KARPAGAM ACADEMY OF HIGHER EDUCATION

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DEPARTMENT OF BIOCHEMISTRY

Subject	**:**	**MOLECULES OF LIFE- Practical**	**Semester**	**:**	**I**
Subject code	**:**	**17BCU111**	**Class**	**:**	**I B.Sc Biochemistry**

TITLE OF THE EXPERIMENTS

1. Safety measures in laboratories.
2. Preparation of normal and molar solutions.
3. Preparation of buffers.
4. Determination of pKa of acetic acid and glycine.
5. Qualitative tests for carbohydrates, lipids, amino acids, proteins and nucleic acids.
6. Separation of amino acids/ sugars/ bases by thin layer chromatography.
7. Estimation of vitamin C.
8. Estimation of vitamin E.

REFERENCE BOOKS

Nelson, D.L. and Cox, M.M., W.H.Freeman., Lehninger: Principles of Biochemistry (2013) 6th ed., and Company (New York), ISBN:13: 978-1-4641-0962-1 / ISBN:10:1-4641-0962-1.

Devlin, T.M., (2011) Textbook of Biochemistry with Clinical Correlations 7th ed., John Wiley & Sons, Inc. (New York), ISBN:978-0-470-28173-4.

Jayaraman, J. (2007). Laboratory Manual in Biochemistry, New Age International Publishers, New Delhi.

Sadasivam, S., and Manickam, A., (2009). Biochemical Methods, New Age International Publishers, New Delhi.

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(Deemed University Established Under Section 3 of UGC Act 1956)

Coimbatore - 641021

(For the candidates admitted from 2015 onwards)

DEPARTMENT OF BIOCHEMISTRY

SUBJECT : **MOLECULES OF LIFE- Practicals**

SEMESTER : **I**

SUBJECT CODE : **17BCU111** **CLASS** : **I B.Sc.BC**

Experiment No: 1

SAFETY MEASURES IN LABORATORIES

GENERAL GUIDELINES

1. Never work alone in the laboratory. No student may work in the science classroom without the presence of the teacher.

2. When first entering a science room, do not touch any equipment, chemicals, or other materials in the laboratory area until you are instructed to do so.

3. Perform only those experiments authorized by your teacher. Carefully follow all instructions, both written and oral. Unauthorized experiments are not allowed.

4. Do not eat food, drink beverages, or chew gum in the laboratory. Do not use laboratory glassware as containers for food or beverages.

5. Be prepared for your work in the laboratory. Read all procedures thoroughly before entering the laboratory. Never fool around in the laboratory. Horseplay, practical jokes, and pranks are dangerous and prohibited.

6. Always work in a well-ventilated area.

7. Observe good housekeeping practices. Work areas should be kept clean and tidy at all times.

8. Be alert and proceed with caution at all times in the laboratory. Notify the teacher immediately of any unsafe conditions you observe.

9. Dispose of all chemical waste properly. Never mix chemicals in sink drains. Sinks are to be used only for water. Check with your teacher for disposal of chemicals and solutions.

10. Labels and equipment instructions must be read carefully before use. Set up and use the equipment as directed by your teacher.

11. Keep hands away from face, eyes, mouth, and body while using chemicals or lab equipment. Wash your hands with soap and water after performing all experiments.

12. Experiments must be personally monitored at all times. Do not wander around the room, distract other students, startle other students or interfere with the laboratory experiments of others.

13. Know the locations and operating procedures of all safety equipment including: first aid kit(s), and fire extinguisher. Know where the fire alarm and the exits are located.

14. Know what to do if there is a fire drill during a laboratory period; containers must be closed, and any electrical equipment turned off.

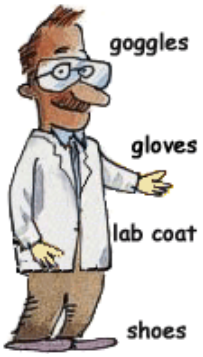
CLOTHING

15. Any time chemicals, heat, or glassware are used, students will wear safety goggles. NO EXCEPTIONS TO THIS RULE!

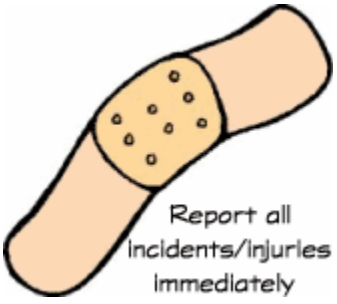
16. Contact lenses may be not be worn in the laboratory.

17. Dress properly during a laboratory activity. Long hair, dangling jewelry, and loose or baggy clothing are a hazard in the laboratory. Long hair must be tied back, and dangling jewelry and baggy clothing must be secured. Shoes must completely cover the foot. No sandals allowed on lab days.

18. A lab coat or smock should be worn during laboratory experiments.



ACCIDENTS AND INJURIES



19. Report any accident (spill, breakage, etc.) or injury (cut, burn, etc.) to the teacher immediately, no matter how trivial it seems. Do not panic.

20. If you or your lab partner is hurt, immediately (and loudly) yell out the teacher's name to get the teacher's attention. Do not panic.

20. If a chemical should splash in your eye(s) or on your skin, immediately flush with running water for at least 20 minutes. Immediately (and loudly) yell out the teacher's name to get the teacher's attention.

HANDLING CHEMICALS

21. All chemicals in the laboratory are to be considered dangerous. Avoid handling chemicals with fingers. Always use a tweezer. When making an observation, keep at least 1 foot away from the specimen. Do not

taste, or smell any chemicals.

22. Check the label on all chemical bottles twice before removing any of the contents. Take only as much chemical as you need.

HANDLING GLASSWARE AND EQUIPMENT

23. Never return unused chemicals to their original container.

24. Never remove chemicals or other materials from the laboratory area.

25. Never handle broken glass with your bare hands. Use a brush and dustpan to clean up broken glass. Place broken glass in the designated glass disposal container.

26. Examine glassware before each use. Never use chipped, cracked, or dirty glassware.

27. If you do not understand how to use a piece of equipment,ASK THE TEACHER FOR HELP!

HEATING SUBSTANCES

28. Do not immerse hot glassware in cold water. The glassware may shatter.

29. Do not operate a hot plate by yourself. Take care that hair, clothing, and hands are a safe distance from the hot plate at all times. Use of hot plate is only allowed in the presence of the teacher.

30. Heated glassware remain very hot for a long time. They should be set aside in a designated place to cool, and picked up with caution. Use tongs or heat protective gloves if necessary.

31. Never look into a container that is being heated.

32. Do not place hot apparatus directly on the laboratory desk. Always use an insulated pad. Allow plenty of time for hot apparatus to cool before touching it.

Experiment No: 2

PREPARATION OF NORMAL AND MOLAR SOLUTIONS

Preparation of standard solutions:

Solution of accurately known strength are called standard solution. A standard solution contains a known weight of reagent in a definite volume of solution. Molecular weight and atomic weight of commonly used chemicals has been in table given below.

Molar solutions:

Molar solution is one, which contains one molecular weight of the reagent in 1 litre of the solution molarity is expressed asm.

Normal solution:

Normal solution is one, which contains one equivalent weight of the reagent in 1 litre of the solution. Normality is expressed as V.

Equivalent weight of acid = Molecular weight/no of replaceable H-ions

There are few standard solutions which are used for analysis.

1. N/10 H_2SO_4
2. N/10 NaoH
3. N/10 $KMNO_4$
4. 0.256 N (1.25 % (w/v)) H_2SO_4
5. 0.313 N (1.25% (w/v)) NaoH
6. 40% NaCl (w/v)
7. 3% KNo_3 (w/v)
8. 20% ammonium wavelength (w/v)
9. 50% Hcl (w/v)

Certain primary standard solutions are also required for standardization of the above solution.

These are

1. N/10 Na_2Co_3
2. N/10 (COOH) $2.2H_2O$

Preparation of H_2SO_4 (N/10)

Equivalent weight of H_2SO_4 =49 g 24

Specific gravity = 1.84 g/ml

Volume of 49 g H_2SO_4 = 26.6 ml 24

Concentrated H_2SO_4 (reagent grade)is about 97% pure.

Therefore, actual amount of conc. H_2SO_4 required for 1 litre of N/10 H_2SO_4 solution = 100/97 x 26.6 = 27.42 ml.

Thus, for 1 litre of N/10 H_2SO_4 solutions 2.74 ml of concentrated.

Procedure

Take 2.74 ml sulphuric acid in a beaker half filled with distilled water. Transfer the contents and washing to a volumetric flask (1 litre) and make volume up to the mark. Shake well and titrate this solution with 10 ml of 0.1 N Naco using mixed methyl orange as an indicator. Repeat the titration to get at least three concordant readings.

Standardization

Suppose 10 ml of 0.1 N Na_2Co_3 = 9.5 ml of H_2SO_4.

V1N1 = V2N2

10 x 0.1 N = 9.5 N2

N2 = 0.10526

To prepare 1 litre N/10 H_2SO_4, the volume of 0.10526 N acid required is 1000 x 0.1/0.10526 = 950 ml. Take 950 ml of 0.10526 N acid and dilute it to 1 litre.Check it again with N/10 equal volume of N/10 Na_2Co_3 solution. Label it as 0.1 N H_2SO_4.

Preparation of N/10 NaoH solution

Molecular weight of NaoH =40

Acidity (No. of replaceable OH group) = 1

Equivalent weight of NaoH = 40

Therefore, 4 g of NaoH in a beaker (as it is hygroscopic) and dissolve it in distilled water (preferably CO_2 free).Transfer the contents and the washing to a volumetric flash (1 litre).

Cool and then make volume up to the mark shake well and standardize this solution against N/10 Oxalic acid using phenolphthalein as an indicator. Label it as o.1 N NaoH solution.

Add 13.16 g of NaoH (95% NaoH) in 1 litre distilled water and shake well. Standardize this solution against know concentration of oxalic acid solution using phenolphthalein as an indicator.

Experiment No: 3

PREPARATION OF BUFFER

A buffer is an aqueous solution containing a weak acid and its conjugate base or a weak base and its conjugate acid. A buffers pH changes very little when a small amount of strong acid or base is added to it. It is used to prevent any change in pH of a solution regardless of solution. Buffer solution are used as a means of keeping pH at a nearly value in a wide variety of chemical application.

For example, blood in the human body is a buffer solution.

Buffer solution are resistant to pH change because of the presence of an equilibrium b/w the acid (H4) and it conjugate base (A). The balanced equation for this reaction is

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

When some strong acid (more H^+) is added to an equilibrium mixture of the weak acid and its conjugate base, the equilibrium is shifted to the left, in accordance with Le-chatelier's principle. This causes the hydrogen ion (H+) concentration to increase by less than the amount expected for the quantity of strong base is added to the mixture, the hydrogen ion concentration decreases by less than the amount expected for the quantity of base added. This because the reaction shits to the right to accommodate for the loss of (H+) in the reaction with the base.

Preparing a buffer solution:

First method:

Prepare a solution with an acid and its conjugate base by dissolving the acid form of the buffer in about 60% of the volume required to obtain the final solution volume.

Then, measure the pH of the solution using a pH probe.

The pH can be adjusted up the desired value using a pH probe.

If the buffer is made with a base and its conjugate acid, the pH can be adjusted using a strong acid like Hcl. Once. The pHis correct dilute the solution to the final desired volume.

Second Method:

Prepared solutions of both the acid form and base form of the solutions.

Both solution must contain the same buffer concentrations as the concentration of the buffer in the final solution.

To get the final buffer, add one solution to the other while monitoring the pH.

Third method:

In a third method, you can determine the extract amount of acid and conjugate base needed to make a buffer of a certain pH, using the Henderson-Hasselach equation

$$pH = pka + \log [(A^-)/[HA]$$

Where pH is the concentration of $[H^+]$ pka is the acid dissociation constant, and $[A^-]$ and [HA] are concentration of conjugate base and starting acid.

Experiment No: 4

DETERMINATION OF PKA OF ACETIC ACID AND GLYCINE

Aim

To determine the pKa values and buffering capacity of buffer solutions.

Requirements

Buffer stock solution (concentrated), pH meter, distilled water, wash bottle, volumetric flasks, measuring cylinders, beakers, and pipettes.

Glycine-NaOH buffer system:

1. 20 mM glycine
2. 200 mM NaOH

Glycine-Acetic acid buffer system

1. 20 mM glycine
2. 200 mM acetic acid

Procedure

pH Measurement:

Mix all solutions thoroughly. The pH measurement may be made in original beakers. Do not change any control on the pH meter except as directed. With the meter on stand by, rinse the electrode with deionized water, gently shake off the excess water, and immerse the electrode in the sample solution. Switch the meter to the pH mode, allow the reading to stabilize, and record the pH. Switch the meter back to the standby mode, rinse the electrode again, and leave the electrode immersed in deionized water. Repeat this procedure for all samples.

Glycine-NaOH buffer system:

Prepare 50 ml 20 mM glycine solution and 100 ml of 200mM NaOH solution. Calibrate the pH meter with standard buffer solution at room temperature. Take 50 ml of glycine solution in a beaker and add 0.5 ml of NaOH solution and shake well to mix. Note the change in pH. Add subsequent quantities of

NaOH with an increment of 0.5 ml each time and note observed pH at regular intervals. Take about 30-35 readings and generate the following observation table:

Volume of NaOH added (ml)	Observed pH
0.0	
1.0	
1.5	
2.0	
2.5	

Results

Plot the titration curve for the given buffer system.

Estimate the pKa for the different ionic species.

Experiment No: 5

QUALITATIVE TESTS FOR CARBOHYDRATES, LIPIDS, AMINO ACIDS AND PROTEINS

Qualitative tests for carbohydrates

S. NO	EXPERIMENT	OBSERVATION	INFERENCE	REACTION
1	**Solubility Test** 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	It indicates the presence of monosaccharides and disaccharides. It indicates the presence of polysaccharides.	
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_2SO_4 along the sides of the test tube. So that the acid forms a layer beneath the test solution.	(a) A deep violet coloured ring was got at the junction of the two layers. (b) No deep violet coloured ring was got at the junction of the two layers.	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.	**Iodine test** To 1.0ml of the test solution added 4-5 drops of iodine solution and contents are mixed gently.	(a) Blue coloured solution was got. (b) No change in colour	Presence of polysaccharide. Absence of polysaccharide	Iodine forms coloured adsorption complexes with polysaccharides.
4.	**Fehling's test** 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.	(a) A reddish brown precipitate is formed. (b) No characteristic precipitate was got.	It indicates the presence of reducing sugar. It indicates the	Fehling's solution contains blue alkaline cupric hydroxide solution, heated with reducing sugars gets reduced to yellow or

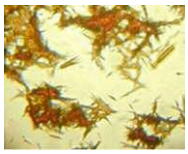
			absence of reducing sugar	red cuprous oxide and is precipitated.
5.	**Benedict's test** To 2.0ml of Benedict's reagent, added 8 drops of the test solution and heated in a boiling water bath for 2 minutes and allowed to cool. spontaneously.	(a) The solution first turned to green and then reddish brown precipitate was formed. (b) No characteristic reddish brown precipitate was formed.	It indicates the presence of reducing sugar It indicates the absence of reducing sugar	free aldehyde or keto group in the reducing sugars reduce cupric hydroxide in alkaline medium to red colored cuprous oxide. Depending on the concentration of sugars, yellow to green color is developed
6.	**Picric acid test** To 2.0ml of sugar solution added 0.5ml of saturated picric acid and 0.5ml of 10% sodium carbonate and heated in a water bath.	(a) A mahagony red coloured solution was got. (b) No characteristic red coloured solution was got.	It confirms the presence of reducing sugar. It shows the absence of reducing sugar and confirms the presence of sucrose which is a non reducing sugar	It is due to the reducing action of the sugar.
7.	**Barfoed's test** To 2.0ml of freshly prepared Barfoed's reagent added 1.0ml of sugar solution and heated in a boiling water bath for 3 minutes. Allow to cool.	(a) A brick red precipitate is formed and settling down at the bottom or sides of the test tube. (b) No characteristic red precipitate was got.	It indicates the presence of monosaccharides. It shows the absence of monosaccharides.	Barfoed's test is used to detect the presence of monosaccharide (reducing) sugars in solution. Barfoed's reagent, a mixture of ethanoic (acetic) acid 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 reagent added 4 drops of sugar solution and heated in a water bath for 3 minutes and cooled the solution at room temperature.	colour was got. (b) No characteristic cherry red colour was got.	presence of keto sugar sucrose and fructose. It indicates the absence of keto sugar sucrose and fructose.	presence of keto sugar sucrose and fructose which on treatment with HCl acid forms 5 hydroxy methyl furfural which then condences with resorcinol to give cherry red coloured complex.
9.	**Tollen's test** 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.	(a) A red coloured solution was got. (b) No characteristic red coloured solution was got.	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.	(a) A greenish blue coloured solution was got. (b) No characteristic greenish blue coloured solution was got.	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	(a) A white precipitate was got and a	It shows the presence of lactose or	

	added 1.0ml of concentrated nitric acid and 1.0ml of distilled water, heated in a boiling water bath for one and a half an hour and left it stand overnight and viewed the crystals under the microscope.	colourless rod shaped crystals were seen under the microscope. (b) No characteristic white precipitate was formed.	galactose. It shows the absence of lactose or galactose.	
12.	**Phenyl hydrazine Test** Took equal volume of sugar solution and phenyl hydrazine mixed, filtered and kept in a boiling water bath for half an hour and cooled at room temperature viewed the crystals under the microscope.	Formation of beautiful yellow crystals of osazone was formed (a) A yellow **needle** shaped crystals like sheeves of corn was observed. (b) A **flower** shaped crystals were observed (c) A **cotton ball or puff** shaped crystals were observed. (d) A small **sunflower** shaped crystals were observed. (e) A **rod** shaped crystals were observed.	It indicates the presence of **glucosazone and fructosazone** It indicates the presence of **galactosazone** It indicates the presence of **lactosazone** It indicates the presence of **maltosazone** It indicates the presence of **xylosazone**	The ketoses and aldoses react with phenylhydrazine to produce a phenylhydrazone which further reacts with another two molecules of phenylhydrazine to yield osazone.

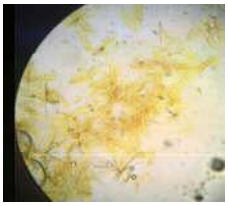
Shapes of different crystals

Glucosazone and Fructosazone



Galactosazone

Lactosazone



Maltosazone

Xylosazone

Qualitative tests for amino acids and proteins

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

		got.	
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.	a. A dark blue or violet coloured solution was got. b. No characteristic blue coloured solution was got.	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.	a. A violet coloured ring was got at the junction of the 2 liquids. b. No characteristic violet coloured ring was got.	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.	a. A red violet coloured ring was got. b. No characteristic red violet coloured ring was got.	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.	a. A violet coloured ring was got at the junction of two liquids. b. No characteristic violet coloured ring was got.	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	**Ehrlich's Test:** 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.

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

	To 2ml of the amino acid solution add 1ml of 10% aqueous lead acetate and 1ml of 40% sodium hydroxide. Heat in a water bath for 5 minutes.	was got. b. No characteristic black precipitate was got.	group which indicates the presence of cysteine. It shows the absence of cysteine
15	**Boiling's Modification (or) Mc Carthy Sullivan Test:** To 1ml of the amino acid solution, add the following reagents in order and mix after each addition. Add 1.5ml of 0.5N sodium hydroxide, 1.5ml of 1% 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.	A red colouration was got.	The methyl group is split to form homocysteine which gives a red colour with sodium nitroprusside solution. It confirms the presence of methionine.

Qualitative tests for lipids

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

4.	**Saponification test:** Take 1 ml of unknown solution in a test tube and add an equal amount of alcoholic KOH solution, mix them thoroughly and keep the mixture during the colour of warming and shake up gently with a little distilled water	Soap formation No soap formation	Indicates the presence of fatty acids Absence of fatty acids
5.	**Test for unsaturated fatty acids:** A clean test tube containing 5 ml of CCl_4 and add a drop of lipid or oil, now bromine water is added drop by drop and number of drops added is recorded	Appearance of permanent yellowish red colour No characteristic colour formation	Indicates the presence of unsaturated fatty acids. Absence of unsaturated fatty acids
6.	**Acrolein test:** For a few drops of oil or lipid, a few crystals of potassium hydrogen sulphate warm gently ta mix and then heated strongly	A pungent colour of acrolein is produced No pungent colour is produced	Indicates the presence of glycerol Absence of glycerol
7.	**Dichromate test:** Take a dry test tube, 3 or 4 ml of oil or lipid, a few drops of 5% potassium dichromate solution and 5 ml of conc. H_2SO_4 mix well	Brown colour is changed to blue No colour change	Indicates the presence of glycerol Absence of glycerol

Experiment No: 6

SEPARATION OF AMINO ACIDS BY THIN LAYER CHROMATOGRAPHY

The separation and identification of organic compounds is a routine work in many service laboratories. Thin layer chromatography (tlc) is an easy technique to adopt for the said purpose. It is highly useful in research laboratories to separate, identify and characterize unknown compounds. A variety of small molecules like amino acids, sugars, organic acids, lipids etc. are separated by tic technique. The greater advantage of tic is the speed at which separation is achieved. When volatile solvents are used the time required to effect separation is only about 30 min and with nonvolatile solvents it is seldom longer than 90 min.

Principle

The general Principle involved in tlc is similar to that of column chromatography i.e. adsorption chromatography. In the adsorption process, the solute competes with the solvent for the surface sites of the adsorbent. Depending on the distribution coefficients, the compounds are distributed on the surface of the adsorbent. Of course, in tic the partition effect in the separation is also not ruled out. The adsorbent normally used contains a binding agent such as calcium sulphate which facilitates the holding of the adsorbent to the glass plate.

Materials

- Glass Plate (20 x 20 cm or 20 x 10 cm)
- Glass Tank with Lid
- Spreader
- Developing Solvents
- Adsorbent Silica GelG/Alumina
- Sample (should be extracted following the procedures indicated for each group of compounds. For e.g., extraction with 80% alcohol for amino acids and sugars)
- Standards Spraying Agent (This also differs as for the group of compounds of interest).

Procedure

Preparation of Plates

1. Place dry, clean glass plates (5 nos, 20 x 20cm) on the plastic base plate over a plane surface.
2. 1. Prepare a slurry of the adsorbent in water (sometimes buffer) in the ratio 1:2 (w/v).
3. Stir the slurry thoroughly for 1-2 min and pour into the applicator positioned on the head glass plate.
4. Coat the slurry over the glass plates at a thickness of 0.25mm for qualitative analysis by moving the applicator at a uniform speed from one end to the other. (One has to gain some experience by practicing to prepare uniformly coated plates.) Leave the plates to dry at room temperature for 15-30 min.
5. Heat the plates in an oven at 100-120°C for l-2h to remove the moisture and to activate the adsorbent on the plate. The dried plates in a rack can be stored in a desiccator over silica gel to prevent moisture absorption.

Sample Application

1. Leave 2.5cm from one end of the glass plate and at least an equal distance from the edges.
2. Apply the sample and standards by means of a micropipette or syringe as small spots. All spots should be placed at equal distance from one end of the plate. See that the adsorbent does not flake off at the sample application point. (Measured volumes are applied for quantitative analysis.)
3. Allow the sample to dry so that spotting can be done repeatedly for a more concentrated sample spot.

Developing Chromatogram

1. Pour the developing solvent into the tank to a depth of 1.5cm. Allow it to stand for at least an hour with a cover plate over the top of the tank to ensure that the atmosphere within the tank becomes saturated with solvent vapor. This is called equilibration.
2. After equilibration, remove the cover plate, and place the thin layer plate (sample applied) vertically in the and so that it stands in the solvent with the spotted end dipping in the solvent.
3. Replace the cover plate. The separation of the compounds occurs as the solvent moves upward. Develop the chromatogram at constant temperature in order to avoid anomalous solvent-running effects.
4. Once the solvent reaches the top of the plate, remove it from the tank, dry and proceed for the identification of the separated compounds.

Result:

The given amino acid was found to be _____.

Experiment No: 7

ESTIMATION OF VITAMIN C

Aim

To estimate the amount of vitamin C in given unknown sample.

Principle

Ascorbic acid is converted to dehydroascorbate by treatment with activated charcoal or bromine. 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% TCA
2. 10% Thiourea
3. 2% DNPH (2g-2,4 Dinitro phenyl hydrazine in 100ml of 0.5N sulphuric acid)
4. 85% Sulphuric acid
5. Standard Ascorbic acid (20-100 µg /ml)-

100 mg of ascorbic acid was dissolved in water and was made up to 100 ml with water. Convert 10mL of stock ascorbic acid solution into dehydro form by bromination. This contains 100µg uric acid /ml.

6. Extraction:

1g of leaves were ground and homogenized in 4% TCA and made up to 10 ml with the same and centrifuged at 2000 rpm for 10 minutes. The supernatant was treated with a pinch of activated charcoal residue, shaken well and kept for 10 minutes. Once again centrifugation was carried out to remove the charcoal residue. The volume of the clear supernatant obtained was noted. Transfer an aliquot (10mL) to a conical flask . and add bromine water drop wise with constant mixing. The enolic hydrogen atoms in ascorbic acid are removed by bromine. When the extract turns orange yellow due to excess bromine, expel it by blowing in air. Make up to a known volume (25 or 50mL) with 4% oxalic acid solution.

Procedure

1. Pipette out 10-100µg standard dehydroascorbic solution into a series of tubes.
2. Similarly pipette out different aliquots (0.1mL-2mL) of brominated sample extract.

3. Make up the volume in each tube to 3mL by adding distilled water.
4. Add one mL of DNPH reagent followed by 1-2 drops of thiourea to each tube.
5. Set a blank as above but with water in place of ascorbic acid solution.
6. Mix the contents of the tubes thoroughly and incubate at 37°C for 3h.
7. After incubation dissolve the orange-red osazone crystals formed by adding 7mL of 80% sulphuric acid.
8. Measure absorbance at 540nm.
9. Plot a graph ascorbic acid concentration versus absorbance and calculate the ascorbic acid content in the sample and expresse as the amount of ascorbic acid in mg/g or 100 g sample.

S.No	Solution	Volume of solution (ml)	Concentration (µg)	Volume of water (ml)	Volume of DNPH (ml)	Volume of Thiourea (ml)	Incubate at 37 ° C for 3 hours	Volume of H_2So_4 (ml)	Allow to standard for 30 minute at room temperature	Optical Density at 630 nm
1	Blank	-	-	3.0	1.0	1-2 drops		7.0		
2	Standard									
	S1	0.2	20	2.8						
	S2	0.4	40	2.6						
	S3	0.6	60	2.4						
	S4	0.8	80	2.2						
	S5	1.0	100	2.0						
3	Sample	0.5	-	2.5						
		0.5	-	2.5						
4	Unknown	1.0	-	-						
		1.0	-	2.5						

Experiment No. 8

ESTIMATION OF VITAMIN-E

Aim

To estimate the amount of vitamin-E present in the given unknown sample.

Principle

Vitamin E can be estimated using reaction of ferric to ferrous ions by tocopherols, which forms a red colour with 2,2'-dipyridyl tocopherols and carotenes were first extracted with xylene and read at 460nm to measure carotenes. A correlation is made for these after adding ferric chloride and read at 520nm.

Reagents

i) 2-2' dipyridyl: 1.2g/L of n-Propanol

ii) Ferric chloride: 1.2g of $FeCl_3$ or 720mg of anhydrous ferric chloride in 1 L of ethanol.

iii) Standard Tocopherol: 10mg/L in absolute ethanol and 1ml of α-tocopherol is equivalent to 100mg of tocopherol acetate.

iv) Absolute ethanol

v) Xylene

Sample preparation

The plant sample (2.5g) was homogenized in 500ml of 0.1N H_2SO_4 and allowed to stand for overnight. The contents of the flask were shaken vigorously and filtered through filter paper. Aliquot of the filtrate were used for the estimation.

Procedure

Into 3 stoppered centrifuge tubes (test, standard and blank) pipetted out 1.5 ml of each tissue extract, 1.5 ml of the standard and 1.5 ml of water respectively. To the test and blank added 1.5 ml ethanol and to the standard added 1.5 ml of water. Added 1.5 ml of xylene to all the tubes, stoppered, mixed well and centrifuged. Transfererred 1 ml of xylene layer into another stoppered tube, taking care not to include any ethanol or protein. Added 1.0 ml of 2, 2' dipyridyl reagent to each tube, stoppered and mixed. Pipetted out 1.5 ml of the mixtures into spectrophotometer cuvettes and read the absorbance of test and standard against the blank at 460 nm. Then in turn beginning with the blank, add 0.33 ml of ferric chloride solution. Mixed well

and after exactly 1.5 minutes read test and standard against the blank at 520 nm.The amount of vitamin E can be calculated using the formula

$$\text{Vitamin E } (\mu g/g) = \frac{(\Delta A520nm - \Delta A450nm \times \text{conc [s]} \times 0.29) \times \text{Total volume}}{(\Delta A520nm \times \text{Vol for experiment} \times \text{wt of sample})}$$

Principle

Hot acidic medium sugar is dehydrated to hydroxy methyl furfural. This compounds found a green colour product with anthrone which was read at 630nm.

Reagents

1. **Anthrone reagent**
   Dissolve 200mg anthrone on 100 ml of ice cold 95% H_2SO_4. It should be prepared freshly before use.
2. **Standard Glucose Solution**
   500 mg of glucose was dissolved in 100 ml of distilled water.
3. **Working Standard**
   10 ml of stock was diluted to 100 ml with distilled water.

Procedure

Into a series of test tubes pipette out 0.1 to 0.5 ml of working standard and labeled as S1 to S5. The given unknown solution was made upto 100 ml with distilled water mix well and from that 0.5 ml was taken in two test tubes and marked as U1 and U2 make up the volume of each test tube to 1 ml. 1 ml of distilled water was serves as blank and add 4 ml of anthrone in all test tubes. Test tubes were treated in a water bath for 8 minutes. Cool rapidly and read the dark green colour solution at 630 nm.

Draw the standard graph by plotting concentration of standard on X-axis and absorption at Y-axis. From the graph, the amount of in the unknown was calculated.

Table

S.No	Solution	Volume of solution (ml)	Concentration (µg)	Volume of water(ml)	Volume of Anthrone (ml)	Heated in a boiling water bath for 8	Optical Density at 630 nm
1	Blank	-	-	1.0			
2	Standard						
	S1	0.2	20	0.8			

	S2	0.4	40	0.6			
	S3	0.6	60	0.4			
	S4	0.8	80	0.2	4.0		
	S5	1.0	100	-			
3	Sample	0.1	-	0.9			
		0.1	-	0.9			
4	Unknown	1.0	-	-			
		1.0	-	0.5			

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DEPARTMENT OF BIOCHEMISTRY

Subject	**:**	**MOLECULES OF LIFE- Practicals**	**Semester**	**:**	**I**
Subject code	**:**	**17BCU111**	**Class**	**:**	**I B.Sc Biochemistry**

Lecture Plan

S.NO	NAME OF THE EXPERIMENT	SUPPORT MATERIALS
1.	Safety measures in laboratories	T1: 4-9
2.	Preparation of normal and molar solutions	T1: 46-49
3.	Preparation of buffers	T1: 49-51
4.	Determination of pKa of acetic acid and glycine	W1
5.	Qualitative tests for carbohydrates, lipids, amino acids, proteins and nucleic acids.	R2: 15-22, 33-36, R1: 33-34
6.	Separation of amino acids/sugars/bases by thin layer chromatography	R2: 199-200
7.	Estimation of Vitamin C	R1: 185-186
8.	Estimation of Vitamin E	J1
9.	Model exam	

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T1: Rajan S. Manual for medical laboratory technology. 2012. 1st edition. Anjana book house, Chennai 600 107.
R1: Sadasivam S and Manickam A. 2009. Biochemical methods, New age International publishers, New delhi.
R2: Singh SR. 2014. Introductory practical biochemistry. Narosa publishing house, New delhi.
J1: Naer PP and Magar NG. 1956. Determination of vitamin E in blood. J.Biol.Chem, 220: 151-159
W1: www.linfield.edu/assets/files/chem/courses/CHEM 322/pKa

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DEPARTMENT OF BIOCHEMISTRY

Subject	**:**	**Basic Biochemistry-Practical**	**Semester**	**:**	**I**
Subject code	**:**	**17BCU111**	**Class**	**:**	**I B.Sc Biochemistry**

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.

17. Define turns.
18. Define domain.
19. Define Ramachandran plot.
20. Define enzyme.
21. List out the source of Vitamin C.
22. Define pKa.
23. What is the molecular weight of water?
24. Define dielectric constant
25. List the sources of vitamin C and vitamin E.