V.V.VANNIAPERUMAL COLLEGE FOR WOMEN
(Belonging to Virudhunagar Hindu Nadars)
An Autonomous Institution Affiliated to Madurai Kamaraj University, Madurai
Re-accredited with 'A' Grade (3rd Cycle) by NAAC
VIRUDHUNAGAR – 626 001

LAB MANUAL ON

BIOCHEMISTRY AND ANIMAL PHYSIOLOGY

(UNDER DBT STAR COLLEGE SCHEME)
No HRD-11011/163/2020-HRD-DBT
Department of Biotechnology, Ministry of Science and Technology
MHRD, New Delhi

Dr. M.TAMILSELVI

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DBT STAR COLLEGE SCHEME

Department of Biotechnology, Ministry of Science and Technology
Government of India, New Delhi

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FOREWORD

The lab manual on Biochemistry and Animal Physiology has been prepared for the benefit of the undergraduate Zoology students as per the revised syllabus under the **Star College Scheme, Department of Biotechnology, Ministry of Science and Technology,** New Delhi. This lab manual will help the students understand the role of biomolecules in biochemical reactions that occur in organisms by performing a series of experiments. These experiments explore the theoretical knowledge obtained in the classroom studies and could be applied to carry out projects in the specific area of the field through the practical. This lab manual is prepared to meet the needs of UG Zoology students .

Despite the fact that the principles of biochemistry and physiology are inextricably linked with one another, the book divides them into two sections. Section 1 deals with the experiments related to biochemistry and Section 2 describes the practicals related to animal physiology. This book covers the laboratory exercises on buffer preparation, pH, qualitative and quantitative estimation of primary metabolites, enzymes, blood clotting, estimation of blood corpuscles, etc. Moreover, the principles, operating techniques and applications of important instruments used in laboratories are included for gaining practical knowledge that would help the students do the projects, create interest in pursuing higher studies, and provide a platform to work in the laboratories after completing the UG degree.

The author acknowledges Dr.H.Abdul Jaffar Ali, Assistant Professor & Head, Department of Biotechnology, Islamiah College, Vaniyambadi, for his support as a reviewer. The Author, the Coordinator, the Principal/Chairman, and the Management of V.V.Vanniaperumal College for Women, Virudhunagar, sincerely acknowledge the **Department of Biotechnology, Ministry of Science and Technology,** New Delhi, for providing financial support for the preparation of this lab manual.

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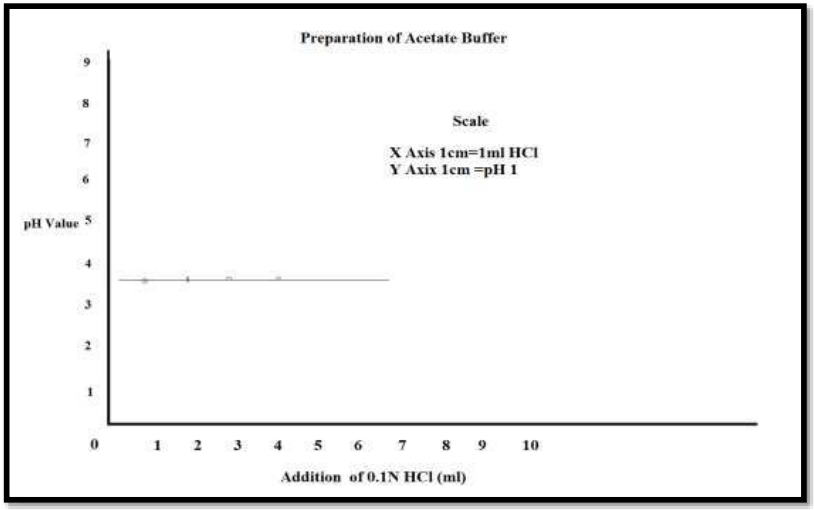
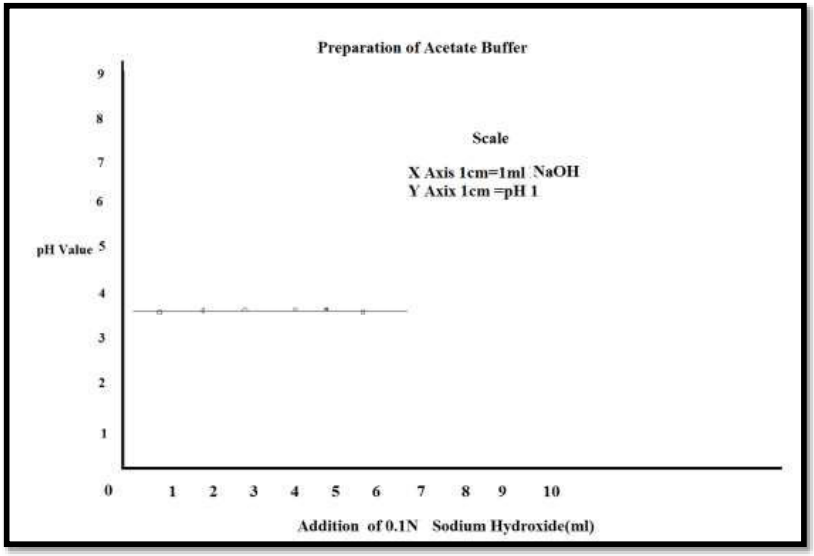
BIOCHEMISTRY & PHYSIOLOGY

PREPARATION OF ACETATE BUFFER





PREPARATION OF ACETIC ACID- SODIUM ACETATE BUFFER

AIM : To prepare acetate buffer at required pH.

PRINCIPLE

A buffer solution resists pH change on the addition of acid or alkali. A typical buffer system consists of a mixture of a weak acid and a salt.

REQUIREMENT

A] Equipment	:	pH meter
B] Apparatus & Glassware	:	beakers, pipettes and Measuring jar
C] Chemicals	:	0.1N Acetic acid, 0.1N HCl, 0.1N sodium acetate and 0.1NaOH

PROCEDURE

A known quantity, 10ml of 0.1N acetic acid is taken in a beaker and 10 ml of 0.1N sodium acetate is added to it and mixed well. This is a buffer solution. The pH of the solution is measured using pH meter. Now add 1 ml of 0.1N HCl. Mix well and measure the pH. Repeat it for three to four times and check the pH. No change in pH of the solution occurs. The titration curve is drawn by taking pH along X axis and quantitative acid/base taken along Y axis.

Take 10ml of 0.1N acetic acid and 100ml of 0.1N sodium acetate in a beaker. Mix well and repeat it for 3 or 4 times and measure the pH of the solution. No change in value of pH occurs. Now add 1 ml of 0.1NaOH and mix well. Repeat it for 3 or 4 times and check the pH value. No change of pH occurs. The titration curve is drawn by taking pH along X axis and quantitative acid/base taken along Y axis.

RESULT : There is no change in pH value with addition of 0.1N HCl/ 0.1N NaOH.

INFERENCE

A buffer solution is one that resists change of pH value on addition of an acid or alkali. A typical buffer system consists of a mixture of weak acid (acetic acid) and its conjugate base (sodium acetate).

OUTCOME: Students are able to

1. understand the theoretical concept of buffer solution.
2. Can prepare buffer solution for the experiments.
3. Acquire skill of preparation of buffer soulutions

BENEDICT TEST FOR CARBOHYDRATE

Negative Reaction Positive Reaction

QUALITATIVE ANALYSIS OF CARBOHYDRATE

AIM: To find the presence or absence of carbohydrate in the given sample by Benedict's test.

REQUIREMENTS

A]Chemicals/Reagents : Benedicts reagent, Glucose

B]Glasswares/Apparatus : Test tubes, Test tubes stand, test tube holders Bunsen burner, measuring cylinders and glass rod

PRINCIPLE

When benedict's reagent is heated with sugar solution, it turns orange red to brick red. This is due to the reducing property of the sugars (Monosaccharides and some disaccharides) as they have free aldehyde or ketone functional groups. This test helps to test the presene of glucose in urine.

PROCEDURE

Take 2 test tubes and marked as A and B. In "A" test tube, 2 ml of Benedict's reagent is taken and added 4-5 drops of water to it. It is boiled for a few minutes and then cooled.

In the "B" test tube, 2 ml of Benedict's reagent is taken and added 4-5 drops of sample (sugar solution) to it. It is boiled for a few minutes and then cooled.

RESULT

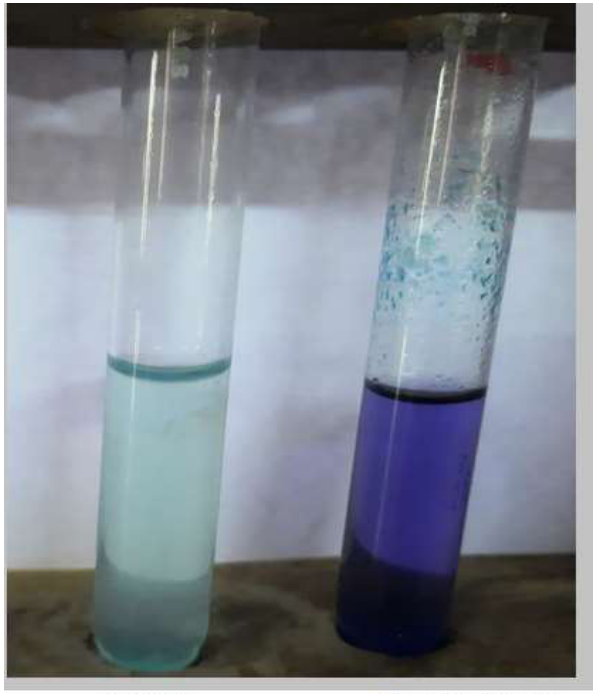
In the B rest tube, the colour is changed from blue to shades of green and then to orange in B test tube.

INFERENCE

The colour change (orange red to brick red) indicates the presence of carbohydrate in the test tube "B"

NOTE: Sucorse will not answer this test. Because sucrose is a non-reducing sugar, which does not react with Benedict's reagent.

BIURET TEST FOR PROTEIN



No Protein Presencce of Protein

QUALITATIVE ANALYSIS OF PROTEIN

AIM: to find the presence or absence of protein in the given sample by Biuret test Method.

REQUIREMENTS

A]Chemicals/Reagents : 5% egg white (Albumen), Biuret reagent (5% copper sulphate, 5% Sodium hydroxide)

B]Glasswares/Apparatus : Test tubes,Test tubes stand, test tube holder, measuring cylinders and glass rod

PRINCIPLE

When biuret reagent is added with the solution containing protein, purple colour appears due to the reaction between the peptide bond of protein and copper to form coloured complexes. This test is used for identification of proteins and aminoacids.

PROCEDURE

Take 2 test tubes and marked as Aand B. In "A" test tube, 2 ml of water is taken. Following, 2 ml of 5% **sodium hydroxide** is added. To this, 5ml of **copper sulphate** solution is added drop by drop and keep it for 2-3 minutes for developing colour.

Take the test tube "B" in which 2 ml of water is taken. Following, 2 ml of 5% **sodium hydroxide and** 5% of **copper sulphate** solution is added drop by drop and keep it for 2-3 minutes for developing colour.

RESULT

The test solution in "B" tube turns to violet colour.

INFERENCE

The colour change indicates the presence of Protein.

EMULSIFICATION TEST FOR LIPIDS

QUALITATIVE ANALYSIS OF LIPIDS

AIM: to find the presence or absence of lipids in the given sample by emulsification test.

REQUIREMENTS

A]Chemicals/Reagents : Coconut oil, 5% sodium carbonate (5 grams of sodium carbonate dissolved in 100 ml of distilled water), distilled water

B]Glasswares/Apparatus : Test tubes, Test tubes stand, test tube holders

PRINCIPLE

Lipids are the organic molecues soluble in non-polar solvents (chloroform, ether, acetone etc). The lipid or oil in water appears as the supernatant. Emlsifying agents emulsify the lipid. As a result, the lipid appears as the tiny droplets suspended in the solution.

PROCEDURE

In a test tube, 2 drops of sample (coconut oil) is taken. To this, 4ml of **water** and 4 drops of 2% **sodium carbonate** is added and shaken well.

RESULT

The formation of emulsion i.e, distribution of lipids uniformly throughout the solution is observed.

INFERENCE

The formation of white effervescence (emulsifying oil droplets) indicates the presence of lipids in the given sample.

OUTCOME: Students are able to

1. Find out the presence of major biochemical components in the given biological samples.
2. Gain the practical knowledge on reactions of biochemical components with other reagents.

Quantitative Estimation of Carbohydrate

S.No	Stock solution((mL))	DH_2O (mL)	Anthrone Reagent (mL)		Concentration of Carbohydrate (µg)	OD value
1	-	1	4			Optical vavlue at 620nm
2	0.1	0.9	4			
3	0.2	0.8	4			
4	0.3	0.7	4			
5	0.4	0.6	4			
6	0.5	0.5	4			
7	0.6	0.4	4			
8	0.7	0.3	4			
9	0.8	0.2	4			
10	0.9	0.1	4			
11	1.0	-	4			
12	Unknown	-	4			
13	Unknown	-	4			
14	Unknown	-	4			
15	Unknown	-	4			
16	Unknown	-	4			

Quantitative Estimation of Carbohydrate

AIM: To estimate the total carbohydrate in unknown samples by Anthrone reagent method.

REQUIREMENTS

A]Equipment	Spectrophotometer and Centrifuge
B]Chemicals	Anthrone reagent, Sulphuric acid, Glucose, Trichloroacetic acid, distilled water
C]Apparatus and Glassware	Pipettes, beaker, Test tube, test tube caps, test tubes stand, Tissue paper and wash bottle

PRINCIPLE

This method can be used for the estimation of reducing as well as non-reducing sugar, i.e. total sugar in the sample. In this method, the sugars in the presence of concentrated sulphuric acid gets dehydrated and produce furfural (from pentoses) or 5-hydroxy methyl furfural (5 HMF). On reacting with anthrone, it produces a coloured compound which can be read at 625nm. Pentoses, hexoses, heptoses and their derivatives yield a coloured product in these reactions whereas trioses, tetroses and amino sugars do not yield any coloured product. Anthrone method is simple, in sensitive to interference and therefore gives reliable result of total carbohydrate in samples.

PREPARATION OF REAGENTS

A] Anthrone reagent: [0.2% prepare a fresh]

2gm of anthrone is dissolved in 1 litre of concentrated H_2SO_4 to prepare 0.2% solution. This reagent needs to be prepared freshly.

B] Standard sugar solution

100mg of sugar (glucose, sucrose, etc) is dissolved in 100 ml distilled water. Dilution factor is 1:10.

Note: Other carbohydrates of the same concentration can also be prepared in preparation of the standared sugar solution.

PROCEDURE

The dry weight of the sample (100mg) is homogenized in a mortar with 2ml of 10% TCA and 8ml of distilled water. The homogenate is centrifuged at 3000rpm for 15 minutes. The supernatant, 0.5ml is taken in a test tube. To this, 4.5 ml of anthrone reagent is added and vortexed rapidly. The tube is covered and kept in a boiling water bath for 10 minutes and cooled to room temperature in a beaker containing tap water. The developed colour is read at 625nm against a reagent and blank in a colorimeter.

The standard curve is obtained using the values read from spectrophotometer with the standard ranging from 0.1ml to 1.0ml. The values are expressed as mg carbohydrate per 100mg dry weight of the sample.

RESULT

The amount of carbohydrate present in unknown sample is -------mg/100mg dry weight of the sample.

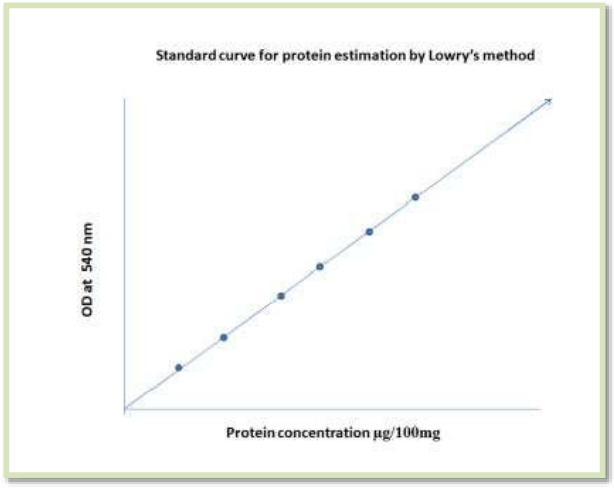
OUTCOME: Students were able to

1. Estimate the total carbohydrate in the given biological samples.
2. Handle the spectrophotometer individually and efficiently.

Quantitative Estimation of Protein

Table shows The OD value of different samples for Protein Estimation

Nature of sample	Volume of sample (ml)	Volume of water (ml)	Volume of copper sulphate (ml)	Volume of Folin Phenol reagent (ml)	Optical Density(OD)
Standard	0.1	0.4	4.0	0.5	
	0.2	0.3	4.0	0.5	
	0.3	0.2	4.0	0.5	
	0.4	0.1	4.0	0.5	
	0.5	----	4.0	0.5	
Unknown	0.1	0.4	4.0	0.5	
	0.2	0.3	4.0	0.5	



Quantitative Estimation of Protein

AIM: To estimate the total protein from the given sample by Lowry *et al.,* method

REQUIREMENTS

A]	**Equipment**	**:** Digital balance Centrifuge and Spectrophotometer
B]	**Chemicals**	**:** Sodium Carbonate, Sodium hydroxide, N –butanol: acetic acid: water (3:1:1), 200mg of Ninhydrin, acetone and acetic acid, Folin Phenol reagent, Trichloro acetic acid, copper sulphate, sodium potassium tartarate, distilled water and phenol reagent
C]	**Apparatus & Glassware**	**:** Test tubes, Beakers, measuring cylinder, pipette, glass rod, test tubes stand, blotting paper

PRINCIPLE

This is the most widely used method in all laboratories. Protein reacts with folin phenol reagent and produces a blue colour. The colour development relies on the formation of a copper-protein complex. In this reaction, Cu^{2+} in alkaline solution complexes with nitrogen atoms of the peptide bonds in proteins. This complex forms a purple colour at high temperature (Cu^{2+} is maintained in alkaline solution). Cu^{2+} also acts as a catalyst in the reduction reaction.

PREPARATION OF REAGENTS

Reagent A

2% Na_2CO_3 in 0.1N NaOH

4.0gm sodium hydroxide is dissolved in 800ml distilled water and then dissolved 20g sodium carbonate (anhydrous) and the volume is made upto 1000ml with distilled water and stored at room temperature.

Reagent B

2% $CuSo_4$ $5H_2O$

20 g copper sulphate is dissolved in 100ml distilled water and stored at room temperature.

Reagent C

2% sodium potassium tartarate

2g of sodium potassium tartarate is dissolved in 100ml distilled water and stored at room temperature.

Reagent D

Mix 0.5 ml of reagent B and 0.5 ml of reagent C and then 99ml of reagent A is added. If the reagent D becomes turbid it should not be mixed (The reagents should be mixed only in the mentioned order).

Reagent E

Commercial phenol reagent (AR grade).

PROCEDURE

A known quantity (100mg) of sample is taken in a mortar and homogenised with 2 ml of 10% Trichloroacetic acid. Then the homogenate is centrifuged at 3000rpm for 15 minutes. The supernatant is decanted and washed with TCA and again centrifuged. The residue is dissolved in 1ml of 1N sodium hydroxide and kept in a water bath at 60ºC-70ºC for 10 minutes. The contents are mixed well with a rod. From this, 0.5ml of the solution is pipetted out and taken in a clean test tube and to this solution 4ml of reagent is added and mixed well thoroughly by gentle shaking. It is kept in room temperature for 15 minutes. To this mixture, 0.5 ml Folin – ciocalteau reagent is added. The test tube is shaken well for uniform mixing and kept in a room temperature for 30 minutes. The blue coloured is read at 540nm against blank in Spectrophotometer. A blank is prepared with 0.5ml of distilled water and all other reagents except the sample. The standared curve is obtained by measuring 0.1% bovine serum albumin (BSA) and the protein content is expressed in mg protein per gram dry weight of the sample.

RESULT

The amount of total protein present in the given sample is ----------/100mg dry weight of the sample.

OUTCOME

Students could estimate protein in any biological samples and they could operate the spectrometer individually .

QUANTITATIVE ESTIMATION OF TOTAL LIPIDS

CALCULATION

Weight of the dry test tube without lipid content	= ----------g
Weight of the test tube with lipid content	= ----------g
Total amount of the lipid	= Weight of the dry test tube without lipid content – Weight of the test tube with lipid content = ----------mg

RESULT: The amount of lipid present in the given sample = -------mg/gm

QUANTITATIVE ESTIMATION OF TOTAL LIPIDS

AIM : To estimate the total lipids from the given sample.

REQUIREMENTS

A] Equipment:	Centrifuge, Digital Balance
B] Chemicals	Chloroform
C] Glassware And Apparatus	test tube, cotton plug, mortar and pestle and water bath

PRINCIPLE

Lipids form a heterogenous group of compounds which have the common property of dissolving in organic solvent such as alcohol, acetone, chloroform, benzene and other hexane. The lipids are generally bound to proteins in biological samples as lipoprotein and hence lipids cannot be efficiently extracted by nonpolar organic solvents alone. The infusion of methanol or ethanol helps in breaking the bonds between the lipids and proteins.

PROCEDURE

Lipid is estimated by following the method of Bragdon (1951). The sample is taken in mortar and homogenized well with 2 ml of chloroform using the pestle. The resultant solution is centrifuged for 15 minutes at 3000rpm and filtered using Whatman No 1 filter paper. The supernatant is collected in a pre-weighed test tube and evaporated to dryness in a water bath. The added weight of the test tube is measured as lipid content. The lipid content is estimated by applying the formula.

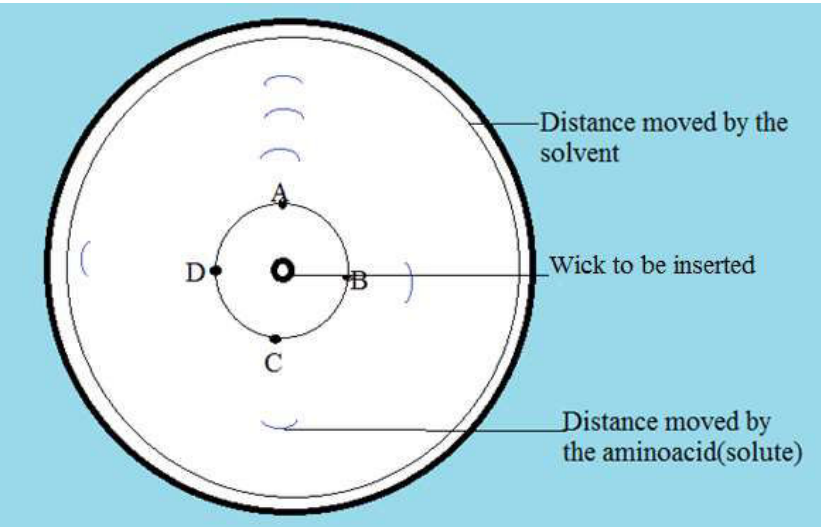
Total amount of the lipid = weight of the test tube with lipid content – weight of the test tube

RESULT

The amount of the lipid present in the given sample = ---mg/gm.

OUTCOME: Students could estimate the total lipids in food/ biological samples.

CHROMATOGRAM



SEPARATION OF AMINOACIDS BY PAPER CHROMATOGRAPHY

AIM: To separate the individual aminoacids from the given sample.

REQUIREMENTS

A]	**Chemicals**	**:** N –butanol: acetic acid: water (3:1:1), 200mg of Ninhydrin, acetone and acetic acid
B	**Apparatus & Glassware**	**:** Petriplates, cotton thread,Whatman No .1 filterpaper, capillary tubes (10µl volume), hot air dryer, disposable plastic gloves, glass sprayer and Chromatography chamber

PREPARATION OF REAGENTS

Solvent System (Mobile Phase)

The solvent system is prepared by mixing 60ml of butanol, 20 ml of acetic acid and 20ml of water in a 250ml glass beaker in the ratio of 3:1:1.

Spray Reagent

200mg of Ninhydrin is dissolved in 99ml of acetone and 1ml of acetic acid and stored in brown bottle.

Standard Amino Acids

2mg/ml of the individual aminoacids (Leucine, alanine, L-Proline) solution is prepared using distilled water. In case of leucine, the soltuon is warmed in a boiling water bath to ensure complete dissolution of the aminaoacids.

PRINCIPLE

The cellulose fibres of chromatography paper act as the supporting matrix for the stationary phase. The stationary phase may be water, a non-polar material such as liquid paraffin, or impregnated particles of solid adsorbent. Aminoacids are separated based on their partition or distribution coefficients between the liquid stationary phase (water held by the chromatographic paper) and the liquid mobile phase. One phase is the liquid (water), whichis held in the pores of the filter paper; and other is the mobile phase which moves over the paper.

TABLE SHOWS THE RF VALUE OF AMINOACIDS

S.No	Aminoacid	Distance travelled by the aminoacid	Distance travelled by the solvent front	Rf value
1	A			
2	B			
3	C			

PROCEDURE

A glass petridish with 100mm diatmeter is cleaned and solvent is added to a height of 1cm.A circular Whatman No.1 filter paper is taken. A circular pencil line is drawn 1cm away from the centre using a pencil. After drawing the circular line, a small hole is made at the centre to fit the wick or cotton thread. This denotes the origin. A mixture containing aminoacids (Proline, Leucine and Isoleucine) are considered as unknown sample and marked as "A" and the known aminoacids (Proline, Leucine and Isoleucine) are spotted at the origin and are marked as "B" "C" and "D" correspondingly with the help of capillary tube (10µl) and allowed to dry. The prepared wick is inserted in the hole. It is placed in the pertirdisih with the lower end of wick touching the solvent solution.

The solvent is allowed to run for about 15 minutes until the solvent reaches about 2cm away from the margin. The solvent front is marked using a pencil and the wick is discarded. Later, the developed chromatogram is air dried at room temperature. The paper is unfolded and the chromatogram is sprayed with ninhydrin reagent.

Aminoacids are observed by the appearance of violet coloured spot. Alpha aminoacids show purple colour while aminoacid (proline) gives yellow colour because it is a secondary amine. Ninhydrin test is highly specific for primary amines and the Proline, a secondary amine give a yellow colour.The distance moved by the aminoacids can be identified using the following formula. The Rf (relative front/ mobility) value is calculated for the separate aminoacids.

$$Rf = \frac{\text{Distance(cm) moved by the solute from the origin(spot)}}{\text{Distance(cm) moved by the solvent from the origin(spot)}}$$

RESULT

The Rf value of aminoacid 1 =
The Rf value of aminoacid 2 =
The Rf value of aminoacid 3=

NOTE

Rf value is constant for a particular aminoacid. The individual aminoacid is identified by its Rf value. The presence of a particular aminoacid in a mixture is confirmd by its Rf value as that of the standard aminoacid. The mixture contains the following aminoacids

1.
2.
3.

OUTCOME

Students learnt the techniques on separation of aminoacids using paper chromatography.

ANALYSIS OF PH IN DIFFERENT WATER SAMPLES

Table shows the pH of different water samples

S.No	Sample	pH	Nature
1	Sample A		Acidic/Alkaline
2	B		
3	C		
4	D		

ANALYSIS OF PH IN DIFFERENT WATER SAMPLES

AIM

To analyse the pH of different water samples using pH meter

REQUIREMENTS

A] **Equipment** : pH meter

B] **Chemicals** : known pH (5.2, 7 and 9.6) buffer solution

C] **Apparatus & Glassware** : Sample containers and beakers

PRINCIPLE

pH is the power of hydrogen ion concentration. It is defined as the negative logarithm of hydrogen ion concentration and denoted as pH = -log $^{(H+)}$

The concentration of H+ ion plays an important role in all biological reactions. The H+ ion concentration in cells and biological fluids is found to vary widely. The pH of pure water is 7. The pH of 1N acid is 0 and the pH of 1N alkali is14. So the pH scale is in between 0 and 14. The pH of an acid solution will be less than 7. The pH of an alkaline solution is more than 7. Any solution capable of donating a hydrogen ion is called a base. Thus the acid or alkalinity is determined based on the electron.

PROCEDURE

Water samples are collected from different places (any aquatic bodies) and depths in wide mouthed glass stoppered bottles. These bottles are marked according to the place of their collection.

In the laboratory, electrodes are rinsed with distilled water and remove the water content using filter paper. Turn the meter on by pressing the “OK” key. Immerse the electrode in the solution to be tested. Stir gently and wait for the reading to stabilize. Now press the C/F Key to measure the readings.

After use, rinse the electrode with water to minimize contamination. Turn the meter by pressing the “OFF” key.

RESULT : The pH of different water samples are given in the table.

The pH of sample A = ----------

The pH of sample B = ----------

The pH of sample C = ----------

OUTCOME

1.Students developed the skill to use pH meter

2.Understand the importance of pH in palatability of water

ESTIMATION OF SALINITY IN DIFFERENT WATER SAMPLES

Table shows the amount of silver nitrate is required to titrate the water samples

S.No	Water Samples (10ml)	Burette reading(ml)		Volume of $AgNo_3$(ml)	Concordant Value(ml)	Indicator
		Initial	Final			
1	Sample [A]					A drop of potassium chromate
2	Sample [B]					
3	Sample [C]					

Volume of titrated water sample =10 ml

CALCULATION

34.55ml of silver nitrate is required to titrate 10ml of standard sea water which has got a chlorinity of 19.38g

$$\text{Therefore, the chlorinity of given water sample} = \frac{19.38}{34.55} \times X\text{ml}$$

Where,

The Chlorinity of normal water (N) = 19.38‰

X is the volume of silver nitrate is required. = --- ml

Then the salinity of the given water sample = 0.03+ (1.805 X chlorinity)

The salinity of water sample (A = ----------g

ESTIMATION OF SALINITY IN DIFFERENT WATER SAMPLES

AIM: To determine the salinity of different water samples by Harvey's Method.

REQUIREMENTS

A] Apparatus & Glassware : Burette (25ml), Pipette (10ml)/measuring cylinder (10ml) and conical flask

B] Reagents : Silver Nitrate(0.1N) and 5% potassium chromate

PRINCIPLE

The chloride present in water samples are precipitated as silver chloride by titrating against silver nitrate using potassium chromate as an indicator. The slightest excess of silver nitrate produces a brick red colour which is taken as the end point. The chlorinity of any given water sample has a definite and close relationship to the salinity and hence the salinity can be determined from its chlorinity.

PROCEDURE

Burette is cleaned with distilled water and rinsed with silver nitrate solution and is filled with same solution. The initial reading is noted. The water sample (10ml) is taken in the conical flask. Two drops of potassium chromate solution are added as an indicator and the colour of the sample changes to yellow. This is now titrated against silver nitrate in the burette till the yellow colour of the solution in the conical flask turns into brick red colour. This is the end point. Now the final reading of the burette is noted. The difference between the initial and final reading gives the amount of silver nitrate required for titrating 10ml of the sample. The titration is repeated till concordant value is obtained. The experiment is repeated with various water samples.

RESULT

Salinity of water sample [A] = ----------gm

Salinity of water sample [B] = ----------gm

VERIFICATION OF BEER –LAMBERT'S LAW

Table 1 shows the different concentration of ($K_2Cr_2O_7$) solution

Test tube number	Potassium dichromate solution (ml)	Distilled water(ml)	Amount of Potassium dichromate (mg)
1	0	5	0
2	0.5	4.5	0.5
3	1.0	4.0	1.0
4	1.5	3.5	1.5
5	2.0	3.0	2.0
6	2.5	2.5	2.5

Table 2 shows the OD value of Potassium dichromate ($K_2Cr_2O_7$) solution

Serial No.	Concentration of Potassium dichromate solution(mg)	Optical Density (OD)
1	0.5	
2	1.0	
3	1.5	
4	2.0	
5	2.5	

VERIFICATION OF BEER –LAMBERT'S LAW

AIM

To verify **Beer-Lambert's Law** using different concentrations of potassium dichromate ($K_2Cr_2O_7$) solution.

REQUIREMENTS

A]Equipment	: Colorimeter with filters
B]Chemicals	: Potassium dichromate
C]Apparatus & Glassware	: test tubes, Test tube stand, pipettes, distilled water and measuring cylinder,

PRINCIPLE

Colorimeter works on the principle of photometry. A colorimeter is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light. The amount of light absorbed or transmitted by coloured solution is in accordance with the Beer's & Lambert's Law.

BEER'S LAW : It states that the intensity of the colour is directly proportional to the concentration of coloured particles in the solution.

LAMBERT'S LAW : It states that the amount of light absorbed by a coloured solution depends on the length of the column or the depth of the liquid through which light passes. The Beer & Lambert Law combines these two laws.

PROCEDURE

In this experiment, six test tubes are taken and they are labelled as 1,2,3,4,5 & 6. In No1 test tube, 5ml of distilled water is taken using a clean 5ml pipette. This serves as the blank solution. Likewise, the test tubes 2,3.4, 5 & 6 are prepared by pouring the solutions as given in table 1.

The blank solution from the test tube No.1 is transferred to the cleaned cuvette. It is inserted into the sample holder. Blue filter is inserted in the meter. Now, the colorimeter is switched on using the knob provided the reading is adjusted to "0" optical density (OD). This is nullify the absorption of light by the solvent. The blank solution is used only for zero setting. Once the zero is set, on any account, the knob should not be disturbed.

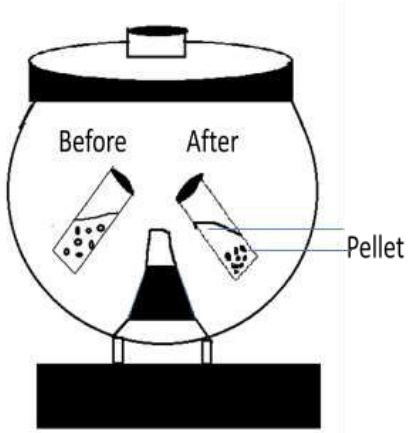
The blank solution in the cuvette is replaced with the solution from the test tube No.2. The optical density (OD) is measured, Similarly the OD of the solution in the remaining test tubes are measured. The readings are noted down in the tabular column as given in Table 2.

A graph is drawn taking concentration of Potassium dichromate on the "X" axis and the OD on the "Y" axis. This graph is known as standard graph. From this standard graph the concentration of unknown Potassium dichromate solution can be measured.

RESULT

The OD increases proportionately to the increase in the concentration of Potassium dichromate solution.

CENTRIFUGE



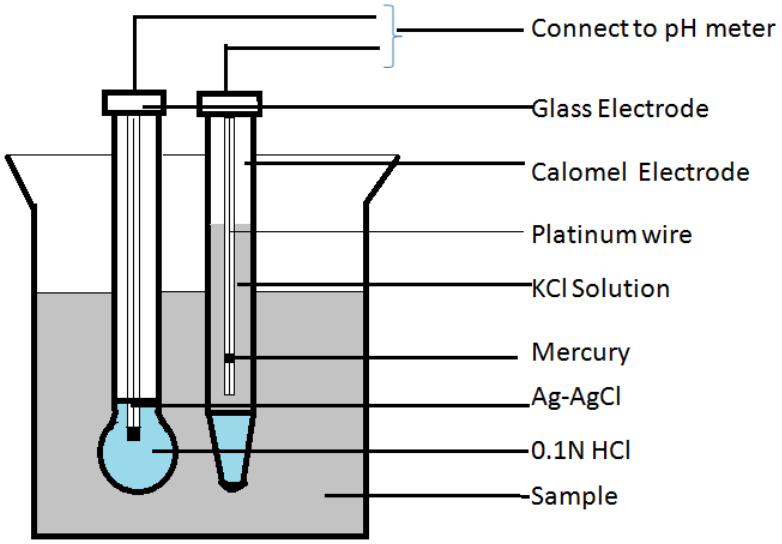
CENTRIFUGE

- It is an apparatus used to separate the particles suspended in a solution by the application of centrifugal force.
- It works on the principle of sedimentation coefficient. Under the influence of gravitational force, substances separate according to their density.
- The centrifuge consists of a motor and a rotor. The motor makes the rotor to spin.
- As a rotor spins in a centrifuge, a centrifugal force is applied to each particle in the sample. The rate of sedimentation of the particles is proportional to the centrifugal force.
- The rate of sedimentation depends on the density of the particles, size of the particles, viscosity of the medium and the gravitational force.

APPLICATIONS

- It is used to separate the sub-cellular components.
- It is used for the determination of molecular weights of proteins.

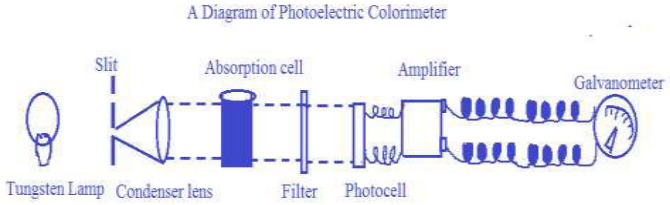
pH METER



pH METER

- This equipment is used to measure the pH of any biological samples eg: water, soil, food etc).
- pH is the measure of acidity or alkalinity of a solution. It is defined as the negative logarithm of the hydrogen ion concentration in a solution.
- The pH meter is of two types, namely Digital pH Meter and Analog pH Meter.
- The pH Meter consists of Power pack and two Electrodes.
- The power pack consists of on/off switch, an indicating meter, a temperature compensation knob, a calibrate knob and a wire with plug pin.
- Glass electrodes and Calomel electrode are the two electrodes.
- The glass electrode has a hard glass tube. At the base, it has thin bulb which contains HCl (0.1mol/lit). It is highly sensitive to H^+ and it allows H^+ to pass through it. A platinum wire is connected to the HCl through a silver-silver chloride electrode (Ag-AgCl). The glass electrode is connected to the power-pack of the pH meter.
- The Calomel electrode is the Reference electrode. It is not sensitive to H+. It contains calomel paste. The calomel paste is connected with a platinum wire through mercury.
- The pH is measured by dipping the electrodes into the test solution and the pH is read from the meter.

COLORIMETER



COLORIMETER

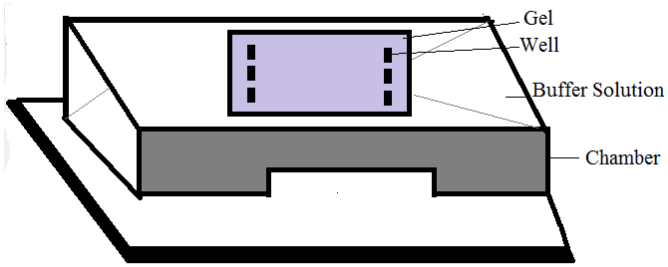
- It is a device used to measure the intensity and wavelength of light absorbed/ transmitted at a particular frequency(colour) by a sample.
- This instrument works under the Principle of Beer-Lambert's Law.
- Beer-Lamberts' Law states that the amount of energy (light) absorbed/transmitted by a colour solution is proportional to the concentration of the solution and the distance that the light travels through the solution.
- Colorimeter consists of the following essential parts.
  a. A light source (a low-voltage filament lamp).
  b. An adjustable aperture (diaphragm to control the intensity of light).
  c. A set of coloured filters (a Prism, providing monochromatic light).
  d. A cuvette (to hold the working sample) and
  e. An indicator (indicates the reading, it includes Galvanometer, Pen recorder, Potentiomenter and oscilloscope).
- In a colorimeter, a beam of light with a specific wavelength is passed through a solution via a series of lenses, which navigate the coloured light in the colorimeter. The colorimeter analyses the reflected light and compares with the predetermined standard.
- Usually, the range wavelength is in between 400 and 700nm.
- The absorption of light transmitted though the medium is directly proportional to the concentration of the substance (chromogens) in the medium.
- If the concentration of the solution is greater, more light will be absorbed as a result the intensity of light fall is lesser.

APPLICATIONS

- It is used to estimate biochemical components in food sample.
- It is used for studying the water quality.
- It is used to monitor bacterial growth or yeast culture.
- It is also used in food industry to monitor the colour intensity in various foods, beverages etc.,

NOTE: A substance to be estimated must have chromogens (coloured substances).

SDS-PAGE



SDS-PAGE

- SDS-PAGE apparatus is used to separate the protein molecules based on their size.
- SDS-PAGE method is composed of gel preparation (Separating gel and Stacking Gel), sample preparation, electrophoresis, protein staining and analysis of generated protein band.
- The principle of SDS-PAGE is that a charged molecule migrates to the electrode with the opposite sign when placed under electric field.
- In this method, Polyacrylamide gel is used as a separating medium and sodium dodecyl sulphate, the detergent is used to neutralize the change of proteins and so this method is called SDS PAGE.
- Sodium dodecyl sulphate denatures the identical proteins into subunits and provides equal charge to mass ratio in their subunits.
- When the denatured protein is electrophoresed through PAGE, proteins migrate towards anode at alkaline pH.
- Smaller proteins move faster than larger proteins.
- Molecular weight of separated proteins can be analysed by comparing molecular weight of standard proteins.
- Each protein unit produces a coloured band on the gel. The intensity of colour bands varies depending on the concentration of proteins.
- This technique is useful to determine the molecular weight of the proteins.

ESTIMATION OF MOISTURE, SODIUM, CALCIUM AND POTASSIUM IN FOOD SAMPLE

AIM: to estimate the moisture content, Sodium, Calcium and Potassium in the given food sample(s).

REQUIREMENTS

A]	**Equipment**	: Flame photometer, Hot Air oven, Electronic weighing balance
B]	**Chemicals**	: Sodium chloride, Calcium carbonate, potassium chloride, perchloric acid, Hydrochloric acid, double distilled water
C]	**Apparatus & Glassware**	: Measuring jar, pipette, conical flask, crucible

PROCEDURE

Estimation of Moisture

About 1 gm of food sample is taken in a weighed dish separately. This sample is spread uniformely in the dish and kept in hot air oven at 100±2℃ for about 16 hours. Then cooled in a disiccator and weighed. The process of heating, cooling and weighing is repeated until to get constant weight of the sample. The differences in weight is calculated and expressed as percentage moisture content in the sample. The percentage (%) moisture content in the given food sample is calculated by applying the given formula

$$\% \text{ of moisture content} = \frac{(b-c)X100}{100}$$

a = weight of the sample in gm

b = weight of the dish + sample

c = weight of the dish + sample after drying

d = drop of weight due to loss of moisture

Take stock solutions as per given table and dilute to 100ml

Na Conc (mEq/lit)	K Conc(mEq/lit)	Stock soln Na (ml)	Stock soln K (ml)
1.0	0.01	0.50	01
1.1	0.02	0.55	0.2
1.2	0.03	0.60	0.3
1.3	0.04	0.65	0.4
1.4	0.05	0.70	05
1.5	0.06	0.75	0.6
1.6	0.07	0.80	0.7
1.7	0.08	0.85	08

ESTIMATION OF SODIUM, CALCIUM AND POTASSIUM

Preparation of sample

Acid Digestion method is adopted to prepare sample. Take 1g of powdered food sample in a crucible. To this, add 3ml of solution containing 1 part of concentrated sulphuric acid and 3 parts of 60% perchloric acid. The crucible is gently heated until the sample is completely digested. Then, stronger heat is applied to facilitate the complete removal of perchloric acid. The sulphuric acid residue is diluted to 20 ml with 2 N HCl acid and any remaining solids is there, it is removed by centrifugation.

PREPARATION OF STANDARD SOLUTIONS

Preparation of stock standard solutions are given below:

For Sodium (Na): Dissolve 2.923 g NaCl in one litre of DD water (50mEq/liter).

For Potassium (K): Dissove 3.728g KCl in one litre of DD water (50mEq/liter).

For Calcium (Ca): Dissolve 2.503 g $CaCO_3$ in 300ml DD Water (50mEq/liter).

a. PROCEDURE FOR ESTIMATION OF NA/K

1. In Flame Photometer, select Sodium and Potassium filters.
2. Following, feed distilled water to atomiser and aspirate distilled water for 30 seconds. Set ZERO on K DPM using SET REF COURSE & FINE controls. No setting is required for Na
3. Feed Standard solutions one by one (1mEq per litre for Na or 1.0/0.01mEqper litre for Na/K to the atomizer and aspirate standard(s) for 30 seconds. Set 100 on Na DPM using SET REF COARSE & FINE adjustment Controls.
4. Feed another standard 1.70/0.080 1mEq per litre for Na/K solution to the atomiser and aspirate it for 30 seconds. Set 170 on Na DPM and 80 on K DPM using respective SET FINE COURSE and FINE controls.
5. Again, feed distilled water to atomizer and aspirate distilled water for 30 seconds. Set/re-set ZERO on K DPM using SET REF COARSE & FINE controls. No setting is required for Na.
6. Repeat steps 3,4, and 5 till satisfactory repeatability is achieved.
7. Now the instrument is ready for estimation of sample.

8. Feed sample to the atomizer and aspirate the sample for 30 seconds and result will be displayed on respective DPMs in 1mEq per litre. After the estimation(s) of the sample, feed idstilled water to the atomizer and aspirate it for 30 seconds.
9. Repeat steps 2-6 for re-calibration after every 10-12 samples for 15 minutes.
10. After the completion of all the estimations, feed distilled water to the atomiser and aspirate it for about 1 minute to wash out remaining from the mixing chamber, atomiser etc.,

b. PROCEDURE FOR ESTIMATION OF CALCIUM(CA)

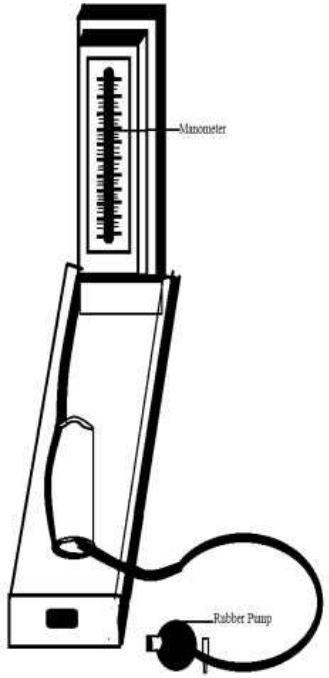
1. In Flame photometer, select appropriate filter for Ca.
2. Feed distilled water to atomizer and aspirate distilled water for 30 seconds. Set ZERO on Ca DPM using SET REF COARSE & Fine controls.
3. Feed 30ppm standard solution for Ca to the atomizer and aspirate it for 30 seconds. Set 100 on CA DPM using SET F.S. COARSE & Fine controls.
4. Again feed distilled water to atomizer and aspirate distilled water for 30 seconds. Set ZERO on K DPM using SET REF COARSE & FINE controls.
5. Repeat steps 3,4, and 5 till satisfactory repeatability is achieved.
6. Now this instrument is ready for estimation of sample.
7. Feed sample to the atomizer and aspirate the sample for 30 seconds and result will be displayed on Ca DPM in ppm. After the estimation(s) of the sample, feed distilled water to the atomizer and aspirate it for 30 seconds.
8. Repeat steps 3 to 6 for re-calibration after every 10- 12 samples or 15 minutes.
9. After the completion of all the estimations, feed distilled water to the atomizer and aspirate it for about 1 minute to wash out remaining from the mixing camber, atomizer etc.

RESULT:

1. The moisture content in the food sample is ----------%
2. The amount of Ca is present in the food sample is ----------ppm/g
3. The amount of Na is present in the food sample is ----------ppm/g
4. The amount of K is present in the food sample is ----------ppm/g

ANIMAL PHYSIOLOGY

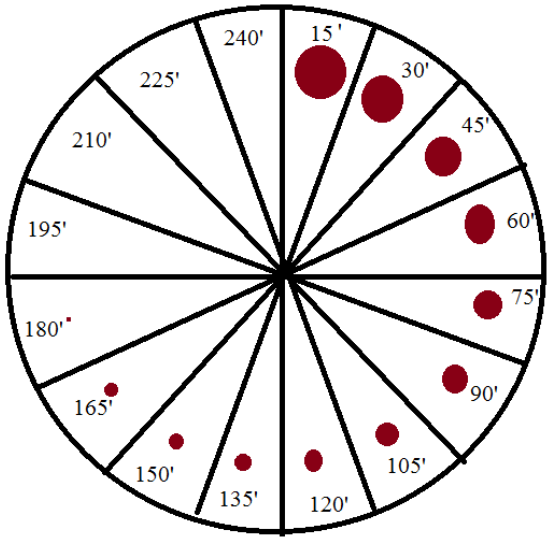
SPHYGMOMANOMETER



SPHYGMOMANOMETER

- Sphygmomanometer is a clinical instrument used to measure the blood pressure in man.
- It consists of an inflatable cuff, a measuring unit (mercury manometer), manually operated bulb and valve, and a stethoscope.
- The inflatable rubber cuff is wrapped around the person's arm. The Stethoscope is placed under the edge of the cuff above the brachial artery.
- Air is pumped into the cuff until the sound of the pulse in the artery is no longer heared through the stethoscope. The air is then released slowly. The point at which the sound appears is noted on the pressure gauge. This is the systolic pressure.
- Then the air is slowly allowed to escape further. At this point, a change in the quality of sound is heared, just before is disappears. At this point, the pressure is observed in the gauge. This is diastolic pressure.
- The air is completely let out and cuff is removed.
- The normal blood pressure of man is 80/120mm Hg and systolic pressure is 120 mm Hg.

BLEEDING TIME OF THE BLOOD



BLEEDING TIME OF THE BLOOD

AIM : To record the bleeding time of the blood in seconds using Duke's Method.

REQUIREMENT : Blotting paper, Sterile Lancet, Cotton wool and Stop clock.

PRINCIPLE : A specific time is required for the formation of a stable clot to stop bleeding of blood from the cut wound.

PROCEDURE

The tip of the finger is cleaned with spirit and skin is allowed to dry. The finger tip is pricked with a sterilized lancet for a drop of blood to appear. Then time is noted. Every 15 seconds, the blood is removed with a blotting paper without touching the actual incision. This is repeated every 15 seconds using different parts of the blotting paper, until there is no more bleeding. The time for the appearance of blood to the time cessation of bleeding is noted, This gives the bleeding time.

RESULT : The bleeding time is found to be --- minutes ------ seconds.

INFERENCE

Normal bleeding time is 2-5minutes. This test is an indicator of the vascular and platelet responses of haemostasis. A prolonged bleeding time is most often due to thrombocytopenia.

NOTE :

- When blood is blotted on the paper, care should be taken to press the wound.
- First drop of blood should be taken only after 30 seconds.

CLOTING TIME OF THE BLOOD

AIM **:** To Determine The Clotting Time of the Blood

REQUIREMENT: Blotting paper, Sterile Lancet, Cotton wool and Stop clock

PRINCIPLE **:** A specific time is required for the blood to clot after it is withdrawn.

PROCEDURE

The tip of the finger is cleaned thoroughly with spirit and allowed to dry. With the sharp sterilized lancet, the finger is prickled. A drop of blood is taken on a clean slide. The time is noted. For every 30 seconds, a clean needle is pulled slowly through the drop of blood. When a fine thread of fibre can be pulled up by the point of the needle, it indicates that the clot has been formed. The time is noted

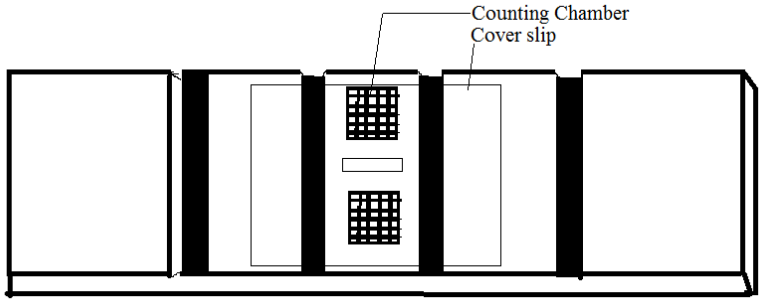
RESULT

The time taken for fibrin thread formation is --------- minutes ---------- seconds.

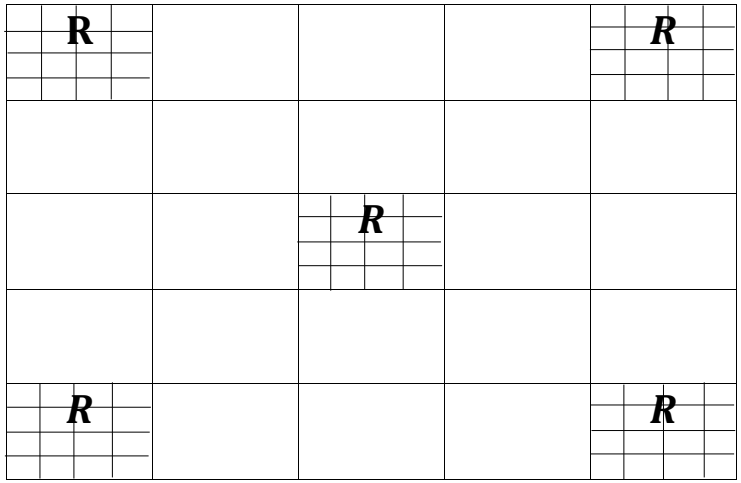
INFERENCE

The clotting time is----------minutes---- seconds which is within normal limits. Normal value is 3 to 8 minutes.

NEUBAURS' COUNTING CHAMBER



RBC COUNTING SQUARE



RBC COUNTING

AIM

To count the total number of RBC in human blood.

REQUIREMENTS

A] Equipment : Compound Microspcope

B] Chemicals : RBC diluting fluid- Hayem's Fluid (Sodium chloride-0.5g, Sodium sulphate-2.5g and Mercuric chloride -0.25g and distilled water-100ml

C] Apparatus & Glassware : Haemocytometer, sterilized needle, coverglass, cotton spirit

PRINCIPLE

Haemocytometer, a microscopic slide having 3 inches long by 1.5 inches wide bears two counting chambers called Neubauer counting chambers used for counting blood cells. The two chambers are separated by a 'H' shaped ridge. A coverslip is placed on the ridge. Each counting chamber is formed by several straight perpendicular and horizontal lines enclosing squares of various measurements, the biggest square is 3X3 mm with an area of 9sq.mm. It is divisible into 9 squares of 1x1 mm each. So that, the total volume of fluid accommodated in the counting chamber is $3x3x0.1x0.1 = 0.9mm^3$. The central square is again divisible into 25 smaller squares and each square is subdivisible ito 16 squares. For total RBC counts, central 1mm square is used.

PROCEDURE

The tip of the index finger is sterilized by rubbing with a cotton soaked in spirit. A gentle prick is made with the help of a sterilized pin or needle. The tip of the finger is pressed till the blood oozes out. The first drop is wiped out with the help of cotton. Then the blood is aspirated into the RBC pipette exactly upto the 101mark. The pipette is rotated between the thumb and forefinger. This will give a dilution of 1:200. The counting chamber and cover glass are cleaned thoroughly.

CALCULATION

Total number of Cells in 80 small squares	=415
The area of small square	=1/400 sq. mm
The depth of counting chamber is	=1/10 mm
Therefore, the volume of a small square	=1/400×1/10
	=1/4000 c.mm
The dilution of blood is	=1/200
Total RBC	= 415×4000×200/80×1×1
	= 41,50,000 per c.mm
Therefore, the total RBCs per c.mm is	= 41,50,000 per c.mm

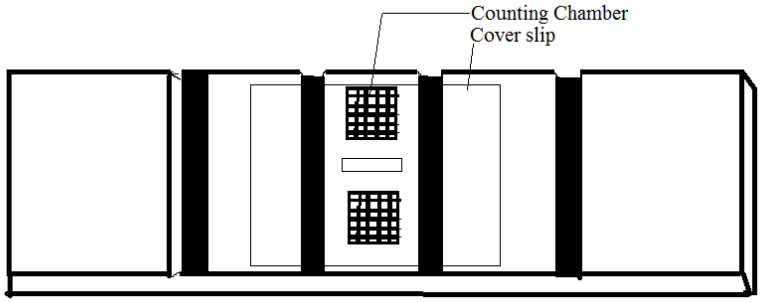
The cover glass is placed in position over the ruled area, using gentle pressure. The suspension is mixed thoroughly by rotating the pipette for about a minute holding it in horizontal position, and finally shaking sidewise. The fluid is expelled from the stem of the pipette at an angle of 45 degrees by lightly touching the tip against the edge of the cover glass. Care should be taken to ensure that the suspension does not flow into the moats on either side, nor should any bubble form under the cover glass. The slide is placed under the microscope. The number of RBC's in 80 small squares (Four squares at the four corners and one at the centre of central area) are counted.

RESULT: 1cmm of human blood contains----------RBCs.

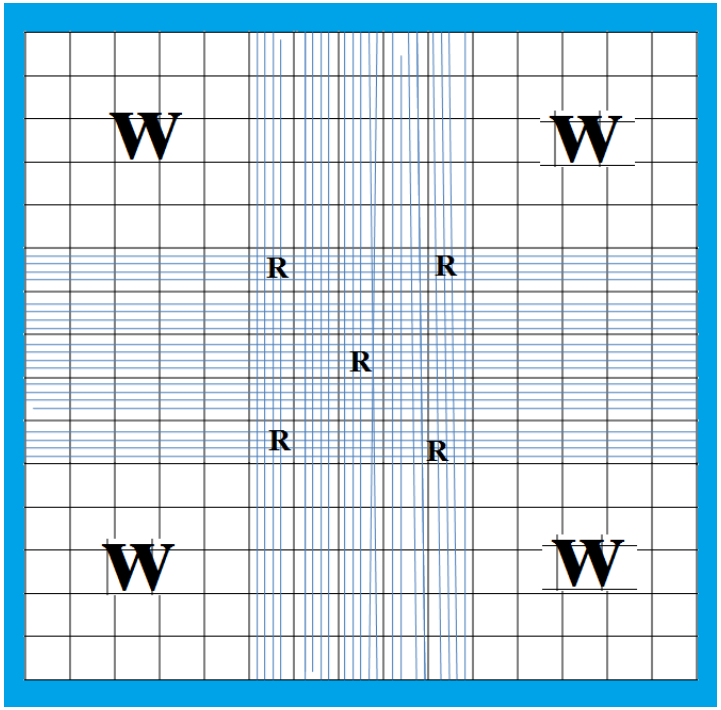
NOTE

It is not necessary to count the RBC in all the 400 smallest squares or even in the 25 smaller squares, count them only in fiver smaller squares. The RBCs lying on the lower and right sides of a square are to be added in the total, while those lying on the upper and left sides are to be rejected.

NEUBAURS' COUNTING CHAMBER



WBC COUNTING SQUARE



WBC COUNTING

AIM: to count the total number of WBC in human blood.

REQUIREMENTS

A] EQUIPMENT	**:** Compound Microspcope
B] CHEMICALS	**:** Haemocytometer, sterilized needle, coverglass, cotton spirit
C] APPARATUS & GLASSWARE	**:** WBC diluting fluid- Turk's solution (Glacial acetic acid-3ml, Distilled water -97ml, and gentian violet to give a pale violet colour)

PRINCIPLE

Haemocytometer, a microscopic slide having 3 inches long by 1.5 inches wide bears two counting chambers called Neubauer counting chambers used for counting blood cells. The two chambers are separated by a 'H' shaped ridge. A coverslip is placed on the ridge. Each counting chamber is formed by several straight perpendicular and horizontal lines enclosing squares of various measurements, the biggest square is 3X3 mm with an area of 9sq.mm. It is divisible into 9 squares of 1x1 mm each, so that, the total volume of counting fluid accommodate in the counting chamber is $3x3x0.1x0.1 = 0.9mm^3$. The central square is again divisible into 25 smaller squares and each square is sub divisible ito 16 squares. For total WBC counts, the four large squares are used.

PROCEDURE

The tip of the index finger is sterilized by rubbing with a cotton soaked in spirit. A gentle prick is madwith the help of a sterilized pin or needle. The tip of the finger is pressed till blood oozes out. The first drop of the blood is wiped out with the help of cotton. Then the blood is aspirated into the WBC pipette exactly upto 0.5 mark. Immediately WBC diluting fluid is loaded upto the mark 11. The pipette is rotated between the thumb and forefinger. Counting chamber and cover glass tare cleaned horoughly. The cover glass is placed in position over the ruled area, using gentle pressure. The suspension is mixed thoroughly by rotating the pipette for about a minute holding it in horizontal position, and finally shaking sidewise.

CALCULATION

Total number of cells contained in 4 squares	**= "X" cells**
The volume of a square	**= 1/10 cu.mm**
The dilution of blood is	**= 1/20**
Therefore the number of cells per cu.mm of undiluted blood is	**= "X" x 10x20 / 4x1x1**
	= ---------- cells /cu.mm

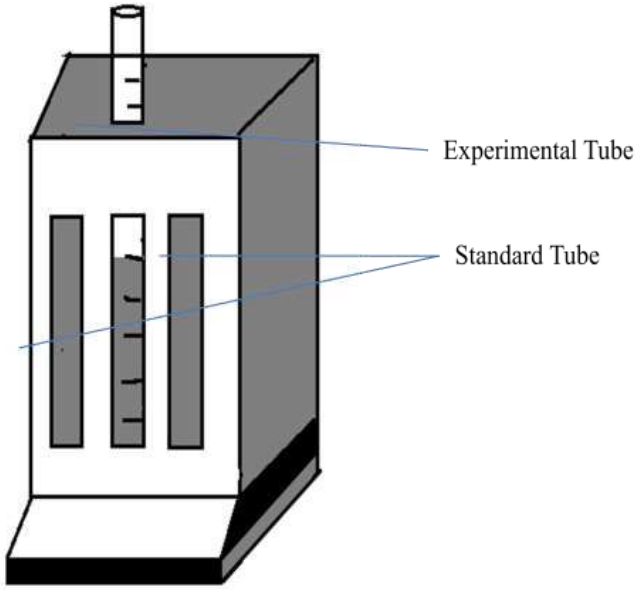
The fluid is expelled from the stem of the pipette at an angle of 45 degrees by lightly touching the tip against the edge of the cover glass. Care should be taken to ensure that the suspension does not flow into the moats on either side, nor should any bubble form under the cover glass. The white blood corpuscles are allowed to settle by waitig for 2-3 minutes. The slide is placed under the microscope. The cells touching on the inner lines on the right and top are counted. The cells touching the lines on the left and bottom are not counted. The difference between the two square millimeter areas should not be more than 10 WBC's.

RESULT: WBC present in human blood is ----------per c.mm.

INFERENCE

Normally healthy man has 4000 to 6000WBC s per cubic millimeter of blood.

HAEMOGLOBINOMETER



ESTIMATION OF HAEMOGLOBIN

AIM:

To estimate the amount of haemoglobin content in the blood by Sahli's haematin method.

REQUIREMENTS

A]	**Chemicals**	**:** Methylated spirit or 90% alcohol, N/10 Hcl, distilled water.
B]	**Apparatus & Glassware**	**:** Haemometer (Haldane's Haemoglobinometer), micropipette with 20 cubic mm mark, sterilized needle or lancet.

PRINCIPLE

The principle of Sahli's Acid Haemtin method is based on the reaction of blood with dilute N/10 Hydorchloric acid. Brown coloured acid haematin is produced after gradual dilution of the suspension of acid haematin in standard tubes. The normal range of haemoglobin in blood of human male is in between 13.5-18.gm/100ml; in women, 11.5-16.5 gm/100ml and for infants 13.6/19.6 gm/100ml.

HAEMOGLOBINOMETER

The apparatus used for haemoglobin estimation is known as haemoglobinometer. It consists of three tubes, two of which are called standard tubes, containing 20 cu. mm of standard (acid haematin) blood (containing 13.8 gms of haemoglobin per 100 ml). The third graduated tube called the diluting tube that is inserted between the standard glass tube which is graduated in G% on one side and % on other side.

PROCEDURE

The graduated tube is first cleaned with distilled water and then with methylated spirit or 90% alcohol. It is thoroughly dried up before being used. Now with the help of a dropper, N/10 HCl solution is filled in the graduated tube upto 20 marks on the percentage side. Then the tip of the finger is pricked with sterilized needle and 20 cubic mm of blood is sucked using micropipette. The blood of micropipette is now transferred to the diluting tube containing N/10 HCl solution by blowing the pipette gently. The pipette should be introduced carefully into the tube and its lower mouth should pass right up to the bottom into HCL solution.

When the blood is expelled out from the pipette, the pipette is immediately rinsed using distilled water several times to avoid blood clotting so that no blood is left in it. The blood is gently mixed with a glass rod. Then, the tube is allowed to stand for 10 minutes so that the haemoglobin is acted upon by HCl and converted into acid haematin. The acid haematin is further diluted by adding distilled water and the solution is stirred continuously with a glass rod till the colour of the blood in the diluting tube matches with that of the standard tubes. Dilution of blood is read in terms of gram percentage.

RESULT

The haemoglobin content of my blood is ____gms/100ml.

NOTE

- The finger and prick(needle) should be sterilized using methylated spirit or alcohol.
- Avoid coagulation of blood inside the micropipette and speed up the experiment.
- The colour of the standard tube should be checked periodically against the standared acid haematin solution.
- For maximum colour intensity, longer time is required.
- Perfect matching with brown glass standard is not possible. Children:

ACTION OF SALIVARY AMYLASE IN MAN

FLOW CHART

Hydrolysis of Starch

⬇

Soluble Starch

⬇

Amylo dextrin (purple colour)

⬇

Erythrodextrin (Brown colour)

⬇

Achromatic dextrin (Light Brown)

⬇

Maltose (No colour change)

ACTION OF SALIVARY AMYLASE IN MAN

AIM: To determine the effect of temperature and pH on salivary amylase activity.

REQUIREMENTS

A] Chemicals	1% starch, iodine and buffer solution
B]Glassware & Apparatus	Test tubes, test tube stand, Measuring cylinder, porcelain tile, stopwatch, thermometer and glass rod.
C]Sample	Human saliva

PRINCIPLE

Salivary amylase is an enzyme produced by salivary glands found in human saliva which functions in the breakdown of starch into simpler compounds. Several factors affect the activity of enzymes, but the activity of enzyme is strongly affected by changes in pH and temperature. At optimum level of these factors, enzymes perform their function best. The optimum temperature and pH of enzymes differ from one to another. Each enzyme works best at optimum pH and temperature in activity. Their activity decreases at values above and below that point due to denaturation that affects the activity of enzymes, The optimum temperature and pH for the action of salivary amylase ranges from 32 °c to 37°c and 5.6 to 6.9 respectively. Extreme temperature causes the native folded structure of protein to uncoil into random configuration. As a result, the protein looses its biological enzymatic activity.

Generally, the activity of amylase is maximum at pH 7 and at the temperature 38°C. The experiment is repeated at different temperature (33° C, 35° C, 38° C, 41° C and 43°C) by keeping the constant pH (pH7). Again, the same experiment is repeated at different pH (3,5,7,9 and 11) by keeping the constant temperature. The salivary amylase (enzyme) present in saliva gradually acts on starch(substrate) and converts it into maltose The starch keeps on producing blue colour with iodine untill it is completely digested into maltose. At this point, no blue colour is formed. This is the end point (or) achromatic point.

Amylase digests starch by catalyzing hydrolysis, which is splitting by the addition of water molecule. The breakdown of starch due to the action of salivary amylase is indicated by the change in colour of the starch solution. The starch is hydrolysed into various dextrins and the colour produced by iodine turns blue, reddish violet, brown and finally no colour change is seen. This point is called achromatic point. The time taken for the complete hydrolysis of a known amount of starch by a known strength of an enzyme is termed as chromatic point. The reciprocal of the chromatic point is the measure of amylase activity.

ACTION OF SALIVARY AMYLASE IN RELATION TO TEMPERATURE

S.No	Temperature	pH	Time Taken(Sec)	1/T	Rate of Reaction
1	32º C	**7**			
2	35º C	**7**			
3	38º C	**7**			
4	41º C	**7**			
5	43ºC	**7**			

ACTION OF SALIVARY AMYLASE IN RELATION TO pH

S.No	Temperature	pH	Time Taken(Sec)	1/T	Rate of Reaction
1	35º C	**4.5**			
2	35º C	**5.6**			
3	35º C	**6.9**			
4	35º C	**7.8**			
5	35º C	**9.2**			

PROCEDURE

1ml of saliva is diluted with 9ml of distilled water to prepare 10% solution. About 2ml of buffer solution of known pH 7 and 3ml of 1% starch solution are taken in a test tube. This is placed in a water bath maintained at 38ºC. To this mixture, 2ml of saliva is mixed, and the stopwatch is started. Now a drop of iodine is placed on a porcelain tile. Following, stir the mixture in the test tube using a glass rod. A drop of saliva mixture is added to the iodine drop in the porcelain tile. Periodically,the same procedure is to be followed at an interval of half a minute. Now the hydrolysis of starch takes place and the colour change of iodine from blue to colourless is observed. At the achromatic point, the stopwatch is stopped, and the time taken for digestion in chromatic point is recorded. The achromatic point indicates that the starch is completely hydrolysed by amylase. The reciprocal of time taken is the rate of amylase activity.

RESULT

Rate of reaction maximum at temperature = -------- º C

Rate of reaction minimum at temperature = -------- º C

Rate of reaction maximum at pH = --------

Rate of reaction minimum at pH = --------

INFERENCE

All enzymes are proteinaceous in nature. At a lower temperature, the enzyme salivary amylase is deactivated. At the higher temperature, the enzyme is denatured. Therefore, maximum time will be taken by an enzyme to digest the starch at lower and higher temperature. Optimum temperature for the enzymatic activity of salivary amylase ranges from 32°C to 37°C. The optimum temperature means that the temperature at which the enzyme shows the maximum activity. At this optimum temperature the enzyme is most active and hence, takes less time to digest the starch.

The rate of enzyme action increases with increase in the H^+ ion concentration upto a certain pH. The optimum pH for the enzymatic activity of salivary amylase ranges from 6 to 7. Above and below this range, the reaction rate reduces and enzymes get denatured. The enzyme salivary amylase is most active at pH 6.8. This pH is called as the optimum pH.

TEST FOR AMMONIA

ANALYSIS OF NITROGENOUS WASTE PRODUCTS (AMMONIA, UREA AND URIC ACID)

TEST FOR AMMONIA

AIM: to find the presence or absence of Ammonia in the given sample

REQUIREMENTS

A] Glassware & Apparatus	Test tubes, test tube holders
B] Chemicals	Nessler's Reagent
C]Sample	Fish tank water,

PRINCIPLE

Nessler Reagent (K_2HgI4) reacts with the ammonia present in the sample (under strongly alkaline conditions) and produce a reddish brown precipitate. The intensity of the colour is directly proportional to the concetnration of ammonia found in the sample.

PROCEDURE

A known quantity of 4 ml of water from fish tank is taken in a test tube. To this, 3 drops of Nessler's reagent is added.

RESULT

Reddish brown precipitate appeared.

INFERENCE

The colour change (orange red to brick red) indicates the presence of ammonia.

TEST FOR UREA

TEST FOR UREA

AIM: to find the presence or absence of Urea in the given sample

REQUIREMENTS

A] Glassware and Apparatus	Test tubes, test tube holders
B] Chemicals	10% NaOH, 5% copper sulphate
C] Sample	Urine sample

PRINCIPLE

Urea is a waste product that is excreted by the kidneys. The urea test determines the amount of urea in the sample (urine) to assess the amount of protein breakdown. The test can help to determine the functioning status of kidneys and to assess the intake of protein.

PROCEDURE

To the given urine sample, 3ml of 10% NaOH is added and 5% of copper sulphate solution is added to it.

RESULT

Purplish violet colour appeared.

INFERENCE

The appearance of purplish violet colour indicates the presence of urea in the sample.

URICACID CRYSTALS

TEST FOR URIC ACID

AIM: To find the presence or absence of Uric acid in the given sample.

REQUIREMENTS

A] Equipment	Compound Microscope
B] Glassware & Apparatus	Watch glass, brush, match box and spirit lamp
C] Chemicals	Na_2Co_3, Con.HCl
D] Sample	Bird's excreta

PRINCIPLE

Bird excretes uric acid as waste product. Producing uric acid instead of urea is advantageous because it is less toxic and reduces water loss and meet out the subsequent need for their survival. Birds, convert toxic ammonia to uric acid or the closely-related compound guanine (guano), rather than urea.

PROCEDURE

To the white portion of Bird's excreta, 2ml of Na_2Co_3 is added and heated for about 3 minutes and cooled. Then 2 drops of concentrated HCl are added and the supernantant is collected in a watch glass. The wastes are removed from the watch glass. A drop of supernantant is taken in a slide and observed under the high power of microscope.

RESULT

The presence of glassy, transparent crystal like structure is observed in the sample.

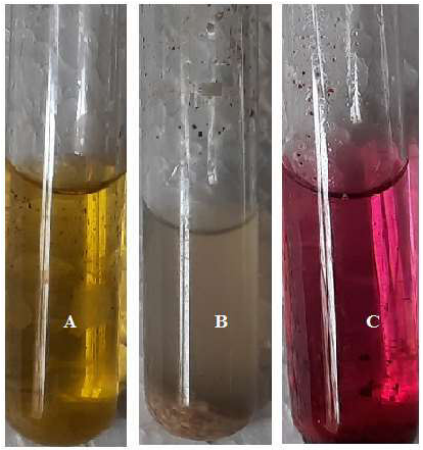
INFERENCE

It indicates the presence of uric acid in the sample.

OUTCOME

Students could find the presence or absence of nitrogen in the biological samples.

ESTIMATION OF UREASE IN HORSEGRAM



ESTIMATION OF UREASE IN HORSEGRAM

AIM

To analyse the activity of an enzyme urease in horsegram.

REQUIREMENTS

A] Biological Material	: Horsegram powder
B] Chemicals	: Phenol red, Urea powder, 2% Acetic acid
C] Glasswares/Apparatus	: Test tubes,Test tubes stand, glass rod, spatula

PRINCIPLE

Urea is the end product of protein metabolism. The enzyme urease present in the horsegram which splits urea into NH_3 and CO_2.

PROCEDURE

Three test tubes are taken and marked these three test tubes as A,B and C. In the test tube A, 5ml of urea is taken and a drop of phenol red is added as indicator.

In tube B, 5 ml of water is taken and a drop of phenol red is added. Following, a spatula full of horsegram powder is added and mixed well.

In tube C, 5 ml of urea is taken and added a drop of phenol red. Subsequently a spatula full of horsegram powder is mixed together. Adjust the colour in A, B and C to orange by using 2% acetic acid or 2% sodium carbonate.

The solutions in tubes A, B and C are neutral when the colour is orange. Mix the components of tube A, B and C and let it stand for 5 – 10 minutes.

RESULT

The colour turns into red only in the tube C not in the other two tubes.

INFERENCE

This is due to the liberation of NH_3 from urea by the action of enzyme urease in horsegram.

ESTIMATION OF OXYGEN CONSUMPTION IN FISH

S.No	Sample	Volume of sample (ml)	Burette Reading(ml)		Volume of $Na_2S_2O^3$ required	Concordant Value(ml)	Indicator
			Initial	Final			
1	Control	25					Starch
2	Experimental water	25					

ESTIMATION OF OXYGEN CONSUMPTION IN FISH

AIM

To determine the amount of O_2 consumed by a fish in known volume of water and period during respiration by Winkler's Method.

REQUIREMENTS

A]	Apparatus & Glassware	: Glass jar, siphon system, measuring jar, reagent bottles, burette, pipette, weighing balance and conical flask.
B]	Chemicals	: Manganous chloride, alkaline iodide, Con. H_2SO_4, Sodium thiosulphate solution, 1% starch solution and kerosene
C]	Biological Sample	: A live fish (Tilapia/ black molly)

PRINCIPLE

The Winkler test is used to determine the concentration of dissolved oxygen in water samples. When manganous chloride is added to the water samples, followed by iodide, manganous hydroxide is formed. It combines with dissolved O_2, which on acidification in the presence of alkaline iodide releases an amount of I_2, that is equivalent to the amount of oxygen in the sample. The amount of oxygen is determined by titration against sodium thiosulphate using starch as an indicator.

PROCEDURE

A live fish is taken gently from the stocking tank without rubbing off the mucous membrane. It must be placed immediately in a jar containing a known volume of tap water. The surface water is covered with a fine layer of kerosene in order to prevent the water as well as the fish from coming in contact with atmospheric air. Time is noted. In this condition, the fish is allowed to respire for half an hour.

A known volume of water is filled in a bottle[A] and kept as "control". After half an hour, the fish is taken out and the water from the jar is siphoned out into experimental bottle[B] of known volume. The oxygen content of both control and experimental water is determined by Winkler's method.

CALCULATION

Weight of the fish	=	------- g
Volume of the bottle(A)	=	--------ml
Volume of the bottle(B)	=	--------ml

CALCULATION

Oxygen content in control water = $\frac{K \times 200 \times \text{vol. of } Na_2S_2O_3 \times 0.698}{\text{Volume of the sample titrated}}$

= ----- cc/litre

K = Volume of the bottle/ volume of the bottle- volume of reagent required

K = -------

Oxygen content in experimental water = $\frac{K \times 200 \times \text{vol. of } Na_2S_2O_3 \times 0.698}{\text{Volume of the sample titrated}}$

= ----- cc/litre

Total uptake of water in one hour = O_2 content in control water –O_2 content in experimental water

= ----cc/litre

Rate of oxygen consumption in one hour = Total uptake of O_2 in one hour/weight of the fish

= ----------cc/gm/hour

The glass stoppered bottle is taken and it is filled with tap water and the stopper is replaced without leaving any bubble inside. The volume of water is measured, The bottle is refilled with experimental water and 1ml of manganous chloride and 1ml of alkaline iodide are added. The stopper is replaced and water is shaken well to ensure maximum chemical reaction and absorption of oxygen is already present in the water sample. This helps in uniform distribution of precipitate. Then the precipitate is allowed to settle down. Then 2 ml of Con. H_2SO_4 is added to the solution and shaken well till the precipitate is dissolved. Now a clear solution of yellow colour is obtained. The burette is cleaned with distilled water and rinsed with sodium thiosulphate. Finally it is filled with sodium thiosulphate of required quantity.

A known quantity, 25 ml of the solution is transferred into conical flask. 2 drops of starch solution is added to this. It is titrated against sodium thiosulphate in the burette. The titration is continually carried out till the blue colour becomes colourless. The tip of the burette is closed and final reading is noted. The difference between the initial and final reading indicates the required amount of sodium thiosulphate utilized for titration. The titration is repeated till concordant values are obtained. The difference between O_2 content of the control and experimental bottle is the amount of oxygen consumed by the experimental fish in 25ml of water in half an hour. The rate of oxygen consumption is found out by dividing the values by the body weight of the fish. This is expressed as ml/gm/per hour.

RESULT

Rate of oxygen consumption of fish in 1 hour = -------ml/gm/hour

INFERENCE

The oxygen content in the experimental water is less than that of control water due to the oxygen consumption by the fish during respiration.

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