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EXPERIMENT-01:

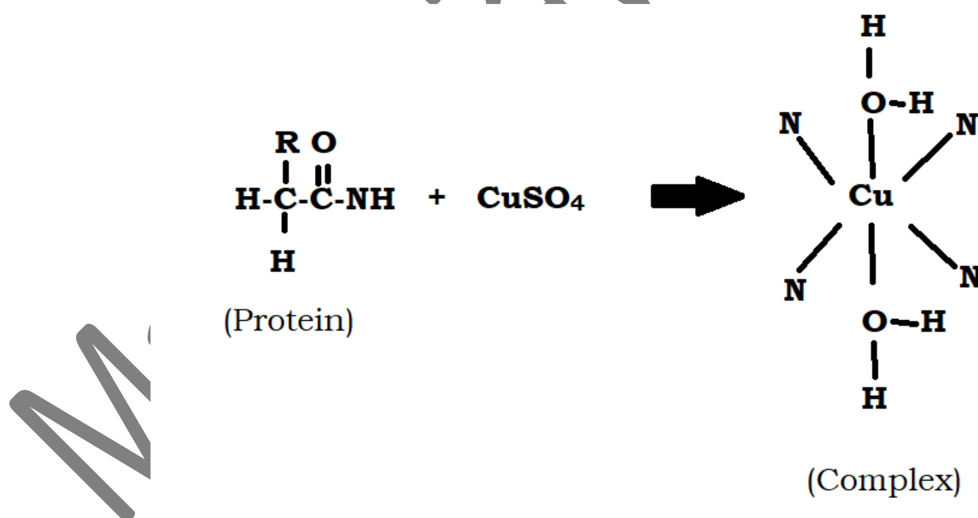
DETERMINATION OF SOLUBLE PROTEIN CONTENT BY LOWRY'S METHOD.

AIM: **DATE:**

To estimate protein by Lowry's biuret method.

WORKING PRINCIPLE:

The method is sensitive to the concentration of about 10μg/ml which is the most widely used protein assay. It uses the biuret reactions in which, Cu^{2+} ions in presence of base reacts with the peptide bond of protein under alkaline conditions, resulting in reduction of cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}) and Lowry's reaction in which the folin ciocalteau reagent which contain phosphomolybdic complex which is a mixture of sodium tungstate, sodium molybdate and phosphate along with $CuSO_4$ solution and the protein, a blue-purple colour is produced which can be assessed by measuring the absorbance rate at **650-770nm.** The blue-purple colour is formed due to the reduction of phospho-molybdotungstate to hetero-polymolybdinum by the Cu^{2+} catalyzed oxidation of aromatic amino acid tryptophan to tyrosine. Thus, the colour intensity depends on the aromatic amino acids present in the protein sample and will thus vary for different proteins.



REAGENTS REQUIRED:

1) **0.1N NaOH solution.**
2) **Alkaline sodium carbonate solution:** Dissolve 20g of sodium carbonate in 100ml of 0.1N NaOH solution (Reagent A).
3) **Copper sulphate-Sodium potassium tartrate solution:** Dissolve 0.5g/100ml of Copper sulphate pentahydrate ($CuSO_4.5H_2O$) in 1% of sodium potassium tartrate solution (Reagent B).

4) **Alkaline copper reagent:** Mix 49ml of reagent A and 1ml of reagent B only on the day of use.
5) **Folin-ciocalteau reagent:** Dilute the folin-ciocalteau reagent with an equal amount of distilled water on the day of use.

PROCEDURE:

1. Take a series of 6 test tubes with different concentrations of proteins.
2. Make up the volume to 1ml with distilled water.
3. Add 5ml of alkaline reagent to each test tube.
4. Incubate the reaction mixture at room temperature for 10 minutes.
5. Add 0.5ml of folin-ciocalteau reagent to each test tube.
6. Incubate the reaction mixture for 30 minutes in room temperature.
7. Read the optical density (O.D) at 750nm.

TABULAR COLUMN:

Test tube No:	Stock of protein (ml)	Distilled water (ml)	Conc. of protein (µg/ml)	Alkaline reagent (ml)	**INCUBATE FOR 10 MINUTES**	Folin-ciocalteau reagent (ml)	**INCUBATE FOR 30 MINUTES**	O.D at 750nm
01.	0.0	1.0		5		0.5		
02.	0.1	0.9		5		0.5		
03.	0.2	0.8		5		0.5		
04.	0.3	0.7		5		0.5		
05.	0.4	0.6		5		0.5		
06.	0.5	0.5		5		0.5		

RESULT:

With reference to the standard graph, _______________ O.D corresponds to ____________ µg/ml of protein.

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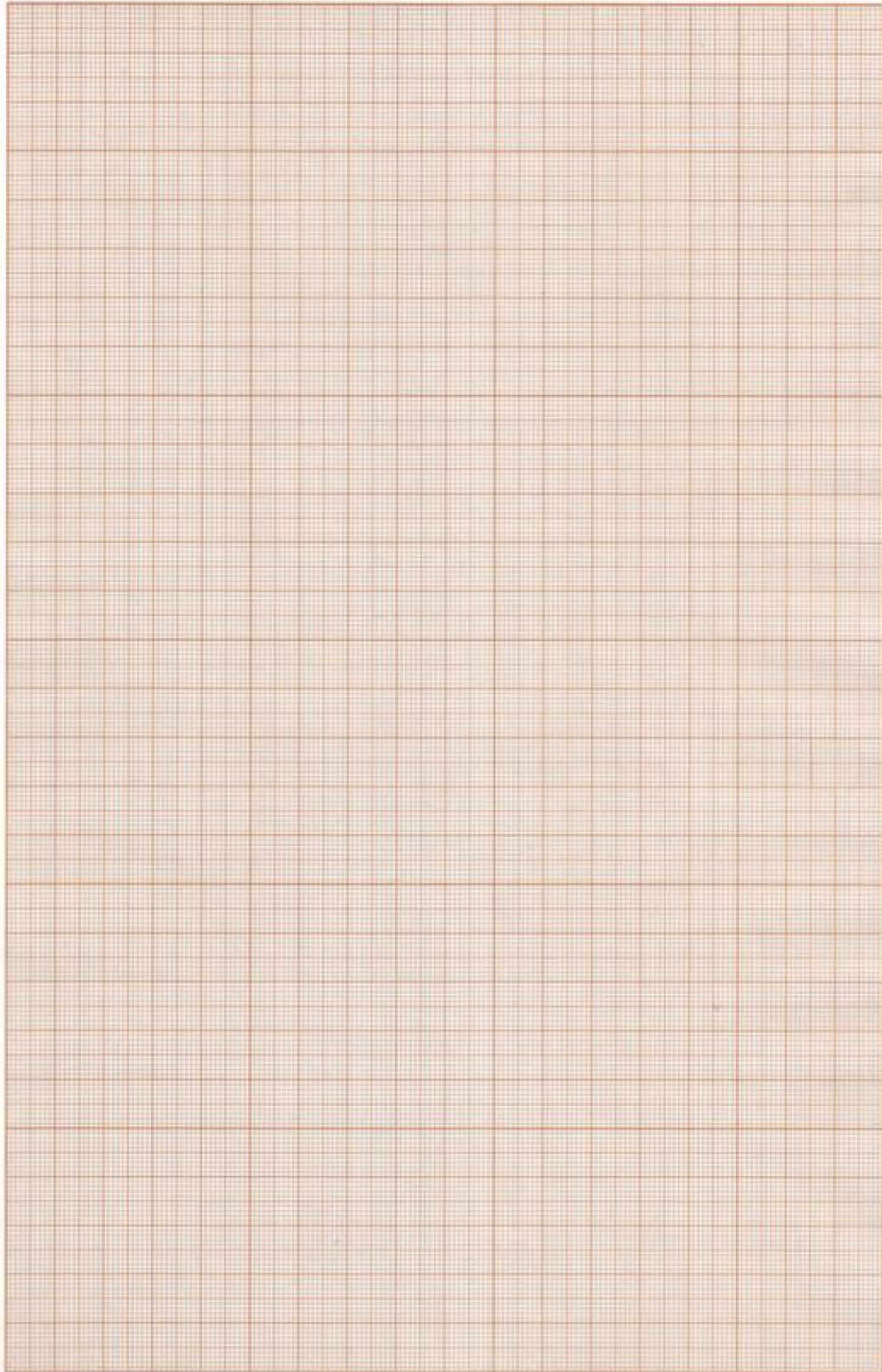

EXPERIMENT-02:

PREPARATION OF STANDARD GRAPH OF CHOLESTEROL BY ZAK'S METHOD.

AIM: **DATE:**

Preparation of standard graph of cholesterol by Zak's method.

INTRODUCTION:

Cholesterol is a steroid lipid and is amphipathic in nature. It consists of basic cyclo-pento-perhydro-phenanthrene nucleus. It is synthesized in the liver from acetyl Co-enzyme A and acts a precursor for steroid hormones and vitamin-D.

Excess cholesterol leads to narrowing of the arteries which in turn causes cardiac problems. Its assessment and understanding are essential for medical complications.

WORKING PRINCIPLE:

The proteins present in the serum sample are first precipitated by adding $FeCl_3.CH_3COOH$ (Ferric chloride acetic acid). The protein free filtrate is treated with concentrated H_2SO_4. In the presence of conc. H_2SO_4, the cholesterol present in the serum get dehydrated to form cholesterol 3,5 diene. In presence of H_2SO_4 and by the catalytic action of Fe^{3+} ions, red coloured complex is formed. The intensity of red colour is measured at 560nm.

REAGENTS:

1. **Ferric chloride acetic acid reagent ($FeCl_3.CH_3COOH$) 0.005%:** 0.05g of ferric chloride ($FeCl_3$) is dissolved in 100 ml of aldehyde free acetic acid.
2. **Concentrated sulphuric acid (H_2SO_4).**
3. **Standard cholesterol.**
4. **Stock solution:** Dissolve 40mg of cholesterol in 100ml of $FeCl_3.CH_3COOH$ solution and store in brown bottle.
5. **Working stock solution:** Dissolve 10ml of stock solution and make up to 100ml with $FeCl_3.CH_3COOH$ reagent (final concentration 4mg in 100ml or 0.04µg/ml).

PROCEDURE:

1. Arrange 5 test tubes in a stand. Pipette out 1-5ml of working stock solution in series of test tube and make it up to 5ml using ferric chloride acetic acid solution ($FeCl_3.CH_3COOH$).

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2. Add 3ml of concentrated H_2SO_4 to each test tube and mix well. Incubate the above mixture for 20-30 minutes in room temperature.
3. The intensity of standard is measured at 560nm against the blank.
4. **Preparation of blank:** 5ml of $FeCl_3.CH_3COOH$ + 3ml of concentrated H_2SO_4 mix well and use as blank.

TABULAR COLUMN:

Test tube No:	Working standard (ml)	$FeCl_3$. CH_3COOH (ml)	Conc. H_2SO_4 (ml)	Conc. of cholesterol (µg/L)		O.D at 560nm
Blank	0.0	5.0	03	0.00	INCUBATE FOR 30 MINUTES	
01.	0.5	4.5	03			
02.	1.0	4.0	03			
03.	1.5	3.5	03			
04.	2.0	3.0	03			
05.	2.5	2.5	03			
06.			03			

RESULT:

With reference to the standard graph, ________________ O.D corresponds to ______________ µg/L of cholesterol.

DISCUSION:

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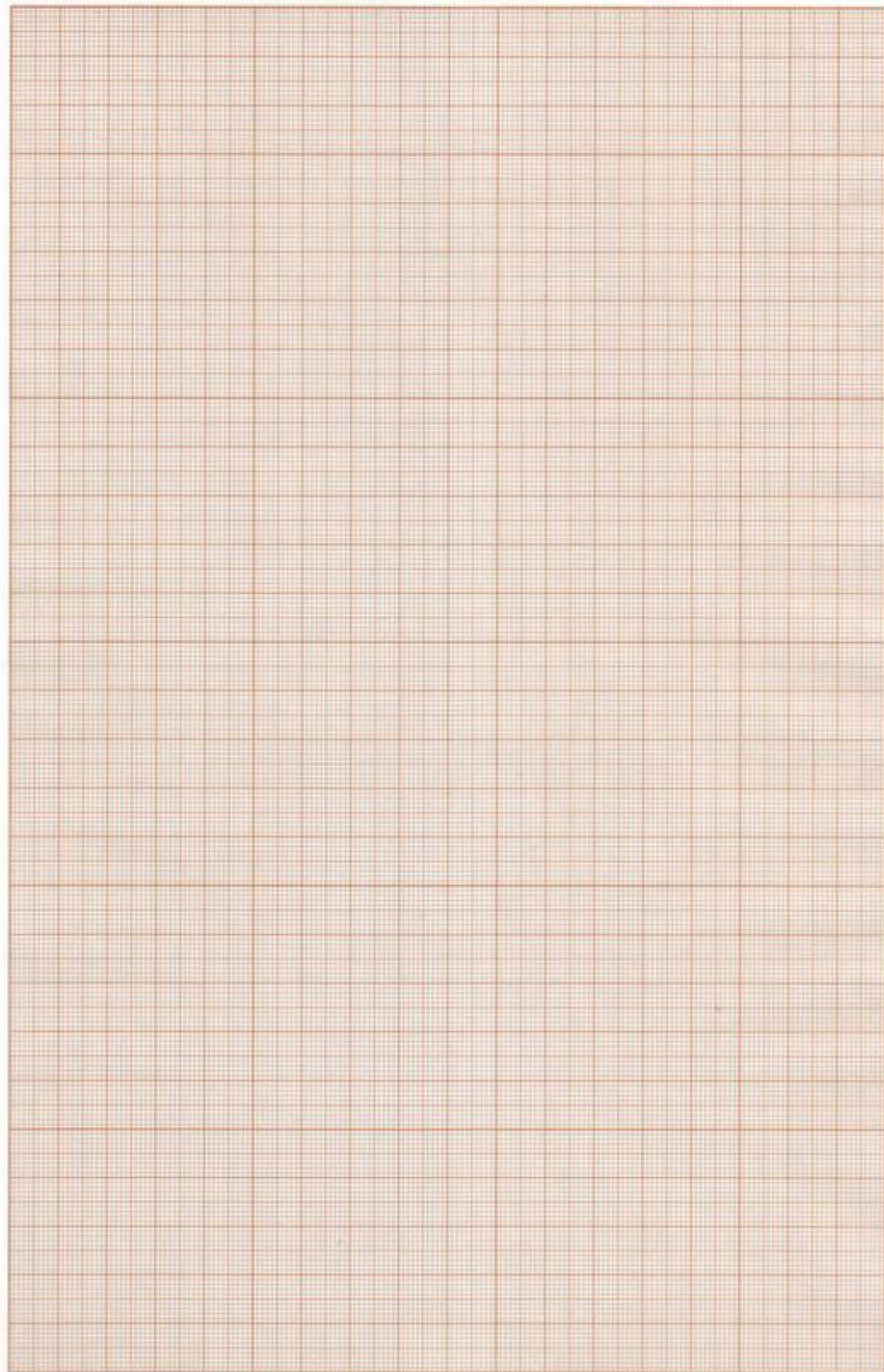
REFERENCE:



EXPERIMENT-03:

ESTIMATION OF AMINO ACID BY SORENSON's TITRATION METHOD.

AIM: **DATE:**

To estimate amino acid by Sorenson's titration method.

PRINCIPLE:

Amino acids react with formaldehyde to form methylene amino acids, when titrated against sodium hydroxide, the end point being appearance of pale pink colour.

Further formaldehyde solution contains formic acid and also amino acids are not exactly neutral. Thus, it is necessary that both the formaldehyde and amino acid solutions should have the same P^H before mixing and for this purpose, each solution is first made just alkaline by adding dilute NaOH solution.

REAGENTS REQUIRED:

1) **10% formaldehyde solution.**
2) **Standard glycine solution:** Accurately weigh 0.8g of glycine and dissolve in 100ml distilled water taken in volumetric flask.
3) **Unknown glycine solution.**
4) **NaOH solution (0.1N):** 0.4g in 100 ml distilled water.
5) **Phenolphthalein indicator:** Commercially available (diluted to 1:10 with alcohol).

PROCEDURE:

1) Transfer 10ml of standard glycine solution into a conical flask and add 1-2 drops of phenolphthalein indicator and titrate against NaOH (0.1N) to get faint pink colour.
2) Similarly, titrate by taking 10 ml of 10% formaldehyde solution and end point is appearance of pale pink colour.
3) Mix the contents of step 1 and step 2 and observe for the disappearance of pink colour. Titrate against NaOH solution till the faint pink colour reappears.
4) Repeat the titration for concordant values.

NOTE: Repeat the above steps for unknown glycine solution.

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FORMULA:

$$\text{Molecular weight (Mw)} = \frac{\frac{Weight}{L}\ of\ amino\ acid\ (8)\ \times\ Volume\ of\ amino\ acid\ used\ (10)}{Normality\ of\ NaOH\ (0.1)\ \times\ Volume\ of\ NaOH\ rundown}$$

TABULAR COLUMN:

1) For neutralization of glycine:

TRIAL NO:	BURETTE READING:		VOLUME OF NaOH RUNDOWN:
	INITIAL READING	FINAL READING	
I			
II			
III			

2) For neutralization of formaldehyde:

TRIAL NO:	BURETTE READING:		VOLUME OF NaOH RUNDOWN:
	INITIAL READING	FINAL READING	
I			
II			
III			

3) For neutralization of reaction mixture:

TRIAL NO:	BURETTE READING:		VOLUME OF NaOH RUNDOWN:
	INITIAL READING	FINAL READING	
I			
II			
III			

RESULT:

The molecular weight of glycine was found to be ____________ g.

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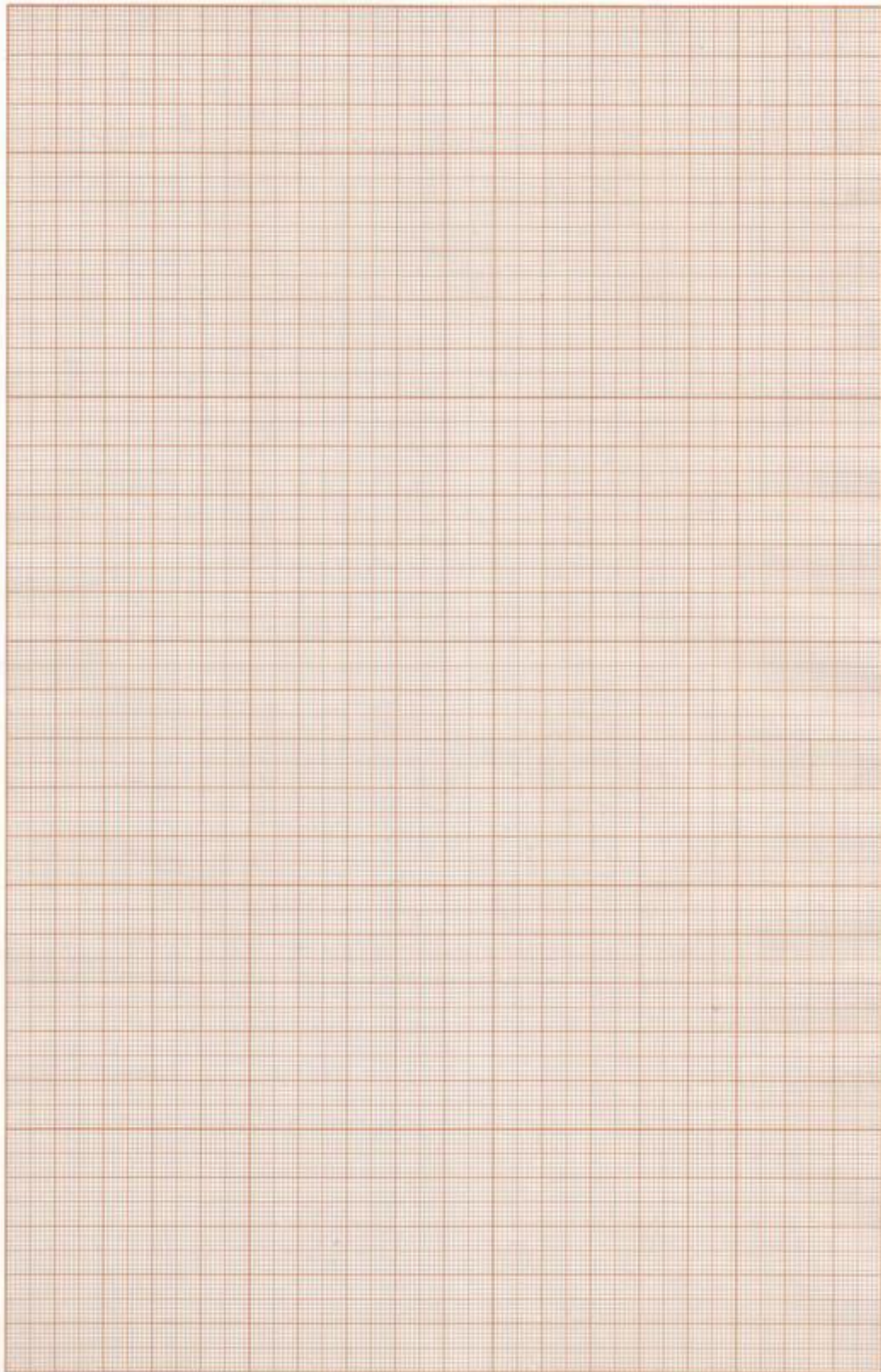


EXPERIMET-04:

ESTIMATION OF TOTAL AMINO ACIDS.

AIM: **DATE:**

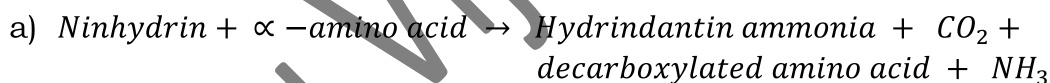
To estimate the total amount of amino acids.

INTRODUCTION:

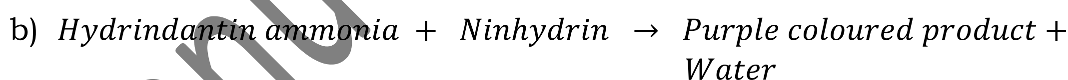
The amino acids are colourless, ionic compounds that form basic building blocks of proteins apart from being bound as protein amino acid. It also exists in free form in many tissues and known as free amino acids. They are mostly water soluble in nature. Very often in animal tissue, during disease condition, the free amino acid composition exhibits a change. Hence, the measurement of total free amino acids gives physiological and health status of an animal.

PRINCIPLE:

Ninhydrin, a powerful reducing agent which decarboxylates the alpha (α) amino acids and yields an intensified colour i.e., bluish-purple colour which is calorimetrically measured at 570nm.



a) $Ninhydrin + \propto - amino\ acid \rightarrow Hydrindantin\ ammonia + CO_2 + decarboxylated\ amino\ acid + NH_3$



b) $Hydrindantin\ ammonia + Ninhydrin \rightarrow Purple\ coloured\ product + Water$

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REAGENTS REQUIRED:

1. **Dissolve 50mg of glycine in 50ml of distilled water in a volumetric flask.** Take 10ml of stock standard solution and dilute to 100ml in another flask and is used as working standard.
2. A series of test tube are arranged with varied volume from 0.2 to 1ml of standard solution gives a concentration range from 10μg to 100μg.
3. **8% ninhydrin reagent:** Weigh 8g of ninhydrin reagent and dissolve in 100ml of acetone.
4. **70% ethanol.**
5. **Distilled water.**

PROCEDURE:

1. Pipette out different volumes (0.1-1ml) of standard amino acid solution to the respective labelled test tubes.
2. Add distilled water to all the test tubes to make the volume to 4ml.
3. Add 4ml of distilled water to the test tube labelled as blank and add 1ml of ninhydrin.
4. Mix the contents of the tube by over-turning and shaking them.
5. Cover the mouth of test tubes with aluminum foil and place the test tubes in water bath for 15 minutes.
6. Cool the test tube in cold water and add 1ml of ethanol to each test tube and mix well.
7. Record the absorbance at 570nm using colorimeter.

TABULAR COLUMN:

Test tube No:	Working Stock of glycine (ml)	Distilled water (ml)	Ninhydrin (ml)		70% Ethanol (ml)	Conc. of glycine (g/ml)	O.D at 570nm
BLANK	0.0	4.0	01	**INCUBATE IN WATERBATH FOR 15 MINUTES AND COOL IT**	01	0.00	
01.	0.2	3.8	01		01	0.02	
02.	0.4	3.6	01		01	0.04	
03.	0.6	3.4	01		01	0.06	
04.	0.8	3.2	01		01	0.08	
05.	1.0	3.0	01		01	0.10	

RESULT:

With reference to the standard graph, ____________________ O.D Corresponds to ____________ g/ml of glycine.

DISCUSSION:

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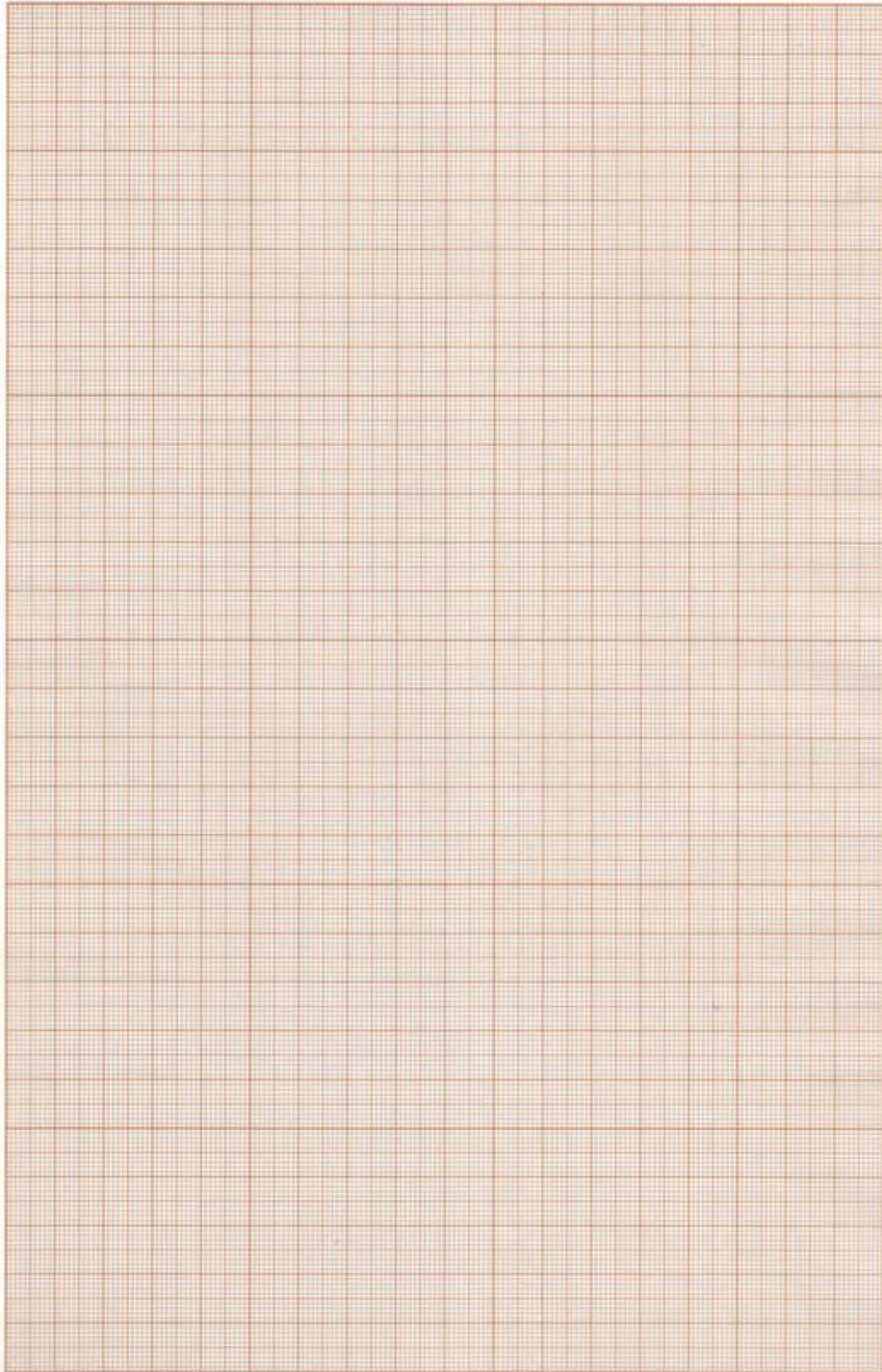

EXPERIMENT-05:

DETERMINATION OF URINE CREATININE CONTENT.

AIM: **DATE:**

To determine urine creatinine content.

CLINICAL SIGNIFICANCE:

Creatinine is synthesized in kidney, liver and pancreas and transported to muscle and brain where it is phosphorylated to phosphor-creatine. Some free creatine in muscle is converted to creatinine. The amount of creatinine produced is proportional to an individual's muscle mass. The waste product creatinine enters the blood supply and is excreted in the urine. In the absence of renal disease, the excretion rate of creatinine in an individual is relatively constant. Thus, urinary creatinine levels are commonly used as an index of standardization for a variety of other tests. Measurement of creatinine clearance is also useful in detecting the renal diseases and estimating the extinct of impairment of renal function.

PRINCIPLE:

The principle of measurement of the creatinine is based on Jaffe's reaction i.e., under the alkaline conditions, creatinine reacts directly with the picric ions forming a reddish complex, the absorbance of which can be measured at 520nm. However, several interfering substances including proteins, ketones, glucose and ascorbic acid also react with picric acid producing similar coloured complexes.

REAGENTS REQUIRED:

1. **STOCK STANDARD:** Weigh 1 gram of creatinine, dissolve in up to 1 liter of 0.1N HCl. This solution is stable at room temperature.
2. **WORKING STANDARD:** Dilute 10ml of stock standard and make up to 100ml with 0.1N HCl (alkaline picrate solution).
3. 0.5M NaOH (20g of NaOH) is dissolved in 1L of distilled water (Reagent A).
4. **Saturated picric acid solution:** 7-8g of picric acid in 500ml of distilled water (Reagent B). Allow excess picric acid to remain in contact with water, shake occasionally. This solution is stable when stored in amber bottle at room temperature.
5. **WORKING REAGENT:** (Alkaline picrate) Mix one part of 0.5M NaOH with one part of saturated picric acid. This solution needs to be prepared fresh daily before assay.
6. **DILUTION OF URINE:** Freshly voided urine was diluted i.e., 01 ml in 99 ml of distilled water

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PROCEDURE:

1. Pipette out working standard solution of varied concentrations into a series of 5 test tubes.
2. Add 0.1M HCl to the test tubes containing samples to make up the volume to 0.5ml.
3. Add 5.5ml distilled water to all the test tubes.
4. Add 1ml of alkaline picrate to all test tubes.
5. Incubate the test tubes in room temperature for 45 minutes.
6. Read the O.D at 520nm.

TABULAR COLUMN:

Test tube No:	Working Solution (ml)	HCl (ml)	Distilled water (ml)	Alkaline picrate (ml)	Creatinine concentration (μg/ml)		O.D at 520nm
BLANK	0.0	0.5	5.5	1	00	**INCUBATE IN ROOM TEMPERATURE FOR 45 MINUTES**	
01.	0.1	0.4	5.5	1	01		
02.	0.2	0.3	5.5	1	02		
03.	0.3	0.2	5.5	1	03		
04.	0.4	0.1	5.5	1	04		
05.	0.5	0.0	5.5	1	05		
06.	unknown						

RESULT:

With reference to standard graph, ________________ O.D corresponds to _____________ μg/ml of creatinine.

DISCUSSION:

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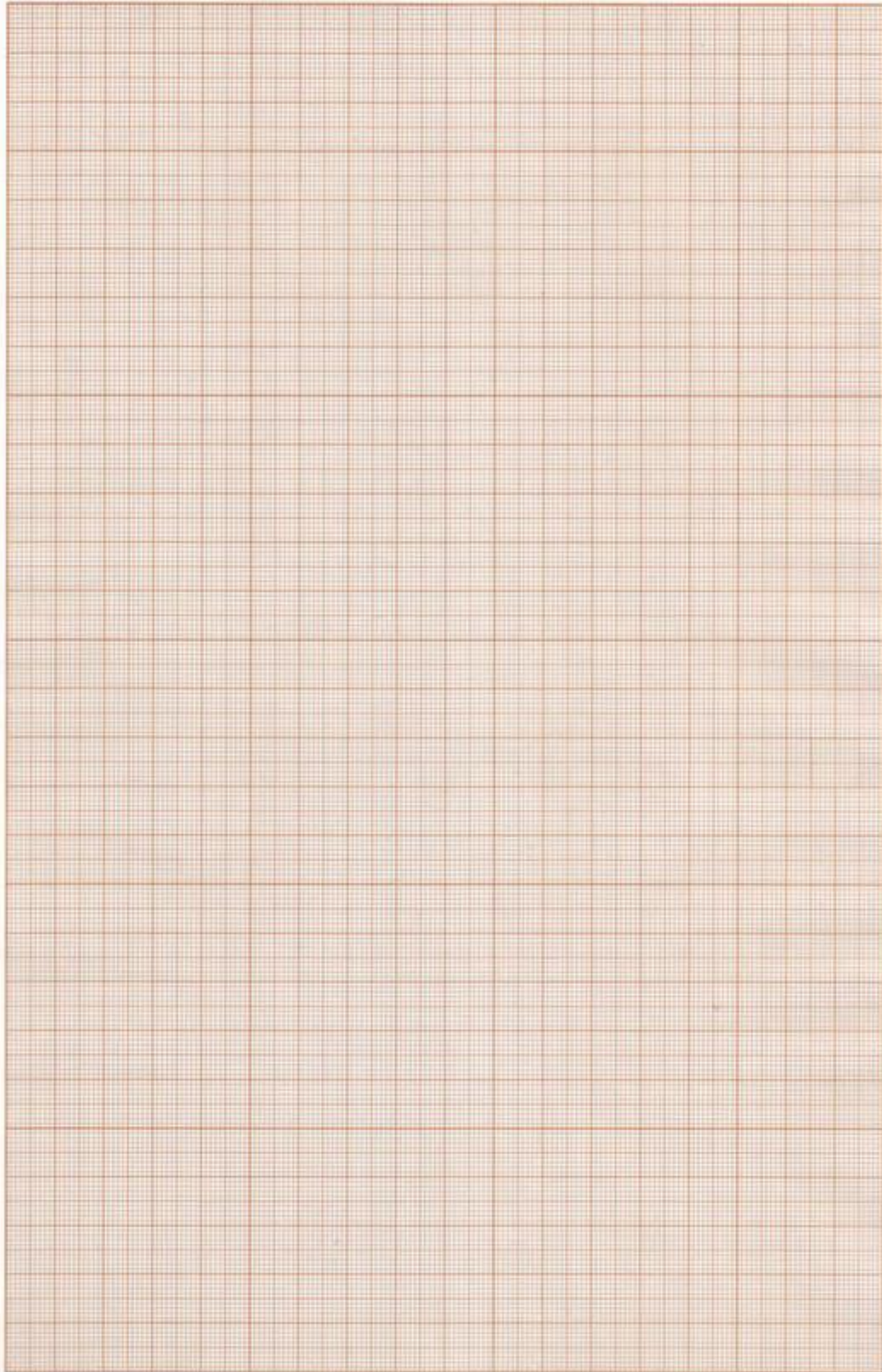

EXPERIMENT-06:

ESTIMATION OF LACTOSE IN MILK

AIM: **DATE:**

Estimation of lactose in milk.

PRINCIPLE:

For the estimation of lactose, the milk is diluted 1:25 and being a reducing sugar as in glucose, may be conveniently estimated by one of the reduction methods commonly used for glucose such as Asatoor and King (1954). The diluted milk is added to sodium sulphate, copper sulphate solution and protein precipitated by the addition of sodium tungstate solution. The mixture is centrifuged and the supernatant liquid is then added to alkaline tartrate. Lactose present reduces the copper sulphate to cuprous oxide. Addition of phospho-molybdic acid solution oxidizes the precipitated cuprous oxide to cupric oxide and is self-reduced to molybdenum blue. The absorbance of resulting blue colour is obtained at 580nm. From this, the concentration of lactose in the diluted milk may be found if parallel tests using standard lactose solutions are carried out.

MATERIALS REQUIRED:

1. Cow's milk, test tube, boiling water bath, aluminum foil, centrifuge, spectrophotometer.
2. **Sodium sulphate copper sulphate solution:** Dissolve 30g of sodium sulphate ($Na_2SO_4.10H_2O$) and 6g of copper sulphate ($CuSO_4.5H_2O$) in water and make up to 1L.
3. **Sodium tungstate solution:** 100g of sodium tungstate ($Na_2WO_4.2H_2O$) is dissolved in water and made up to 1 liter.
4. **Alkaline tartrate:** Dissolve 25g of sodium carbonate in about 600ml of water. Add 20g of anhydrous sodium carbonate and stir until its dissolved. Dissolve 18g of potassium oxalate in little water and add the above solution. Dissolve 12g of sodium potassium tartrate in a small amount of water and add to above solution.
5. **Phospho-molybdic acid reagent:** Dissolve 35g of molybdic acid and 5g of sodium tungstate in 200ml of 10% NaOH solution. Add 200ml of water and boil off any ammonia present in molybdic acid. This usually takes 30-40 minutes. Cool and dilute to about 350ml water, then add 125ml of phosphoric acid, make up to 500ml with water.
6. **STOCK STANDARD: (Lactose Solution)** Dissolve 0.1g of lactose in 100ml of sodium sulphate copper sulphate solution.
7. **WORKING STANDARD: (Lactose Solution)** Make 1:10/10mg per deciliter of stock lactose solution using sodium sulphate copper sulphate solution.

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PROCEDURE:

1. Make 1:25 dilution of cow's milk in the water.
2. Add 0.2ml of diluted cow's milk to 7.6ml of sodium sulphate copper sulphate solution.
3. Add 0.2ml of sodium tungstate solution mixture and centrifuge the cow's milk.
4. Set up the test tubes from A-G (A=blank, B-E=standards, F and G milk samples), cover the tubes with aluminium foil. Keep in boiling water bath for 10 minutes. Cool and add 3ml of phospho-molybdic acid to each test tube followed by 3ml of distilled water, mix well.
5. After 5 minutes, read the absorbance against the lactose concentration at 680nm against the blank.
6. Plot the standard graph against the lactose concentration.

TABULAR COLUMN:

Test tube No:	Working Standard (ml)	Na_2SO_4 $CuSO_4$ (ml)	Super - natant (ml)	Na_2WO_4 (ml)	Alkaline tartrate (ml)	INCUBATE IN WATERBATH FOR 10 MINUTES	Phospho molybdic acid (ml)	D W (ml)	O.D at 680 nm
A	0.00	1.00	-	-	1		3	3	
B	0.25	0.75	-	-	1		3	3	
C	0.50	0.50	-	-	1		3	3	
D	0.75	0.25	-	-	1		3	3	
E	1.00	-	-	-	1		3	3	
F	0.20	7.60	1.0	0.2	1		3	3	
G	0.20	7.60	1.0	0.2	1		3	3	

RESULT:

The amount of lactose concentration present in the given milk sample was found to be ______________ mg/ml.

DISCUSSION:

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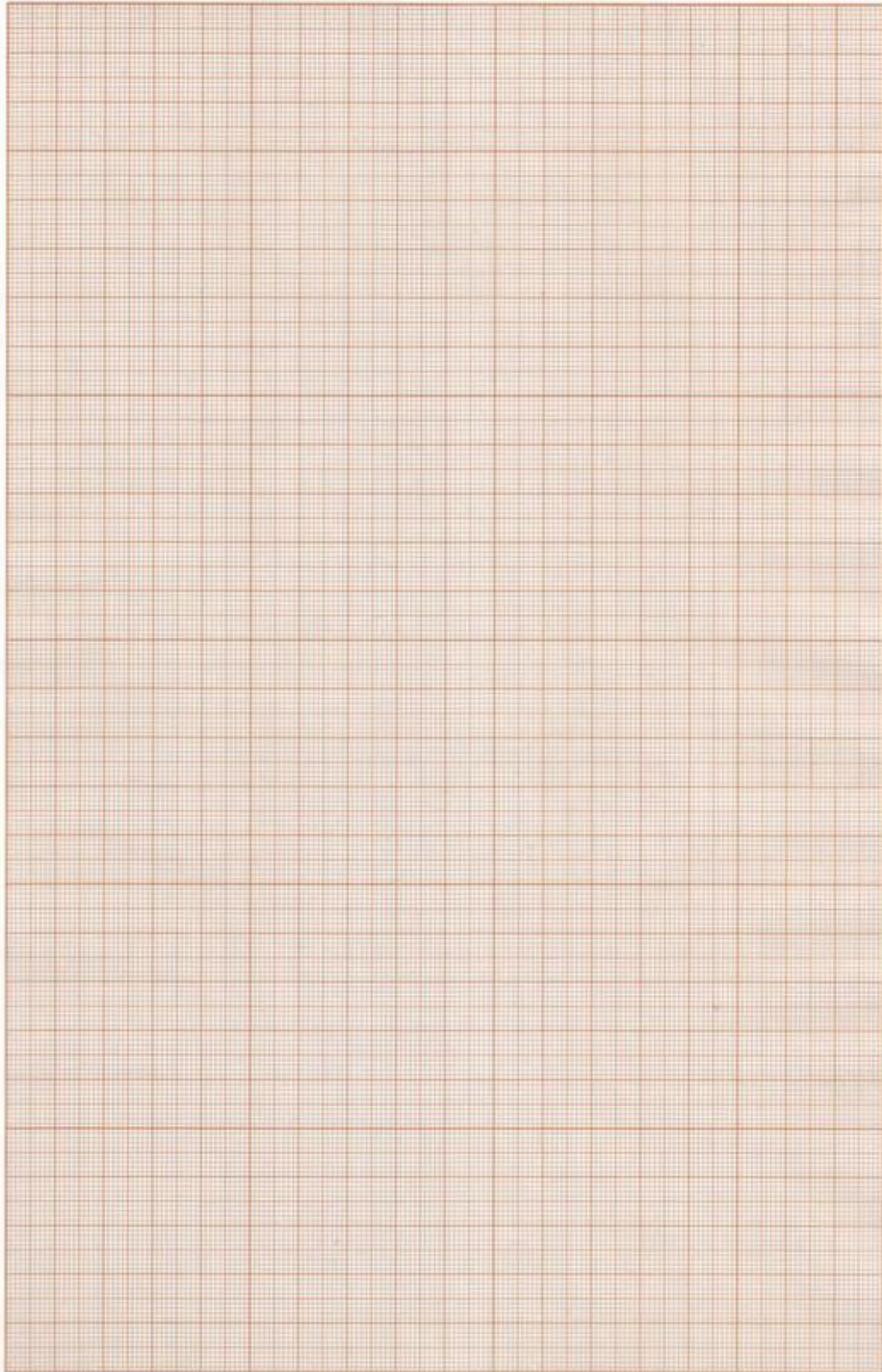
REFERENCE:



EXPERIMENT-07:

ESTIMATION OF TOTAL PROTEIN IN MILK

AIM: **DATE:**

To estimate the total protein content of the milk.

INTRODUCTION:

The major content of milk is lactose, fat and proteins. The protein includes casein (a phosphoprotein and 80% of total protein), Lacto-albumin and little lactoglobulin. There are also important amounts of calcium, phosphorous and vitamin A, B2 with small quantities of vitamin B1, C and D3 and iron. The white colour of milk is due to emulsified liquid and to the salts of casein.

The fat content of human milk is similar to that of cow's milk but there is more lactose and less protein, calcium and phosphorous. Colostrum is more yellowish and contains about twice as much protein including immunoglobins. The protein content falls with time reaching average levels after about 1 month. Fat and lactose change little than earlier.

PRINCIPLE:

Total protein in milk is estimated as casein by biuret reaction, this reaction is given by peptides containing at least two peptide bonds i.e., it is not given by dipeptides and amino acids in general although histidine, serine and threonine do give a reaction. Under strongly alkaline conditions, such compounds give violet-coloured complexes with cupric salts, which may be Spectrophotometrically estimated. Reaction takes its name from fact that biuret itself obtained by heating urea giving a similar coloured complex with cupric ions.

$$2H_2N\text{-}CO\text{-}NH_2 \longrightarrow NH_3 + HN_2\text{-}CO\text{-}NH\text{-}CO\text{-}NH_2$$

UREA **BIURET**

MATERIALS REQUIRED:

1. Cow's milk, test tube, volumetric flask, colorimeter, water bath (37° C), pipette.
2. Casein stock solution: 0.8g in 100ml of distilled water.
3. **SOLUTION A:** Copper sulphate (7.5g) and sodium potassium tartrate (30g) are dissolved in 500ml of distilled water.
4. **SOLUTION B:** 1N NaOH solution (40g in 1 liter of distilled water).

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5. **COLOURED REAGENT:** (Biuret reagent) Mix 1 volume of solution A with 4 volumes of solution B. Prepare freshly.

PROCEDURE:

1. Dilute the milk to 1:10 by pipetting 10ml into 100ml volumetric flask and makeup to the mark with distilled water.
2. Set up the test tubes in following order; A=blank, B to F=standard casein solution and G and H are samples in duplicate.
3. Add casein stock solution in varied concentrations to each test tube.
4. Incubate in water bath at 37°C for 5 minutes.
5. Add 5ml of coloured reagent to each tube, mix well and allowed to stand for 20 minutes in the water bath.
6. Blanking with solution A, read the optical density of B to H at 540 nm.
7. Plot the standard curve for absorbance against casein concentration using the results for solutions B to F.
8. From this standard curve, estimate the concentration of casein in solutions G and H.

TABULAR COLUMN:

<table>
<tr><th>Test tube No:</th><th>Distilled water (ml)</th><th>Stock casein solution (ml)</th><th>Diluted milk (ml)</th><th rowspan="9">INCUBATE IN WATERBATH FOR 5 MINUTES</th><th>Alkaline reagent (ml)</th><th rowspan="9">INCUBATE IN WATERBATH FOR 20 MINUTES</th><th>Conc. of casein (g/ml)</th><th>O.D at 540 nm</th></tr>
<tr><td>A</td><td>2.50</td><td>-</td><td>-</td><td>5</td><td></td><td></td></tr>
<tr><td>B</td><td>2.00</td><td>0.50</td><td>-</td><td>5</td><td></td><td></td></tr>
<tr><td>C</td><td>1.50</td><td>1.00</td><td>-</td><td>5</td><td></td><td></td></tr>
<tr><td>D</td><td>1.00</td><td>1.50</td><td>-</td><td>5</td><td></td><td></td></tr>
<tr><td>E</td><td>0.50</td><td>2.00</td><td>-</td><td>5</td><td></td><td></td></tr>
<tr><td>F</td><td>-</td><td>2.50</td><td>-</td><td>5</td><td></td><td></td></tr>
<tr><td>G</td><td>-</td><td>-</td><td>2.5</td><td>5</td><td></td><td></td></tr>
<tr><td>H</td><td>-</td><td>-</td><td>2.5</td><td>5</td><td></td><td></td></tr>
</table>

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RESULT:

Amount of casein in the milk with reference to standard graph was found to be _____________ g/ml with reference to ______________ O.D.

DISCUSSION:

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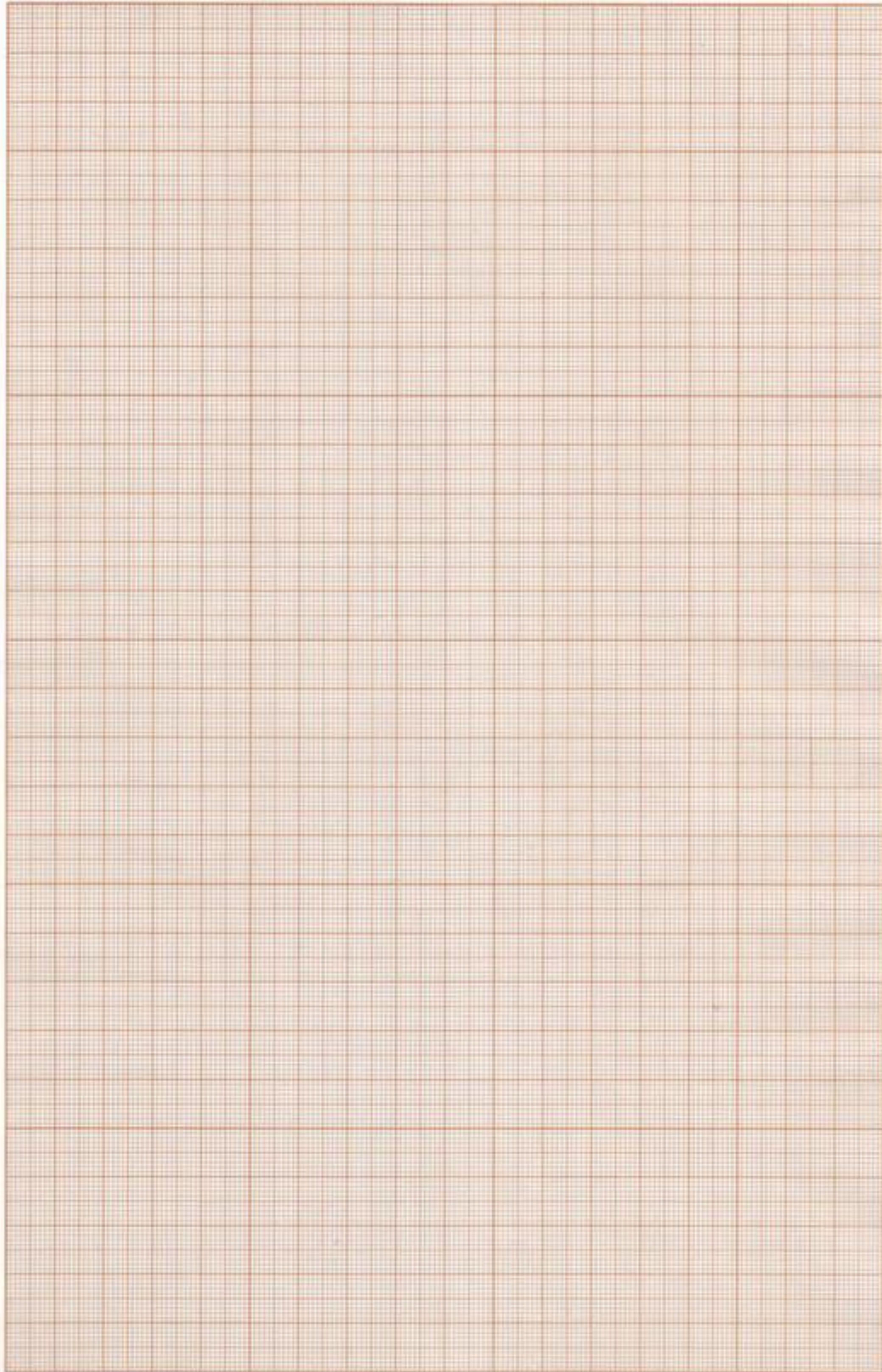
REFERENCE:



EXPERIMENT-08:

ESTIMATION OF DNA BY DIPHENYL AMINE METHOD

AIM: **DATE:**

To estimate the amount of DNA present in the given unknown solution by diphenylamine method.

PRINCIPLE:

When DNA is heated with Diphenylamine under acidic condition, a bluish-green coloured complex which has an absorbance peak at 540nm is obtained. This reaction is given by 2-deoxypentose. In general, in acidic solution deoxy pentose is converted into a highly reactive β-hydroxy levulinic aldehyde which reacts with diphenyl amine (DPA) giving bluish-green coloured complex. The colour intensity is measured at 590nm.

REAGENTS:

1. **STOCK STANDARD SOLUTION:** 50mg of DNA is dissolved in 50ml of saline sodium citrate buffer (concentration 1mg/ml).
2. **WORKING STANDARD:** 5ml of stock solution is diluted to 50ml with distilled water.
3. **DIPHENYL REAGENT:** 10g of pure diphenyl amine was dissolved in 25ml of concentrated sulphuric acid (H_2SO_4), which is made up to 100ml with glacial acetic acid. This solution must be prepared fresh.
4. **BUFFER SOLUTION: (P^H 7.4)**
   - It is 0.15M sodium chloride (NaCl). 0.877g of NaCl in 100ml of distilled water.
   - 0.015M sodium citrate (0.441g of sodium citrate in 100ml distilled water).
   - Equal volumes of above solutions are adder and then 1ml of mixture is taken and its diluted with 10ml of distilled water.
5. **STANDARD DNA:** 100mg of DNA is added to diluted saline citrate solution.

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PROCEDURE:

1. 0.5 to 2.5 ml of working standard solution is pipetted out in to 5 test tubes labelled as 1 to 5 where concentration ranges from 50-250μg.
2. 1 ml and 2 ml of unknown solution is pipetted out into 2 test tubes and labelled as U_1 and U_2.
3. The volume in all test tubes is made up to 3ml with distilled water and 3ml of 3ml of distilled water alone serves as blank.
4. 4ml of diphenylamine reagent is added to all test tubes and kept in water bath at 36° C for 20 minutes.

5. Test tubes are cooled and bluish coloured complex develops and the O.D is read at 590nm.
6. A standard graph is drawn taking concentration of DNA in x-axis and absorbance of DNA on y-axis.
7. From the standard graph, the amount of DNA present in unknown solution is calculated.

TABULAR COLUMN:

Test tube No:	Working solution (ml)	Distilled water (ml)	DPA (ml)		Conc. of DNA (µg/ml)	O.D at 590nm
00	0.0	3.0	← 4 ml →	**INCUBATE IN WATER BATH FOR 20 MINUTES at 36°C**	00	
01	0.5	2.5			50	
02	1.0	2.0			100	
03	1.5	1.5			150	
04	2.0	1.0			200	
05	2.5	0.5			250	
U_1	1.0	2.0			-	
U_2	1.0	1.0			-	

RESULT:

The amount of DNA present in given unknown solution was found to be _______________µg/ml and unknown solution 2 is __________________µg/ml.

DISCUSSION:

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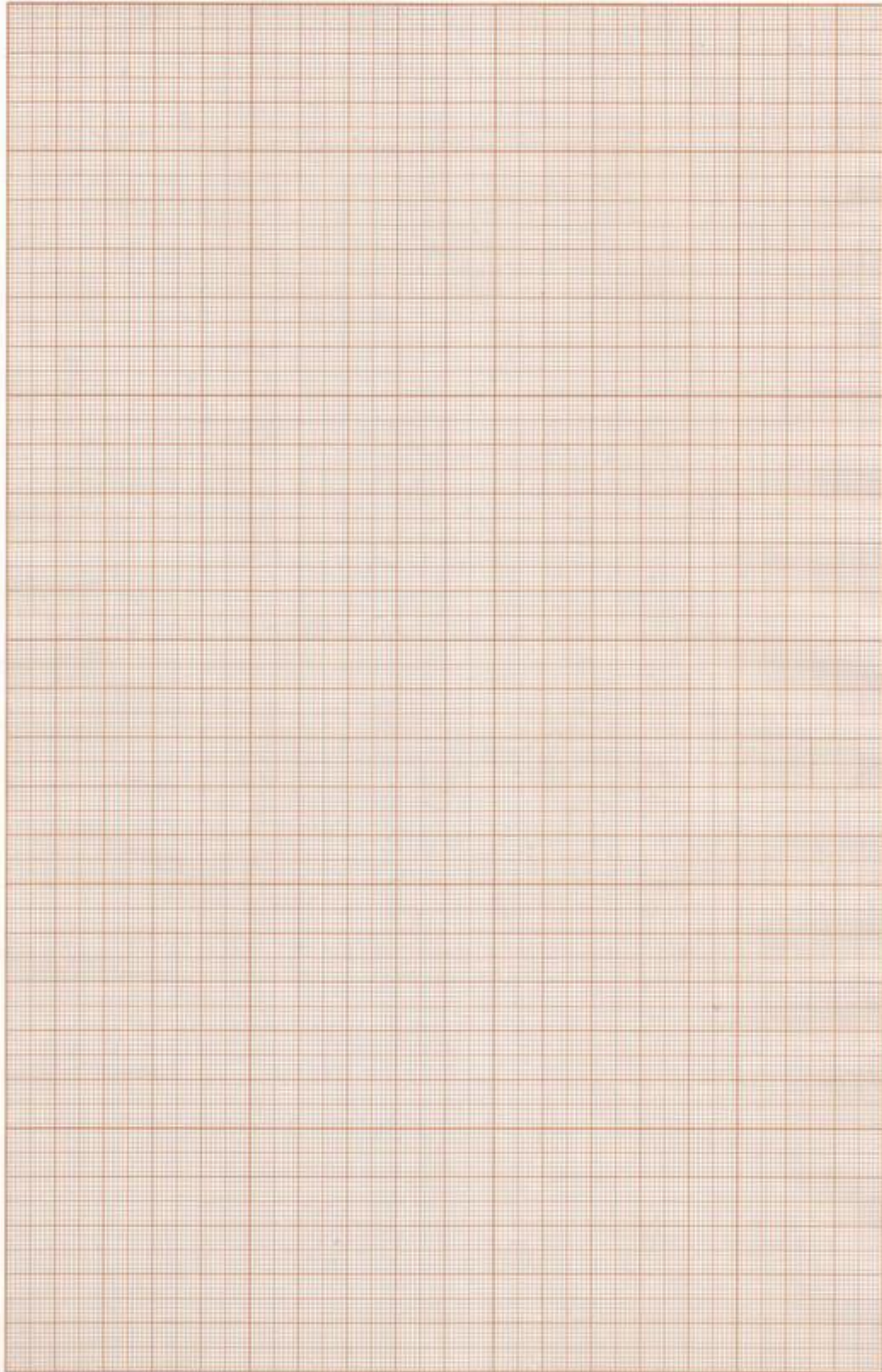
REFERENCE:



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EXPERIMENT-09:

ESTIMATION OF RNA BY ORCINOL METHOD.

AIM: **DATE:**

To estimate RNA by Orcinol reagent method.

PRINCIPLE:

It's a general reaction for pentoses and depends on the formation of furfural. When the pentose is heated with concentrated HCl, the orcinol reacts with furfural in the presence of ferric chloride as a catalyst, giving green colour. Only the purine nucleotides give significant reactions.

REAGENTS REQUIRED:

1. 5% perchloric acid.
2. Standard RNA.
3. ORCINOL REAGENT: Dissolve 100mg of perchloride in 100ml of concentrated HCl and add 3.5ml of 6% solution of orcinol prepared in alcohol (N-butanol).
4. STANDARD RNA SOLUTION: Dissolve 0.05g of RNA in 100ml of perchloric acid (5%) to give final solution containing 500 µg/ml.
5. 7.14ml of perchloric acid is made up to 100ml with distilled water.
6. Orcinol reagent: Dissolve 1g of orcinol in 100ml of conc. HCl containing 0.3g of $FeCl_3$.

PROCEDURE:

1. Take 2ml of solution of each of dilutions of RNA standard solutions.
2. Test samples and 2ml of 5% perchloric acid as a blank in different tube.
3. Add 3ml of orcinol reagent to all test tubes and mix thoroughly.
4. Keep test tubes in boiling water bath for 20 minutes and read O.D at 660nm against blank.

TABULAR COLUMN:

Test tube No:	Std. RNA solution (ml)	Conc. of RNA (mg/ml)	5% PCA (ml)	Orcinol reagent	INCUBATE FOR 10 MINUTES IN WATER BATH	O.D at 660nm
01	0.0	0.00	2.0	← 3 ml →		
02	0.2	0.02	1.8			
03	0.4	0.04	1.6			
04	0.6	0.06	1.4			
05	0.8	0.08	1.2			
06	1.0	1.00	1.0			

RESULT:

The concentration of the given unknown solution was found to be ___________________ mg/ml.

DISCUSSION:

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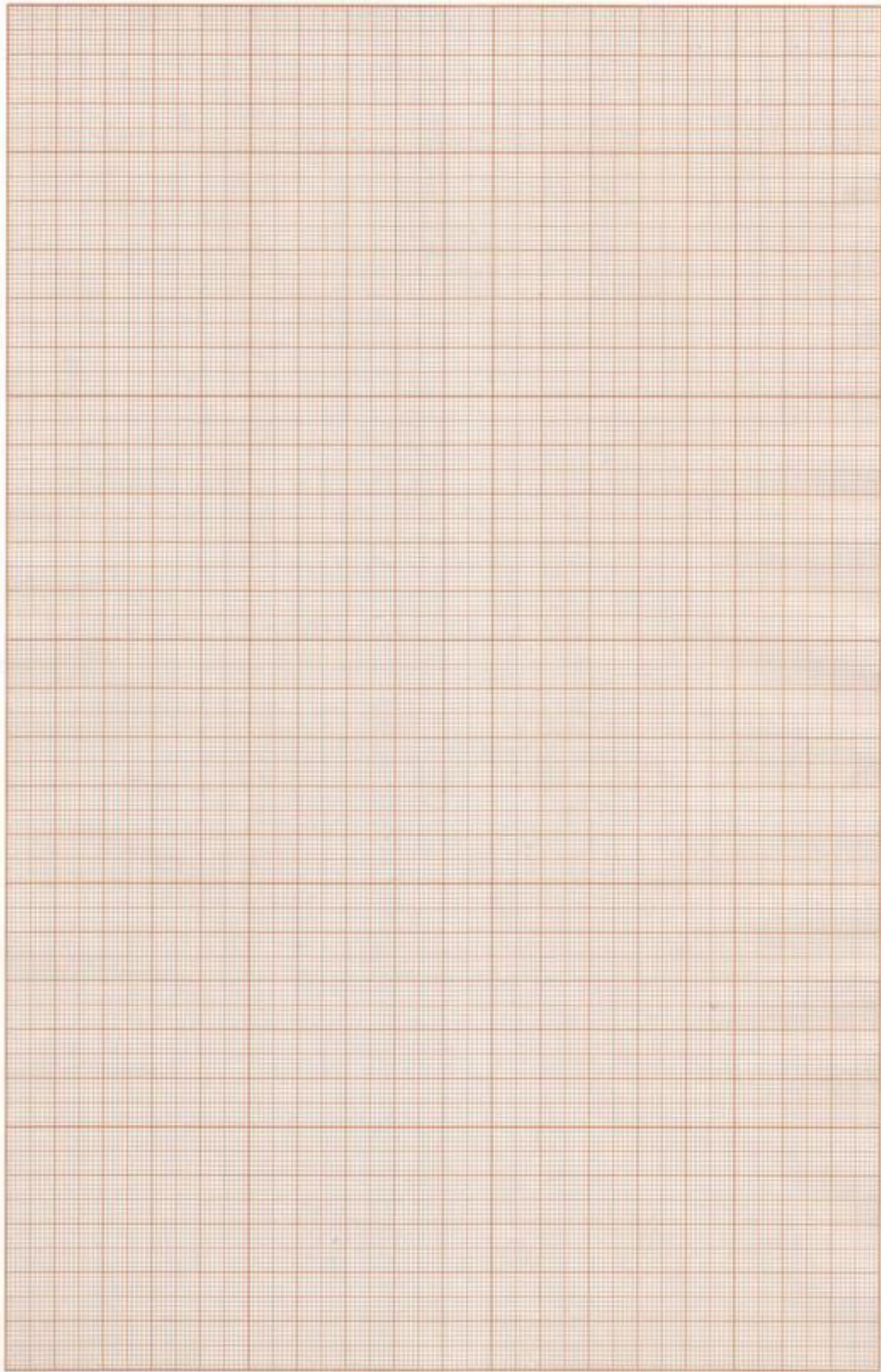

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EXPERIMENT-10:

EFFECT OF TEMPERATURE AND pH ON THE ENZYME ACTIVITY OF SALIVARY AMYLASE.

AIM: **DATE:**

To determine the narrow range of temperature and pH values at which salivary amylase exhibits its optimum activity.

INTRODUCTION:

An enzyme is a protein molecule that is biological catalyst with 3 characteristics. First, the basic function of an enzyme is to increase the rate of reaction. Second, most enzymes act specifically with only one reactant called a remarkable characteristic in that enzymes and are regulated from a state of low activity of enzymes and are strongly affected by change in pH and temperature. Its activity decreases its value above and below that point due to the denaturation of enzymes. Denaturation can be defined as the loss of enough structure rending the enzyme inactive. This is net surprisingly considering the importance of tertiary structure in enzyme function and non-covalent forces in determining the shape of enzyme.

Salivary amylase is the enzyme produced by the salivary glands. Formally known as ptyalin, it breaks down starch into maltose and isomaltose. Amylase, like any other enzymes works as a catalyst. All catalysts are enzymes, but not all enzymes are catalysts. A catalyst is a substance that involves in a chemical reaction but does not become part of the product. Amylase digest starch by catalyzing hydrolysis, which is splitting by the addition of a water molecule. The presence and absence of starch can be confirmed by several tests such as the iodine test, Benedict's and Fehling test. In general, a blue-black colour indicates the presence of starch.

The objectives of the experiment are to examine the enzymatic activity and specificity of salivary amylase depending on changes in temperature and pH. This experiment also aims to determine the narrow range of temperature and pH values at which salivary amylase exhibit its optimum activity.

MATERIALS AND METHOD:

PART-I: EFFECT OF TEMPERATURE:

An enzyme solution was prepared by mixing 1ml of saliva with 9ml of distilled water and 30ml of 0.5% sodium chloride solution. 2ml of enzyme solution was transferred into a large test tube and labelled as 4°C. 2ml of buffer starch solution (01% starch in phosphate buffer pH-6.7) was added in a separate large test tube. Both the test tubes were incubated for 10 minutes in ice bath (4°C). The solutions were immediately mixed. 3 drops of the

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mixture was taken quickly and 2 drops of 0.001M iodine solution was added simultaneously onto a spot plate (first well). This is marked as zero minute. After 1 minute interval (incubation continued), 3 drops of the mixture was taken again and 2 drops of iodine solution was added simultaneously onto the second well. This is marked as test at 1 minute. Step 5 was repeated until a light-yellow coloured solution was observed. The time (t) was noted for the other temperature (room temperature- 37, 50 and 70°C). Step 1 to 6 were repeated following the desired incubation temperature. The reciprocal time (1/time in min^{-1}) in 6 steps versus the temperature (T) was plotted. The optimum temperature of the amylase was determined.

TABULAR COLUMN:

Temperature (T°C)	Time (t) min	1/t (min^{-1})
04		
25		
37		
50		

PART-II: EFFECT OF pH

1ml of acetate buffer (pH-4) and 1ml of 2% unbuffered starch were mixed in a large test tube. 2ml of the enzyme solution was added in a separate large test tube. Both test tubes were incubated for 10 minutes in 37°C water bath. The solutions were immediately mixed. 3 drops of the mixture was taken quickly and 2 drops of 0.001M iodine solution was added simultaneously onto a stop plate (first well). This was the zero-minute reading. After 1 minute interval (incubation continued), 3 drops of the mixture was taken again and 2 drops of the iodine solution was added on to the second well simultaneously. This is recorded as minute one. Step 5 was repeated until a light-yellow coloured solution was observed. The time (t) was noted for other pH (5,6,7,8,10). Step 1-6 were repeated using the appropriate buffer. Acetate buffer solution for pH-5, phosphate buffer solution for pH 6.7 and 8 and bicarbonate buffer for pH 10 were used. The reciprocal of time (1/time, min^{-1}) in step 6 versus the buffer pH was plotted. The optimum pH of the amylase was determined.

TABULAR COLUMN:

pH	Time (t) min	1/t (min^{-1})
04.0		
06.8		
08.0		
10.0		

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RESULT:

The effect of temperature and pH on the enzymatic activity of salivary amylase was determined by measuring the rates of reaction in varying temperature and pH. The 0.5% NaCl added in the enzyme solution activates the salivary amylase to perform its function to hydrolyze starch. The hydrolysis or breakdown of starch due to the action of salivary amylase is indicated by the change in colour of the starch solution from a blue-black colour to light yellow coloured solution.

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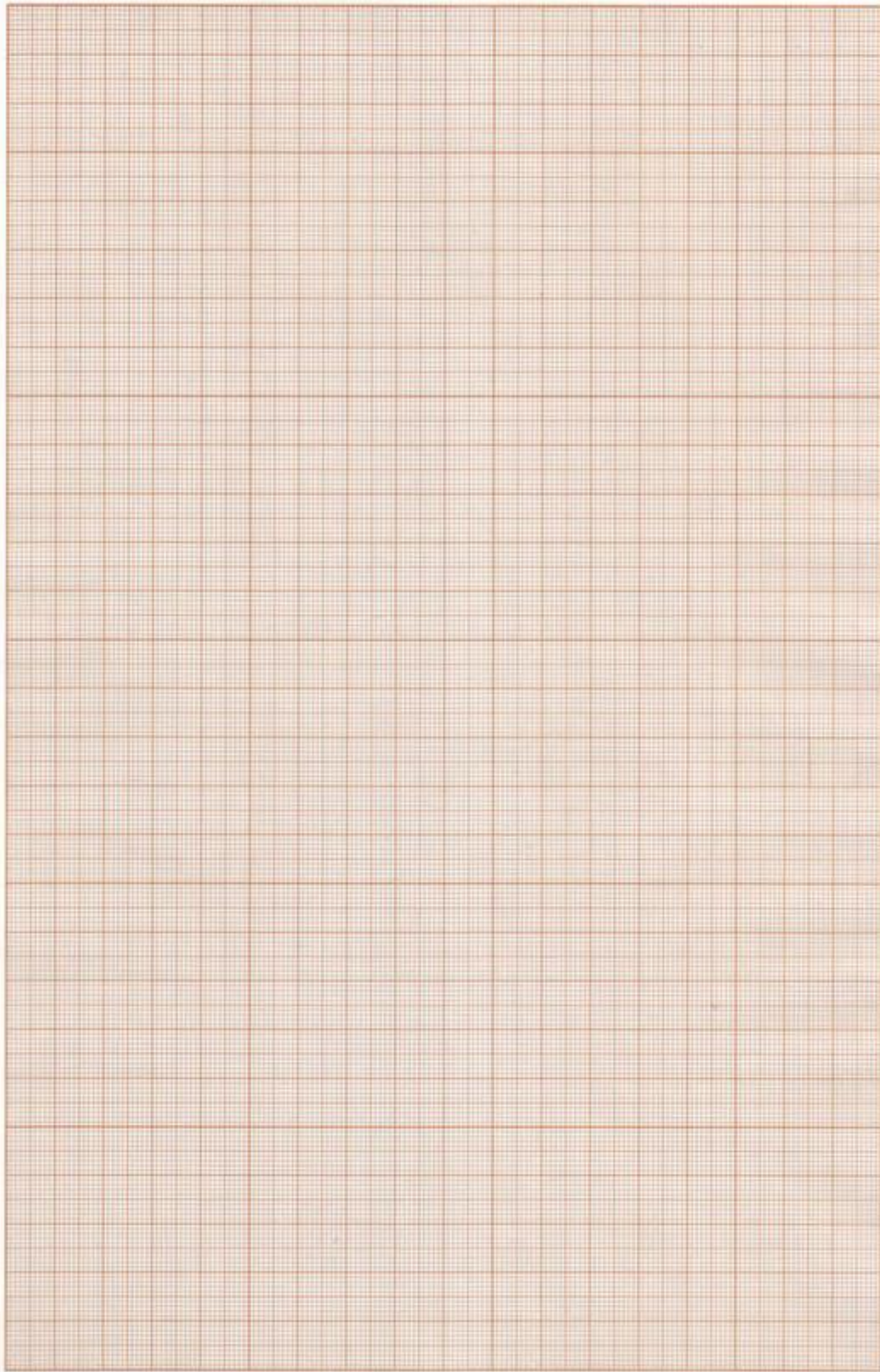
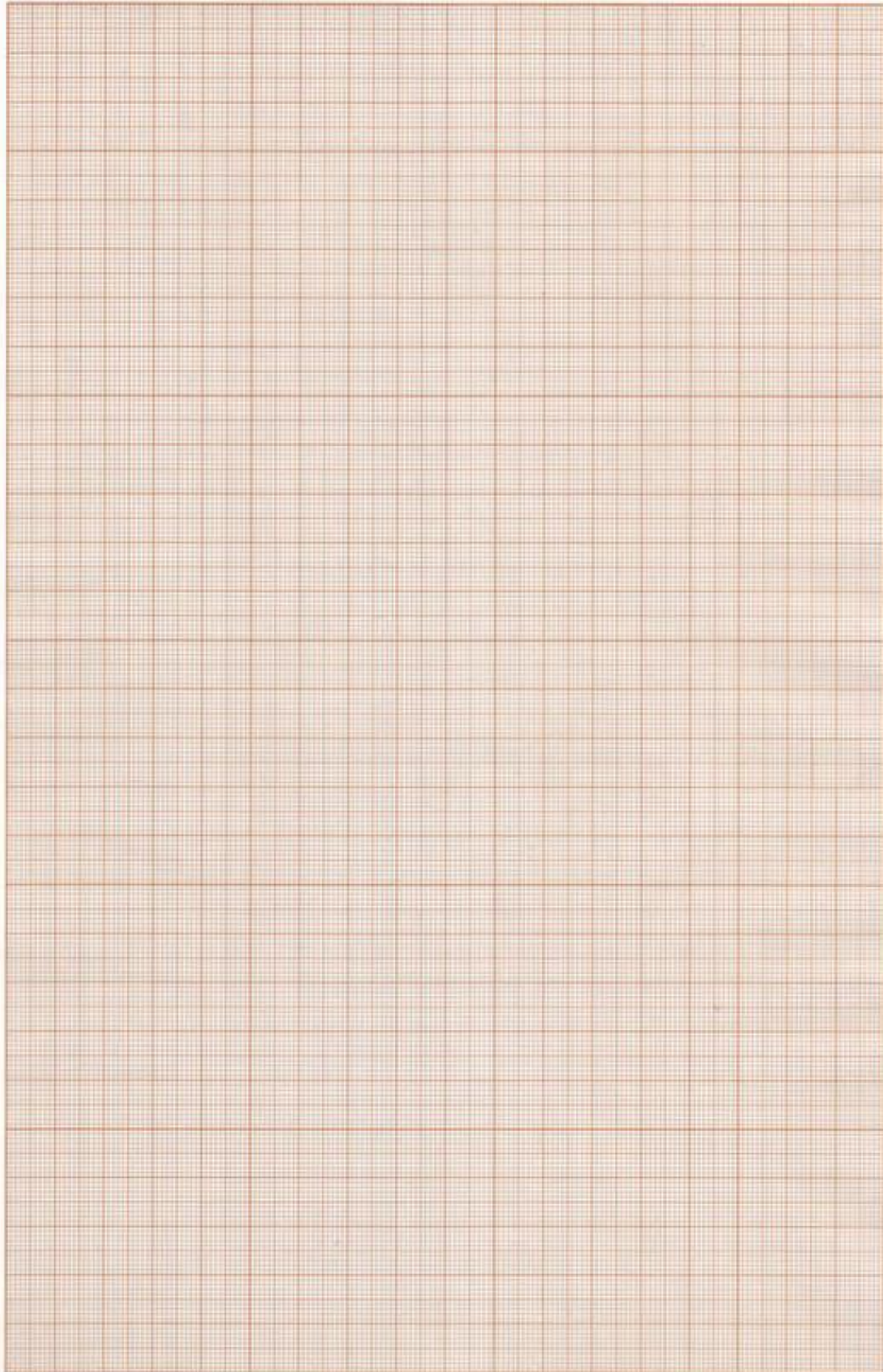
CONCLUSION:

Several factors affect the activity of enzymes. Among these are the temperature and pH. At optimum levels of these factors, enzymes perform their function best. Optimum temperature and pH differ from one enzyme to another. Salivary amylase is an enzyme found in human saliva which functions to break down starch to simpler compounds. Through the experiment, it was found that the optimum temperature of salivary amylase ranges from ________________ to _______________ and its optimum pH ranges from ___________ to ____________.

REFERENCE:





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