KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)

(Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

SYLLABUS

DEPARTMENT OF CHEMISTRY

STAFF NAME : Dr.M.R.EZHILARASI
SUBJECT NAME : Molecules of life practicals
SUB.CODE :16CHU613B
SEMESTER : V I
CLASS : III B.Sc (CHEMISTRY)

Semester-VI

16CHU613B MOLECULES OF LIFE - PRACTICAL 4H 2C

Instruction Hours/week: L:0 T:0 P:4 Marks: Internal: 40 External: 60 Total:100

Scope

The lab course deals with the Separation, determination of concentration and action of enzymes, characterisation of lipids and carbohydrates.

Objectives

The lab course enables a student to

1. Separate amino acids by paper chromatography
2. To determine the concentration of glycine solution by formylation method.
3. Study of titration curve of glycine
4. Study the action of salivary amylase on starch
5. Study the effect of temperature on the action of salivary amylase on starch.
6. To determine the saponification value of an oil/fat.
7. To determine the iodine value of an oil/fat
8. Differentiate between a reducing/ nonreducing sugar.
9. Extract of DNA from onion/cauliflower
10. To synthesise aspirin by acetylation of salicylic acid and compare it with the ingredient of an aspirin tablet by TLC.

Methodology
Paper chromatography, titrations, extraction of DNA, TLC

1. Separation of amino acids by paper chromatography
2. To determine the concentration of glycine solution by formylation method.
3. Study of titration curve of glycine
4. Action of salivary amylase on starch
5. Effect of temperature on the action of salivary amylase on starch.
6. To determine the saponification value of an oil/fat.
7. To determine the iodine value of an oil/fat
8. Differentiate between a reducing/ nonreducing sugar.
9. Extraction of DNA from onion/cauliflower
10. To synthesise aspirin by acetylation of salicylic acid and compare it with the ingredient of an aspirin tablet by TLC.

Suggested Readings

Text Books:

1. Furniss, B.S., Hannaford, A.J., Rogers, V., Smith, P.W.G. & Tatchell, A.R. (1978). *Vogel's Textbook of Practical Organic Chemistry*. ELBS.
2. Ahluwalia, V.K. & Aggarwal, R.(2004). *Comprehensive Practical Organic Chemistry.* Universities Press.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)

(Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

LECTURE PLAN

DEPARTMENT OF CHEMISTRY

Staff Name : Dr.M.R.Ezhilarasi
Subject Name : Molecules of Life Practical
Sub.Code :16CHU613B
Semester : VI
Class : III B.Sc (Chemistry)

Total Lecture Hours: 40 Hours

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
1	4	Writing the experimental procedure	
2	4	Separation of amino acid by paper chromatography	R1: 220
3	4	Aspirin synthesis and compare with the tablet by TLC	R1: 220
4	4	Determination of saponification value of an oil/fat	R1: 150
5	4	Determination of the iodine value of an oil/fat	R2: 130
6	4	Differentiate between a reducing and non reducing sugar	R1: 47
7	4	Determine the concentration of glycine solution by formylation method	R1:146

8	4	Study of titration curve of glycine	
9	4	Viva-voce questions discussion	
10	4	Model Practical examination	
	Total No of Hours Planned For Practical= 40 hours		

References:

R1: Raj K Bansal. Laboratory Manual of Organic Chemistry .4 th edition.2001. New age international (p) ltd. New Delhi.

R2: V.V.Ramanujam. Organic Chemistry lab manual. The National publishing co. Chennai

Website:

W1: http// www. Study of titration Curve of glycine.

DEPARTMENT OF CHEMISTRY

PRACTICAL MANUAL

MOLECULES OF LIFE PRACTICAL

Karpagam Academy of Higher Education
(Deemed to be University)
Established Under Section 3 of UGC Act, 1956)
Pollachi Main Road,Eachanari Post,
Coimbatore – 641 021,Tamil Nadu,India.

LIST OF PRACTICALS

Experiment No-1: Separation of amino acid by paper chromatography

Name of the experiment:

Separation of amino acids by ascending paper Chromatography .

Introduction:

Chromatography is a method of separating and analyzing mixtures of chemicals. The paper chromatography is used for the testing of purity of compounds and identifying substances and a very useful method as it is quick and very small quantities of materials used It is used to separate the mixture of substances into their components. They all have a stationary phase and a mobile phase. The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates. In this chromatography the stationary phase is a very uniform absorbent paper. The mobile phase is a suitable liquid solvent or mixture of solvents.

Principle:

In this experiment a small spot with amino acid onto a strip paper is made out. The bottom of the strip will then be placed in a wide-mouth jar of mixture of solvent, 40% Et-OH (ethanol) and 60% H_2O and the solvent then shook up into the paper. The solvent is the mobile phase and the paper is the stationary phase. The attraction of the solvent to the paper is larger than the attraction of the water too itself hence the mixture of solvent moves up to the paper. The amino acid which also be attracted to the paper to itself and to the water differently and thus a different component will move a different distance depending upon the strength of attraction to each of these objects we can calculate retention factor, R_f of the sample

$$R_f = \frac{distance\ travelled\ by\ the\ sample\ component}{distance\ travelled\ by\ the\ solvent}$$

Materials and instrument:Apparatus:

1. Ruler

2. Pencil , (two)

3. Capillary tube

4. A wide moth jar

5.10-15 identically sized strips of paper

Solvent and sample:

1. Mixture of Et-OH (ethanol) and water $(H_2O)_2$. Amino acids.

Locating reagent:

1.Ninhydrine solution.

Procedure:

1.The paper strips are cut about one by four (4) inches in area.

2.Then drawn a line across it horizontally to one cm (1 cm) from thebottom of one of the paper strips by using a ruler and pencil. This is theoriginal line.

3.Then a small amount of solvent is pour into the jar (there be barelyenough for the paper strips to hang inside of the jar and just touch thesolvent)

4.Then place a small dot (.) of the sample onto the line by using one of thecapillary tubes.

5. Then the paper is tapped with a pencil and hung it into the jar of solvents o that the bottom edge of the paper which is just barely touching.

6.Then it to be keep for some time for rising up onto the strip.

7.Then the strip needed to be remove from the jar and a mark is to be made to how far the solvent rise with a pencil.

8.Then the paper strip is to be dry in normal sunlight and dip in locating reagent (Ninhydrine solution).

9.Then the strip is to be expos UV light and then dry and indicate the spot.

10.Then the distance of the component and solvent travel from the starting point is to be measure and calculate the**Rf** valuefor eachcomponent.

11. Repeat the experiment for each type of component.

Experimental data:

Determination of distance:

Experiment no.	Sample	Distance travelled by the sample,d_1 (cm)	Distance travelledby the solvent,d_2 cm
1	A		
2	B		
3	C		
4	D(mixture)		

Determination of R_f Values of each amino acid sample

Experiment no.	Sample	$\frac{d1cm}{d2\ cm}$	R_f
1	A		
2	B		
3	C		
4	D(mixture)		

Calculation:

We know,

Retention factor,$\mathbf{R_f} = \frac{\textbf{Distance travelled by the Sample Component}}{\textbf{Distance travelled by the Solvent}}$

Result:

1.The $\mathbf{R_f}$ value for the sample, A =2.

The $\mathbf{R_f}$ value for the sample, B =3.

The **R_f** value for the sample, C=4.

The **R_f** value for the mixed sample, D =

By comparing the above **R_f** value the component which present in the mixture is=

Experiment No-2:

Aspirin synthesis and compare with the tablet by TLC

INTRODUCTION

Since ancient times, the bark and leaves of willow trees have been used as a pain killer. The active component, salicylic acid (SA), can, however, cause stomach upset because its acidity (pKa = 2.97) can be higher than the pH of the human stomach (pH ~ 4 after digestion is complete).

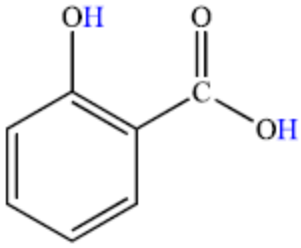


Figure 1. Salicylic Acid

Salicylic acid is a diprotic organic acid with two acidic functional groups: a *carboxylic acid* and a *phenol.* (Acidic hydrogen atoms are blue.) In comparison, the monoprotic acetylsalicylic acid(ASA, aspirin) is less acidic (pKa = 4.57). The reason: an ester has replaced the acidic phenol in ASA.

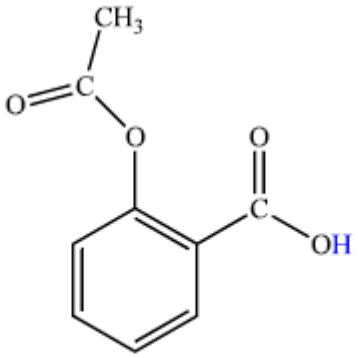


Figure 2. Acetylsalicylic Acid (ASA)

The synthesis of ASA from salicylic acid results in the formation of an ester functional group and, therefore, is called an **esterification**. The first step in this esterification is to create a **suspension** of salicylic acid (a solid at room temperature) in an excess of acetic anhydride (a liquid at room temperature). Acetic anhydride serves as both a reactant and a solvent. A **catalyst** is required for this reaction. Phosphoric acid, H3PO4, donates a H+ which binds to the reaction complex. As a catalyst, H+ is regenerated (not consumed) by the end of the reaction. As the reaction proceeds, the solid salicylic acid disappears and the acetylsalicylic acid productremains dissolved in the hot solution. Once all solid has disappeared (all the salicylic acid has been consumed) the reaction is complete.

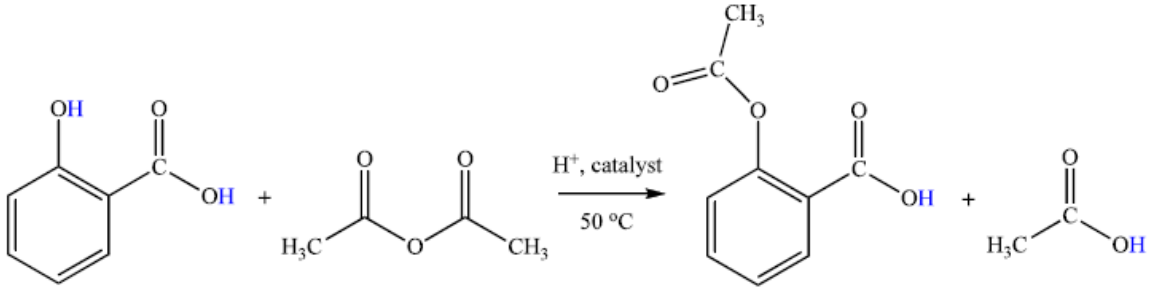


Figure 3. Formation of Acetylsalicylic Acid (ASA, a.k.a., Aspirin)

At this point the excess acetic anhydride must be **hydrolyzed** (split apart by the addition of water) to acetic acid. Acetic anhydride is *very* reactive toward water, so the hydrolysis must be done slowly – water should be added drop-wise.

More water is then added and the flask is placed in an ice bath to lower the solubility and precipitate the ASA product. The product is then collected by filtration.

The product's purity is analyzed in chromatographic techniques:

The number of impurities in different samples will be determined by **thin layer chromatography**. Silica will serve as the *stationary phase* and an organic solvent will be the *mobile phase*. The different compounds in aspirin move up the TLC plate at different rates based

the competing intermolecular interactions of the polar silica plate and the less polar solvent. More polar compounds *adsorb* more strongly to the silica and do not move as far up the TLC plate. Less polar components will favor the solvent and move higher. The TLC of the ASA you synthesize in lab will be compared with the TLCs of commercially prepared ASA, the salicylic acid starting material, caffeine, and an aspirin tablet

A commercially prepared aspirin tablet contains mostly ASA, but other components such as caffeine, buffers, and starch binding agents are also added.

Synthesis & TLC Analysis:

TLC Preparation

1. Create two TLC plates (labeled #1 & #2). Each will be spotted with three samples.

• Plate #1: *Salicylic Acid (SA) reactant, Reaction Mixture before heating (Sample A) & after heating (Sample B)*

• Plate #2: *Pure ASA, Aspirin tablet, & Caffeine*

2. Round the bottom corners of the two TLC plates with scissors so the liquid eluent travels evenly up the plates. Use a pencil to lightly mark the origin line where the sample spots will be made.

3. Take a small amount of the salicylic acid (SA) starting material and dissolve as much as you can in 3 drops of ethyl acetate. Add another 10 drops of ethyl acetate. Spot the SA solution on the bottom left of Plate #1. Make the spot as small but as concentrated as possible (place a drop of solution, let it dry, and then repeat at least 3 times). Use a UV lamp to see if the spot is the right size (~1 mm diameter) before moving on. **Do not look directly into the UV lamp**. The other two spots on TLC plate #1 will be added later.

4. Prepare one TLC developing chamber: Add ethyl acetate into the TLC developing chamber until the liquid surface is below the origin line once the plate is placed in the container. A piece of filter paper should be stood against one wall inside of the flask. The purpose of the filter paper is to ensure that the atmosphere inside the chamber is saturated. Any vessel containing ethyl acetate should be capped when not in use to prevent its vapors from escaping into the laboratory.

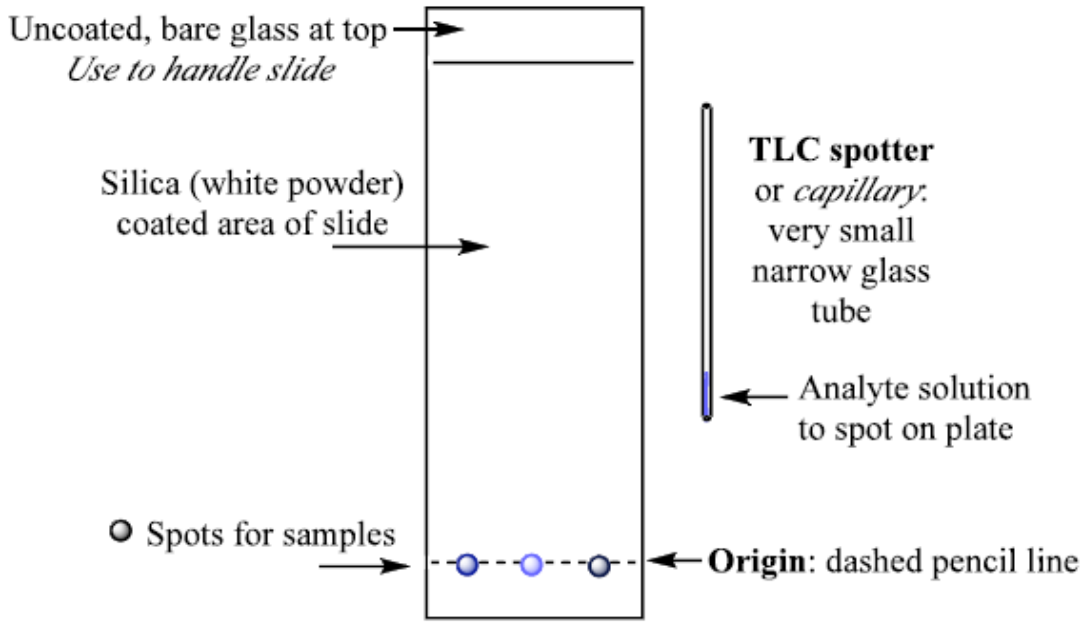


Figure 6. *TLC Plate spotted with Samples.*

5. Repeat the spotting process on Plate #2 with the aspirin tablet, pure ASA, & caffeine. • Crush an aspirin tablet and place in a labeled, pre-weighed vial or test tube. Record the mass of the aspirin tablet. Do not lose this container or its contents, you will use them later.

• Take a small amount of the aspirin tablet and dissolve as much as you can in 3 drops of ethyl acetate. Add another 10 drops of ethyl acetate. Spot the solution on the left side of Plate #2 on the origin line.

• Repeat the process with a small portion of pure ASA & caffeine (separately). Spot these two solution in the middle and right side of the origin line of Plate #2. ***Synthesis &TLC Development***

6. In the fume hood and wearing gloves, combine ~ 1.5 g salicylic acid (record an accurate mass) and 3.5 mL acetic anhydride (from a graduated cylinder – cap the graduated cylinder when transferring between hoods) into a DRY vial. Add 5 drops H3PO4 to the suspension. Use a pipet to remove 2-3 drops of solution (Sample A).

7. Use a *new* TLC spotter and spot the removed solution on the middle of the origin line of Plate #1. (Spots should be evenly spaced and the right size so samples do not run together.)

8. Begin gently stirring and heating the mixture in a 50 ℃ water bath. Continue heating and stirring until all solid disappears (about 10 minutes).

9. Develop Plate #2 while the reaction goes to completion.

• Place the plate in the prepared developing chamber. Make sure no edge of the TLC plate touches the filter paper. (If the filter paper touches any side of the TLC plate, the spots will be drawn sideways.) Solvent will begin to move up the plate. Once the 'solvent front' is within 1/2

cm of the top of the plate, remove the plate from the chamber and mark the **solvent front line** with a pencil. Do not let your solvent front travel to the top of the TLC plate.

• Circle any visible spots on the TLC plate with a pencil, then use a UV lamp to see spots not otherwise visible. Measure the distance from the origin to the solvent front and from the origin to each spot. Take a digital picture of the plate to include with your report. Record data neatly in a table and use the following equation to determine the Rf values of the various spots on the TLC plate:

$$\text{Rf} = \frac{\text{Distance from the origin to the centre of the TLC spot(cm)}}{\text{Distance from the origin to the solvent front (cm)}}$$

10. After all solid disappears from the reaction mixture, use a pipet to remove 2-3 drops of solution. Use a *new* TLC spotter and spot the removed solution on the right of the origin line of Plate #1 (Sample B). Place the plate aside, you can develop it once you have the your ASA product drying in the oven (step #14).

11. Remove the vial from the water bath and carefully add 15 drops distilled H_2O. Wait one minute. If precipitation does not begin add 15 more drops of H_2O. If precipitation does not occur after 5 minutes, go on to the next step.

12. Once solid begins **precipitating** out of solution, add 15 mL distilled H_2O. The flask and its contents may now be removed from the fume hood. Break up any solids and cool in ice bath for 10 minutes.

13. To complete this step, read the document titled *Vacuum Filtration* on the lab manual website. Record the mass of a piece of filter paper. Collect the precipitate by **vacuum filtration** and wash with 2 mL chilled distilled H2O. Discard the **filtrate** in the sink.

14. Dry the crystals for 15 minutes in the filter and then place them on a labeled preweighed watch glass in the oven for at least 10 minutes. You should check the temperature on the oven… if the temperature exceeds 65 °C, your product will decompose. (Note: While waiting for your product to dry in the oven, continue on with the next sections of the procedure.) Place Plate #1 into the developing chamber (repeat step #9).

15. Remove from the oven. Once cooled to room temperature, accurately record the mass.

Collect this sample in a new vial labeled with the contents (ASA), your name, day and time of you lab section, and the date (with the year).

Result:

Determine the Rf for all of the components on each slide. What is the Rf of ASA? SA?

Experiment No-3

Determination of Saponification value of an oil/fat:

Materials Required:

1) Fats and Oils [coconut oil, sunflower oil]
2) Conical Flask
3) 100ml beaker
4) Weigh Balance
5) Dropper
6) Reflux condenser
7) Boiling Water bath
8) Glass pipette (25ml)
9) Burette

Reagents Required:

1) Ethanolic KOH (95% ethanol, v/v)
2) Potassium hydroxide [0.5N]
3) Fat solvent
4) Hydrochloric acid[0.5N]
5) Phenolphthalein indicator
6) Sodium carbonate
7) Methyl orange indicator

Procedure:
Titration-I

Standardization of Hydrochloric acid:

Titration-II

Saponification value of an oil/fat:

1) Weigh 1g of fat in a tared beaker and dissolve in about 3ml of the fat solvent [ethanol /ether mixture].
2) Quantitatively transfer the contents of the beaker three times with a further 7ml of the solvent.
3) Add 25ml of 0.5N alcoholic KOH and mix well, attach this to a reflux condenser.

4) Set up another reflux condenser as the blank with all other reagents present except the fat.
5) Place both the flasks in a boiling water bath for 30 minutes .
6) Cool the flasks to room temperature.
7) Now add phenolphthalein indicator to both the flasks and titrate with 0.5N HCl .
8) Note down the endpoint of blank and test.
9) The difference between the blank and test reading gives the number of millilitres of 0.5N KOH required to saponify 1g of fat.
10) Calculate the saponification value using the formula:

Saponification value or number of fat = mg of KOH consumed by 1g of fat.
Weight of KOH = Normality of KOH * Equivalent weight* volume of KOH in litres

Volume of KOH consumed by 1g fat = [Blank – test]ml

Experiment No-4

Determination of iodine value of oil

Introduction:

The iodine value is a measure of the degree of unsaturation in oil. It is constant for particular oil or fat. Iodine value is a useful parameter in studying oxidative rancidity of oils since higher the unsaturation the greater the possibility of the oils to go rancid.

Principle

The oils contain both saturated and unsaturated fatty acids. Iodine gets incorporated into the fatty acid chain wherever the double bond exist. Hence, the measure of the iodine absorbed by an oil, gives the degree of unsaturation. Iodine value/number is defined as the 'g' of iodine absorbed by 100g of the oil.

Materials

» *Hanus Iodine Solution*

Weigh 13.6g of iodine and dissolve in 825mL glacial acetic acid by heating, and cool. Titrate 25mL of this solution against 0.1N sodium thiosulphate. Measure another portion of 200mL of glacial acetic acid and add 3mL of bromine to it. To 5mL of this solution add 10mL of 15% potassium iodide solution and titrate against 0.1N sodium thiosulphate. Calculate the value of bromine solution, to double halogen content of the remaining 800mL of the above iodine solution as follows:

» X = B/C,

Where, X = mL of bromine solution required to double the halogen content,

B = 800 x thiosulphate equivalent of 1mL of iodine solution C = thiosulphate equivalent of one mL of bromine solution.

» 15% Potassium Iodide Solution
» 0.1% Sodium Thiosulphate
» 1% Starch

Procedure

1. Weigh 0.5 or 0.25g of oil into an iodine flask and dissolve in 10mL of chloroform.
2. Add 25mL of Hanus iodine solution using a pipette, draining it in a definite time. Mix well and allow to stand in dark for exactly 30min with occasional shaking.
3. Add 10mL of 15% KI, shake thoroughly and add 100mL of freshly boiled and cooled water, washing down any free iodine on the stopper.
4. Titrate against 0.1N sodium thiosulphate until yellow solution turns almost colorless.
5. Add a few drops of starch as indicator and titrate until the blue color completely disappears.
6. Towards the end of titration, stopper the flask and shake vigorously so that any iodine remaining in solution in $CHCl_3$ is taken up by potassium iodide solution.
7. Run a blank without the sample.

Calculation

The quantity of thiosulphate required for blank minus the quantity required for sample gives thiosulphite equivalent of iodide adsorbed by the fat or oil taken for analysis.

$$\text{Iodine number} = \frac{(B-S) \times N \times 12.69}{\text{Weight of sample (g)}}$$

where,
B = mL thiosulphate for blank
S = mL thiosulphate for sample
N = normality of thiosulphate solution

Amount of fat/oil taken should be adjusted such that the excess iodine in the added 25mL of Hanus iodine solution has about 60% of excess iodine of the amount added, i.e., if (B – S) is greater than B/2, repeat the smaller amount of sample.

Experiment No-5

Differentiate between a reducing and non reducing sugar

Introduction:

Sugar is a type of carbohydrate. There are many different types of sugars. Some sugars are simple sugars with a simple structure. They are known as monosaccharides. Some common examples for monosaccharides include glucose, fructose, and galactose. Some sugars are formed by the bonding of two monosaccharides. They are known as disaccharides. Some common disaccharides are sucrose, maltose, and lactose. What we use as table sugar is obtained from plants such as sugar cane. Sugars can be divided into two groups depending on their chemical behaviors: reducing sugars and nonreducing sugars. The main difference between reducing and nonreducing sugar is that **reducing sugars have free** aldehyde or ketone **groups whereas nonreducing sugars do not have free aldehyde or ketone groups.**

1. **Benedict's Test (positive for reducing sugars)**

Principle:

Benedict's reagent contains cupric ions, which in an alkaline environment, oxidize the aldehyde group to a carboxylic acid. Cupric ions are reduced to cuprous oxide, which forms a red precipitate

$$RCHO + 2Cu^{2+} + 4OH^{-} \longrightarrow RCOOH + Cu_2O + 2H_2O$$

Reagents

- Set up 1 % solutions of:
  - glucose,
  - sucrose,
  - starch,
  - maltose,
  - fructose,
  - lactose.
- Benedict's reagent (ready to use)

Procedure

1. Place 15 drops of the following 1% carbohydrate solutions in separate, labeled test tubes: glucose, fructose, sucrose, lactose, maltose, and starch.
2. Also place 1 ml of distilled water in another tube to serve as a control.
3. To each tube, add 1 ml of Benedict's reagent and heat the tubes in a boiling water bath for 5 minutes.
4. Remove the tubes from water bath. Note and record the results.

❖ In the presence of a reducing sugar a precipitate which may be red, yellow or green will form.

2. **Barfoed's Test**

(Used to distinguish between mono- & di-saccharides)

Principle

Barfoed's reagent reacts with mono-saccharides to produce cuprous oxide at a faster rate than disaccharides do:

$RCHO + 2Cu^{2+} + 2H_2O$ -----> $RCOOH + Cu_2O + 4H^+$

Reagents

❖ Set up 1 % solutions of the following solutions separately:

- glucose,
- maltose,
- fructose,
- lactose,
- sucrose
- Barfoed's reagent.

- **Procedure:**

1. Place 15 drops of the following 1% carbohydrate solutions in separate, labeled test tubes: glucose, fructose, sucrose, lactose, and maltose.
2. To each tube, add 1 ml of Barfoed's reagent, and heat in a boiling water bath for 10 minutes.
3. Remove the tubes from water bath. Note and record your observations.

A red precipitate will form if the test is positive.

Picric Acid Test
(for reducing sugars)

Principle

Picric acid (2,4,6-trinitrophenol) or TNP reacts with reducing sugars to give a red colored picramic acid $C_6H_2.OH.NH_2(NO_2)_2$

Reagents

- Set up 1 % solution of:
- maltose,
- sucrose
- Saturated solution of picric acid
- 1N NaOH solution

Procedure:

1. Into a test tube add 1 ml of maltose solution, into the second tube, 1ml of sucrose solution.
2. Add into each tube 1 ml of a saturated solution of picric acid, and then add into each tube 0.5 ml of sodium hydroxide solution.
3. Heat both samples in a boiling water bath.

❖ In the presence of reducing sugars, the solution stains red; a sodium salt of picric acid is formed.

Experiment No-6

Determine the concentration of glycine solution by formylation method

Principle:

Neutral amino acids like Glycine which contain equal number of amino and carboxylic groups , cannot be estimated by directly titrating with standard sodium hydroxide solution due to the formation of zwitter ions in aqueous solution. Hence, the estimation is done by protecting NH2 group with excess of neutralized formaldehyde solution(Which reacts with the amino group of amino acid , leaving the carboxylic group free) and then titrating with standard sodium hydroxide solution.

$H_3N^+ - CH_2 - COO + OH \rightleftharpoons H_2N - CH_2 - COO + H_2O$
Zwitter ion

$H_2N - CH_2 - COO + HCHO \longrightarrow HOCH_2 - HN - CH_2 - COO \longrightarrow CH_2 = NCH_2COOH + H_2O$

$CH_2 = NCH_2COOH + NaOH \longrightarrow CH_2 = NCH_2COONa + H_2O$

Chemicals required:

AR Oxalic acid---------- 1.6 g/ 250 ml

Sodium hydroxide---------- 4g/1 litre

Formalin solution (40%)---------- 60ml

Phenolphthalein indicator

Glycine---------- 38g/ 1litre

(20 ml volume range may be given to each student to be made up into 100 ml in a standard flask)

Reagents required:

Preparation of standard Oxalic acid solution: About 1.6 g of crystalline Oxalic acid is weighed accurately, transferred into 250 ml standard flask and the solution is made up to the mark with distilled water.

Preparation of neutral formaldehyde solution: To the formaldehyde solution about 5 drops of phenolphthalein indicator is added followed the addition of dilute sodium hydroxide solution till the solution is just faintly pink.

Procedure:

Standardization of sodium hydroxide solution:

20 ml of the standard oxalic acid solution is pipette out in to a clean conical flask, a drop of phenolphthalein indicator is added and the solution is titrated against sodium hydroxide taken in the burette. The end point is the appearance of pale permanent pink colour. From the strength of oxalic acid solution, the strength of sodium hydroxide solution is calculated.

Standardization of glycine solution:

The given glycine solution is made up in to 100ml in a standard flask. 20 ml of the solution is pipette out in to a clean conical flask. About 20 ml of neutral formalin solution is added slowly with constant shaking. 2 or 3 drops of phenolphthalein indicator is added and the solution is titrated against sodium hydroxide solution taken in the butrette. The end point is the appearance of pale permanent pink colour.

Blank titration:

A blank titration is performed with all solutions as described above except glycine solution.

Calculation

From the strength of oxalic acid solution, calculate the strength of sodium hydroxide solution.

Let the strength of sodium hydroxide solution = x N

Let the volume of NaOH reqiuered by glycine solution = V1 ml

Let the blank titre value without glycine solution = V2 ml

Let the volume of glycine solution taken = 20 ml

Therefore ,

Volume of sodium hydroxide required by glycine solution = (V1-V2) ml

Srength of glycine solution $= \frac{N\ NaOH\ X\ V\ NaOH}{V\ Glycine}$

$$= \frac{N\ NaOH\ X\ (V1-V2)}{20!}$$

Amount of glycine present in the given 100 ml of the solution

$$= \frac{\text{N NaOH x (V1}-\text{V2)}}{20}\ X\ \frac{75}{10}\ \text{g}$$

Experiment No-7

Study of titration curve of glycine

Aim:

1. To determine the titration curve for an amino acid.
2. To use this curve to estimate the pKa values of the ionizable groups of the amino acid.
3. To understand the acid base behaviour of an amino acid.

Theory:

Titration curves are obtained when the pH of given volume of a sample solution varies after successive addition of acid or alkali. The curves are usually plots of pH against the volume of titrant added or more correctly against the number of equivalents added per mole of the sample.

This curve empirically defines several characteristics. The precise number of each characteristic depends on the nature of the acid being titrated:

1) the number of ionizing groups, 2) the pKa of the ionizing group(s), 3) the buffer region(s).

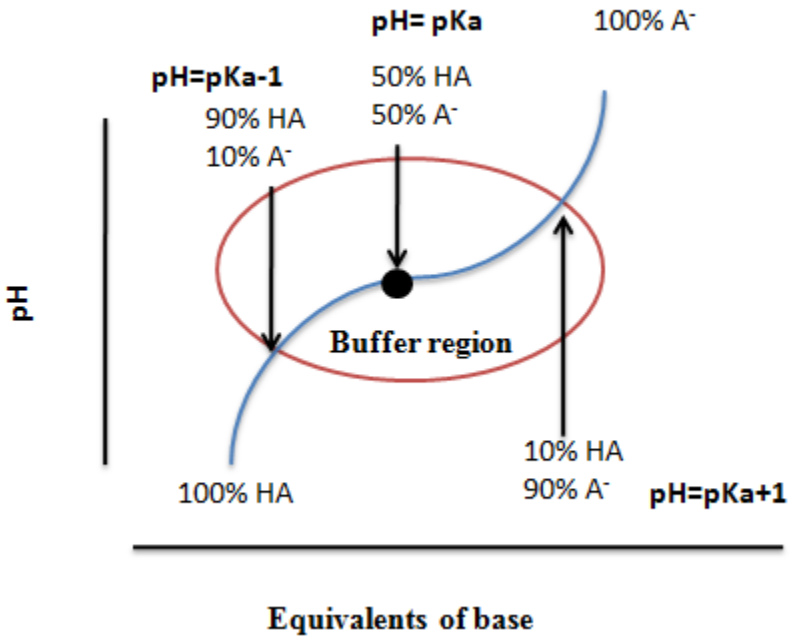


Fig: 1: Titration curve

Amino Acids are weak Polyprotic Acids. They are present as zwitter ions at neutral pH and are amphoteric molecules that can be titrated with both acid and alkali. All of the amino acids have an acidic group (COOH) and a basic group (NH_2) attached to the α-carbon, and also they contain ionizable groups that act as weak acids or bases, giving off or taking on protons when the pH is altered.

The strong positive charge on the amino group induces a tendency for the carboxylic acid group to lose a proton, so amino acids are considered to be strong acids. Some amino acids have other ionizable groups in their side chains and these can also be titrated.

When an amino acid is dissolved in water it exists predominantly in the isoelectric form. The isoelectric point, pI, is the pH of an aqueous solution of an amino acid at which the molecules have no net charge. In other words, the positively charged groups are exactly balanced by the negatively charged groups. When this dissolved amino acid is titrated with acid, it acts as a base, and with base, it acts as an acid which makes them an amphoteric molecule.

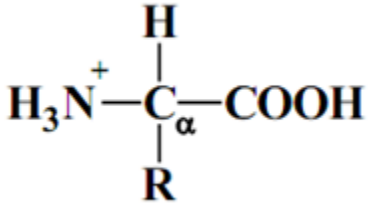
These ionizations follow the Henderson-Hasselbalch equation:

$$pH = pKa + log\frac{[unprotonated\ form\ (base)]}{[Protonated\ form\ (acid)]}$$

When the concentration of the unprotonated form equals that of the unprotonated form, the ratio of their concentrations equals 1, and log 1=0. Hence, pKa can be defined as the pH at which the concentrations of the protonated and unprotonated forms of a particular ionizable species are equal. The pKa also equals the pH at which the ionizable group is at its best buffering capacity; that is the pH at which the solution resists changes in pH most effectively.

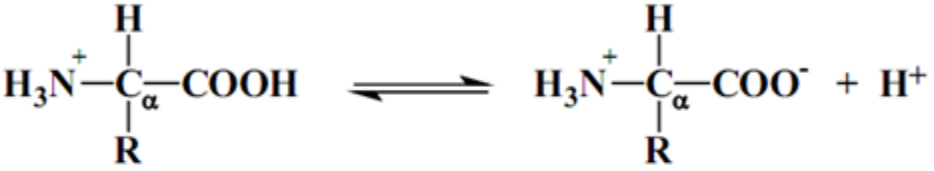
The pK is the pH at the midpoint of the buffering region (where the pH changes only slightly upon addition of either acid or base). The pK is the pH corresponding to the inflection point in the titration curve. The end point of a titration curve represents the observed end of the titration. The isoelectric point (isoelectric pH; pI) is the pH at which the amino acid has a net zero charge. For a simple diprotic amino acid, the pI falls halfway between the two pK values. For acidic amino acids, the pI is given by ½(pK1 + pK2) and for basic amino acids it's given by ½(pK2 + pK3).

In this experiment we are finding out the titration curve of the amino acid Glycine.

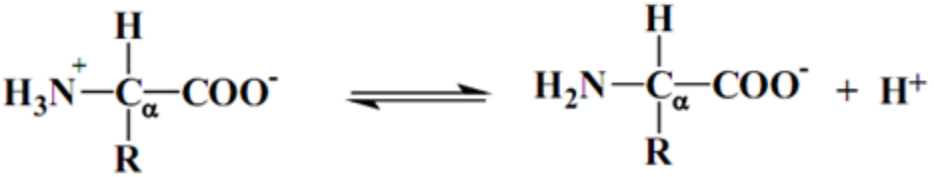


Glycine is a diprotic amino acid which means that it has two dissociable Protons, one on the α amino group and the other on the carboxyl group. In the case of Glycine,the R group does not contribute a dissociable Proton.

Dissociation 1:



Dissociation 2:



The dissociation of proton proceeds in a certain order which depends on the acidity of the proton: the one which is most acidic and having a lower pKa will dissociate first. So, the H+ on the α-COOH group (pKa1) will dissociate before that on the α-NH3 group (pKa2).

Materials Required:

1. 0.1M Hydrochloric acid
2. 0.1M Sodium Hydroxide
3. pH Meter
4. 0.1M Glycine
5. Burette -2
6. Beaker
7. Stirrer
8. Standard Buffer of pH=4, pH= 7, pH=10

Working steps:

1. Pipette out 20ml of the amino acid solution into a 100ml beaker.
2. Standardize the pH meter using the standard buffer solutions.
3. Determine the pH of the amino acid solution.
4. Add 0.3ml of 0.1M HCl from the burette and record the pH after each addition.
5. Continue adding the acid until the pH falls to 1.6

6. Wash thoroughly the pH electrode in distilled water.
7. Take 20 ml of amino acid solution in another beaker and check its pH.
8. Now titrate the amino acid solution by adding 0.3ml of 0.1M NaOH until the pH reaches 12.5.
9. Plot the titration curve using the values recorded and find the pKa values.

Tabular Column:

Volume of NaOH added [ml]	pH =6.22
0.3	
0.3	
0.3	

Volume of HCl added [ml]	pH =6.22
0.3	
0.3	
0.3	

Experiment No-8

Effect of Different Temperatures on the Activity of Salivary Amylase on Starch

Materials Required

Three series of test tubes having iodine solution in each, test tubes, ice cubes, water, 15 ml 1% starch solution + 3 ml 1% NaCl, saliva solution, droppers, thermometer, Bunsen burner and wire gauze.

Real Lab Procedure

- Take beaker containing 15 ml of 1% starch solution + 3 ml of 1% NaCl solution.
- Divide and pour this solution into three test tubes and mark them as A, B and C.
- Maintain the temperature of the beaker containing ice cubes at 5°C.
- Take beaker containing ice cubes and keep it on the table.

- Take another two beakers containing water and heat over the Bunsen burner.
- Now transfer experimental tube A into a beaker containing ice.
- Transfer the second experimental tube B into water bath set at 37°C and third experimental tube C into the beaker maintained at 50°C.
- Using a dropper, take 1 ml saliva solution and transfer the solution into test tube A.
- Similarly, add 1 ml saliva solution into test tube B and test tube C.
- Immediately, using a dropper, take few drops from experimental tube A and transfer this into first series of test tubes having iodine solution.
- Similarly, using fresh droppers, do the same procedure for test tube B and test tube C and transfer the solution into second and third series of test tubes having iodine solution.
- Note this time as zero minute reading.
- After an interval of 2 minutes, again take a few drops from each tube and add to the iodine tubes and note the change in colour of iodine.
- Keep on repeating the experiment at an interval of every 2 minutes till colour of iodine does not change.

Results

It takes less time to reach achromic point at 37°C, as the enzyme is maximum active at this temperature, while at higher and lower temperatures more time is taken to reach the achromic point.

Conclusion

All enzymes are proteinaceous in nature. At lower temperatures, the enzyme salivary amylase is deactivated and at higher temperatures, the enzyme is denaturated. Therefore, more time will be taken by enzyme to digest the starch at lower and higher temperatures. At 37° C, the enzyme is most active, hence, takes less time to digest the starch.

Effect of Different pH on the Activity of Salivary Amylase on Starch

Materials Required

Three series of test tubes having iodine solution in each, test tubes, pH tablets of 5, 6.8 and 8, beaker containing water with thermometer, 15 ml 1% starch solution + 3 ml 1% NaCl, saliva solution, droppers, Bunsen burner and wire gauze.

Real Lab Procedure

- Take a beaker containing 15 ml of 1% starch solution + 3 ml of 1% NaCl solution.
- Divide and pour this solution into three test tubes and mark them as A, B and C.

- Add pH tablet 5 into test tube A, pH tablet 6.8 into test tube B and pH tablet 8 into test tube C.
- Now transfer experimental tube A, B and C into a beaker containing water and a thermometer for recording temperature. Temperature of this beaker is to be maintained at 37°C.
- Using a dropper, take 3 ml saliva solution and add 1 ml of solution to each of the three test tubes.
- Immediately using a dropper, take few drops from experimental tube A and transfer this into the first series of test tubes having iodine solution.
- Similarly, do the same procedure for test tube B and test tube C and transfer the solution into second and third series of test tubes having iodine solution.
- Note this time as zero minute reading.
- After an interval of 2 minutes, again take a drop from each tube and add to the iodine tubes and note the change in colour of iodine.
- Keep on repeating the experiment at an interval of every 2 minutes till colour of iodine does not change.

Results

pH 5 is acidic and pH 8 is alkaline, therefore salivary amylase did not act in these tubes. Whereas, the enzyme acted in the tube with pH 6.8 (i.e., slightly acidic) and digested the starch.

Note: If we add saliva on starch, the salivary amylase present in saliva gradually acts on starch and converts it into maltose. Starch keeps on giving blue colour with iodine till it is completely digested into maltose. At this point, no blue colour is formed. This is the end point or achromic point.