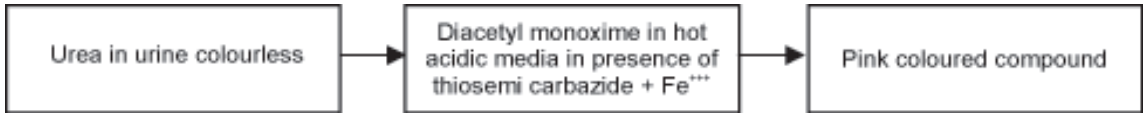
Urea is principle end product of proteins, amino acid metabolism in body. Urea is filtered at glomeruli and after partial reabsorption excreated in urine. In 24 hours about 20 g to 30 g urea is excreted. Excretion of urea in urine varies directly with protein intake. It is increased in fever, diabetes, excess adrenocortical activity. While decreased in liver diseases and acidosis.

3.0 NEW CONCEPTS:

Proposition 1 : Diacetyl Monoxime Method (DAM method)

Urea reacts with diacetyl monoxime in hot acidic medium and in the presence of thiosemicarbazide and ferric ions to form a pink coloured compound which can be measured at green filter/ wavelength 530nm.

Concept Structure



Proposition 2 :

Estimation of urinary urea is important to evaluate kidney function by urea clearance test.

4.0 LEARNING OBJECTIVES:

Intellectual Skills

1. To understand concept of diacetyl monoxime method.
2. To calculate urea nitrogen in g/lit of urine.

Motor Skills

1. Ability to prepare test, standard and blank solutions.
2. Ability to operate spectrophotometer / photoelectric colourimeter.

5.0 APPARATUS:

Glasswares

Test tubes, pipettes, measuring cylinder, conical flask, waterbath, stop watch, photoelectric colourimeter / spectrophotometer.

Chemicals

Urine sample, Diacetyl monoxime reagent (0.2% w/v diacetyl monoxime in distilled water), Thiosemicarbazide reagent (40 mg thiosemicarbazide in 100 ml water), Acid reagent (Mix 60 ml concentrated sulphuric acid, 10 ml of orthophosphoric acid and 10 ml of 1 % ferric chloride, make volume to 1000 ml with distilled water) urea nitrogen standard 20 mg/100 ml (dissolve 42.8 mg of urea in 1000 ml of saturated benzoic acid, as urea = 2.14 mg x urea N since molecular weight of urea is 60 and that of urea nitrogen is 28 according to molecular formula NH_2CONH_2)

6.0 STEPWISE PROCEDURE:

1.0 Preparation of working reagent – Prepare it fresh by mixing one part of diacetyl monoxime reagent, one part of thiosemicarbazide reagent and two parts of acid reagent.

2.0 Dilute urine 1:10 in distilled water i.e. to 1.0 ml of urine add 9.0 ml distilled water.

3.0 Prepare test, standard and blank as follows

	Test	Standard	Blank
Working reagent	5.0 ml	5.0 ml	5.0 ml
Diluted urine	0.05 ml	—	—
Urea nitrogen standard 20 mg / 100 ml	—	0.05ml	—
Distilled water	—	—	0.05 ml

4.0 Mix the contents of tubes thoroughly and keep in boiling water bath for 20 minutes. Cool the tubes to room temperature

5.0 Set the spectrophotometer at 530nm or photoelectric colourimeter at green filter.

6.0 Adjust 0% T,

7.0 Adjust 100% T with Blank.

8.0 Read absorbance for standard and test solution

Urea nitrogen can also be estimated by Berthelot reaction method and Karr's direct nesselerization method (using urease as reagent)

7.0 Observation:

Absorbance of standard solution A_S =

Absorbance of test solution A_T =

8.0 Calculation:

$$C_T = \frac{A_T}{A_S} \times C_S$$

Where C_T = quantity of urea nitrogen in 0.05 ml diluted urine

C_S = quantity of urea nitrogen in 0.05 ml urea nitrogen standard

= 0.01 mg.

As urine is diluted 1:10 in water, 0.05 ml diluted urine represents 0.005 ml of undiluted urine

Therefore quantity of urea nitrogen in 0.005 ml undiluted urine = C_T

Therefore quantity of urea nitrogen in 1000 ml urine = $\frac{C_T \times 1000}{0.005}$

$$= \frac{A_T}{A_S} \times 0.01 \times \frac{1000}{0.005} \text{ mg}$$

Therefore quantity of urea nitrogen in 1000 ml urine = $\frac{A_T}{A_S} \times 20 \times 100$ mg

$$= \frac{\text{.....}}{\text{.....}} \times 20 \times 100 \text{ mg}$$

= ..... mg

therefore quantity of urea nitrogen in 1000 ml urine $= \frac{\text{..... g}}{1000}$

therefore quantity of urea nitrogen in given urine sample = …….. g/ lit.

9.0 Result:

Quantity of urea nitrogen in given sample of urine is …… g/ lit

Normal value urea nitrogen in urine is 10g/ lit

10.0 Questions

Note : Students to answer Q....., Q....., Q....., and the question numbers shall be allotted by teacher.

1. What is principle involved in estimation of urea nitrogen in urine by DAM method?
2. State the two biomolecules which are metabolized to urea in body.
3. State the name of organ in which urea formation takes place.
4. What is urea cycle ?
5. Which filter of photoelectric colourimeter is used for estimation of urea nitrogen in urine by DAM method.
6. Which colour is obtained after addition of working reagent to test and sample solution and boiling for 20 minutes in estimation of urea nitrogen in urine by DAM method and why ?
7. What is role of urea in body ?
8. State two diseases in which urea excretion in urine is increased than normal value.
9. State other one method which can be used for estimation of urea nitrogen in serum and urine.
10. What is normal value of urea nitrogen in urine ?

(Space for answers)

(Space for answers)

Date : Signature of Teacher

Experiment No. 26

1.0 TITLE:

To estimate quantity of total cholesterol in given sample of blood plasma by modified Libermann Burchard reaction.

2.0 PRIOR CONCEPTS : Cholesterol

It is present in all cells, all body fluids (except cerebrospinal fluid) brain, blood, muscles etc.

3.0 NEW CONCEPTS:

Proposition 1 : Hypercholesterolemia

It is increase in total cholesterol value in serum than normal. It is observed in nephrosis, diabetis mellitus, obstructive jaundice, myxedema, gout, atherosclerosis

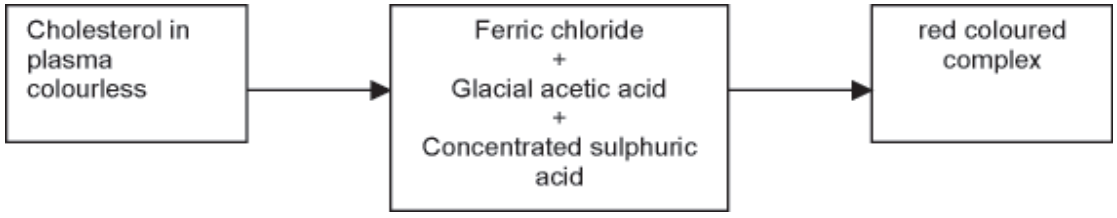
Proposition 2 : Hypocholesterolemia

Decrease in total cholesterol value in serum is observed in hyperthyroidism, pernicious anaemia, mal absorption syndrome, acute infections and in terminal states.

Proposition 3 : Watson - Colourimetric method Modified Libermann - Burchard reaction.

Cholesterol reacts with ferric chloride and concentrated sulphuric acid in the presense of glacial acetic acid to form red coloured complex. Intensity of colour is proportional to the cholesterol concentration and can be measured at 625nm (red filter)

Concept Structure



4.0 LEARNING OBJECTIVES:

Intellectual Skills

1. To understand concept of modified Libermann Burchardt reaction.
2. To calculate quantity total cholesterol in plasma in mg/100ml

Motor Skills

1. Ability to prepare test, standard and blank solution.
2. Ability to operate spectrophotometer/ Photoelectric colourimeter

5.0 APPARATUS:

Glasswares

Test tubes, pipettes, measuring cyclinders, water bath, stop watch, photoelectric colourimeter/ spectrophotometer.

Chemicals

Blood plasma, cholesterol standard (200mg cholesterol in 100 ml glacial acetic acid), glacial acetic acid, ferric chloride solution 10% w/v in distilled water, colour reagent (add 1ml ferric chloride solution in 99 ml concentrated sulphuric acid and mix well).

6.0 STEPWISE PROCEDURE:

1.0 Prepare Test Standard and blank as follows.

		Test	Standard	Blank
1.	Colour reagent	4.0 ml	4.0 ml	4.0 ml
2.	Blood plasma	0.1 ml	--	--
3.	Cholesterol Standard 200 mg/100ml	--	0.1 ml	--
4.	Distilled water	--	--	0.1 ml
5.	Glacial acetic acid	3.0 ml	3.0 ml	3.0 ml

Shake well to mix colour reagent and ensure even heat distribution.

2.0 Allow to cool in water bath at room temperature for 20 minutes.

3.0 Set the spectrophotometer at 625 nm/photoelectric colourimeter, at red filter.

4.0 Adjust 0% T,

5.0 Adjust 100% T using blank.

6.0 Read absorbance (optical density) for standard and test solution.

Note - Use dry cuvettes for the reading. Colour reagent and cholesterol working standard, Concentrated sulphuric acid, glacial acetic acid are corrosive, do not pipette by mouth. Avoid contact with skin and clothing. Serum cholesterol can also be estimated by enzymatic method, photoelectric colourimeter methods (Watson method / Salkowaski method)

7.0 Observation:

Absorbance of standard solution A_S =

Absorbance of test solution A_T =

8.0 Calculation:

$$C_T = \frac{A_T}{A_S} \times C_S$$

Where C_T = quantity of cholesterol in 0.1 ml plasma

C_S = quantity of total cholesterol in 0.1 ml standard

= 0.2 mg

As quantity of cholesterol in 0.1 ml blood plasma = C_T

quantity of Cholesterol in 100 ml blood plasma $= \frac{C_T \times 100}{0.1}$ mg

$$= \frac{\frac{A_T}{A_S} \times 0.2 \times 100}{0.1} \text{ mg}$$

$$= \frac{A_T}{A_S} \times 200 \text{ mg}$$

$$= \frac{\text{.....}}{\text{.....}} \times 200 \text{ mg}$$

= ..... mg

therefore quantity of total cholesterol in given sample of blood plasma = ... mg/100ml

9.0 Result:

Quantity of total cholesterol in given sample of blood plasma = ……. mg/100ml

Normal value of total cholesterol in blood plasma is 150 - 250 mg/100ml.

10.0 Questions

Note : Students to answer Q....., Q....., Q....., and the question numbers shall be allotted by teacher.

1. What is principle involved in modified Libermann Burchardt reaction for estimation of total cholesterol in blood plasma.
2. State two steps involved in preparation of blood plasma from blood.
3. State the two risk diseases patient may be suffering from, If total cholesterol in blood plasma is 400 mg/ 100 ml
4. State name of the organs where cholesterol is synthesized.
5. Draw structure of cholesterol.
6. State difference between HDL cholesterol and LDL cholesterol in respect of density.
7. State which type of elevated cholesterol value HDL or LDL is harmful to body.
8. What is antherosclerosis ?
9. Which wavelengh of spectrophotometer is selected for estimation of total cholesterol value in blood plasma by modified Libermann Burchardt method ?
10. State the unit in which total cholesterol value in blood plasma is expressed.

(Space for answers)

(Space for answers)

Signature of Teacher

COLOURIMETER

Colourimeter – A colourimeter is used to measure the concentration of substance in coloured sample by comparing the amount of light it absorbs, with that absorbed by a standard preparation that contains a known amount of substance being tested.

In colourimetric determination a specific reagent or reagents are used which react with substance under determination to form a coloured complex. The concentration of coloured complex is directly proportional to the concentration of substance under determination. The depth of coloured complex is measured on photometer or spectrophotometer.

Most colourimetric analytical tests are based on Beer-Lambert's law which states that the absorbance of a solution at the appropriate wavelength is directly proportional to its concentration and the light path through the solution. This law can be applied for measuring the concentration of substance in an unknown (test) solution by using formula.

$$\text{Concentration of test } C_T = \frac{\text{Absorbance of test } A_T}{\text{Absorbance of standard } A_S} \times \text{Concentration of standard } C_S$$

Formerly the amount of light absorbed by a coloured solution i.e. absorbance was referred as optical density (O.D.)

$$\text{Absorbance } A = 2 - \log \%T$$

Absorbance is zero when there is 100% transmission.

In colourimetric tests, the light path is kept constant by using optically matched cuvettes usually of 10mm light path distance or tubes of known light path distance.

Basic components of a photometer and spectrophotometer are

a) A light source b) Monochromator / filter
c) Cuvette d) Photodetector and e) Galvanometer.

The instrument which uses a light filter for wavelength at which photometric measurements are made is called photometer, while the instrument which uses a prism or diffraction grating with a slit to get monochromatic light is called spectrophotometer.

The colourimeter is supplied with colour filters which ranges between wavelength 400-700 nm.

Wavelength	Filter
400-419	Deep violet
420-449	Violet
450-479	Blue
480-504	Blue green
505-534	Green

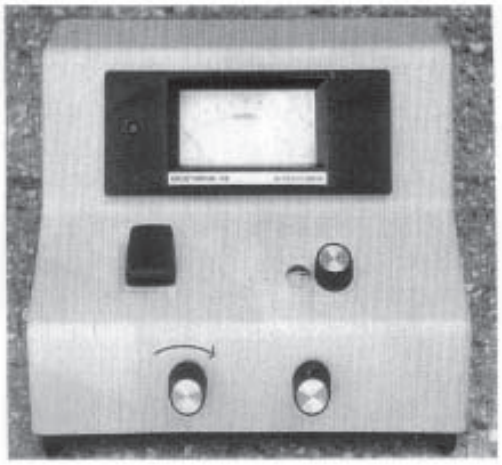
Wavelength	Filter
535-564	Yellow green
565-589	Yellow
590-639	Orange
640-689	Red
690-700	Deep red

Basic steps involved in use of colourimeter / spectrophotometer are

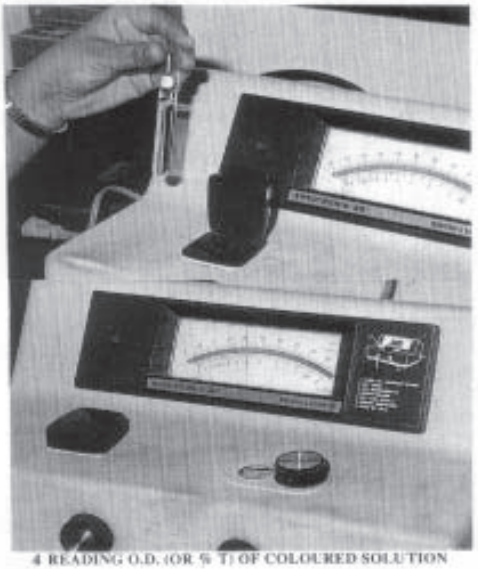
1. Put on the main switch
2. Select a proper filter / wavelength
3. Adjust 0% without cuvette
4. Place a cuvette filled with blank / distilled water and adjust 100%T.
5. Replace the blank / distilled water with test and standard solution and record their absorbance (optical density).

Reagent blank is used to correct any absorbtion of light by reagents. It contains reagents and chemicals used for development of colour but do not contain the substance being tested.

Working of Spectrophotometer



3 ADJUSTING 100% T AGAINST DISTILLED WATER



4 READING O.D. (OR % T) OF COLOURED SOLUTION

Experiment No. 27

1.0 TITLE:

To estimate quantity of serum alkaline phosphatase in given sample of serum by King Armstrong method.

2.0 PRIOR CONCEPTS: Serum.

3.0 NEW CONCEPTS:

Proposition 1 : Phosphatases.

Phosphatases are group of enzymes characterized by their ability to hydrolyse organic phosphates.

Clinically three types of phosphatases are recognized.

1. Alkaline phosphatase (of serum, bone, liver) with optimum pH 9.8.
2. Acid phosphatse (of prostate, liver and serum) with optimum pH 4.9 and
3. Red cell phosphatase with optimum pH 5.5 – 6.0

Proposition 2 : Alkaline phosphatase.

Alkaline phosphatase is present in all tissues of body. It occurs at high levels in kidney tubules, intestinal epithelium, bones, placenta and liver. This enzyme is associated with calcification process in bones and with lipid transport in intestine. The form present in serum of normal adults originates mainly in liver with about 50% of total activity coming from skeleton.

Proposition 3 : Estimation of serum alkaline phosphatase

serum alkaline phosphatase estimations are of interest in diagnosis of two group of conditions.

a) hepatobiliary disease

b) bone disease associated with increased osteo blastic activity.

Moderately elevated levels are observed in hepatic condition, osteomalacia, rickets, hyper-parathyroidism. The elevations are more marked in post hepatic conditions very high levels are found in bone cancer.

Serum alkaline phosphatase is decreased in cretinism, scurvey, severe anaemia, kwashiorkar.

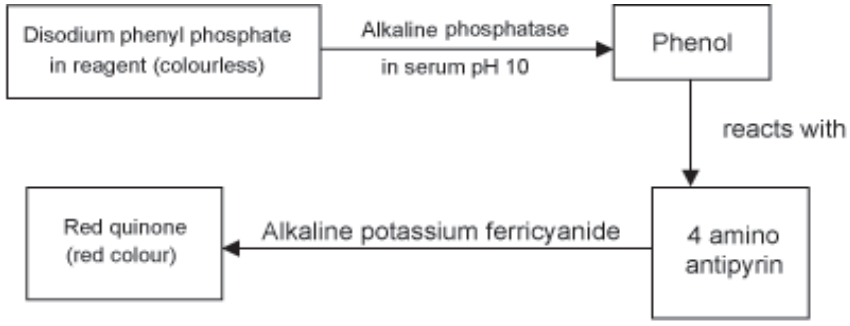
Methods of estimation of serum alkaline phosphatase includes, King and Armstrong method using 4 amino antipyrine, p-nitro phenyl phosphate (PNP) method.

Proposition 4 : Method of King and Armstrong using 4 amino antipyrine.

Serum alkaline phosphatase is maximally active at pH 10. The activity is measured by amount of phenol hydrolysed from diphenyl phosphate. The formed phenol reacts with 4 amino antipyrin in presence of alkaline potassium ferricyanide with the formation of red or purple colour which is measured photometrically at 510 nm/ green filter.

Enzyme activity is measured in King and Armstrong (K.A.) unit. Where one unit is equal to activity which releases 1.0 mg of phenol in 15 minutes at pH 10 by 100 ml of serum under test.

Concept Structure :



4.0 LEARNING OBJECTIVES:

Intellectual Skills

1. To understand concept of King Armstrong method.
2. To calculate amount of serum alkaline phosphatase in given sample of serum.

Motor Skills

1. Ability to add proper amount of reagents in blank, standard, control and test solution respectively.
2. Ability to operate colourimeter / spectrophotometer
3. Ability to measure % transmittance and absorbance.

5.0 APPARATUS:

Glasswares

Conical flask, pipettes, test tubes, volumetric flask, incubator, cuvettes photo electric colourimeter / spectrophotometer.

Chemicals

Serum sample, 0.01M disodium phenyl phosphate (dissolve 1. 0 g in 500ml water, boil quickly but briefly cool and preserve with 2ml chloroform in cold.
0.1 M buffer pH-10 (dissolve 3.18 g sodium carbonate and 1.68 g sodium bicarbonate in 500 ml water). 0.5 N sodium hydroxide (20 g sodium hydroxide in 100 ml water)
0.5N sodium bicarbonate (42 g sodium bicarbonate in 1000 ml water)
0.6% w/v 4 amino antipyrine (stored in brown bottle)
2.4% w/v potassium ferricyanide (stored in brown bottle)
0.1% phenol stock (1mg/ ml, dissolve 1 g pure crystalline phenol in 1000ml 0.1 NHCl) store at 4^0c
0.001% phenol working standard (1mg / 100 ml, dilute 1 ml of stock phenol to 100 ml with water) store at 4^0c.

6.0 STEPWISE PROCEDURE:

1.0 Prepare blank, standard, test and control as mentioned in following table.

Reagents	Blank	Standard	Test	Control
Buffer pH 10.0	1.1 ml	1.1ml	1.0ml	1.0ml
0.01 M disodium phenyl phosphate	-	-	1.0ml	1.0ml
Serum	-	-	0.1ml	-
Phenol working Standard	-	1.0ml	-	-
Distilled water	1.0ml	-	-	-
Incubate at 37^0c for 15 minutes				
0.5N sodium hydroxide	0.5ml	0.5ml	0.5ml	0.5ml
serum	-	-	-	0.1ml
0.5N sodium bicarbonate	1.2ml	1.2ml	1.2ml	1.2ml
Mix thoroughly				
4 amino antipyrine	1.0ml	1.0ml	1.0ml	1.0ml
Potassium ferricyanide	1.0ml	1.0ml	1.0ml	1.0ml

Mix thoroughly after each addition as failure to mix leads to irregular result. Avoid exposure to strong sunlight.

2.0 Set photoelectric colourimeter / spectrophotometer at green filter / 510 nm.

3.0 Adjust 0% T

4.0 Adjust 100% T with distilled water

5.0 Measure absorbance or calculate absorbance by using formula A=2-log%T for blank, standard, test and control.

7.0 OBSERVATION

Absorbance of blank = A_B =
Absorbance of standard = A_S =
Absorbance of test = A_T =
Absorbance of control = A_C =

8.0 CALCULATIONS

Quantity of serum alkaline phosphatase in 0.1 ml serum = C_T

$$C_T = \frac{A_T - A_c}{A_s - A_B} \times C_s$$

Where C_s = Quantity of phenol in 1.0 ml phenol working standard = 0.01 mg

$$C_T = \frac{A_T - A_c}{A_s - A_B} \times 0.01 \text{ mg}$$

As enzyme activity is measured in K.A. where one unit activity is, which releases 1.0 mg phenol in 15 minutes at pH 10 by 100 ml of serum.

Quantity of serum alkaline phosphatase in 100ml serum

$$= C_T \times \frac{100}{0.1} \text{ K.A. units}$$

$$= \frac{A_T - A_c}{A_s - A_B} \times 0.01 \times \frac{100}{0.1}$$

$$= \frac{A_T - A_c}{A_s - A_B} \times 10 \text{ K.A. units}$$

$$= \frac{\text{.....}}{\text{.....}} \times 10 \text{ K.A. units}$$

$$= \text{..... K.A. units}$$

9.0 RESULT :

Activity of serum alkaline phosphatase in give sample of serum = ... K.A. units / 100 ml
Normal range is 3-13 K.A. units / 100 ml in adults, 18-33 K.A. units / 100 ml children.

10.0 QUESTIONS:

Note : Students to answer Q....., Q....., Q....., and the question numbers shall be allotted by teacher.

1. Name three organs where serum alkaline phosphatase is present in high level.
2. Name two diseases which can be diagnosed by estimation of serum alkaline phosphatase.
3. Name the disorders in which serum alkaline phosphatase is elevated.
4. Discuss the principle behind King and Armstrong method using 4amino antipyrine used for estimation of serum alkaline phosphatase.

5. Give the unit in which serum alkaline phosphatase activity is measured.
6. What is one King Armstrong unit of enzyme activity?
7. Name the reagents used in estimation of serum alkaline phosphatase by King Armstrong method.
8. Which filter of photoelectric colourimeter / wavelength of spectrophotometer is used in estimation of serum alkaline phosphatase by King Armstrong method?
9. What is normal range of serum alkaline phosphatase in adults?
10. The serum sample of adult shows serum alkaine phosphatase 25KA units / 100 ml state the possible diseases he may be suffering from ?

(Space for answers)

(Space for answers)

Signature of Teacher

Experiment No. 28

1.0 TITLE :

To estimate quantity of Calcium in a given sample of blood serum. (C.P.C. method)

2.0 PRIOR CONCEPTS:

It is a major constituent of bone and teeth.

3.0 NEW CONCEPTS:

Proposition 1: Rickets
It is a calcium deficiency disease in a children.

Proposition 2: Osteomalacia
It is a calcium deficiency disease in a adults.

Proposition 3: Hypocalcemia
It is a calcium deficiency disease in a body.

Proposition 4: Cresolphthalein complexone method
Calcium in alkaline medium reacts with cresolphthalein complexone to form purple coloured complex. The absorbance of colour developed is proportional to calcium concentration in a sample.

4.0 LEARNING OBJECTIVES:

Intellectual skills

1. To understand concept of C.P.C. method used to estimate quantity of calcium in a serum.
2. To understand diseases Rickets, Osteomalacia, Hypocalcemia.
3. To calculate amount of calcium in surum

Motor skills

1. Ability to add reagents in blank, standard and tests.
2. Ability to handle and operate photoelectric colorimeter.
3. Ability to measure absorbance of Test, Standard and Blank.

5.0 APPARATUS:

Glassware:

Test tubes, Beakers, Test tube stand, Glass rod, Graduated pipette,Centrifuge, Photoelectric Colorimeter etc.

Chemicals:

Reagent 1. Calcium Reagent 1. It is prepared by mixing 40 mg of Cresolpthalein complexone in ml Concentrated HCl followed by 2.5 g of 8 hydroxy quinoline. 100 ml of dimethyl Sulphoxide and Final volume is made up to 1 litre using distilled water.

Reagent 2. Calcium Reagent 2. It is prepared by mixing 500 mg of KCN and 40 ml of diethyl amine in 960 ml of distilled water.

Reagent 3. (Calcium standard) Calcium Standard – 10 mg/100ml contains 25 mg of Calcium carbonate in a 50 % V/V HCL.

6.0 STEPWISE PROCEDURE:

1. Preparation of working reagent.
   It is prepared by mixing equal amount of of Reagent 1 and reagent 2. (10 ml each.) Shake properly .
2. Preparation of test, standard and blank solutions as follows.

Sr. No.	Reagent	Blank	Standard	Test
1.	Working Reagent	6 ml	6 ml	6 ml
2.	Serum	———-	———-	0.05 ml
3.	Calcium standard	———-	0.05 ml	———-
4.	Distilled water	0.05 ml	———-	———-

3. Mix well keep at a room temp. for 15 minute.
4. Set the spectrophotometer at 530 nm or photoelectric colourimeter at yellow filter.
5. Adjust 100 % Transmission with a Blank.
6. Read absorbance for standard and test.

7.0 OBSERVATIONS:

1. Absorbance (optical density) of Standard : A_S ———————
2. Absorbance (optical density) of Test : A_T ———————

8.0 CALCULATION:

Quantity of calcium in 0.05 ml serum = C_T

$$C_T = \frac{As}{A_T} \times C_s$$

C_S = Quantity of calcium in a 0.05 ml of calcium standard = 0.005

$$C_T = \frac{As}{A_T} \times 0.005$$

As quantity of calcium in 0.05 ml serum = C_T

$$\text{Quantity of calcium in 100 ml of serum} = \frac{C_T \times 100}{0.05}$$

$$= \frac{\frac{A_S}{A_T} \times 0.005 \times 100}{0.05}$$

$$= \frac{A_S \times 10}{A_T}$$

9.0 RESULT:

Given sample of serum contains Calcium ———————————— mg% Calcium.

Normal values : Serum Calcium 8.7 - 11 mg /100 ml

10.0 QUESTIONS:

(Note – Student to answer Q…,Q…,Q… and Question number shall be allotted by teacher.)

1. State two conditions in which serum calcium level is increased .
2. Write three functions of calcium.
3. Differentiate between Rickets and Osteomalacia.
4. State two conditions in which serum calcium level is decreased.
5. Write daily requirement of calcium.
6. Name deficiency diseases of calcium.
7. Name organs of body in which calcium is present as major constituent
8. Name the filter at which estimation of calcium in serum is done by CPC method
9. Give principle involved in cresolpthalein complexone method used in estimation of calcium in blood serum

Space for answer

Signature of Teacher

Experiment No. 29

1.0 TITLE :

To estimate quantity of glucose in given sample of serum. (GOD / POD method).

2.0 PRIOR CONCEPTS:

Glucose is major carbohydrate present in blood.

3.0 NEW CONCEPTS:

Proposition 1: Diabetes Mellitus

Increase in sugar (glucose) level in blood than normal level is known as diabetes mellitus.

Proposition 2: Hypoglycemia

Decrease in blood glucose level than normal level is known as hypoglycemia.

Preposition 3: GOD / POD method

Glucose oxidase (GOD) oxidises glucose to gluconic acid and hydrogen peroxide.

$$Glucose + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} Gluconic\ Acid + H_2O_2$$

In presene of enzyme peroxidase . hydrogen peroxide reacts with phenol and 4-amino antipyrine (4 AAP)to form red coloured Quinoneimine dye absorbance of coloured dye is measured at 510 nm and directly proportional to glucose concentration in sample.

$$H_2O_2 + Phenol + 4\ Amino\ antipyrine \xrightarrow{\text{Peroxidase}} Quinoneimine\ dye + H_2O$$

4.0 LEARNING OBJECTIVES:

Intellectual skills

1. To understand concept of oxidation of glucose to gluconic acid by enzyme glucose oxidase.
2. To understand diseases diabetes mellitus and hypoglycemia.
3. To Calculate amount of glucose in a serum.

Motor skills

1. Ability to add reagents in standard, blank and test.
2. Ability to handle and operate photoelectric colorimeter.
3. Ability to measure absorbance of Test, Standard and Blank.

5.0 APPARATUS:

Glassware :

Test tubes, Beakers, Test tube stand, Glass rod, Photoelectric Colorimeter, Centrifuge, Incubator etc.

Chemicals:

1. (Buffer Enzyme) This reagent is prepared by mixing following constituents in a 100 ml of phosphate buffer (M/10, pH 7).
   1. Glucose Oxidase : 650 unit
   2. Peroxidase : 500 unit
   3. 4 amino phenozone : 20 mg
   4. Sodium azide : 30 mg
2. Phenol reagent : 100 mg/100 ml
3. Glucose Standard : 100 mg/100 ml

6.0 STEPWISE PROCEDURE:

1. Preparation of glucose reagent.
   Mix 2 parts of buffer enzyme reagent and one part of phenol reagent to gate glucose reagent.
2. Preparation of test, standard and blank solutions as follows.

Sr. No.	Reagent	Blank	Standard	Test
1.	Glucose reagent	3.0 ml	3.0 ml	3.0 ml
2.	Serum / Plasma	--	--	0.02 ml
3.	Glucose standard	--	0.2 ml	--
4.	Distilled water	0.02 ml	--	--

3. Mix well incubate at a 370c for 15 minutes. The final colour is stable for one hour.
4. Set the spectrophotometer at 530 nm or photoelectric colourimeter on green filter.
5. Adjust 100 % Transmission and 0 absorbance with a distilled water.
6. Read absorbance for standard test and blank

7.0 OBSERVATION TABLE :

1. Absorbance (optical density) of Blank : A_B _____
2. Absorbance (optical density) of Standard : A_S _____
3. Absorbance (optical density) of Test : A_T _____

8.0 CALCULATION :

$$C_T = \frac{A_T - A_B}{As - A_B} \times Cs$$

When Cs = 0.02 mg

C_T = quantity of glucose in 0.02 ml serum.

Hence quantity of glucose in a 100 ml serum

$$= \frac{C_T \times 100}{0.02} \text{ mg/100 ml}$$

$$= \frac{\dfrac{A_T - A_B \times 0.02}{As - A_B} \times 100 \text{ mg/100 ml}}{0.02}$$

$$= \frac{A_T - A_B}{A_s - A_B} \times 100 \text{ mg/100 ml}$$

$$= \frac{..... - .....}{.....} \times 100 \text{ mg/100 ml}$$

9.0 RESULT :

Given sample of serum/plasma contains Glucose ---------- mg/100 ml
Normal values : 70 - 105 mg /100 ml

10.0 QUESTIONS : (Note - Student to answer Q.....,Q.....,Q..... and question number shall be allotted by teacher.)

1. Define Diabetes Mellitus.
2. State normal level of glucose in a blood fasting and post prandial .
3. State the colour produced in a estimation of glucose in serum by GOD/POD method.
4. Write the use of glucose oxidase in a above estimation.
5. Define Hypoglycemia ?
6. Write two examples of drugs used in treatment of diabetes mellites.
7. Name the harmone secreted by pancreas.
8. State the use and route of administration of Insulin.
9. State the causes of diabetes mellitus.
10. Define Glycosuria ?

Space for answer

Signature of Teacher

Experiment No. 30

Prepared / permanent slides be obtained from pathology laboratory for microscopic examination of sputum.

1.0 TITLE:

To study microscopic examination of sputum.

2.0 PRIOR CONCEPTS: Sputum

It is excretory product of respiratory tract.

3.0 NEW CONCEPTS:

Proposition 1 : Examination of sputum.

It reveals infections of lower respiratory tract.

Proposition 2 : Gram staining method.

It differentiates bacteria into gram positive and gram negative bacteria.

Proposition 3 : Acid fast staining

It stains organisms such as Mycobacterium tuberculosis, Mycobacterium leprae which are difficult to stain by ordinary staining methods because of lipid containing cell walls. They bind carbol fuchsin tightly and resist destaining with strong decolouring agents like alcohols and strong acids. In this carbol fuchsin is used as primary stain while phenol is used as mordant.

4.0 LEARNING OBJECTIVES:

Intellectual Skills

1. To understand concept of gram staining and acid fast staining.
2. To identify colours observed in gram staining and acid fast staining.

Motor Skills

1. Ability to handle and use microscope.

5.0 APPARATUS:

Glasswares

Prepared / permanent slides, (oil immersion) microscope

Chemicals

Crystal violet stain (solution A – crystal violet 2g, ethyl alcohol 20 ml, solution B- Ammonium oxalate 0.8g, distilled water 80ml, mix solution A and B keep for 24 hours and filter)

Gram's iodine solution (iodine 1.0 g potassium iodine 2.0 g distilled water to 100 ml)

Decolourizer (mix 95% alcohol and acetone in equal proportion)

Safranine solution (safranine 'O' 0.34 g in absolute alcohol 10 ml and distilled water 90 ml)

Stock carbol fuchsin solution (solution A – Basic fuchsin powder 3g, 95% ethyl alcohol upto 100 ml, dissolve powder in alcohol by using mortar and pestle, if necessary heat in boiling water bath, solution B- 50% phenol solution).

Working carbol fuchsin staining solution (mix 10 ml of solution A with 90 ml of solution B) 20% v/v sulphuric acid, methylene blue 0.3% w/v in water.

6.0 Stepwise Procedure:

1. Collection of sample.

   For routine common investigations sputum is collected in wide mouth bottle (30ml /60ml) with a tight fitting screw caps. Collection is done early in the morning.

   The patient is instructed to clean mouth with water to avoid contamination by food particle. Patient is asked to cough sputum from bronchi or lungs and not saliva.

   For (suspected tuberculosis) tuberculosis investigations sample is collected over a period of 24 hours.

2. Grams staining : The gram staining of sputum sample involves following steps.
   1. Preparation of smear of sputum sample and fixing it by heating.
   2. Staining of smear with crystal violet for 1 minute.
   3. Washing it under running tap water and then treatment with gram's iodine for one minute.
4. Decolourization of smear with alcohol – acetone for 20 to 30 seconds it is continued till purple stain just stops coming on the slide.
5. Washing of the slide under running tap water.
6. Staining with safranine. The stained smear is allowed to drain and dry and observed under high power objective and finally under oil immersion objective.

   Gram positive bacteria retain the colour and observed as violet coloured bacteria, while gram negative bacteria do not retain colour after treatment with alcohol / acetone and are counterstained by safranine and observed as pink coloured bacteria.

   Diagram showing procedure for gram staining.

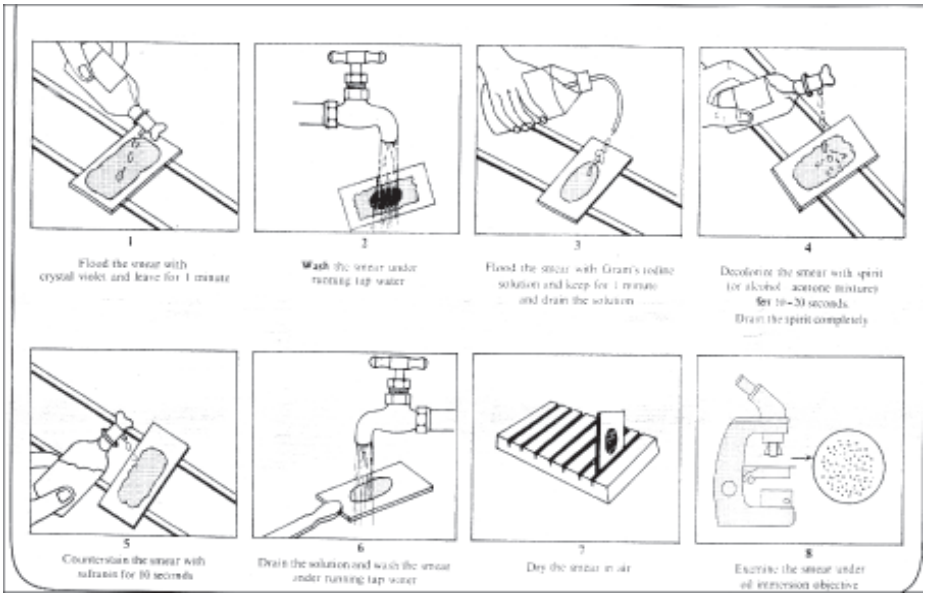


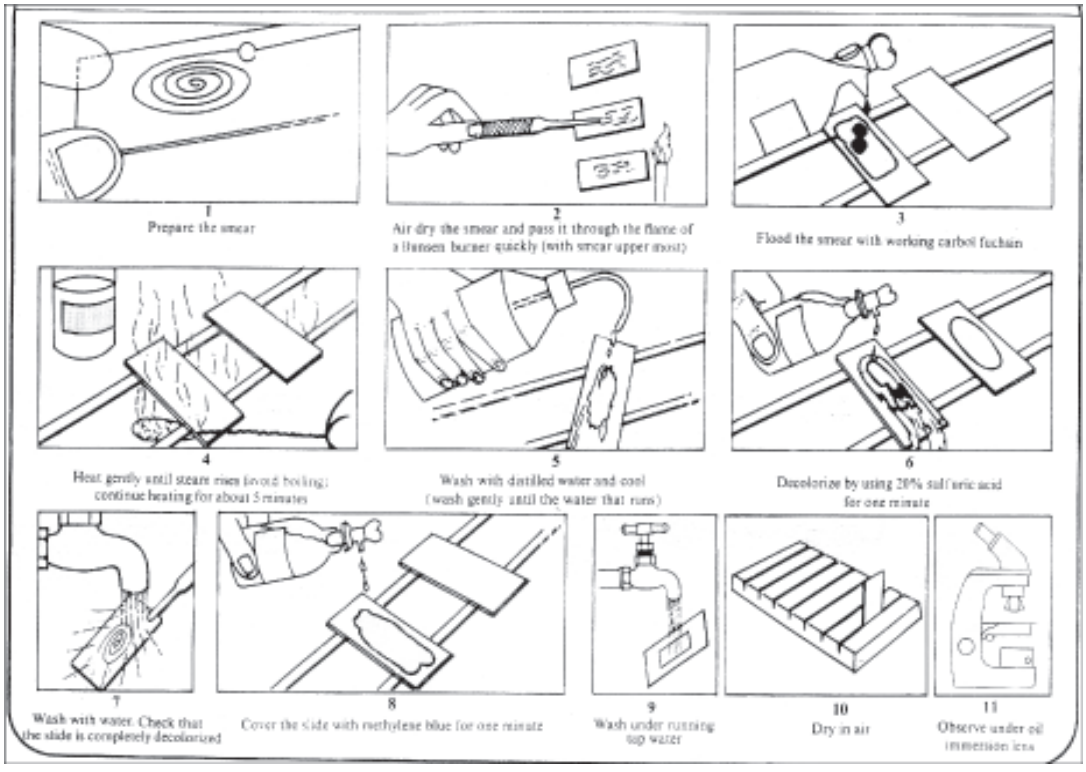
Fig 30.1

Streptococcus and staphylococcus are gram positive organisms observed violet coloured when stained with gram staining. These are normal habitats of nasal membrane and can cause disorders like pneumonia, phyaryngitis.

Few examples - Gram positive cocci in clusters of staphylococcus aures. Gram positive cocci in chains streptococcus pneumoniae. Gram positive cocci in pairs streptococcus pyogens. Gram negative rods of klebshella pneumoniae.

3. Acid fast staining : Acid fast staining is done by ziehl-Neelsen hot stain method in which heat is applied for detection of Mycobacterium tuberculosis. It involves following steps ,
   1. Preparation of smear from sputum specimen on glass slide and fixing it by heating on bunsen burner flame.
   2. Staining the heat fixed smear with working carbol fuchsin stain.
   3. Heating gently by bunsen flame until steam rises for 5 minutes, the stain is not allowed to dry.
   4. Washing off the stain with water till water that runs off is colourless.
   5. Decolourisation using 20% sulphuric acid for one minute.
   6. Washing with water and counterstaining with methylene blue stain for one minute.
   7. Washing the slide with tap water, allowing it to drain finally observed under oil immersion objective.

   The acid fast organisms are observed as bright red bacilli on blue background.
   Diagram showing procedure for acid fast staining.



7.0 OBSERVATION :

Student to observe prepared / permanent slides obtained, under microscope and report observations in following table.

1) Microscopic examination of prepared / permanent slide of gram staining (sputum smear)

Test	Colour	Observation Present / absent
1. Gram negative bacteria (rods) size 1 µm	Pink	
2. Gram positive bacteria (spherical and few rods)	Violet	
3. Yeast cells (Oval, buddy or filaments)	Violet	
4. Pus cells	Pink	
5. Epithelial cells	Pink	

2) Microscopic examination of prepared / permanent slide of acid fast staining (sputum smear)

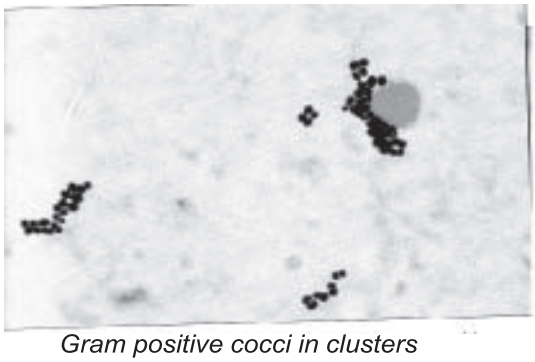
Test	Colour	Observation Present / absent
1. Acid fast organisms	Bright red bacilli on blue background	
2. Other organisms	Dark blue	

8.0 RESULT:

Microscopic examination of given sample of slides of sputum shows presence of gram negative bacteria/ gram positive bacteria/ pus cells/ yeast cells/ Epithelial cells/ acid fast organisms.

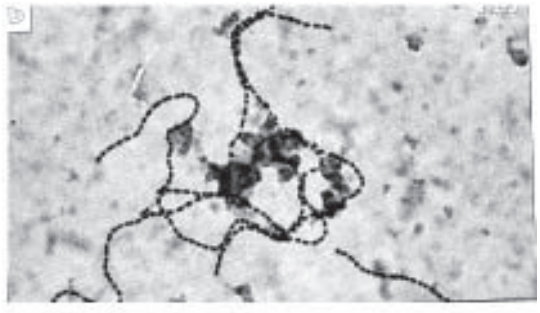
9.0 REFERENCE :

Slides showing gram staining



Gram positive cocci in clusters staphylococcus aureus

Fig 30.3



Gram positive cocci in chains pyogenes

Fig 30.4

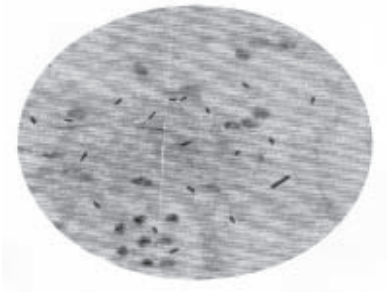
Gram positive cocci in pairs streptococcus pneumoniae

Fig 30.5

Gram negative rods of klebshela pneumoniae.

Fig 30.6

Slide showing acid fast staining



Bright red bacilli of Mycobacterium tuberculosis

Fig 30.7

10.0 QUESTIONS:

Note : Students to answer Q....., Q....., Q....., and the question numbers shall be allotted by teacher.

1. State the purpose of examination of sputum.
2. Sputum is obtained from which organ of body.
3. State the procedure of gram staining.
4. Name two gram positive organisms.
5. Name two gram negative organisms.
6. State the principle of acid fast staining.
7. Write procedure of acid fast staining.
8. Name two acid fast organisms and the diseases they cause in human being.
9. Draw the slide showing microscopic observation of gram positive bacteria and gram negative bacteria.
10. Draw the slide showing acid fast staining of mycobacterium tuberculosis.

Space for answer

Space for answer

Space for answer

Signature of Teacher

Experiment No. 31

Prepared/permanent slides be obtained from pathology laboratory for microscopic examination of faeces.

1.0 TITLE:

To study microscopic examination of faeces

2.0 PRIOR CONCEPTS: Faeces or stool.

It is end product of digestive system of body. Normally composed of undigested food, various products of digestion, bile, leucocytes and non pathogenic bacteria.

3.0 NEW CONCEPTS:

Proposition 1 : Parasite

It is an organism that is entirely dependant on another organism referred to as its host, for all or part of its life cycle and metabolic requirements.

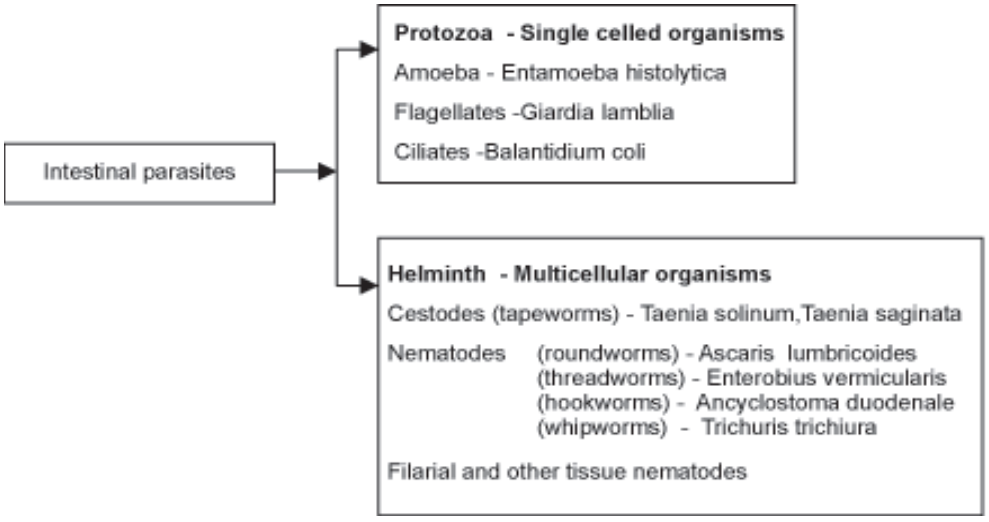
Proposition 2 : Parasitic infection

Parasitic infections in human being primarily involve intestine hence the common specimen used for diagnosis of intestinal parasitic infection is faeces or stool sample.

Proposition 3 : Parasitic infections

It includes amoebiasis, giardiasis, infections caused by nematodes, cestodes, trematodes, filaria etc

Concept Structure :



4.0 LEARNING OBJECTIVES:

Intellectual Skills

1. To understand concept of microscopic examination and staining.
2. To identify various shapes observed under microscope.
3. To interpret parasitic infection from the observations.

Motor Skills

1. Ability to handle and use microscope.

5.0 APPARATUS:

Glasswares

Prepared / permanent slides, microscope

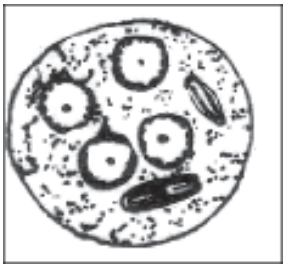
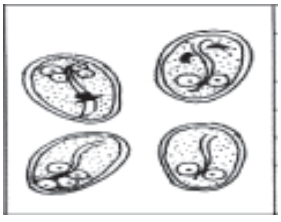
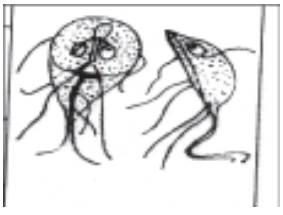
Chemicals

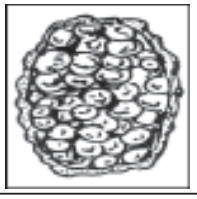
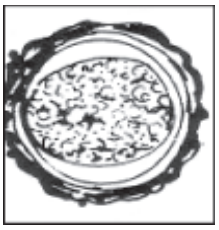
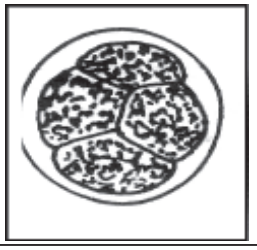
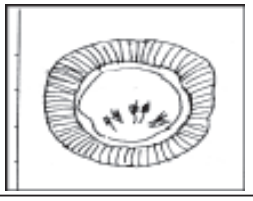
Lugol's iodine solution.

6.0 Stepwise Procedure:

1. Stained preparation: It involves following steps.
   1. A drop of Lugol's iodine is placed on the slide.
   2. Little fecal material which is concentrated by fecal concentration techniques is mixed with drop of iodine solution.
   3. A coverslip is placed on it.
   4. Observed under microscope with 40x objective.

Microscopic examinations of slides of faeces reveals following details.

Observation	Physiological / pathological significance	Diagram
1. Parasites 1) Round in shape of size 12-15 µm, four nuclei, cytoplasm stained yellowish grey in iodine solution. Cell contains cytoplasm with chromatin nucleus and inclusion of RBC, leucocytes, tissue debris and bacterias absent.	Entamoeba histolytica cyst	
2) Oval shape of size 8-12 µm with a double wall, granular cytoplasm stained yellow in iodine solution. 2-4 oval nuclei and two parallel hair like flagella.	Giardia lamblia cyst.	
3) Elongated shape of size 10-18 µm. It has a large concave sucking disk on ventral surface. It has four pairs pear shaped flagella and two nuclei.	Giardia lamblia trophozoite	
2. Intestinal helminths 4) Barrel shape of size 33-40 µm with a mucus plug at each pole. Yellow brownish colour double shell. It contains granular mass.	Ova of whipworm Trichuris tri chiura	

Observation	Physiological / pathological significance	Diagram
5) Round oval in shape brownish in colour with thin outer shell of size 80-90 µm contains central mass of large granules.	Unfertilized eggs of round worm (Ascaris lumbricoides)	
6) Oval or round shape of size 70 µm. Shell is stained brown with yellow contents. It contains granular mass which is unsegmented fertilised ovum.	Fertilized eggs of round worm, Ascaris lumbricoides.	
7) Oval or elliptical colourless surrounded by thin transparent shell which appears as black line around ovum. It contains ovum which is segmented 4-8 cell stage.	Ova of hookworm Ancylostoma duodenale	
8) Alternate irregular slide, longer than broad, yellow to brown colour.	Eggs of tapeworm Taenia solinum Taenia saginata	
Adult worms found in faeces 1. Pinkish white of size 15cm in length taper at both ends. The tail of male is curved and has small rod like projections. There is a small mouth surrounded by three lips.	Large round worm Ascaris lumbricoides.	
2. White and opaque of length 20mm and diameter 6mm. It has a central stem which has 0-20 side branches on each side. Main side branches are subdivided into smaller branches.	Tapeworms Taenia saginata and Taenia solinum	

7.0 Observation

Student to observe prepared / permanent slides obtained, under microscope and report observations in following table.

Sr. No.	Observation	Physiological / pathological significance

8.0 RESULT:

The microscopic examination of given slides shows presence of

1) ..... 2) .....

3) ..... 4) .....

5) ..... 6) .....

7) ..... 8) .....

9) ..... 10) .....

9.0 QUESTIONS:

Note : Students to answer Q....., Q....., Q....., and the question numbers shall be allotted by teacher.

1. State the purpose of examination of faeces.
2. State the name of parasites of human intestine.
3. Name at least five diseases caused by helminths in human being.
4. Name two diseases caused by protozoa in human beings.
5. Draw a diagram of microscopic examination of Entamoeba histolytica.
6. Draw a diagram of microscopic examination of Giardia lamblia.
7. Draw a diagram of microscopic examination of roundworm.
8. Draw a diagram of microscopic examination of Tapeworm.
9. Classify the types of parasites of human intestine.

Space for answer

Space for answer

Signature of Teacher

Experiment No. 32

1.0 TITLE:

To visit a hospital to study methods of injecting drugs.

2.0 PRIOR CONCEPTS: Hospital

3.0 NEW CONCEPTS:

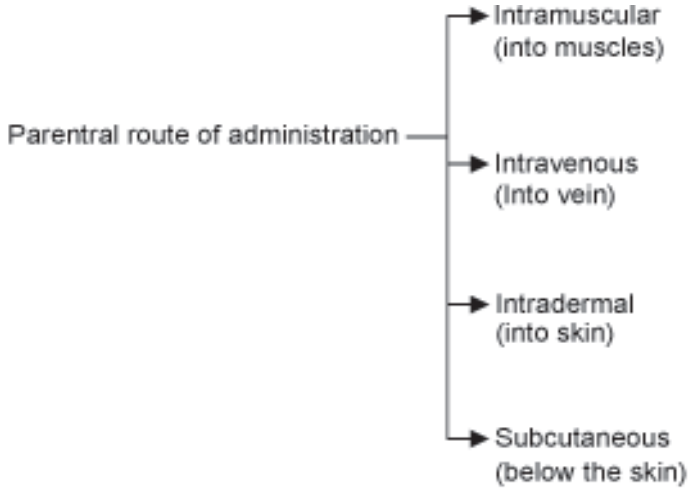
Proposition 1 : Injection

It is introduction of fluid (under pressure) into tissues, a vessel, cavity or hollow organ. It is one of the route of administration of drug.

Proposition 2 : Parentral route of administration of drug.

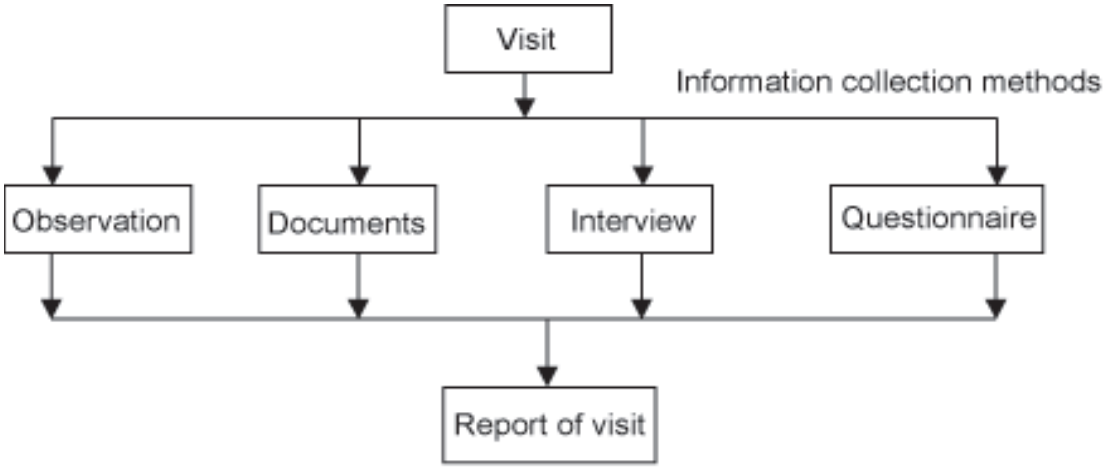
It is administration of drug by route other than enteral i.e. related to gastrointestinal tract

Concept Structure :



Proposition 3 : Visit.

Methods of information collection during visit includes observations, documents, interviews and questionnaire. Information collected should be used for writing report on visit.



4.0 LEARNING OBJECTIVES:

Intellectual Skills

1. To understand concept of injecting drugs.
2. To understand various routes of parentral administration of drug.

Motor Skills

1. Ability to observe the procedure of injecting drugs into body.

5.0 STEPWISE PROCEDURE:

1. Fix the date and time of visit to a hospital by taking permission from appropriate authority.
2. Before the visit to a hospital teacher shall explain methods of injecting drugs.
   - Intramuscular
   - Intravenous
   - Intradermal
   - Subcutaneous

Drugs are administered parentrally in the form of solution by means of syringe and needle or intravenous infusion apparatus.

Syringes, needles, intravenous administration set.

1. A syringe consists of a plunger, a barrel and the main body. They are available in different sizes like 1 ml, 2 ml, 5 ml, 10ml, 20ml, 30ml, or 40 ml. One ml syringe graduated by 0.01 ml division is known as tuberculin syringe and syringe about 1ml size graduated according to insulin unit dose is known as insulin syringe. The needle is attached to the thick neck of barrel. Some syringes have metal tip while some has luer lock system. Luer locking has advantage of locking the needle in position so that it is not dislodged when pressure is applied to the plunger or syringe is withdrawn from site of injection.
2. The needle used for administration of drugs is known as hypodermic needle. It is made up of stainless steel, hypochrome steel, chromium, nickel, platinum etc. It is strong, slightly flexible and rust resistant. The size of needle is defined by two measures guage and length of needle (shaft) in inches. Guage indicates the outer diameter of hypodermic needle and may range from 13 guage to 27 guage. Higher the number smaller is the diameter. Length of needle or shaft is measured from the junction of hub to the tip of point in inches. Needle length ranges between 1/4 inch to 3 ½ inches. For intramuscular injection a needle of 20 to 24 guage and of length 3/4 to 1 inches is used depending on volume of fluid and nature of drug. For intravenous injection 20/22 guage needle and 1 to 1/2 inch length is preferred while for intradermal injection needle of 24 guage and 1/4 to 3/4 inch length is used.
3. For intravenous administration of transfusion fluids, the intravenous administration set or IV set is used. It consist of spike, chamber, flow regulator, a latex tube and needle. The length of tube is 1.7 meter with 3 mm of internal diameter. The tube is sterile, pyrogen free, smooth, flexible and non toxic. The spike is strong needle like structure made up of plastic material having 3.4 mm internal diameter. It is inserted into bottle from where the infusion of fluid is done. The chamber is transparent, clear, collapsible with nylon microfilter of 3/4 cm diameter. Length of chamber is about 3 inches. Flow regulator regulates flow rate of transfusion fluid. A latex tube of 6cm length is provided in between needle and flow regulator to facilitate injection of extra medication without leakage. Needle is usually siliconised 20/22 guage and 1/4-1/2 inches long.

   The syringes, needle, intravenous administration set used for injections must be sterile

- **Intramuscular injection**

  In this technique, drug is injected in the layers of muscle tissue. The injections are made with longer and heavier needle. The irritant drugs, suspensions are administered by this route. small volumes upto 2 ml are injected into deltoid muscle, while large volumes upto 10 ml are injected into gluteal muscle. It is utilized for administration of esters of sex harmones, steroids, poorly soluble salts like benzathine penicillin.

- **Intravenous injection** :

  In this technique, drug is injected directly into vein, usually cubital vein at the bend of elbow is selected. This route bypasses all barriers of drug absorbtion hence 100% absorbtion is achieved. Insoluble drugs, oily substances, drugs in suspensions, acid or alkaline salts are never administered by this route. This route is utilized for administration of potent drugs when rapid action is desired. Transfusion fluids are administered by this route.

- **Intradermal injection** :

  In this technique, drug is injected in the layers of the skin in small quantities i.e. not more 1 ml. It is used when local or systemic effect of drug is desired. It is utilized for diagnostic tests like shick test for diphtheria, for BCG vaccine, to test allergic test.

- **Subcutaneous injection** :

  In this technique, injection is made into loose connective tissue – subcutaneous tissue under the skin, site of injection is outer surface of arm or front of the thigh. Solution of drug upto quantity 2ml is administered by this route. Drugs like adrenaline, morphine, insulin are administered by this route.

  Other parentral route includes :

  Intrathecal injection : This technique involves injection of spinal anaesthetics into subarchanoid space. The drugs act directly on central nervous system.

  Intraarticular and intralesional injection.

  In this technique, drugs are injected into a joint for local treatment. Drugs like Hydrocortisone acetate is injected in joint for treatment of rheumatoid arthritis. Glucocorticoids, local anaesthetics are injected intraleisonally into painful and tender spots.

  3. Visit a hospital and report your observations in given format.

6.0 OBSERVATION :

1. Name of the hospital :- .....
2. Address of the hospital :- .....

   .....

   .....

   .....

3. Student to write the observations in following table

Route of injection of drug	Site of injection	Name of the drug	Dose (quantity injected in ml)	Age of patient in years	Needle used having siz		Syringe and needle used sterile disposable /if nondisposable method of sterilisation used for sterilisation
					Guage	Length in Inches	
1. Intramuscular							
2. Intravenous							
3. Intradermal							
4. Subcutaneous							

7.0 RESULT :

Injection of drugs is observed by 1) ..... 2) .....

3) ..... and 4) ..... routes

8.0 QUESTIONS:

Note : Students to answer Q....., Q....., Q....., and the question numbers shall be allotted by teacher.

1. State the routes of parentral administration of drug.
2. Name the site of intravenous injection of drug.
3. State the precautions to be taken during intramuscular injection of drug.
4. State three disadvantages of parentral route of administration of drug.
5. State five advantages of parentral route of administration of drug.
6. State the procedure of subcutaneous injection of drug.
7. State the procedure of intravenous injection of transfusion fluids.
8. Give reason why the needle, syringes, intravenous administration set used for injection must be sterile.
9. Give method of sterilisation of needle and syringes used in hospitals for injecting drugs.
10. Which route of injection is used for administration of insulin ?

Space for answer

Space for answer

Signature of Teacher

Experiment No. 33

1.0 TITLE :

To visit a pathology laboratory to study methods of withdrawal of blood.

2.0 PRIOR CONCEPTS : Pathology laboratory

Pathology laboratory is a place where samples of blood, urine, sputum, faeces etc. are collected and examined for diagnostic purpose.

3.0 NEW CONCEPTS:

Proposition 1 : Blood, Examination of blood

The common examinations done on blood are hematological, biochemical, serological and cultural examinations.

Proposition 2 : Withdrawal of blood

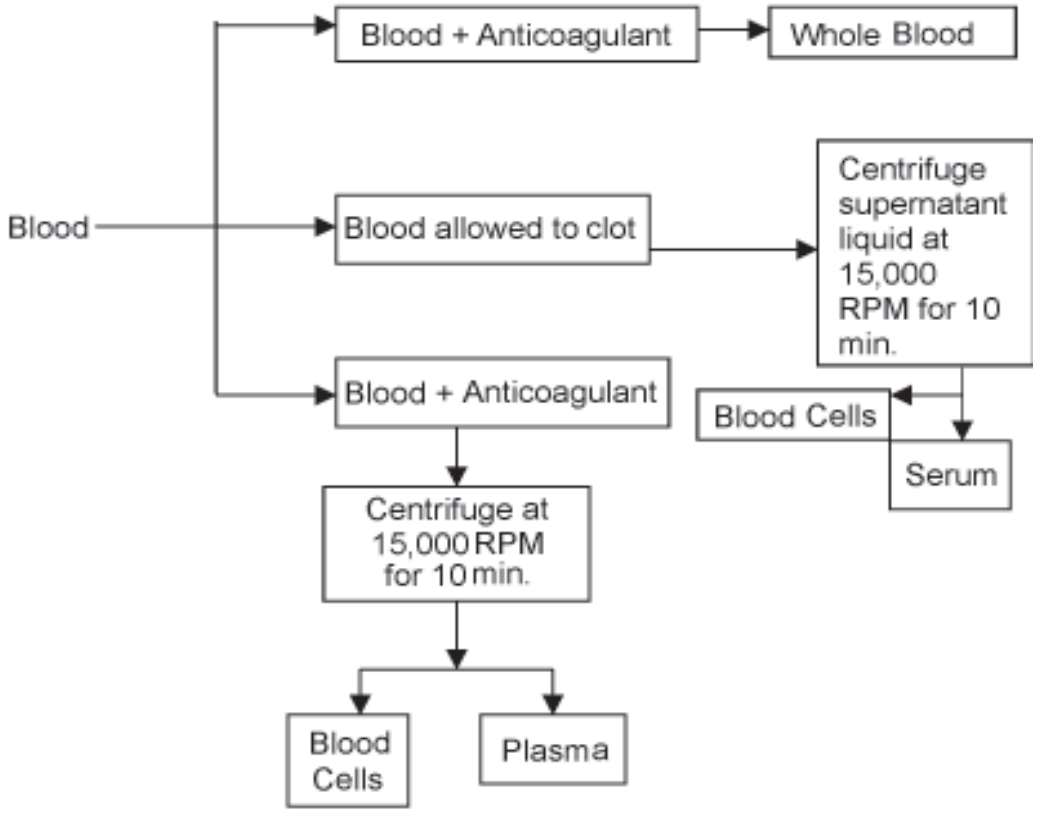
Blood can be withdrawn from body by two methods

1) Capillary withdrawal 2) Vein blood withdrawal

Proposition 3 :

Blood is used in the form of whole blood, serum, plasma for various investigations.

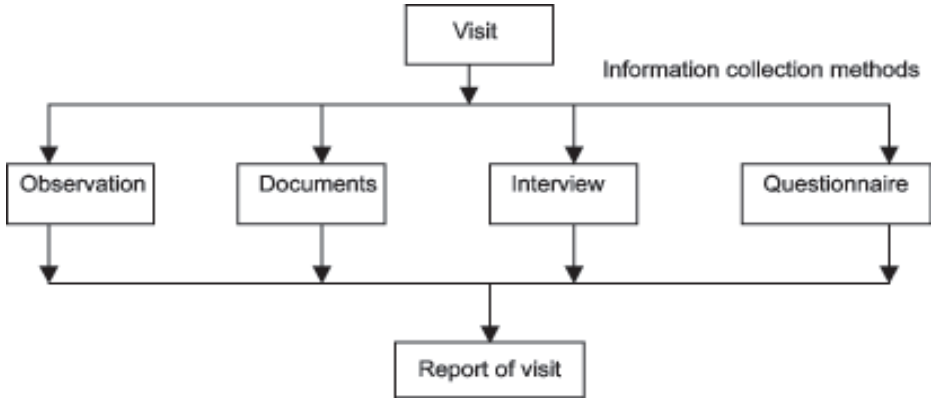
Concept Structure :



Note Plasma differs from serum as plasma contains fibrinogen and anticoagulant.

Proposition 4 : Visit.

Methods of information collection during visit includes observations, documents, interviews and questionnaire. Information collected should be used for writing report on visit.



4.0 LEARNING OBJECTIVES :

Intellectual skills :

1. To understand the concept of withdrawal of blood
2. To understand the procedure of preparation of plasma and serum from blood.

Motor skills :

1. Ability to observe various methods of withdrawal of blood.

5.0 STEPWISE PROCEDURE :

1. Fix the date and time of visit to pathology laboratory by taking permission from appropriate authority.
2. Before the visit to a pathology laboratory teacher shall explain methods of withdrawal of blood listed below.

➢ **Capillary blood withdrawal :**

The capillary blood is obtained by pricking the skin. In this method few drops of blood is collected.

Blood is directly collected in pipette. For capillary blood withdrawal, blood is withdrawn from either ball of finger tip or ear lobule. In infants blood is withdrawn from either ball of thumb or the great toe of the heel. This method is used for haemoglobin estimation, R.B.C. count, differential leucocyte count or blood group determination where amount of blood required is less.

In this method

1. Site of puncture is examined to make sure that there is no edema or congestion.
2. Site is cleaned with alcohol and allowed to dry.
3. Prick is obtained at site using sterile needle or with sterile lancet.
4. The first drop of blood is wiped off and sample is collected in pipette.
5. Once the blood is collected, piece of cotton wool is moistened with alcohol and pressure is applied with it for a minute.

➢ **Venous method for withdrawal of blood :**

This method is obtained by vein puncture, collected blood is delivered slowly in bulbs or tubes containing anticoagulant like disodium EDTA, heparin, ammonium oxalate, potassium oxalate, sodium fluoride, etc. In this method a few ml of blood is collected, it is withdrawn

with sterile syringe and transferred in a bulb or tube containing anticoagulant. Withdrawal of blood from veins is used for donation of blood or haematogical examinations and diagnostic purpose.

In this method

- Vein in antecubital fossa is chosen, it is made prominent by applying tourniquet.
- Site is cleaned with alcohol, it is allowed to dry.
- The vein is punctured with needle and plunger of the syringe is pulled back gently.
- Torniquet is relesed and after sufficient collection of blood, needle is withdrawn.
- Punctured site is pressed with cotton pad moistened with alcohol.

3. Visit a pathology laboratory and report your observations in given format.

6.0 OBSERVATION :

1. Name of the pathology laboratory.

   .....

2. Address of pathology laboratory.

   .....

3. Name of the method observed for withdrawal of blood.

   .....

   .....

4. Site form which blood is withdrawn.

   .....

   .....

   Purpose of withdrawal of blood.

   .....

5. Age of the patient in years .....
6. Instruments used during withdrawal of blood.

   .....

   .....

7. Quantity of blood withdrawn ..... drops/ml
8. Name and quantity of anticoagulant if any, added to withdrawn blood .....
9. General procedure in five steps of the observed method of withdrawal of blood.

   .....

   .....

   .....

   .....

   Stamp of pathology laboratory and registration No. .....

7.0 RESULT:

Blood withdrawal is observed by 1. ..... method

and 2. ..... method

8.0 QUESTIONS:

Note : Students to answer Q....., Q....., Q..... and the question numbers shall be allotted by teacher.

1. State four purposes of withdrawal of blood
2. Give composition of blood.
3. State at least two differences between whole blood and plasma.
4. State one difference between plasma and serum.
5. Name two investigations on blood involving use of blood plasma.
6. Name two investigations on blood involving use of blood serum.
7. Name methods of withdrawal of blood.
8. State four advantages of capillary method of withdrawal of blood.
9. State precautions to be taken during venous method of withdrawal of blood.
10. State four advantages of venous method of withdrawal of blood.
11. State two disadvantages of capillary method of withdrawal of blood.
12. State two disadvantages of venous method of withdrawal of blood.
13. Draw a labelled diagram of pricking needle or lancet.
14. Draw a neat labelled diagram of syringe used for withdrawal of blood from vein.

Space for Answers

Space for answer

Date : Signature of Teacher

REFERENCE DIAGRAM :

Fig 2.1 Glucosazone

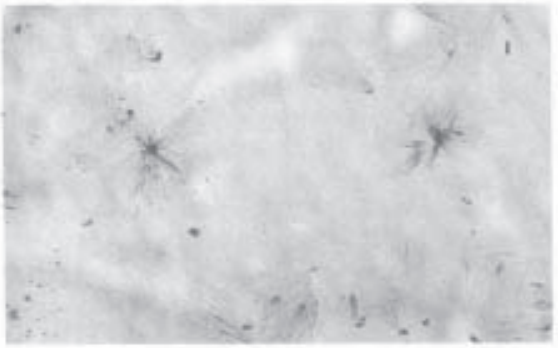


Fig 2.2 Maltosazone

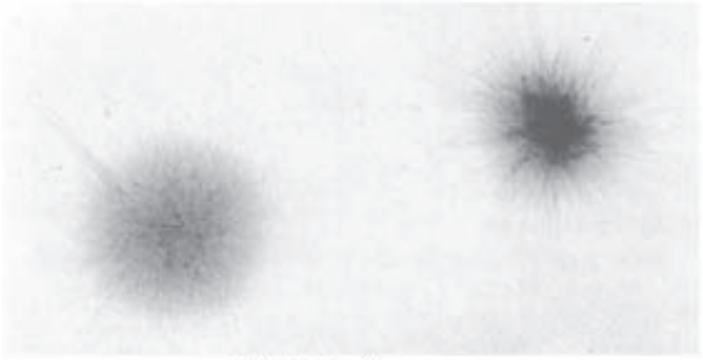


Fig 2.3 Lactosazone

PLATE (COLOURS OF THE TESTS)
REACTIONS OF CARBOHYDRATES

TEST	OBSERVATION	COLOR	CONFIRMS
Molisch's Test	Violet Ring		Carbohydrate
	Blue Negative		Absence of reducing sugar 0%
	Green Color (+)		Presence of reducing sugar 0.1 – 0.5 g %
Benedict's Test	Yellow (++)		Presence of reducing sugar 0.5 – 1.0 g %
	Orange (+++)		Presence of reducing sugar 1.0 – 2.0 g %
	Brick red (++++)		Presence of reducing sugar > 2.0 g %
Barfoed's Test	Deep Blue Color		Monosaccharides
Fouiger's Test	Blue Color		Keto Sugar
Selivanoff's Test	Red Color		Keto Sugar
Iodine Test	Purple		Dextrine

Fig. 2.4

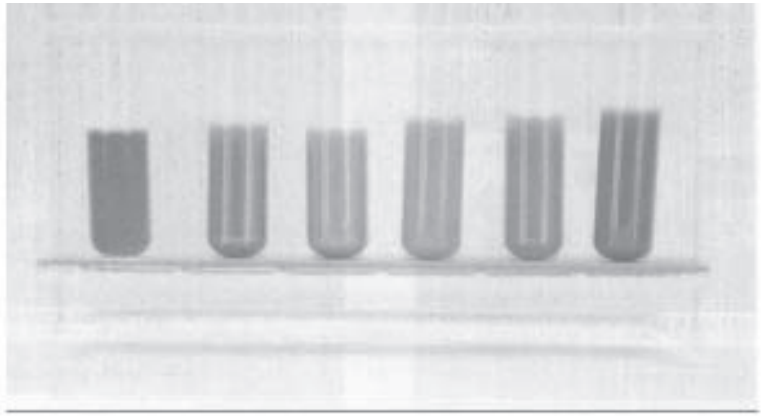
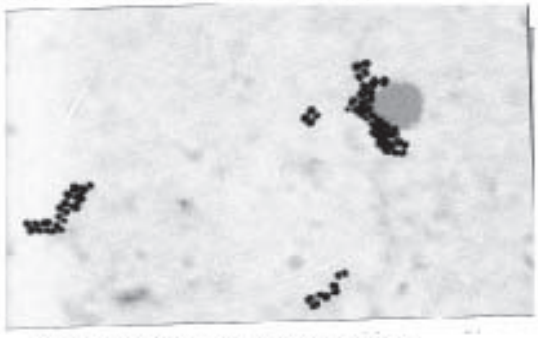


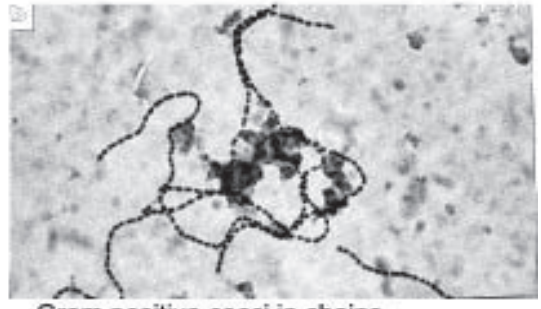
Fig 23.2

Slides showing gram staining



Gram positive cocci in clusters staphylococcus aureus

Fig 30.3



Gram positive cocci in chains pyogenes

Fig 30.4

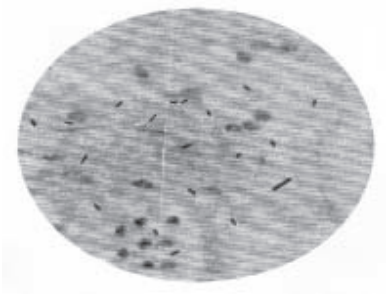
Gram positive cocci in pairs streptococcus pneumoniae

Fig 30.5

Gram negative rods of klebshela pneumoniae.

Fig 30.6

Slide showing acid fast staining



Bright red bacilli of Mycobacterium tuberculosis
Fig 30.7

Pattern of M.S.B.T.E.'s Practical Examination

Class : First Year D. Pharm. Subject : Biochemistry and Clinical Pathology (E.R. 91)

Time : 3 hours Marks : 80

Q. 1 Synopsis (10)

Q. 2 Major experiment (40)

Identification of given sample of natural product by qualitative analysis.

Q. 3 Minor experiment

a) Detection of abnormal constituents present in given sample of urine. (20)

OR

a) Colourimetric estimation of biochemical constituent.

b) Microscopy

Identify sketch and label the pathogenic micro organism observing slide under microscope.

Q. 4 Viva Voce (10)

(Note : Experiment on colourimetric estimation shall be allotted to few students of each batch.

Guidelines for M.S.B.T.E.'s Annual Practical Examination

Q. 1 Synopsis (10)

Any five questions from Lab Manual for two marks for each.

Q. 2 Identification of natural product (5)

I] Identification of type

Carbohydrates / protein / lipid

II] Identification of group (10)

Reducing / non-reducing sugar

Coagulated / non-coagulated protein

Aliphatic /Aromatic amino acid

Fat soluble / water soluble lipid

III] Classification of group

Reducing sugars

Glucose / Fructose / Maltose / Lactose

Non-reducing sugars

Sucrose / Starch

Precipitation and colour reaction of proteins of amino acids (10)

Saponification and emulsification tests of lipids

iv] Confirmatory tests (5)

Q. 3 a) Two abnormal constituents can be given during examination each for 10 marks (20)

OR

a) Colorimetric estimation

1. Preparation of solution
2. Colorimetric reading
3. Calculation

b) Microscopy (10)

Two slides can be given for abservation each of 5 marks

Q. 4 Viva (10)

List of Laboratory Manuals Developed by MSBTE For Diploma In Pharmacy

First Year

1.	Pharmaceutics - I	(0805)
2.	Pharmaceutical Chemistry - I	(0806)
3.	Pharmacognosy	(0807)
4.	Biochemistry and Clinical Pathology	(0808)
5.	Human Anatomy and Physiology	(0809)

Second Year

1.	Pharmaceutics - II	(0811)
2.	Pharmaceutical Chemistry - II	(0812)
3.	Pharmacology and Toxicology	(0813)
4.	Hospital and Clinical Pharmacy	(0816)

PHARMACIST'S OATH

- I swear by the Code of Ethics of Pharmacy Council of India in relation to the community and shall act as an integral part of health care team.
- I shall uphold the laws and standards governing my profession.
- I shall strive to perfect and enlarge my knowledge to contribute to the advancement of pharmacy and public health.
- I shall follow the system, which I consider best for pharmaceutical care and counseling of patient.
- I shall endeavour to discover and manufacture drugs of quality to alleviate sufferings of humanity.
- I shall hold in confidence the knowledge gained about the patients in connection with my professional practice and never divulge unless compelled to do so by the law.
- I shall associate with organizations having their objectives for betterment of Profession of Pharmacy and make contribution to carry out the work of those organizations.
- While I continue to keep this oath unviolated, may it be granted to me to enjoy life and practice of pharmacy respected by all, at all times!
- Should I trespass and violate this oath, may the reverse be my lot!

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