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VI Semester B.Sc.,
Paper-VIII
Bio Chemistry Laboratory Manual

CONTENTS

PREPARATION OF BUFFERS

EXPERIMENT NO.1

AIM: PREPARATION OF BUFFERS AND DETERMINATION OF THEIR pH VALUES USING pH METER

PRINCIPLE: A solution which resists the change in pH with a small addition of an acid or a base is called a buffer solution. There are three types of buffers-

1. Acidic buffer: Mixture of a Weak acid and Its salt with a strong base.
   Example: $CH_3COOH + CH_3COONa$ (acetic acid + sodium acetate)
2. Basic buffer: Mixture of a Weak base and its salt with a strong acid.
   Example- NH_4OH+NH_4Cl (ammonium hydroxide + Ammonium chloride)
3. Neutral buffer- salt of a weak acid and a weak base.
   Example – CH_3COONH_4 (Ammonium acetate)

The pH of a buffer can be determined by using the Henderson-Hasselbatch equation

$$pH = pK_a + \text{Log } \frac{[\text{Salt}]}{[\text{Acid}]} \quad ; \quad pOH = pK_b + \text{Log } \frac{[\text{Salt}]}{[\text{base}]}$$

By properly adjusting the concentrations of the `acid, base and salt with their pK_a/pK_b values, pH can be calculated.

PROCEDURE: Preparation of an acetate buffer of pH 4.4 (or any pH)

Step 1: Two solutions required for the acetate buffer ie, acetic acid and sodium acetate of the same concentration can be prepared (0.2M)

Step 2: using the normality equation N_1V_1 (conc. base) = N_2V_2 (dil. base)and substituting any volume of 0.2M sodium acetate the concentration of the diluted sodium acetate solution can be calculated.

Step 3: Knowing the pH and the pKa of the acetic acid (from tables) and the concentration of the base and using the Henderson-Hasselbatch equation the concentration of the acid can be calculated.

Step 4: Using the normality equation N_1V_1 (conc acid) = N_2V_2 (dil acid). The volume of 0.2 M acid required for dilution can be calculated.

Step 5: The calculated volumes volumes of the two solutions (0.2M acetic acid and 0.2M sodium acetate) were pipetted out into 100 volumetric flask and made up to 100 ml with distilled water. Mixed well and the pH of that buffer solution was determined using pH meter. If a difference in pH reading as compared to the required may be to get the required pH value of 4.4.

RESULT: The pH of the prepared buffer solution was found to be ---------

SEPARATION OF α-AMINO ACIDS BY PAPER CHROMATOGRAPHY

EXPERIMENT NO.2

AIM: **TO SEPARATE AND DETECT THE AMINO ACIDS FROM THE GIVEN MIXTURE BY PAPER CHROMATOGRAPHY.**

PRINCIPLE: In this technique the compounds to be separated and spotted on one end of the whattman filter paper and paper is kept dipped in a mixture comprised of a hydrophilic and hydrophobic solvent. The solvent mixture starts wetting the paper by capillary action but the cellulose component of the paper can absorb only the hydrophilic solvent and not the other. Thus, there exists a phase separation at the micro level. The separated amino acids are located by ninhydrin reagent which gives a purple color.

REAGENTS: Whattmann no.1 Chromatographic paper. Solvent: 200ml of solvent is prepared by mixing, 80ml of butanol, 20ml of acetic acid and 100ml distilled water in the ratio of 4:1:5. The three solutions are shaken in a separating funnel. Two layers separate out, an aqueous layer and an organic layer. The upper layer is the organic layer and the lower layer is the aqueous layer. The aqueous layer is drained out and the organic layer is taken to be the solvent (mobile phase) and taken in the chromatographic chamber. Locating reagent: (0.2% Ninhydrin) 200mg of ninhydrin is dissolved in 100 ml of alcohol. Standard amino acids: A small amount of standard amino acids are dissolved in ml of distilled water, some times a drop of acid or alkali are needed to bring the compound into solution.

PROCEDURE: The chromatography paper is taken and a line of about 5cm from one end is drawn. The standard amino acid like arginine, tryptophan, tyrosine, histidine, methionine are spotted at 3 cm interval. The given unknown mixture is also applied in the same way. The sample solution is applied using capillary tubes on the labeled points by repeated applications. The wet area should not be bigger than the encircled point of application. After drying the paper for 5mins the paper is then placed in the chromatographic chamber overnight. The paper is then removed and the solventfront is marked and the paper is allowed to dry in an oven of 50 degree centigrade . Ninhydin is sprayed and the paper is dried in an even at 100”c till the spots appear, The sample are visualized as purple color spots and distance moved by each amino acid is measured. Rf value is the ratio of the distance moved by each amino acid to the distance moved by the solvent. The different amino acids present in the given mixture move at different level and they are identified by comparing with the standard Rf value.

$$Rf = \frac{\text{Distance traveled by solute in cm}}{\text{Distance traveled by solvent cm}}$$

Result: The amino acids present in the mixture is …….

ESTIMATION OF AMINO ACID USING NINHYDRIN BY COLORIMETRIC METHOD

EXPERIMENT NO.3

AIM: TO ESTIMATE THE AMOUNT OF AMINO ACID BY USING NINHYDRIN REAGENT.

PRINCIPLE: Ninhydrin, also chemically known as triketohydrindene hydrate reacts with amino acids ti give a purple coloured complex (Ruhemann's purple) with an absorption maximum at 570nm. However, imino acids like proline and hydroxyl proline yield a yellow colour with an absorption maximum at 440nm. Ninhydrin oxidizes the amino acid to aldehyde, releasing carbondioxide and ammonia. During the course of the reaction, ninhydrin gets reduced to hydrindantin. The hydrindantin. Formed condenses with ninhydrinin the presence of ammonia to yield a purple coloured complex – Ruhemann's complex.

REAGENTS REQUIRED:

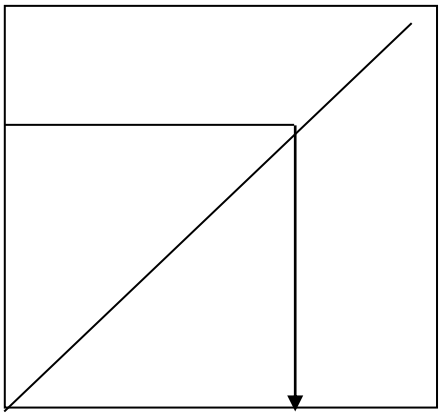
1. Preparation of stock amino acid solution: 100mg of leucine is weighed accurately and dissolved in little amount of water in a 100ml standard flask and the solution was made upto the mark with distilled water.The concentration is 1mg/1ml. 2. Preparation of working standard solution: 10ml of the stock standard solution is made upto 100ml with distilled water. The concentration of working standard is 100µg/ml.

PROCEDURE:

Standard solution of leucine in the range of 0.5 to 2.5 is pipetted out into five different test tubes. From the prepared unknown solution 0.6 and 1.2ml are pipetted out into two test tubes. The volume in all the tubes is made upto 4ml with distilled water. 4ml of distilled water alone is taken as blank. Then 1ml of ninhydrin is added to all the tubes. And the tubes are shaken well and kept in a boiling water bath for 15 mins and allowed to cool. 1ml of ethanol is added to all the tubes and the intensity of purple colour developed is read at 550nm. A standard graph is obtained by plotting the concentration of amino acid on x axis and the absorbance on the y axis. From the standard graph , the amount of amino acid present in the given unknown solution can be calculated

PROTOCOL:

Sl.No.	Vol. of Leucine. (cm^3)	Vol. of dist. Water (cm^3)	Vol. of Ninhy-drin (cm^3)	**MIXED WELL KEPT ON A BOILING WATER BATH FOR 15 MINS** AND ALLOW-ED TO COOL	Vol. of Ethanol (cm^3)	Optical density at 550nm
1.	0	4.0	1		1	
2.	0.5	3.5	1		1	
3.	1	3	1		1	
4.	1.5	2.5	1		1	
5.	2	2	1		1	
6.	2.5	1.5	1		1	
7.	U.K-1(1)	0	-		1	
8.	U.K-2 (2)	0	-		1	



$1cm^3$ of (unknown) soln.contains … µg/ml.
100 cm^3 of the soln. contains…..100x……
= ……µg/ml.

RESULT: The amount of amino acid present in the given unknown solution is ---µg.

ISOLATION OF LACTOSE AND CASEIN FROM MILK AND ESTIMATION OF LACTOSE COLORIMETRICALLY

EXPERIMENT NO.4

AIM: **ISOLATION OF LACTOSE AND CASEIN FROM MILK AND ESTIMATION OF LACTOSE BY COLORIMETRIC METHOD.**

PRINCIPLE: Casein is the main protein present in milk. By adjusting the pH of milk to 4.8 (isoelectric pH) casein can be precipitated. The unwanted fat with casein can be eliminated using alcohol as casein is insoluble in alcohol. The filtrate after separation of casein contains lactose which can be quantitatively estimated by DNS method. Lactose reduces alkaline 3, 5-dinitro salicylic acid to a red product. The intensity of the red colour is read at 540 nm which is a measure of the amount of lactose.

PROCEDURE: Isolation of casein: Exactly 50 ml of milk is taken in a 250ml beaker and warmed. To this sodium acetate buffer is slowly added with stirring until the pH of the mixture reaches 4.8 (read using pH -meter). The precipitate of casein obtained is cooled and filtered (using muslin cloth or Whatman no. 1 filter paper). The precipitate is washed with water and about 15ml of ethanol is added. It is filtered again, washed with 1:1 ethyl ether – ethanol mixture, dried and weighed.

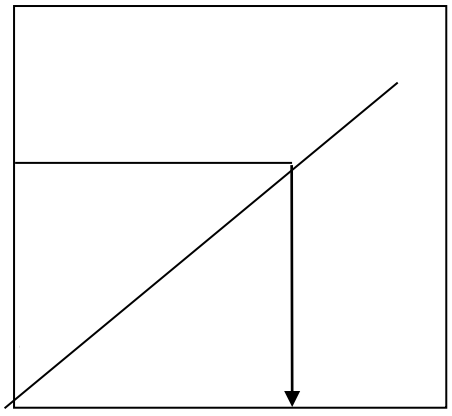
Isolation of lactose: The filtrate obtained after the separation of casein from milk is heated with a gram of $CaCO_3$ to remove the remaining protein. It is filtered, cooled and the amount of lactose in the filtrate is determined quantitatively by the DNS method.

Estimation of lactose by DNS method: Exactly 0.2, 0.4, 0.6, 0.8 and 1.0 ml each of standard lactose solutions (1mg/ml) are pipetted out into clean and dry boiling tubes labelled as 2, 3, 4, 5 and 6 respectively. The lactose filtrate obtained after the separation of proteins is made up to 100ml by adding distilled water. Exactly 1ml of this solution is pipetted out into boiling tube labelled as 7. Exactly 2ml of distilled water is pipetted out into boiling tube labeled as 1 (blank). Also the solution in all other tubes is made up to 2ml by adding distilled water. To each of the tubes from 1 to 7, 2ml of DNS reagent is added. Each of the solutions is thoroughly shaken, heated in a boiling water bath for about 15 minutes when a red colour is developed in all except the blank. After cooling, to each of the tubes 16ml of distilled water is added and the absorbance is measured at 540nm against blank solution.A graph of amount of glucose in milligrams versus absorbance is drawn. A straight line graph passing through the origin is obtained, from which the amount of lactose in 1ml of milk filtrate is determined. Amount of reducing sugar in 100ml of milk filtrate or 50ml of milk is calculated.

PROTOCOL:

Sl.No.	Vol. of Lactose (cm^3)	Amount of Lactose (mg)	Vol. of Water (cm^3)	Vol. of D.N.S reagent (cm^3)		Vol. of water (cm^3)	Optical density at 540nm
1.	0	0	2	2	BOIL FOR 15 MINS. AND COOL	16	
2.	0.2	0.2	1.8	2		16	
3.	0.4	0.4	1.6	2		16	
4.	0.6	0.6	1.4	2		16	
5.	0.8	0.8	1.2	2		16	
6.	1.0	1.0	1.0	2		16	
7.	U.K-1(1)	-	1.0	2		16	
8.	U.K-2 (2)	-	1.0	2		16	

Observations and Calculations:



1 cm^3 of Milk (unknown).contains …mg of lactose
100 cm^3 of Milk contains…..100x….. mg
=……mg of lactose

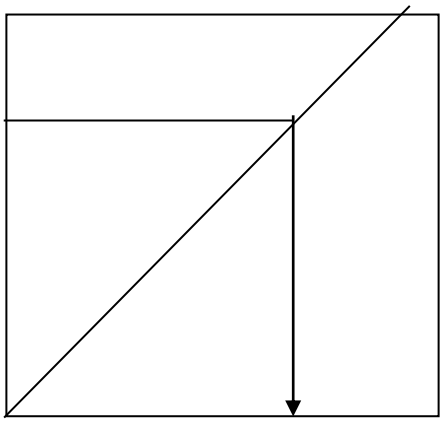
Result: The amount of lactose present in the given sample of milk is ……

OBSERVATIONS

Reaction: $2K_3Fe(CN)_6 + 2KI \rightarrow 2K_4Fe(CN)_6 + I_2$

PROTOCOL:

Sl.No.	Vol. of Glucose (cm^3)	Amount of Glucose (mg)	Vol. of Water (cm^3)	Vol. of Alk.ferri cyanide (cm^3)		Vol. of 5%acetic acid+KI +NaCl (cm^3)	Vol. of Sodium thiosul phate (cm^3)	Blank -Titre Value (cm^3)
1.	-	-	2	**1**	BOIL	1+4=5		
2	-	-	2	1	FOR	1+4=5		
3.	0.4	0.4	1.6	1	15	1+4=5		
4.	0.8	0.8	1.2	1	MINS.	1+4=5		
5.	1.2	1.2	0.8	1		1+4=5		
6.	1.6	1.6	0.4	1	AND	1+4=5		
7.	2.0	2.0	0	1		1+4=5		
8.	U.K.1	-	1	1	COOL	1+4=5		
9.	U.K.2	-	1	1		1+4=5		



$1cm^3$ of glucose (unknown)soln.contains .. mg
$100\ cm^3$ of glucose contains…..100x….. mg
=……

EXPERIMENT NO. 5

AIM: TO ESTIMATE THE AMOUNT OF GLUCOSE PRESENT IN THE GIVEN SOLUTION USING STANDARD GLUCOSE SOLUTION (1mg/ml), POTASSIUM FERRICYANIDE AND POTASSIUM IODIDE BY HEGEDORN JENSON'S METHOD TITRIMETRICALLY

Principle: This method is based on the principle that reducing sugars are oxidized by Potassium ferricyanide. The sugar solution is heated with an excess of potassium ferri-cyanide reagent. The excess is determined by treating with potassium iodide in acid medium. The iodine liberated is titrated against N/40 sodium thiosulphate using starch as indicator. The reverse reaction is prevented by precipitation of the ferrocyanide formed as a zinc salt by addition of zinc sulphate.

Procedure: Different aliquots of standard glucose(1mg/ml) varying from 0.4 to 2 ml are pipetted out into different test tubes. The solutions are made up to 2 ml in each case with distilled water. 1ml of alkaline ferricyanide reagent (equal volume of ferri-cyanide and sodium carbonate solution are mixed) is added to each tube. The test tubes are heated in a boiling water bath for exactly 15 mins. The tubes are cooled, 1ml of 5% acetic acid solution followed by 4 ml of $ZnSO_4$-NaCl-KI mixture are added to each tube. The contents of the tubes are mixed well and the liberated iodine is titrated against N/40 sodium thiosulphate solution using 1% starch solution as indicator. A blank titration is carried out under similar condition using 2ml distilled water instead of sugar solution.

Result: The unknown solution (given) contains: 1…….. 2……..mgs of glucose.

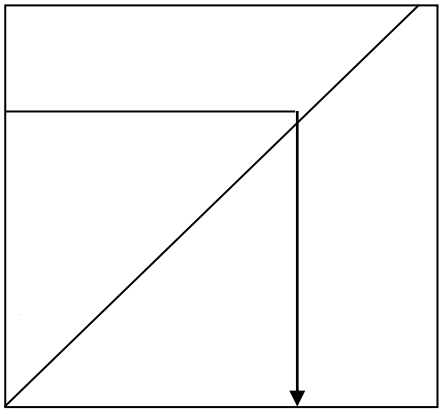
OBSERVATIONS:

Reactions: $C_6H_{12}O_6 + 2CuO \rightarrow C_5H_{11}O_5COOH + Cu_2O$

$5KI + KIO_3 + 3H_2SO_4 \rightarrow 3K_2SO_4 + 3H_2O + 3I_2$

PROTOCOL:

Sl.No.	Vol. of Glucose (cm^3)	Amount of Glucose (mg)	Vol. of Water (cm^3)	Vol. of Copper reagent (cm^3)		Vol. of 1 N H_2SO_4 (cm^3)	Vol. of Sodium thiosul-phate (cm^3)	Blank -Titre Value (cm^3)
1.	0	-	2	2	BOIL	2		
2.	0	-	2	2	FOR	2		
3.	0.2	0.2	1.8	2	10	2		
4.	0.4	0.4	1.6	2	MINS.	2		
5.	0.6	0.6	1.4	2	AND	2		
6.	0.8	0.8	1.2	2	COOL	2		
7.	1.0	1.0	1.0	2		2		
8.	U.K.1	-	1.0	2		2		
9.	U.K.2	-	1.0	2		2		



Blank -Titre Value (Cm^3)

Amount of glucose(mg)

1 cm^3 of glucose (unknown)soln.contains .. mg

100 cm^3 of glucose contains…..100x….. mg

=……mg

EXPERIMENT NO. 6

AIM: TO ESTIMATE THE AMOUNT OF GLUCOSE PRESENT IN THE GIVEN SOLUTION USING STANDARD GLUCOSE SOLUTION (1mg/ml), AND COPPER REAGENT BY NELSON- SOMOGY'S METHOD TITRIMETRICALLY

Principle: Reducing sugars selectively reduce a copper salt in alkaline medium. The oxidation of cuprous oxide by atmospheric oxygen is prevented by the addition of sodium sulphate. On acidification of the reaction mixture, potassium iodide and potassium iodate present in the reagent liberates iodine. In acid medium cuprous oxide goes into solution giving cuprous ions and these ions are oxidized by the liberated iodine. The excess iodine is titrated against N/200 sodium thiosulphate using starch as indicator.

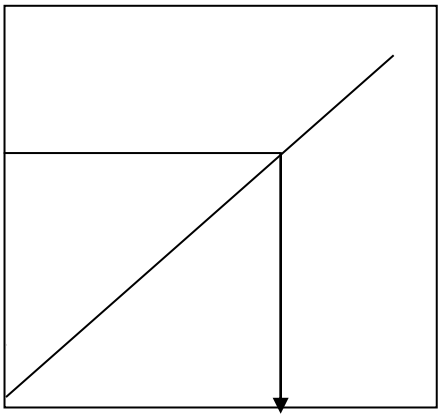
Procedure: Different aliquots of standard glucose solution (1mg/ml) varying 0.2ml to 1ml are pipetted out into different test tubes. The solutions are made up to 2ml in each case with distilled water. 1ml of the copper reagent is added to each test tube. The tubes are heated in a boiling water bath for exactly 10 minutes and cooled. 2ml of 1N sulphuric acid is added to each tube and kept aside for 2 to 3 minutes. The liberated iodine is titrated against N/200 sodium thiosulphate solution using 1% starch as indicator. A blank titration is carried out under the same conditions using 2ml of distilled water instead of sugar solution.

Result: The unknown solution (given) contains: 1……. 2…….mgs of glucose.

OBSERVATIONS

PROTOCOL:

Sl.No.	Vol. of Glucose (cm^3)	Amount of Glucose (mg)	Vol. of Water (cm^3)	Vol. of D.N.S reagent (cm^3)		Vol. of water (cm^3)	Optical density at 540nm
1.	0	0	2	2	BOIL	16	
2.	0.2	0.36	1.8	2	FOR	16	
3.	0.4	0.72	1.6	2	15	16	
4.	0.6	1.08	1.4	2	MINS.	16	
5.	0.8	1.44	1.2	2	AND	16	
6.	1.0	1.80	1.0	2	COOL	16	
7.	U.K-1(1)	-	1.0	2		16	
8.	U.K-2 (2)	-	1.0	2		16	



1 cm^3 of glucose (unknown)soln.contains .. mg
100 cm^3 of glucose contains…..100x….. mg
=……mg

EXPERIMENT NO. 9

AIM: TO ESTIMATE THE AMOUNT OF GLUCOSE PRESENT IN THE GIVEN SOLUTION USING STANDARD GLUCOSE SOLUTION(1.8mg/ml), AND DINITRO SALICYLIC ACID REAGENT(DNS) BY DNS METHOD COLOURIMETRICALLY

Principle: An alkaline solution of 3,5 dinitro salicylic acid(DNS) oxidizes a reducing sugar in hot condition and a red color is obtained since the acid is reduced by the sugar. This test is finds importance since it demonstrates the reduction of a non-metallic compound by a reducing sugar. The reaction can be used successfully as a quantitative method to estimate the amount of reducing sugar.

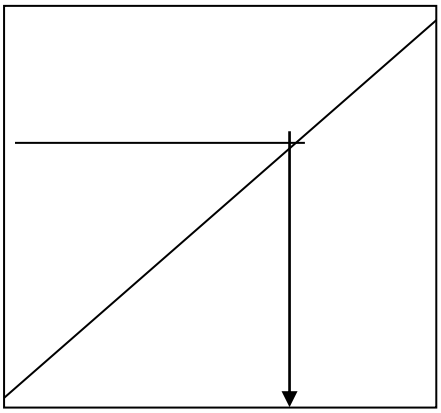
Procedure: Different aliquots of standard glucose solution (1.8mg/ml) varying 0.2ml to 1ml are pipetted out into different test tubes. The solutions are made up to 2ml in each case with distilled water. 2ml of the DNS reagent is added to each test tube.The tubes are heated in a boiling water bath for exactly 15 minutes and cooled. 16ml of water is added to each tube. The contents of the tubes are thoroughly mixed well and the absorbance of the solutions are measured in a colorimeter at 540nm against the blank solution.

Result: The unknown solution (given) contains: 1…….. 2……..mgs of glucose

OBSERVATIONS

PROTOCOL:

Sl.No.	Vol. of Creatin-ine (cm^3)	Amount of Creati-nine(µg)	Vol. of Water (cm^3)	Vol. of Picric-acid (cm^3)	Vol. of NaOH (cm^3)		Vol. of Water (cm^3)	O.D at 540nm
1.	0	0	2.0	2.0	0.2	**ALLOW THE TUBES TO STAND FOR 15 MIN-UTES**	5.8(tot-al 10ml	
2.	0.2	40	1.8	2.0	0.2		5.8	
3.	0.4	80	1.6	2.0	0.2		5.8	
4.	0.6	120	1.4	2.0	0.2		5.8	
5.	0.8	160	1.2	2.0	0.2		5.8	
6.	1.0	200	1.0	2.0	0.2		5.8	
7.	U.K-1 (1)	-	1.0	2.0	0.2		5.8	
8.	U.K-2 (1)	-	1.0	2.0	0.2		5.8	



1 c 1 cm^3 of unknown soln.contains ……. **µg.**

100 cm^3 of unknown contains…..100x….. **µg.**

=…….µg.

EXPERIMENT NO. 7

AIM: TO ESTIMATE THE AMOUNT OF CREATININE PRESENT IN THE GIVEN SOLUTION USING STANDARD CRETININE SOLUTION (1µg)/ml), AND PICRIC ACID BY JAFFE'S METHOD

Principle: Creatinine is one of the end products of nitrogen metabolism in human beings. The daily excretion of creatinine ranges from 1 to 1.8 microgram in normal adults. In the presence of strong alkali, creatinine yields a red color on reaction with picric acid. The color may be due to the formation of picramic acid or enolized form of the picric acid.

Procedure: 0.2 ml to 1 ml aliquots of standard creatinine solution are pipetted out into different test tubes and 1ml of unknown solution in to two separate test tubes. The volume in each tube is made up to 2 ml with distilled water. 2 ml of picric acid followed by 0.2ml of 10% NaOH are added into tubes and allowed to stand for 15 minutes. The volume in each case is made up to 10 ml with distilled water. The contents are mixed well and the absorbance is measured at 540nm against blank solution.

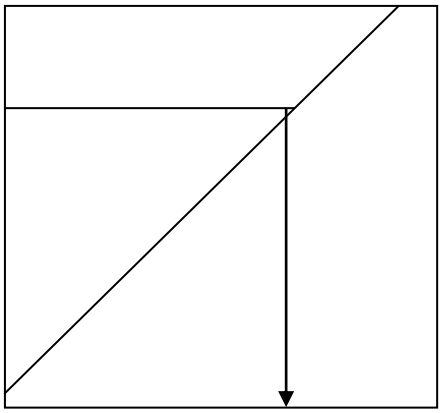
Result: The given sample of unknown solution contains 1…… **µg,** 2…… **µg** of creatinine.

OBSERVATIONS

PROTOCOL:

Sl.No.	Vol. of Inor.Phos. (cm^3)	Amount of Inor.phos. (µ.mol)	Vol. of Water (cm^3)	Vol. of Acid molybdate +red.agent (cm^3)		Vol. of water (cm^3)	Optical density at 660nm
1.	0	0	1.0	1+1 = 2	**MIXED WELL & ALLOW -ED TO STAND FOR 30 MINS**	7	
2.	0.2	0.2	0.8	1+1 = 2		7	
3.	0.4	0.4	0.6	1+1 = 2		7	
4.	0.6	0.6	0.4	1+1 = 2		7	
5.	0.8	0.8	0.2	1+1 = 2		7	
6.	1.0	1.0	0	1+1 = 2		7	
7.	U.K-1(1)	-	0	1+1 = 2		7	
8.	U.K-2 (2)	-	0	1+1 = 2		7	

Optical density



Amount of Inog.Phos.(µ.mol)

1cm^3 of (unknown) soln.contains … µ.mol.
100 cm^3 of the soln. contains…..100x……
= ……µ.mol

EXPERIMENT NO. 8

AIM: TO ESTIMATE THE AMOUNT OF THE GIVEN INORGANIC-PHOSPHATE USING ACID MOLYBDATE & REDUCING AGENT BY MODIFIED FISKE SUBBA -RAO METHOD

Principle: Phosphate reacts with molybdic acid to form Phospho-molybdic acid. On treatment with 1,2,4 amino naphthol sulphonic acid or p-methyl amino phenol phosphor molybdic acid is selectively reduced to produce a deep blue color, which is probably a mixture of lower oxides of molybdenum. This color is then read in a colorimeter against a suitable blank at 660nm.

Procedure: Different aliquots of standard inorganic phosphate solution(1µ.mol/ml) varying from 0.2 to 1 ml are pipetted out intocdifferent test tubes. The volumes are made up to 1 ml in each case with distilled water, 1ml of acid molybdate and 1ml of the reducing agent are added to each tube. The contents of the tubes are mixed well and allowed to stand for 30 minutes at room temperature for color development. At the end of 30 minutes, the solution in each tube is diluted to 10 ml with distilled water and the absorbance is measured in colorimeter at 660 nm against the blank solution.

Result: The given unknown solution of phosphate contains:

1 ……. 2. …… micromoles of phosphate.

ESTIMATION OF SERUM CHOLESTEROL BY ZAK'S METHOD

EXPERIMENT NO.10:

AIM: **TO ESTIMATE THE AMOUNT OF CHOLESTEROL PRESENT IN THE GIVEN SERUM SAMPLE.**

INTRODUCTION: The estimation of serum cholesterol is important since increased serum cholesterol is one of the causative factors for atherosclerotic diseases leading to myocardial infarction, cerebral haemorrhage and thrombosis. Cholesterol is the most abundant sterol in the human tissues and body7 bfluids. It is present in blood in two forms – free(30%) and esterified (70%). It is transported bound to lipoproteins mainly HDL and LDL. It has many important functions in our body.

PRICIPLE: In strong acidicmedium the cholesterol molecules first undergoes dehydration to form cholesta 3, 5 – dien and then oxidation and sulphonation by conc. sulphuric acid in prescence of Fe 3+ ions as catalyst to form red coloured bis – cholesta 3,5 – dien. This is proportional to cholesterol concentration and is measured optically at 540nm.

REAGENTS REQUIRED:

Preparation of stock solution: 100mg of cholesterol is accurately weighed, dissolved in glacial acetic acid and made upto 100ml with the same in the standard flask. Concentration is 100mg/ml.

Preparation of working standard solution: 10ml of the stock solution is diluted and made upto 100ml with 0.05% ferric chloride solution. Conc. is 100µg/ml Stock ferric chloride reagent: 500mg of ferric chloride is dissolved in 100ml of aldehyde free acetic acid (0.5%).

Working ferric chloride reagent: 10ml of the stock ferric chloride is diluted to 100ml with glacial acetic acid (0.05%) Analar sulphuric acid:

Preparation of serum supernatant: To 0.3ml of serum 6.7ml of ferric chloride acetic acid reagent is added and incubated for 10min at room temp. and centrifuged. The clear supernatant was used for estimation.

PROCEDURE: 0.5 to 2.5ml of standard cholesterol solution is pipetted into different test tubes with corresponding concentration ranging from 25µg to 125µg of cholesterol. 1ml of serum supernatant is taken in a separate test tube. The volume in each tube is made upto 5ml with ferric chloride acetic acid reagent. 5ml of ferric chloride acetic acid reagent alone serves as the blank. 3ml of conc. sulphuric acid was added to all the test tubes from the burette. The solution was mixed well and allowed to stand for 20mins at room temperature. A pinkish colour was developed and was read at 540nm. From the values obtained a standard calibration curve is drawn with concentration of cholesterol on X axis and optical density on the Y axis. From the std. graph, the amount of cholesterol present in the given serum sample is calculated.

RESULT: The amount of cholesterol present in the given serum sample is _____mg in 100ml.

Normal Value: 150 – 250mg in 100ml

EXPERIMENT NO.11

AIM: EXTRACTION OF DNA FROM ONIONS

The procedures involved in biotechnology implementation are predicated on the isolation of DNA from a tissue sample. This laboratory exercise is designed to give you the opportunity to extract DNA from onion tissue. The technique used is quick and easy for both you and your students. The DNA that is isolated can be digested using various endonucleases, followed with an electrophoresis of the digest. We use an onion because of its' cost, abundance and low starch content. You will make the onion filtrate from onion treated with salt, distilled water, and dishwashing DNA to be more clearly seen. The SDS detergent causes the cell membrane to break down by emulsifying the lipids and proteins of the cell and disrupting the polar interactions that hold the cell membrane together. The detergent then forms complexes with these lipids and proteins, causing them to precipitate out of solution. The use of NaCl salt shields the negative phosphate ends of the DNA which allows these ends to come closer so they can precipitate out of a cold 95% ethyl alcohol solution. You will be altering the filtrate so that you can "spool" DNA out when it precipitates. The DNA is soluble in the detergent solution but is insoluble in the alcohol. When you add the chilled alcohol, the DNA will come out of solution and easily spool on a glass rod. At the end of the exercise you will find a flow chart summarizing the steps and solutions required.

THE PROCEDURE HAS THREE BASIC STEPS: Homogenization which involves heating and blending the onion tissue in order to break down the cells. The heat treatment softens the phospholipid in the cell membrane and denatures the DNAse enzymes which if present, would cut the DNA into small fragments so that it would not spool. The onion tissue is mixed in a blender with homogenization media, which breaks down the cell wall, cell membrane and nuclear membrane allowing the release of DNA.

Deproteinization which involves adding a protease enzyme Papain - a common enzyme used to clean soft contact lenses. This will denature the proteins clinging to the DNA making the molecule flexible and easy to spool. Precipitation of DNA which involves adding ethanol alcohol which causes every component in the filtrate to stay in solution except DNA. The DNA will gather at the interface of the filtrate and ethanol and can be spooled out with a glass rod.

Homogenization media: SDS (Sodium Dodecyl Sulfate) is a biological detergent which causes the cell membrane to break down further and emulsifies the lipids and proteins of the cell by disrupting the polar interactions that hold the cell membrane together. The detergent forms complexes with these lipids and proteins causing them to precipitate out of the solution. SDS is the major ingredient in laundry detergent.

EDTA (Ethylenediamine tetracetic acid) weakens the cell by binding the divalent cations (Mg++ and Ca++) which are needed for membrane stability. This further aids in breaking open the cells of the onion.

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NaCl (Sodium chloride) enables nucleic acids to precipitate out of an alcohol solution because it shields the negative phosphate end of DNA causing the strands to come closer together and coalesce.

1. DNA is found in the nucleus of membrane-bound cells. The membranes are lipid and protein in composition. The cell membranes must be lysed in order to release the DNA.
2. DNA is a polymer made up of repeating chains of nucleotides. The sugar and phosphate components of DNA (the backbone) are both readily soluble in water.
3. The phosphate groups on the outside of DNA carry a negative charge. These negative charges are attracted to and are neutralized by cations such as sodium. When sodium is added to DNA it forms a protective "shell" around it. On the other hand, protein molecules precipitate from solution in the presence of salt.
4. DNA is insoluble in ethanol (ethyl alcohol). As ethanol is added to a solution containing DNA, the DNA will come out of solution and stick to whatever is around.
5. At room temperature DNA begins to denature by the action of DNase (present in cell extracts). DNA extraction procedures must be carried on in ice.

PROCEDURE:

1. Place 100 ml of homogenizing solution in a beaker heat the solution in a water bath until it reaches 60 C
2. Mince the onion and add to the solution when it has reached 60 C. Stir and let sit for 15 minutes. Try not to let the temperature go much above 60 C. The temperature is intended to denature proteins that would break up the DNA into small segments.
3. After the heat treatment, immediately place the beaker into an ice bath for 5 minutes. Swirl the solution gently to allow even cooling throughout. This step slows down the break down of DNA.

4. Pour the contents of the beaker into the homogenizer and blend as per the flow chart.
5. Filter the homogenate through cheesecloth draped over a clean beaker.
6. Pour some of the filtrate into a large test tube. Hold your test tube with filtered homogenate at an angle, gradually pour twice the volume of ice cold alcohol down the wall of the test tube as there is homogenate present.
7. Watch what happens. You should see some stringy substance precipitate out. This is DNA. When it looks very stringy, place a glass rod into the tube so that the end of the rod is just bellow the upper layer of liquid(alcohol) and try to spool the DNA. It should look very clear and glistens around the glass rod. Using a glass stirring rod, gently but quickly twirl the rod into and out of the 2 layers. Gently lift the rod out of the tube and observe any substance attached to the rod.
8. This DNA represents all the DNA found in the onion cells. The chromosomes were broken in the process and the DNA precipitated due to the chemical treatment.

To use DNA for cloning or restriction digests, wash with 95% ethanol, then 70% ethanol. Air dry and re suspend in 500ul TE buffer. Heat in a 60 C water bath for 10 minutes to denature potential DNases. Store at -20 C. TIPS: DNA clings to glass - negative charge of DNA is attracted to positive charge in the silica of glass. Therefore, use plastic tubes for the spooling part of this lab You can make simple glass rods by heating the ends of glass pasteur pipettes and pushing the end to make a small hook. These make dandy rods to spool and hook up the DNA. A similar procedure to this one can be used to extract DNA from animal tissue such as calf thymus. Thymus glands from calves are sold in butcher shops and gourmet grocery stores as "sweetbreads". You can use either fresh or frozen.
PROTOCOL
Extraction of DNA from Onion: Dice an onion into small pieces, weigh out 50g of onion ,place onion into a 250 ml beaker + add 100 ml of homogen. medium incubate in a 60 C water bath for 15 min. chill quickly in an ice bath (15-20 C) handle the DNA gently, not rough pour chilled preparation into blender homog. for 45 sec. at low speed homog. for 30 sec. at high speed pour into a 1000 ml beaker allow to stand in ice bath for 15-20 min. pour through 4 layers of cheesecloth over a 500 ml beaker in ice slowly add cold 5% ethanol down the side of the beaker 80 ml spool out DNA in one direction only, dry the DNA with paper towel and suspend in TE buffer, store in freezer.
Homogenization Medium:
5% SDS (50g/L) ---------- 0.15M NaCl (8.8g/L)
0.15M sodium citrate (43.7g/L) ---------- 0.001M EDTA (0.5M stock, 2ml/L)
TE Buffer pH 8.0
0.01M Tris-HCl pH 8.0 ----------5 mls of 2M stock
0.001M EDTA pH 8.0 ----------2 mls of 0.5M stock