

## **KARPAGAM ACADEMY OF HIGHER EDUCATION**

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Subject	:	<b>Basic Biochemistry-Practical</b>	Semester	:	Ι
Subject code	:	17MBU113	Class	:	I B.Sc Microbiology

### <u>SYLLABUS</u>

#### S. No

#### **TITLE OF THE EXPERIMENTS**

- 1. Properties of water, concept of pH and buffers, preparation of buffers and numerical problems to explain the concepts.
- 2. Numerical problems on calculations of standard free energy change and equilibrium constant
- 3. Standard free energy change of coupled reactions
- 4. Qualitative/Quantitative tests for carbohydrates, reducing sugars, and non-reducing sugars.
- 5. Qualitative/Quantitative tests for lipids and proteins.
- 6. Study of protein secondary and tertiary structures with the help of models.
- 7. Study of enzyme kinetics calculation of Vmax, Km, Kcat values.
- 8. Study effect of temperature, pH and heavy metals on enzyme activity.
- 9. Estimation of any one vitamin.

#### SUGGESTED READING

- 1. Campbell., M.K. (2012) Biochemistry, 7<sup>th</sup> edition, Published by Cengage Learning.
- 2. Campbell, P.N., and Smith, A.D. (2011) Biochemistry illustrated, 4<sup>th</sup> edition, Published by Churchill Livingstone.
- 3. Tymoczko, J.L., Berg, J.M., and Stryer, L. (2012) Biochemistry: A short course, 2<sup>nd</sup> edition. W.H. Freeman.
- 4. Berg, J.M., Tymoczko, J.L., and Stryer, L. (2011) Biochemistry, W.H. Freeman and Company.
- Nelson, D.L., and Cox, MM. (2008) Lehninger Principles of Biochemistry, 5<sup>th</sup> edition.
   W.H. Freeman and Company.
- 6. Willey, M.J. herwood, L.M. & Woolverton, C.J. (2013) Prescott, Harley and Klein's Microbiology 9<sup>th</sup> edition. McGraw Hill.
- 7. Voet, D., and Voet, J.G. (2004) Biochemistry 3<sup>rd</sup> edition, John Wiley and Sons.



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## **DEPARTMENT OF BIOCHEMISTRY**

Subject	:	Basic Biochemistry-Practical	Semester	:	Ι
Subject code	:	17MBU113	Class	:	I B.Sc Microbiology

#### **Experiment No: 1**

## Properties of water, concept of pH and buffers, preparation of buffers and numerical problems to explain the concepts

#### **Properties of water**

Water is a major, unique, ubiquitous substance that is a major component of all living things. Its nature and properties have intrigued philosophers, naturalists and scientists since antiquity. Water continues to engage the attention of scientists today as it remains incompletely understood inspite of the intense study over many years. This is primarily because water is anomalous in many of its physical and chemical properties. Some of water's unique properties are literally essential for life, while others have profound effects on the size and shape of living organisms, how they work, and the physical limits or constraints within they must operate. This was recognized by Lawrence Henderson in 1913 in his classic and still very readable book, The fitness of the environment: An Inquiry into the Biological Significance of the Properties of Matter. Since then more has been learned about the structure and properties of water at the molecular level, much of its through spectroscopic and thermodynamic experiments. The more recent discipline of computer simulation has also played a role, having achieved a level of sophistication in the study of water in which it can be used to interpret experiment. Many of water's basic physical properties can now be explained, at least semi-quantitatively in molecular and structural terms.

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#### Selected physical properties of water

Formula	Water (H <sub>2</sub> O)
Molecular weight (g mol <sup>-1</sup> )	18
Density (kg L <sup>-1</sup> )	0.998
Boiling point (K)	373
Molecular volume (nm <sup>3</sup> )	0.0299
Volume of fusion (nm <sup>3</sup> )	0.0027
Liquid density maximum (K)	277
Specific heat (Jk <sup>-1</sup> g <sup>-1</sup> )	4.18
(JK <sup>-1</sup> mol <sup>-1</sup> )	75.2
Heat of vaporization (kJ g <sup>-1</sup> )	2.3
(kJ mol <sup>-1</sup> )	41.4
Surface tension (mN m <sup>-1</sup> )	72.8
Viscosity (µPa s)	1002
Dielectric constant	78.6
Dipole moment (Cm x 10 <sup>30</sup> ) <sup>a</sup>	6.01

#### **Biological relevance of water's physical properties**

Water, owing to its high boiling point, exists predominantly in its liquid form in the range of environment where life flow flourishes, although the other two phases, ice and vapour, play an essential role in shaping the environment. The high specific heat and heat of vaporization of water have important consequences for organisms at the cellular and physiological level, in particular for the efficiency of processes involving heat transfer temperature regulation, cooling etc. Viscosity is the major parameter of water that determines how fast molecules and ions can be transported and have rapidly they diffuse in aqueous solution. It thus, provides a physical upper limit to the rates of many molecular level events, within which organisms must live and evolve. These include the rates of ion channel conductance, association of substrates with enzymes, binding rates, and rates of macromolecular assembly.

#### **Dielectric constant**

The dielectric constant is a measure of how easily a material is polarized by an electric field relative to vacuum. It is defined by the magnitude of the dielectric polarization induced by a unit field. Water has nearly 80 times the dielectric constant of vacuum and it is an order of magnitude more polarizable than most organic solvents.

#### Ionization

Because the O-H bond of water is strongly polarized, the electron density around the hydrogen atom is very low and the O-H bond is rather weak compared with most covalent bonds. Thermal fluctuation in the liquid often (every 20  $\mu$ s or so) result in sufficient further polarization of the O-H bond that the hydrogen nucleus can dissociate as a proton, or H<sup>+</sup> ion. Water being an excellent solvent for ions, it can solvate the resulting OH<sup>-</sup> and H<sup>+</sup> ions, the latter primarily as H<sub>3</sub>O<sup>+</sup>. As a consequence dissociated water has a relatively long diffusion lifetime of about 100  $\mu$ s in pure water before recombination. The spontaneous ionization of water is characterized by a dissociation constant, derived using equation.

$$\frac{[\text{H}^+][\text{OH}^-]}{[\text{H}_2\text{O}]} = 1.82 \times 10^{-16} \text{ mol } \text{L}^{-1}$$

#### Hydrogen bonding

Hydrogen bonding between water molecules explains many of its physical and chemical properties. The strong hydrogen bonding in water explains why water has such a high melting and boiling point, high latent heat condensation and crystallization and low vapor pressure.

#### **Structure models**

Several models for the structure of water have been proposed each attempting too much either experimental evidence from spectroscopy or modeling results.

#### Ice I

- Tetrahedral coordinated regular structure requiring 6 units to return to the origin.
- 0.....0 distances are equal at 2.76 Å.
- The protons are exactly in the O......O plane.

#### **Flickering cluster model**

Flickering clusters are short-lived groups of water molecules that are interlinked by hydrogen bonds in liquid water. These clusters are representative of the fact that hydrogen bonds are easily broken and reformed.

#### **Broken ice model**

After ice melts, basic structure is retained and each other is bonded to 4 others.

#### **Network structure**

Most probable structure for cold water.

#### **Concept of pH**

#### рН

The negative logarithm of the hydrogen ion concentration, the pH is expressed on follows:

#### $pH = -log [H^+]$

The pH scale is a measure of hydrogen ion concentration that eliminates dealing with large powers of 10 and compresses a large range of concentrations onto a more convenient scale, for aqueous solution between 1 and 14. At a high concentration of H<sup>+</sup>, (10<sup>-1</sup> M), the pH value is low, pH =1 while at low concentration (10<sup>-12</sup> M), the pH is high= 12. Hydrogen ions in solution arise from the dissociation of acids. (HA represents undissociated acid, H<sup>+</sup> hydronium ion and A<sup>-</sup> conjugate base).

## $HA \rightarrow H^+ + A^-$

Strong acids (or bases) are considered to be completely dissociated into ions in dilute solution. However, weak acids (or bases) are only partially dissociated in solution, and thus equilibrium is established between ions and the undissociated molecules.

 $HA \leftrightarrow H^+ + A^-$ 

The equilibrium constant K<sub>a</sub>, is defined as follows.

## $K_a = [H^+][A^-] / [HA]$

From this expression, we can observe the Henderson-Hasselbach equation, commonly called the "buffer equation" which relates the pH of solution to the  $pK_a$  of the acid and the relative concentration of the undissociated acid and the conjugate base forms. Solving first for the hydrogen ion concentration:

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 $[H^+] = K_a [HA] / [A^-]$ 

Converting to the logarithmic form and multiplying by -1, we obtain:

-log [H<sup>+</sup>] = -log K<sub>a</sub> - log ([HA]/[A<sup>-</sup>])

Defining operator 'p = - log' we have:

 $pH = pK_a - log ([HA]/[A])$ 

Finally, by inverting the log ([HA]/[A<sup>-</sup>]) term, we obtain:

 $pH = pK_{a} + log([A] / [HA])$ 

or

pH = pK<sub>a</sub> + log( [conjugate base] / [acid] )

This is the Henderson-Hasselbach equation. It is most useful in the preparation of buffer and in understanding how the concentration of the acid and conjugate base forms of a weak acid effect the pH.

#### **Concept of buffer**

A buffer, by definition, resists changes in the pH of the solution. A buffer must contain the chemical species for "neutralizing" added amounts of acid or base. Generally, a buffer is a solution of a weak acid and its conjugate base or a weak base and its conjugate acid. A buffer is selected on the basics of the pK<sub>a</sub> value and its chemical nature.

The Handerson-Hasselbach equation gives the relationship between pH,  $pK_a$ 's and the ratio of the concentration of the salt and acid forms of the buffer. As shown by this equation, when the concentration of the conjugate base and the undissociated acid are equal, [Conjugate base] = [acid], the pH of the solution equals the  $pK_a$  of the buffer. When [conjugate base] = 10x[acid], then log ([conjugate base]/[acid]) = log 10 = 1 and therefore:

## $pH = pK_a + 1$

When [conjugate base] = 1/10x[acid], then log ([conjugate base]/[acid]) = log 1/10 =-1 and therefore:

 $pH = pK_a - 1$ 

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The buffers are most effective in the range  $pH = pk_a \pm 1$ . Outside that range, the concentration of either the acid of the conjugate base is too small to effectively resist the effect of added hydrogen or hydroxide ion.

Once the desired pH range for an experiment has been decided, one can select a buffer on a basic of pK value. Since temperature affects the dissociation of some weak acids and bases the pK value for buffers is temperature dependent. Therefore, pH of buffers should always be adjusted for the temperature at which they will be used.

# Numerical problems on calculations of standard free energy change and equilibrium constant

Calculate the standard free energy changes of the following metabolically important enzyme-catalyzed reactions at 25°C and pH 7.0 from the equilibrium constants given.

(a) Glutamate + oxaloacetate  $\leftrightarrow$  asparate +  $\alpha$ -ketoglutarate K'eq = 6.8 (b) Dihyroxyacetone phosphate  $\leftrightarrow$  glyceraldehydes-3-phosphate K'eq = 0.00475 (c) Fructose-6-phosphate + ATP  $\leftrightarrow$  F-1,6-biphosphate + ADP K'eq = 254

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\Delta G^{\circ} = -RT In K'eq R=8.315 J/mol°K T= 298°K
a. -8.315 (298) (In 6.8)
-4.757 J/mol = -4.76 kJ/mol
b. -8.315 (298) (In0.0475)
7.5kJ/mol
c. -8.315 (298) (In 254)
-13.7 kJ/mol
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Calculate the equilibrium constants K'eq for each of the following reactions at pH 7.0 and 25°C using the  $\Delta G^{\circ}$  values given:

(a). Glucose-6-phosphate + H<sub>2</sub>O → glucose + P<sub>1</sub> ΔG°' = -13.8 kJ/mol
(b). Lactose + H2O → Glucose + Galactose ΔG°' = -15.9 kJ/mol
(c). Malate → fumarate + H<sub>2</sub>O ΔG°' = + 3.1 kJ/mol
(a). -13,800 = -8.315 (298) In K'eq K'eq = 262
(b) -15,900 = -8.315(298) In K'eq K'eq = 612
(c) + 3,100 = -8.315 (298) In K'eq K'eq = 0.286 *Prepared by Mr. S. Rajamanikandan, Dept. of Biochemistry, KAHE* Page 7/32

#### Standard free energy change of coupled reactions

This problem explores the consequences of coupling ATP hydrolysis under physiological conditions to a thermodynamically unfavorable biochemical reaction. Because we want to explore these consequences in stages, we shall consider the hypothetical transformation, X  $\rightarrow$  Y, a reaction for which  $\Delta G^{\circ} = 20 \text{ kJ/mol}$ .

(a) what is the ration [Y]/[X] at equilibrium?

(b) Suppose X and Y participate in a sequence of reactions during which ATP is hydrolyzed to ADP and Pi. The overall reaction is

 $X + ATP + H_2O \longrightarrow Y + ADP + Pi$ 

Calculate [Y]/[X] for this reaction at equilibrium. Assume for the purposes of this calculation that the concentrations of ATP, ADP and Pi are all 1 M when the reaction is at equilibrium.

(c) We know that [ATP], [ADP], and [Pi] are not 1 M under physiological conditions. Calculate theration [Y]/[X] for the ATP-coupled reaction when the values of [ATP], [ADP], and [Pi] are 8.05 mM, 0.93 mM, and 8.05 mM, respectively.

a. 20,000 = -8.315 (298) in Keq  
Keq = 3.2 10<sup>-4</sup> M  
b. ATP + H<sub>2</sub>O 
$$\rightarrow$$
 ADP + Pi  $\Delta G^{\circ}$  = -30.5 kJ/mol when coupled to the reaction gives  
 $\Delta G^{\circ}$  = -10.5 kJ/mol  
-10.500 = -8.315 (298) in K  
K = 69.2  
c. Keq = [ADP] [Pi] [Y] 69.2= [0.93 10<sup>-3</sup>] [8.05 10<sup>-3</sup>] [Y]  
[ATP] [X] [9.05 10<sup>-3</sup>] [X]

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## Qualitative/Quantitative tests for carbohydrates, reducing and non-reducing

sugars

S. NO	EXPERIMENT	OBSERVATION	INFERENCE	REACTION
1	<b>Solubility Test</b> To a little amount of the given substance in a test tube added few drops of distilled water and shook well.	(a) Soluble (b) Insoluble	Itindicatesthepresenceofmonosaccharidesand disaccharides.Itindicatesthepresenceofpolysaccharides.	
2	Molisch's Test To 2.0ml of the sugar solution added 2-3 drops of Molisch's reagent, mixed well and added 2.0ml of Conc. H <sub>2</sub> SO <sub>4</sub> along the sides of the test tube. So that the acid forms a layer beneath the test solution.	<ul> <li>(a) A deep violet coloured ring was got at the junction of the two layers.</li> <li>(b) No deep violet coloured ring was got at the junction of the two layers.</li> </ul>	It indicates the presence of carbohydrates. It indicates the absence of carbohydrates.	Sugar is dehydrated to hydroxyl methyl furfural by concentrated sulphuric acid. The hydroxyl methyl furfurol then condenses with α - naphthol to give a violet coloured ring shows the presence of carbohydrates.
3.	<b>Iodine test</b> To 1.0ml of the test solution added 4-5 drops of iodine solution and contents are mixed gently.	<ul><li>(a) Blue coloured solution was got.</li><li>(b) No change in colour</li></ul>	Presence of polysaccharide. Absence of polysaccharide	Iodine forms coloured adsorption complexes with polysaccharides.
4.	<b>Fehling's test</b> To 2.0ml of Fehling's reagent added 0.5ml of the given sugar solution mixed and heated in a boiling water bath for two minutes.	<ul> <li>(a) A reddish brown precipitate is formed.</li> <li>(b) No characteristic precipitate was got.</li> </ul>	It indicates the presence of reducing sugar. It indicates the absence of reducing sugar	Fehling's solution contains blue alkaline cupric hydroxide solution, heated with reducing sugars gets reduced to yellow or red cuprous oxide and is precipitated.

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5.	Benedict's test	(a) The solution first	It indicates the	free aldehyde or keto
	To 2.0ml of Benedict's	turned to green and	presence of	group in the reducing
	reagent, added 8 drops of the	then reddish brown	reducing sugar	sugars reduce cupric
	test solution and heated in a	precipitate was		hydroxide in alkaline
	boiling water bath for 2	formed.		medium to red
	minutes and allowed to cool.	(b) No characteristic		colored cuprous
	spontaneously.	reddish brown	It indicates the	oxide. Depending on
		precipitate was	absence of reducing	the concentration of
		formed.	sugar	sugars, yellow to
				green color is
				developed
6.	Picric acid test	(a) A mahagony red	It confirms the	It is due to the
	To 2.0ml of sugar solution	coloured solution	presence of	reducing action of the
	added 0.5ml of saturated	was got.	reducing sugar.	sugar.
	picric acid and 0.5ml of 10%	(b) No characteristic		
	sodium carbonate and heated	red coloured solution	It shows the	
	in a water bath.	was got.	absence of reducing	
			sugar and confirms	
			the presence of	
			sucrose which is a	
			non reducing sugar	
7.	Barfoed's test	(a) A brick red	It indicates the	Barfoed's test is used
	To 2.0ml of freshly prepared	precipitate is formed	presence of	to detect the presence
	Barfoed's reagent added	and settling down at	monosaccharides.	of monosaccharide
	1.0ml of sugar solution and	the bottom or sides		(reducing) sugars in
	heated in a boiling water bath	of the test tube.	It shows the	solution. Barfoed's
	for 3 minutes. Allow to cool.	(b) No characteristic	absence of	reagent, a mixture of
		red precipitate was	monosaccharides.	ethanoic (acetic) acid
		got.		and copper(II)
				acetate, is combined
				with the test solution
				and boiled. A red
				copper(II) oxide
				precipitate is formed
				will indicates the
				presence of reducing
				sugar. This test is
				specific for
				monosaccharides
8.	Seliwanoff's test	(a) A cherry red	It indicates the	It indicates the
	To 3.0ml of Seliwanoff's	colour was got.	presence of keto	presence of keto
	reagent added 4 drops of		sugar sucrose and	sugar sucrose and

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	sugar solution and heated in a water bath for 3 minutes and cooled the solution at room temperature.	(b) No characteristic cherry red colour was got.	fructose. It indicates the absence of keto sugar sucrose and fructose.	fructose which on treatment with HCl acid forms 5 hydroxy methyl furfural which then condences with resorcinol to give cherry red coloured complex.
9.	<b>Tollen's test</b> To equal volume of sugar solution and concentrated hydrochloric acid added a pinch of phloroglucinol and heated in a boiling water bath for 2 minutes.	<ul><li>(a) A red coloured solution was got.</li><li>(b) No characteristic red coloured solution was got.</li></ul>	It confirms the presence of pentose, lactose and galactose. It shows the absence of pentose, lactose and galactose.	
10.	Bial's test To 2.0ml of Bial's reagent added 0.5ml of the test solution and heated in a boiling water bath for 3 minutes.	<ul> <li>(a) A greenish blue</li> <li>coloured solution</li> <li>was got.</li> <li>(b) No characteristic</li> <li>greenish blue</li> <li>coloured solution</li> <li>was got.</li> </ul>	It indicates the presence of pentoses. It indicates the presence of hexoses.	Bial's test is used to distinguish between pentoses and hexoses. They react with Bial's reagent and are converted to furfural. Orcinol and furfural condense in the presence of ferric ion to form a colored product. Appearance of green colour or precipitate indicates the presence of pentoses and formation of muddy brown precipitate shows the presence of hexoses.
11.	Mucic acid test To 50mg of sugar in a test tube added 1.0ml of concentrated nitric acid and	(a) A white precipitate was got and a colourless rod shaped crystals were	It shows the presence of lactose or galactose.	

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for one and a half an hour and (b	(b) No characteristic	It shows the	
left it stand overnight and w	white precipitate	absence of lactose	
viewed the crystals under the w	was formed.	or galactose.	
microscope.       Formation         12.       Phenyl hydrazine Test       Formation         Took equal volume of sugar       bergan bergan       bergan         solution       and       phenyl       cr         hydrazine       mixed, filtered       and       weight with weight with the second at the s	Formation of beautiful yellow crystals of osazone was formed (a) A yellow <b>needle</b> shaped crystals like sheeves of corn was observed. (b) A <b>flower</b> shaped crystals were observed (c) A <b>cotton ball or</b> <b>puff</b> shaped crystals were observed. (d) A small <b>sunflower</b> shaped crystals were observed. (e) A <b>rod</b> shaped crystals were observed.	It indicates the presence of <b>glucosazone and fructosazone</b> It indicates the presence of <b>galactosazone</b> It indicates the presence of <b>lactosazone</b> It indicates the presence of <b>maltosazone</b> It indicates the presence of <b>maltosazone</b>	The ketoses and aldoses react with phenylhydrazine to produce a phenylhydrazone which further reacts with another two molecules of phenylhydrazine to yield osazone.

## Shapes of different crystals



## Qualitative/Quantitative tests for lipids and proteins

S. NO	EXPERIMENT	OBSERVATION	INFERENCE
1	Solubility Test:		
	a. Cold water	a. Insoluble	The amino acid may be tyrosine The amino acid may be
		b. Soluble	Arginine, Methionine, Cysteine, Tryptophan or Histidine
	b. Hot water	Soluble	The amino acid may be
	c. C. Dilute potassium hydroxide	Soluble	Arginine, Methionine, Cysteine Tryptophan
	e. Dilute hydrochloric acid	Soluble	Tyrosine or Histidine.
	f. Alcohol	Soluble	The amino acid may be
		a. Soluble	Tryptophan or Histidine. The amino acid may be Arginine, Methionine, Cysteine or Tyrosine
		<b>b.</b> Insoluble	
2	Ninhydrin Test:	A violet colouration	This is due to the formation of
	To 5ml of amino acid solution taken	was got.	Rheumann's purple which
	in a test tube, add 0.5ml of 1%		indicates the presence of
	ninhydrin reagent. Place the test tube		amino acid.
	in a boiling water bath for 1-2 minutes		
	and cool to room temperature.		
3	Pauly's diazo Test:	a. A red colouration	The amino acid with
	To 2ml of amino acid solution add	was got.	diazotized sulphanilic acid in
	1ml of 1% sulphanilic acid in 10%		alkaline medium forms the
	hydro chloric acid and then cool in ice.		highly coloured azo
	Add 1ml of 5% sodium nitrite solution		compound. This indicates the
	and leave in cold water for 3 minutes.		presence of aromatic amino
	Make the solution alkaline by the		acid tyrosine, tryptophan or
	addition of 2ml of 1% sodium		histidine.
	carbonate solution and note the colour	b. No characteristic	It shows the absence of
	tormed.	red colouration	aromatic amino acids

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		was got.	tyrosine, tryptophan or
		Was goa	histidine.
4	Acetic Acid Test: To 2ml of amino acid solution add 1% of bromine in 33% acetic acid until an yellow colour was got. After 10 minutes add 5 to 10 drops of 5% ammonium carbonate solution.	<ul> <li>a. A dark blue or violet coloured solution was got.</li> <li>b. No characteristic blue coloured solution was got.</li> </ul>	This confirms the presence of Histidine. This shows the absence of histidine.
5	Hopkin's Cole Test: To 1ml of the amino acid solution add 2ml of glacial acetic acid (exposed to sun light) and add 2ml of concentrated sulphuric acid along the sides of the test tube.	<ul> <li>a. A violet coloured ring was got at the junction of the 2 liquids.</li> <li>b. No characteristic violet coloured ring was got.</li> </ul>	It is due to the condensation of aldehyde group with indole group in the presence of concentrated sulphuric acid. This test confirms the presence of tryptophan. It shows the absence of tryptophan.
6	Voisent Rhode Test: Add 1ml of amino acid solution to 1ml of 5% para dimethyl amino benzaldehyde in concentrated sulphuric acid.	<ul> <li>a. A red violet coloured ring was got.</li> <li>b. No characteristic red violet coloured ring was got.</li> </ul>	It is due to the condensation of aldehyde group with indole group in the presence of concentrated sulphuric acid. This test confirms the presence of tryptophan. It shows the absence of tryptophan.
7	Aldehyde Test: To 1ml of amino acid solution add one drop of 1 in 500 formalin and 1 drop of 15% mercuric sulphate in 10% sulphuric acid and add 3-4 drops of concentrated sulphuric acid.	<ul> <li>a. A violet coloured ring was got at the junction of two liquids.</li> <li>b. No characteristic violet coloured ring was got.</li> </ul>	It is due to the condensation of aldehyde group with an indole group in presence of concentrated sulphuric acid. This test confirms the presence of tryptophan. It shows the absence of tryptophan.
8	<b>Ehrlich's Test:</b> Add 2ml of Ehrlich's reagent to 2ml of the amino acid solution.	a. A red coloured solution was got.	It is due to the condensation of aldehyde group with indole ring in the presence of hydrochloric acid. It confirms the presence of tryptophan.

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r			
		b. No characteristic	It shows the absence of
		red coloured	tryptophan.
		solution was got.	
9	Hoffmann's Test (or) Millon's Test:	a. A deep red	It is due to the presence of
	To 1ml of amino acid solution add 1	colouration was	phenolic hydroxyl group. This
	drop of Millon's reagent and place it in	got.	test confirms the presence of
	a water bath for 30 seconds.		tyrosine.
		b. No red colouration	It shows the absence of
		was got.	tyrosine.
10	Folin's Phenol Test:	a. A blue colouration	It is due to the presence of
	To 1ml of amino acid add an equal	was got.	phenolic hydroxyl group. This
	volume of Folin's phenol reagent and		test confirms the presence of
	treat with 6 drops of saturated sodium		tyrosine.
	carbonate.	b. No characteristic	It shows the absence of
		blue colouration	tyrosine
		was got	
11	Xanthonroteic acid Test	a An vellow colour	It is due to nitration of amino
11	To 2ml of amino acid solution add	was got in acid	acid to form vellow nitro
	an equal volume of concentrated nitric	solution which	derivative. This confirms the
	an equal volume of concentrated milit	turned to bright	prosonce of tyrosine which
	400/ acdium hydrovide to make the	aranga with	bes the phonolic group
	40% southin hydroxide to make the	of alige with	has the phenone group.
	solution strongly alkaline	alkaline solution.	Le altre altre altre a C
		b. No orange colour	It shows the absence of
10		was got.	tyrosine.
12	Ferric Chloride Test:	a. A transitory violet	It is due to the presence of
	To the amino acid solution add one	colour appeared	thiol group. This test confirms
	drop of dilute (0.05%) ferric chloride	on the addition of	the presence of cysteine.
	solution and a drop of 0.5% copper	copper sulphate.	
	sulphate solution.		
		b. No characteristic	It shows the absence of
		violet colour was	cysteine.
		got.	
13	Nitroprusside Test:	a. A red coloured	It is due to the presence of
	Treat2ml of amino acid solution with	solution was got	thiol group. This indicates the
	0.5ml of 2% dilute sodium	which faded away	presence of cysteine.
	nitroprusside solution and add a drop	after 2 minutes.	
	of dilute solution of sodium		
	hydroxide(1%).		
14	Lead Acetate Test:	a. A black precipitate	It is due to the presence of
	To 2ml of the amino acid solution add	was got.	thiol group which indicates
	1ml of 10% aqueous lead acetate and		the presence of cysteine.

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	1ml of 40% sodium hydroxide. Heat in		
	a water bath for 5 minutes.	b. No characteristic	It shows the absence of
		black precipitate was	cysteine
		got.	
15	Boiling's Modification (or) Mc	A red colouration was	The methyl group is split to
	Carthy Sullivan Test:	got.	form homocysteine which
	To 1ml of the amino acid solution, add		gives a red colour with
	the following reagents in order and		sodium nitroprusside
	mix after each addition. Add 1.5ml of		solution. It confirms the
	0.5N sodium hydroxide, 1.5ml of 1%		presence of methionine.
	glycine, 0.3ml of 10% sodium nitro		
	prusside solution and place it in a		
	incubator at 37-40°C for 15 minutes		
	and then added hydrochloric acid and		
	let it stand at room temperature for 10		
	minutes.		

## Lipids

S .No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Greese spot test:	A greesy spot	Indicates the presence of
	Take a small amount of oil on a piece of	penetrating the	lipids
	paper	paper will be formed.	
		No greesy spot was	Absence of lipids
		formed	
2.	Test for free fatty acids:	_	
	Take a few drops of phenolphthalein	Disappearance of	Presence of fatty acids since
	solution in a test tube and in it one to	colour	the alkali is neutralized
	two drops of very dilute alkaline	No diamagna of	
	solution just sufficient to give the	No disappearance of	Absence of fatty acids
	drops of oil and shake	colour	
3	Fmulsification:		
5.	Take a clean dry test tube add 2 ml of	Minutes dronlets	Presence of oil or linid
	dilute hile salt solution and few drops	suspended in the	reserve of on or upid
	of unknown solution is added and	liquid	
	shaken vigorously for 1 minutes.	No droplets	Absence of oil or lipid
		suspended in the	*
		luquid	
4.	Saponification test:		
	Take 1 ml of unknown solution in a test	Soap formation	Indicates the presence of fatty

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-			
	tube and add an equal amount of		acids
	alcoholic KOH solution, mix them		
	thoroughly and keep the mixture	No soap formation	Absence of fatty acids
	during the colour of warming and shake		
	up gently with a little distilled water		
5.	Test for unsaturated fatty acids:		
	A clean test tube containing 5 ml of CCl <sub>4</sub>	Appearance of	Indicates the presence of
	and add a drop of lipid or oil, now	permanent yellowish	unsaturated fatty acids.
	bromine water is added drop by drop	red colour	
	and number of drops added is recorded	No characteristic	Absence of unsaturated fatty
		colour formation	acids
6.	Acrolein test:		
	For a few drops of oil or lipid, a few	A pungent colour of	Indicates the presence of
	crystals of potassium hydrogen	acrolein is produced	glycerol
	sulphate warm gently ta mix and then	No pungent colour is	Absence of glycerol
	heated strongly	produced	
7.	Dichromate test:		
	Take a dry test tube, 3 or 4 ml of oil or	Brown colour is	Indicates the presence of
	lipid, a few drops of 5% potassium	changed to blue	glycerol
	dichromate solution and 5 ml of conc.	No colour change	Absence of glycerol
	H <sub>2</sub> SO <sub>4</sub> mix well		

# Study the protein secondary and tertiary structures with the help of models Aim:

To study the secondary and tertiary structures of proteins with the help of models.

## 3D structures are proteins sources of information:

- Shape and domain structures.
- Protein classification.
- Prediction of function for uncharacterized proteins.
- Interactions with other macromolecules.
- Interaction with small ligands, metal ions, nucleotides, substrates, cofactors and inhibitors.
- Evidence for enzyme mechanism.
- Structure based drug development.
- Post translational modification: disulfide bonds, N-glycosylations.
- Experimental evidence for transmembrane domains.

## **Basics of protein structures**

- Primary structure
- Secondary structure
- Tertiary structure
- Quaternary structure

## Secondary Structure

- a) Helices
- b) Strands
- c) Turns and loops

## Alpha-helix

#### **Characteristics**

Helical structures have negative phi and psi angles in typical values being -60° and -50°. Every main chain C=O and N-H group is hydrogen bonded to a peptide bond 4

residues away (i.e O to N, +4). This gives a very regular stable arrangement 3.6 residues per turn 5.4 Å repeat along the helix axis. Each residue corresponds to a rise of ca 1.5 Å. **Beta structure (strands):** 

Characteristics: Positive psi angles typically ca 130° and negative phi values typically ca -140°. No hydrogen bonds amongst backbone atoms from the same strand.

#### Beta strand can form parallel or anti-parallel beta sheets:

Characteristics: Stabilized by hydrogen bonds between backbone atoms from adjacent chains. The axial distance between adjacent residues is 3.5 Å. These are two residues per repeats unit which gives the beta strand a 7 Å pitch.

#### **Turns and Loops:**

#### Loops:

General name for a motile part of the polypeptide with no fined secondary structure.

#### Turns:

Several types, defined structure, requirement for specific or  $\alpha$  a at key position meaning they can be predicted. The polypeptide chains it makes a u-turn over 2-5 residues.

#### Super secondary structures:

Compounds of 2-3 secondary structure elements.

**Example:** Helix turn helix motils, frequent in DNA binding proteins, coiled coils. Eg. From myosin.

#### Folding pattern of polypeptide chain:

- The folding pattern of a polypeptide chain can be described in turns of the angles of rotation around the main chain bonds.
- Phi and psi describe the main chain conformation omega corresponds to the trans (omega-180) or cis (omega = 0) conformation.
- Except pro, trans is the more stable conformation.

#### Key facts about a polypeptide chain:

- Chemical bonds have characteristic lengths.
- The peptide bond has partial double bond character, meaning it is shorter and rigid.
- Other bonds are single bonds (but restriction of rotation due to steric hindrance).

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#### Ramachandran plot (1):

Each type of secondary structure has a characteristic combination of phi and psi angles.

#### Ramachandran plot (2):

For each positive conformation, the structure is examined for close contacts between atoms. Atoms are treated as hard spheres with dimensions corresponding to their van der Waals radii angles, which causes spheres to collides corresponds to sterically disallowed conformation of the polypeptide backbone.

#### **Tertiary structures:**

- Domains
- Repeats
- Zinc fingers

#### **Domain**:

Independently folded part of a protein, arrange size, about 150  $\alpha$  a residues lower limit ( $\alpha$  50 residues).

#### **Repeats:**

- Several types: LRR, ANK, Heat ...... Compose of view
- Secondary structure elements, sterilized by interactions between repeatesion form large structures.

#### Zinc fingers:

- Several types of structure is sterilized by bound zinc ion.
   E7 hands:
- Structure is sterilized by bound calcium.

#### Study of enzyme kinetics - calculation of Vmax, Km, Kcat values

#### Catalase

Catalase represents a special type of peroxidase which has a specific property of very rapidly catalyzing the decomposition of hydrogen peroxide.

 $2H_2O_2$   $\longleftarrow$   $2H_2O+O_2$ 

It may be considered that one molecule of hydrogen peroxide acts as a substrate and electron donor and the other molecule serves as a electron acceptor or oxidizing agent. One molecule of water is reduced to OH ions giving a red product of two molecules of water and one molecule of oxygen.

#### Aim:

To prepare the crude enzyme from the given source and to study the effects of varying pH, temperature, enzyme and substrate concentration on the activity of catalase.

#### **Principle:**

Sodium perborate activity may serve as a suitable for catalase. Sodium perborate (NaBO<sub>3.</sub>4H<sub>2</sub>O) in aqueous solution is acted acted upon by catalase, the remaining  $H_2O_2$  is determined by titration with KMno<sub>4</sub> in the presence of  $H_2SO_4$ .

 $2KMno_4+H_2O_2+2H_2SO_4 \longrightarrow 2KHSO_4+2Mno4+8H_2O+3O_2$ 

#### **Reagents:**

> 1.54% sodium perborate solution:

Molecular weight of sodium perboric acid is 154. The pH is adjusted to 6.8 with concentrated HCL (1,54g in 100ml of water).

- $\succ$  2N H<sub>2</sub>SO<sub>4</sub>
- > 0.01N KMno<sub>4</sub> prepared by diluting in KMno<sub>4</sub> (0.1N KMno<sub>4</sub> = 3.16g of KMno<sub>4</sub>/litre)
- > M/15 phosphate buffer
- Solution A: M/15 disodium hydrogen phosphate (11.877g/ litre)
- Solution B: M/15 potassium dihydrogen phosphate (9.072g /litre)

X ml of the solution A was mixed with Y ml of the solution B as shown in the tabular column to prepare phosphate buffer of different pH.

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#### **Enzyme preparation:**

The cucumber or chow was cut into pieces and weighed 10g accurately. It was then homogenized in a mixie by adding 100ml of water and filtered the filtrate was kept in ice then used for the experiment.

S. No	pН	X ml of NA <sub>2</sub> HPO4	Y ml of KH <sub>2</sub> PO4
1.	6.0	12.0	88.0
2.	6.4	26.5	73.5
3.	6.8	50.0	50.0
4.	7.2	71.5	28.5
5.	7.6	86.8	13.2

#### **Preparation of buffer:**

#### **Effect of Substrate Concentration on Catalase Activity**

#### Aim:

To prepare the crude enzyme from the given source and to study the effects of varying substrate concentration on the activity of catalase.

#### **Principle:**

Sodium perborate activity may serve as a substrate for catalase. Sodium perborate (NaBo3.4H2O) in aqueous solution is acted upon by catalase, the remaining H2O2 is determined by titration with KMno4 in the presence of sulphuric acid.

 $2KMno_4+7H_2O_2+2H_2SO_4 \longrightarrow 2KHSO_4+2MnO_4+8H_2O+3O_2$ 

#### **Reagents:**

- 1. 1.54% sodium perborate (substrate)
- 2. 2N sulphuric acid
- 3. 0.01N KMnO4
- 4. M/15 phosphate buffer
- 5. Enzyme (source)

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#### **Procedure:**

Into a series of clean conical flasks pipetted out varying volume of substrate (0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0) and made up the total volume of each to 9ml with M/15 phosphate buffer of optimum pH 6.8. Then added 1.0ml of the enzyme to the experimental flask alone and mixed well. Incubated all the tubes at room temperature for 15mts. Stopped the reaction by adding 10.0ml of 2N H2SO4 at the end of incubation period. Controls were also conducted along with the experiments by adding the enzyme at the end of incubation period. The contents of the flask were then titrated against 0.01N KMno4 in the burette. The end point is the appearance of pale pink colour. The difference between the controls and experiments gives the volume of 0.01N KMno4 equivalent to the enzyme activity.

A graph was then drawn by plotting substrate concentration on X axis and volume of 0.01N KMno4 on y axis. From the graph Km value ie, the substrate concentration at half of the maximal velocity (Vmax) is determined. A line weaver burk plot was drawn by plotting 1/v on y axis and 1/[s] on x axis. From which the Km value is determined.

#### **RESULT:**

The Km values for the given enzyme catalase at room temperature at pH 6.8 for 15mts by Michaleis menton plot Km= ------

Line weaver burk plot Km=-----

S. No	S	1/[S]	V	1/V
1.	0.5	2.0	0.1	10.00
2.	1.0	1.0	0.2	5.00
3.	2.0	0.5	0.3	3.33
4.	3.0	0.33	0.4	2.50
5.	4.0	0.25	0.5	3.33
6.	5.0	0.20	0.6	1.67

Effect of substrate concentration on catalase activity:

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	Volume Volume Volume Volume					ume of 0	.01N KM					
S. No	of	of	of	of	control			E	Experimer	Difference		
	substrate (ml)	buffer (ml)	enzyme (ml)	2NH <sub>2</sub> SO <sub>4</sub>	Initial	Final	Final Titre value		Final	Titre value		
1.	0.5	8.5	1.0	10.0								
2.	1.0	8.0	1.0	10.0								self
3.	2.0	7.0	1.0	10.0								
4.	3.0	6.0	1.0	10.0								
5.	4.0	5.0	1.0	10.0								
6.	5.0	4.0	1.0	10.0								

Effect of substrate concentration on catalase activity:

#### Effect of pH on Catalase Activity

#### Aim:

To prepare the crude enzyme from the given source and to study the effects of varying pH on the activity of catalase.

#### **Principle:**

Sodium perborate activity may serve as a substrate for catalase. Sodium perborate (NaBo3.4H2O) in aquous solution is acted upon by catalase, the remaining H2O2 is determined by titration with KMno4 in the presence of sulphuric acid.

 $2KMno_4+7H_2O_2+2H_2SO_4 \longrightarrow 2KHSO_4+2MnO_4+8H_2O+3O_2$ 

#### **Reagents:**

- 1. 1.54% sodium perborate(substrate)
- 2. 2N sulphuric acid
- 3. 0.01N KMnO4
- 4. M/15 phosphate buffer
- 5. Enzyme (source)

#### **Procedure:**

Into a series of clean conical flask, 5ml of M/15 phosphate buffer of varying pH 6.0, 6.4, 6.8, 7.2, 7.6) was pipette out. Add 4.0ml of the substrate (1.54% sodium perborate) into each of the flask and then 1.0ml of enzyme to the experimental flask alone and mixed well. Incubated all the tubes at room temperature for 15mts. Stopped the reaction by adding 10.0ml of 2N H2SO4 at the end of incubation period. Controls were also conducted along with the experiments by adding the enzyme at the end of incubation period. The contents of the flask were then titrated against 0.01N KMno4 in the burette. The end point between the controls and experiments gives the volume of 0.01N KMno4 equivalent to the enzyme activity.

A graph was then draw by blotting pH variations on X axis and volume of 0.01N KMno4 on y axis, from which the optimum pH is calculated.

#### **RESULT:**

The optimum pH for catalase activity was found to be ------ at room temperature for an incubation period for 15mts.

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Effect of pH on catalase activity:

	Volumo	<b>X</b> 7 <b>I</b>				Volume of 0.01N KMnO <sub>4</sub>								
pН	of buffer	Of	of	15mts	Volume	n 1ml		Control			Experime	Difference		
	(ml)	substrate (ml)	enzyme (ml)	e for	of 2NH-SO	each	Initial	Final	Titre	Initial	Final	Titre	-	
				ture	21112304	ls of			value			value		
6.0	5.0	4.0	1.0	npera	10.0	ontro]								
6.4	5.0	4.0	1.0	m ten	10.0	the co								self
6.8	5.0	4.0	1.0	it rooi	10.0	ne to								
7.2	5.0	4.0	1.0	oate a	10.0	enzyn								
7.6	5.0	4.0	1.0	Incul	10.0	ydd e								

#### **Effect of Temperature on Catalase Activity**

#### Aim:

To prepare the crude enzyme from the given source and to study the effects of varying temperature on the activity of catalase.

#### **Principle:**

Sodium perborate activity may serve as a substrate for catalase. Sodium perborate (NaBo3.4H2O) in aqueous solution is acted upon by catalase, the remaining H2O2 is determined by titration with KMno4 in the presence of sulphuric acid.

 $2KMno_4+7H_2O_2+2H_2SO_4 \longrightarrow 2KHSO_4+2MnO_4+8H_2O+3O_2$ 

#### **Reagents:**

- 1. 1.54% sodium perborate(substrate)
- 2. 2N sulphuric acid
- 3. 0.01N KMnO4
- 4. M/15 phosphate buffer
- 5. Enzyme (source)

#### **Procedure:**

Into a series of clean conical flask, 5ml of M/15 phosphate buffer of optimum pH 6.8was pipette out. Add 4.0ml of the substrate (1.54% sodium perborate) into each of the flask and then 1.0ml of enzyme to the experimental flask alone and mixed well. Incubated at varying temperature (10°, 20°, 28°, 37° and 40°C) for 15mts. Stopped the reaction by adding 10.0ml of 2N H2SO4 at the end of incubation period. The contents of the flask were then titrated against 0.01N KMno4 in the burette. The end point is the appearance of pale pink colour. The difference between the controls and experiments gives the volume of 0.01N KMno4 equivalent to the enzyme activity.

A graph was then drawn by plotting temperature variations on X axis and volume of 0.01N KMno4 on y axis, from which the optimum temperature is calculated.

#### **Result:**

The optimum temperature for catalase activity was found to be ------ for an incubation period for 15mts at the pH 6.8.

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Effect of temperature on catalase activity:

7					S		l	Volume of 0.01N KMno4								
S. No	Temp °C	Volume of	Volume of	Volume of	: 15mt	of		Control		Control		Experiment				
		buffer (ml)	substrate (ml)	enzyme (ml)	ature for	2NH <sub>2</sub> SO <sub>4</sub>	ols of ea	Initial	Final	Titre value	Initial	Final	Titre value			
1	10 <sup>0</sup>	5.0	4.0	1.0	mper	10.0	contr									
2	20 <sup>0</sup>	5.0	4.0	1.0	om te	10.0	o the								self	
3	28 <sup>0</sup>	5.0	4.0	1.0	at roo	10.0	yme t									
4	37 <sup>0</sup>	5.0	4.0	1.0	ıbate	10.0	d enzy									
5	45 <sup>0</sup>	5.0	4.0	1.0	Incu	10.0	Add									

#### Estimation of Ascorbic Acid in Citrus Fruits (Vitamin C)

#### Aim:

To estimate the amount of ascorbic acid present in the given unknown sample solution

#### **Principle:**

Ascorbic acid is first dehydrogenated by bromination. The dehydroascorbate then reacts with 2, 4-dinitrophenyl hydrazine to form osazones, which dissolves in sulphuric acid to give an orange coloured solution whose absorbance can be measured spectrophotometrically at 540 nm.

#### **Reagents:**

- 1. 4% Oxalic acid
- 2. 0.5N Sulphuric acid
- 3. 10% Thiourea
- 4. 2% DNPH (2g-2,4 Dinitro phenyl hydrazine in 100ml of 0.5N sulphuric acid filtered and used)
- 5. 80% Sulphuric acid.
- 6. Bromine water: 1 2 drops in 100ml of cold water.
- Ascorbic acid: Stock standard 100mg of ascorbic acid in 100ml of 4% oxalic acid in a standard flask.
- 8. Working standard

Dilute 10ml of stock standard solution with 4% oxalic acid solution after bromination to concentration of working standard in 100mg/100ml.

#### Sample extraction:

2g of amla sample is taken in 25-50ml of 4% Oxalic acid solution. Centrifuged at 1000 rpm for 2 minutes, filtered and collected the liquid transformed and aliquote of 20ml to a conical flask and added bromine water dropwise with constant shaking. The phenolic hydrogen atoms in the extract turn orange yellow to the extent of bromine. Expel it by blowing in air made up to the known with 4% oxalic acid solution. Similarly converted 10ml of stock ascorbic acid in hydro form by bromination.

#### **Procedure:**

Pipetted out 0.2-1ml of ascorbic acid solution corresponding to  $\mu$ g value of 20-100. Similarly pipetted out 0.5ml of brominated sample extract and made up the volume in all tubes to 3ml by adding distilled water and add 1ml of DNPH reagent followed by 1-2 drops of thiourea into each tube. A blank was set as above but with distilled water.

Mix the contents of the tube thoroughly and incubated at 37°C for 3hours. After incubated the tubes were kept in the ice bath and dissolve the orange red colour osazone crystals formed by adding 7ml of 50% Sulphuric acid dropwise while tube in the ice bath. Then the tubes were removed from ice and allowed to stand for 30mts at room temperature and optical density is taken at 540nm.

A standard graph was drawn by taking the concentration of ascorbic acid on x-axis and optical density on y-axis. From the graph the concentration of the ascorbic acid in the unknown and sample was calculated.

#### **Result:**

1. a) The amount of ascorbic acid 2gm of amla (sample) = -----mg of ascorbic acid.

b) The amount of ascorbic acid 2gm of green leaves (sample) = -----mg of ascorbic acid. The amount of ascorbic acid in 100ml of unknown sample = ----- mg of ascorbic acid.

2017-2020 Batch

Estimation of Ascorbic Acid in Citrus Fruits (Vitamin C)

S.No	Solution	Volume of solution (ml)	Concentration (µg)	Volume of water (ml)	Volume of DNPH (ml)	Volume of Thiourea (ml)		Volume of H <sub>2</sub> So <sub>4</sub> (ml)	emperature	Optical Density at 630 nm
1	Blank	-	-	3.0	<b>≜</b>	<b></b>		<b>▲</b>	om t	
2	Standard								t r0(	
	<b>S1</b>	0.2	20	2.8					ite a	
	S2	0.4	40	2.6					minu	
	<b>S3</b>	0.6	60	2.4			at 37		301	
	<b>S4</b>	0.8	80	2.2	1.0	1-2 drops	ate :	7.0	l for	
	<b>S</b> 5	1.0	100	2.0			lcub		dard	
3	Sample	0.5	-	2.5			Ir		stan	
		0.5	-	2.5					v to	
4	Unknown	1.0	-	-					Allov	
		1.0	-	2.5		•		•	ł	

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# **KARPAGAM ACADEMY OF HIGHER EDUCATION**

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## **DEPARTMENT OF BIOCHEMISTRY**

Subject	:	Basic Biochemistry-Practical	Semester	:	Ι
Subject code	:	17MBU113	Class	:	I B.Sc Microbiology

#### **Viva-Questions**

#### S. NO

#### Questions

- 1. Define pH.
- 2. How will you confirm the given sugar as glucose.
- 3. How will you confirm the given sugar as fructose.
- 4. How will you confirm the given sugar as polysaccharide.
- 5. What is use of ninhydrin reaction.
- 6. Define amino acids.
- 7. List out the sulphur containing amino acids.
- 8. List out the aromatic amino acids.
- 9. What is the basic structure of amino acid.
- 10. How are peptide bond forms.
- 11. What are the confirmatory tests for cysteine.
- 12. What is the confirmatory test for tryptophan.
- 13. List out the bonds involved in the tertiary structure of protein
- 14. Define protein.
- 15. List the basics of protein structures.
- 16. Define Loop.

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- 18. Define domain.
- 19. Define Ramachandran plot.
- 20. Define enzyme.
- 21. List out the source of Vitamin C.
- 22. Define MM equation.
- 23. What is the molecular weight of water.
- 24. Define dielectric constant
- 25. List out the properties of water.