SYLLABUS **Semester-IV**

17CHU414B ANALYTICAL CLINICAL BIOCHEMISTRY - PRACTICAL 3H 1C

Instruction Hours/week: L:0 T:0 P:3 Marks: Internal: 40 External: 60 Total:100

Scope

The course deals with the identification and estimation of carbohydrates, lipids and proteins

Objectives

The course enables the student to

1. Identify and estimate carbohydrates, lipids, iodine number and saponification number of oils
2. Determine proteins and cholesterol

Methodology

Identification and estimation of the following:

1. Carbohydrates – qualitative and quantitative.
2. Lipids – qualitative.
3. Determination of the iodine number of oil.
4. Determination of the saponification number of oil.
5. Determination of cholesterol using Liebermann- Burchard reaction.
6. Proteins – qualitative.
7. Isolation of protein.
8. Determination of protein by the Biuret reaction.
9. Determination of nucleic acids

Suggested Readings

Text Books:

1. Cooper, T.G. (1977). *Tool of Biochemistry*. John Wiley and Sons.
2. Keith Wilson & John Walker.(1994). *Practical Biochemistry*. Cambridge University Press.
3. Alan H Gowenlock,(2005). Varley's. *Practical Clinical Biochemistry*.CBS Publisher.
4. Thomas M. Devlin.(2009). *Textbook of Biochemistry*. Academic Internet Publishers.
5. Berg, J.M., Tymoczko, J.L. & Stryer, L. (2002). *Biochemistry*. W.H. Freeman.

Reference Books

1. Nelson, D. L. & Cox, M. M.(2008). *Lehninger's Principles of Bioch*emistry. 7th Ed. W. H. Freeman.
2. Harwood. (1990). *Series on Analytical Chemistry*. John Wiley & Sons.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)

(Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021

DEPARTMENT OF CHEMISTRY

Name of the Faculty: B. Prabha
Department : CHEMISTRY
Semester IV
Year : II
Course Code : 17CHU414B
Class : B.Sc., Chemistry

PHYSICAL CHEMISTRY PRACTICAL-I

Total no. of hour's: 10 Hours

S. No.	Duration Hours	Name of the Experiment	Support Material
1.	3	Introduction , Procedure writing and demonstration	
2.	3	Carbohydrates- Qualitative and quantitative method	T_2 & T_4
3.	3	Lipids- Qualitative Test	T_2 & T_4
4.	3	Determination of the iodine number of oil.	T_2 & T_4
5.	3	Determination of the saponification number of oil.	T_2 & T_4
6.	3	Determination of cholesterol using Liebermann- Burchard reaction.	T_2 & T_4
7.	3	Proteins – qualitative, Determination of protein by the Biuret reaction.	T_2 & T_4
8.	3	Determination of nucleic acids	T_2 & T_4
9.	3	Revision	
10.	3	Model Exam	

Suggested Materials:

1. T_1: Keith Wilson & John Walker.(1994). *Practical Biochemistry*. Cambridge University Press.
2. T_2: Alan H Gowenlock,(2005). Varley's. *Practical Clinical Biochemistry*.CBS Publisher.
3. T_4: Thomas, M.Delvin, (2009). *Text book of biochemistry.*

ANALYTICAL CLINICAL BIOCHEMISTRY- LAB MANUAL

FOR

III B.Sc., CHEMISTRY STUDENTS

DEPARTMENT OF CHEMISTRY

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)

(Established Under section 3 of UGC Act, 1956)

Eachanari Post, Pollachi main road,

Coimbatore-641021

Tamilnadu, India

CONTENS

QUALITATIVE TEST FOR CARBOHYDRATES, LIPIDS, AMINO ACIDS AND PROTIEN

Qualitative test 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 monosacchraides and disaccharides. It indicates the presence of polysaccharides.	_____
2	MOLISCH'S TEST: To 2.0 ml of the sugar solution added 2-3 drops of Molisch's reagent, mixed well and added 2.0 drops of Conc.H_2SO_4 along the sides of the test tube. So that 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 furfurol by concentrated sulphuric acid.The hydroxyl methyl furfurol then condenses with □-naphthol to give a violet coloured ring shows the presence of carbohydrates.
3	IODINE TEST: To 1.0 ml of the test solution aadded 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 2.0 ml of Fehling's reagent added 0.5 ml 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 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.
5	BENEDICT'S TEST: T o 2.0 ml 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 coloured cuprous oxide.Depending on the concentration of sugars,yellow to green colour is developed.
6	PICRIC ACID TEST:	a)A mahagony red	It confirms the	It is due to the reducing

	To 2.0 ml of sugar solution added 0.5 ml of saturated picric acid and 0.5 ml of 10% sodium carbonate and heated in a water bath.	coloured solution was got. b)No characteristic red coloured solution was got.	presence of reducing sugar. It shows the absence of reducing sugar and confirms the presence sucrose which is a non reducing sugar.	action of the sugar.
7	BARFOED'S TEST: To 2.0 ml of freshly prepared Barfoed's reagent added 1.0 ml 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 ed copper(II) oxide precipitate is formed will indicates the presence of reducing sugar.This test is specific for monosaccharides.
8	SALIWANOFF'S TEST: To 3.0 ml of Saliwanoff's reagent added 4 drops of sugar solution and heated in a water bath for 3 minutes and cooled the solution at room temperature.	a)A cherry red colour was got. b)No characteristic cherry red colour was got.	It indicates the presence of keto sugar sucrose and fructose. It indicates the absence of keto sugar sucrose and fructose.	It indicates the presence of keto sugar sucrose and fructose which on treatment with HCl acid forms 5 hydroxyl methyl furfural which then condenses 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 0.5 ml of the test solution and heated in a boiling water	a)A greenish blue coloured solution was got. b)No characteristic greenish blue	It indicates the presence of pentose. It indicates the presence of	Bial's test is used to distinguish between pentoses and hexoses.They react with Bial's reagent and are

	bath for 3 minutes.	coloured solution .	hexoses.	converted to furfural.Orcinol and furfural condense in the presence of ferric ion to form a colored product.Appearance of green colour precipitate indicates the presence of pentoses and formation of muddy brown precipitate shows the presence of hexoses.
11	MUCIC AID TEST: To 50 mg of sugar in a test tube added 1.0 ml of concentrated nitric acid and 1.0 ml 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.	a)A white precipitate was got and a colourless rod shaped crystals were seen under microscope. b)No characteristic white precipitate was formed.		
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.		

ESTIMATION OF GLUCOSE (BERTRAND'S METHOD)

Weight of the $K_2Cr_2O_7$ present in the whole of the given solution = W g

Normality of standard $K_2Cr_2O_7$ $= \frac{W \times 10}{Eq.mass\ of\ K_2Cr_2O_7}$

$= \frac{W \times 4}{49}$

N_1 = ------- N

= y N

Calculation:

Burette Solution: $KMnO_4$

Pipette Solution: $K_2Cr_2O_7$

Reagents: dil.H_2SO_4

Indicator: Self ($KMnO_4$)

S.No.	Volume of $K_2Cr_2O_7$ solution	Burette Reading (ml)		Volume of $KMnO_4$ Solution (ml)	Concordant Value (ml)
		Initial	Final		
	x				z

Volume of Potassium dichromate Solution(V_1) = x ml

Normality of Potassium dichromate Solution (N_1) = y N

Volume of Potassium permanganate Solution (V_2) = z ml

Normality of Potassium permanganate Solution (N_2) = V_1N_1 / V_2

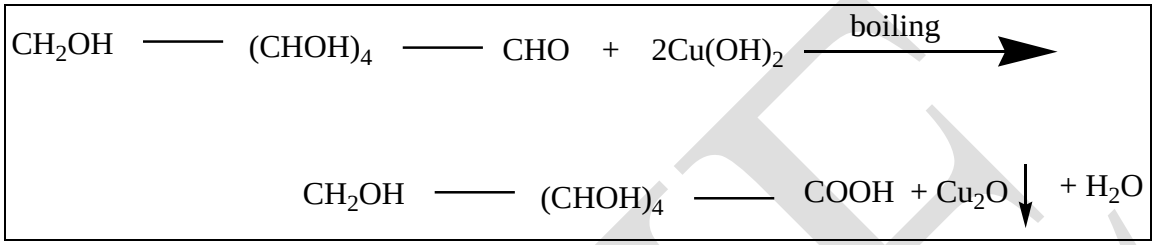
= x X y / z

= a N.

ESTIMATION OF GLUCOSE (BERTRAND'S METHOD)

Principle:

Glucose is a reducing sugar. When glucose is boiled with an excess of alkaline copper hydroxide, it is oxidized to gluconic acid and $Cu(OH)_2$, gets reduced to cuprous oxide.



The precipitated red-coloured Cu_2O is dissolved in a warm acidic solution of ferric alum. Cu_2O reduces ferric sulphate (in ferric alum) to ferrous sulphate. The reduced $FeSO_4$ is titrated against standard $KMnO_4$.

$$Cu_2O+Fe_2(SO_4)_3+H_2SO_4 \rightarrow 2CuSO_4+2FeSO_4+H_2O$$

$$10FeSO_4+8H_2SO_4+2KMnO_4 \rightarrow K_2SO_4+2MnSO_4+5Fe_2(SO_4)_3+8H_2O.$$

$2Cu \equiv 2FeSO_4 \equiv$ 2 liters of 1N $KMnO_4$

1 atom of copper (63.6g) ≡ 1liter of 1N $KMnO_4$

1000ml of 1N $KMnO_4$ ≡ 63.6g of copper

1ml of N/10 $KMnO_4$ ≡ 0.00636g of copper

≡ 6.36mg of copper

From the amount of copper, the equivalent amount of sugar is calculated from the given conversion table.

CHEMICALS REQUIRED

AR Oxalic acid =1.6 g/250 ml water

Potassium permanganate =0.75 g/250 ml of water (about N/10)

Cupric sulphate (Fehling A) =8 g/200 ml of water

CALCULATION:

Burette Solution: $KMnO_4$

Pipette Solution: Unknown Solution

Reagents: dil.H_2SO_4

Indicator: Self ($KMnO_4$)

S.No.	Volume of $K_2Cr_2O_7$ solution	Burette Reading (ml)		Volume of $KMnO_4$ Solution (ml)	Concordant Value (ml)
		Initial	Final		
	X				**z**

Calculate the strength of $KMnO_4$ from the standard oxalic acid = a N

Volume of sugar solution taken = 20 ml

Volume of $KMnO_4$ required to react with the reduced $FeSO_4$ = z ml

1 ml of N/10 $KMnO_4$ = 6.36 mg of Cu

A ml of standard $KMnO_4$

$$= \frac{6.36 * A * \text{strength of } KMnO_4}{0.1}$$

=B mg of Cu

From the conversion table, the glucose equivalent is noted corresponding to Cu equivalent.

B mg of Cu = Z mg of sugar

20 ml glucose solution contains Z mg of sugar.

Amount of glucose present in 100 ml of the made up solution = 100/20 Z

Rochelle salt - 40 g
Caustic soda - 30 g } (Fehling B) = dissolved in 200ml water

Ferric alum - 24 g
Con. H_2SO_4 - 20 ml } = dissolved in 200ml

Dil. H_2SO_4 – 100ml, AR Glucose = 13 g/1 liter of water

(20 ml volume range may be given to each student in a bottle)

PROCEDURE

TITRATION I: Standardisation of potassium permanganate solution.

A standard solution of N/10 oxalic acid is prepared by weighing about 1.6 g of analar acid crystals (equivalent weight: 63) accurately in a chemical balance and dissolving in water. The solution is then made upto 250 ml in a standard flask.

20 ml of standard oxalic acid solution is pipette out in a clean 250 ml conical flask. 20 ml of dilute sulphuric acid is added 60^0C. The hot solution is then titrated against $KMnO_4$ solution taken in the burette. The end point is the appearance of pale permanent pink colour. The titration is repeated to get concordant values. The strength of $KMnO_4$ is calculated.

$2MnO_4^- + 16H^+ + 5C_2O_4^{2-} \rightarrow 2Mn^{2+} + 10CO_2 + 8H_2O$

TITRATION II: Estimation of Glucose

The given glucose solution in the bottle is quantitatively transferred and made upto 100 ml in a standard flask. 20 ml is pipette out into a clean 250 ml conical flask. About 20 ml of $CuSO_4$ solution (Fehlings Solution A) is added, followed by the addition of 20 ml of tartrate solution (Fehlings Solution B). The solution is then heated to boiling, maintained at this temperature for 2-3 mins. The red cuprous oxide precipitate formed is allowed to settle. The clear supernatant liquid is carefully decanted.

The precipitate is washed with water to remove the excess of $CuSO_4$ solution (it is advisable to filter the precipitate through G-4 sintered glass crucible). To the precipitate, acidified ferric alum is added slowly with shaking, till the red precipitate completely dissolves. About 20ml of

dil.H_2SO_4 is added and the solution is titrated against $KMnO_4$ taken in a burette. The end point is the appearance of pale permanent pink colour. A duplicate is also conducted.

Result:

The amount of glucose present in the whole of the given solution = g

IODINE VALUE OF AN OIL(HANUS METHOD)

Weight of the $K_2Cr_2O_7$ present in the whole of the given solution = W g

Normality of standard $K_2Cr_2O_7$ $= \frac{W \times 10}{Eq.mass\ of\ K_2Cr_2O_7}$

$= \frac{W \times 4}{49}$

N_1 = ------- N

= y N

Calculation:

Burette Solution: Sodium thiosulphate

Pipette Solution: $K_2Cr_2O_7$

Reagents: dil.H_2SO_4

Indicator: Starch

S.No.	Volume of $K_2Cr_2O_7$ solution	Burette Reading (ml)		Volume of thio Solution (ml)	Concordant Value (ml)
		Initial	Final		
	x				z

Volume of Potassium dichromate Solution (V_1) = x ml

Normality of Potassium dichromate Solution (N_1) = y N

Volume of Sodium thiosulphate Solution (V_2) = z ml

Normality of Sodium thiosulphate Solution (N_2) = V_1N_1 / V_2

= x X y / z

= a N.

IODINE VALUE OF AN OIL(HANUS METHOD)

Aim:

To determine the iodine value of given oil

Principle

Iodine value is an defined as the number of parts by weight of iodine reacting with 100 parts by weight of an oil or fat. The drying power of an oil is generally proportional to its iodine value. Cocount oil(a non-drying oil) has low iodine value of 6-10; linseed oil (a drying oil) has high iodine of 77-88 value. Iodine value indicates the degree of unsaturation of the fatty acids present in the oil or fat. The determination of the iodine value is of great importance in characterizing an oil ,and also in finding the proportion of an adulterant in a sample of the oil.

In Hanus method of determination of the iodine value, a known weight of oil is dissolved in CCl_4, and treated with a known excess volume of iodine monobromide solution. The unused IBr is back titrated against standard sodium thiosulphate solution.

Chemicals required

AR potassium dichromate = 1.2 g/ 250 ml water

Sodium thiosulfate =6g/250 ml water (about N/10)

Potassium iodide =10g/ 100ml water

Starch solution (freshly prepared) =10ml

IBr – Iodine (13.2g/ 1 litre HOAc) = 3ml liquid bromine.(100ml required for each student)

Carbon tetrachloride = 20ml.

Procedure

Titration 1: Standardisation of thio

A standard solution of N/10 $K_2Cr_2O_7$ is prepared by weighing about 0.49 g of analr crystals (eq.wt = 49)accurately in a chemical balance, dissolving in water and making up to 100ml in a standard flask.

20 ml of standard $K_2Cr_2O_7$ solution is pipetted out into a clean 250 ml conical flask. About 5 ml con.HCl is added, followed by 10 ml of 10% aq. KI solution. The liberated iodine is immediately titrated against thio taken in a burette. When the solution acquires a straw-yellow colour, 1 ml of freshly prepared starch is added and the titration is continued (in drops) with constant shaking. The end point is the change in colour from blue to green (due to Cr^{3+}). The titration is repeated to get concordant values. The strength of thio is calculated.

$$Cr_2O_7^{2-} + 14H^+ + 6I^- \rightarrow 2Cr^{3+} + 7H_2O + 3I_2$$

$$2S_2O_3^{2-} + I_2 \rightarrow 2I^- + S_4O_6^{2-}$$

Calculation:

Burette Solution: Sodium thiosulphate

Pipette Solution: $K_2Cr_2O_7$

Reagents: dil.H_2SO_4

Indicator: Starch

S.No.	Volume of $K_2Cr_2O_7$ solution	Burette Reading (ml)		Volume of thio Solution (ml)	Concordant Value (ml)
		Initial	Final		
	x				z

Volume of Sodium thiosulphate Solution (V_2) = z ml

Normality of Sodium thiosulphate Solution (N_2) =

Calculate the strength of ratio.

Let A ml be the volume of $Na_2S_2O_3$ required for the excess IBr solution.

Let B ml be the blank titre value

Iodine value = (B-A) * 127/1000* 100/w * standard of thio, where w is the weight of oil taken, and 127 is the atomic weight of iodine.

Titration 2 Determination of iodine value

About 0.5 to 1g of the given oil weighed accurately in a chemical balance and dissolved in 10ml of CCl_4 in a stopper 500ml bottle. 25 ml of iodine monobromide solution is burette out into a bottle and the time is noted. The resulting mixture, if turbid is cleared by adding a small additional known volume of CCl_4. The stopper is moistened with a few drops of aq. KI solution and inserted into the bottle. The bottle is kept aside for about 40-60 minutes, with occasional shaking. Then, the reaction mixture is diluted with 200ml of water, followed by the addition of 20ml of 10% aq. KI solution. The mixture is titrated against thio taken in a burette, using starch as a indicator, added near the end point (disappearance of blue colour). A duplicate is also conducted,

A blank titration is carried out without the oil, using exactly the same quantity of CCl_4 (10 ml lus any additional amount used) and 25ml of iodine monobromide solution, using starch as an indicator.

Result

The Iodine Value of oil is =

SAPONIFICATION VALUE OF AN OIL

Principle:

The saponification value is defined as the number of milligram of KOH required for the hydrolysis of 1g of an oil or a fat. To determine the saponification value, a weighed quantity of the given oil is refluxed with a known volume of alcoholic potassium hydroxide solution. The unused alkaline is then back-titrated against standard acid.

Saponification values of some common oils are given below

(1) Mustard oil = 174	(5) Linseed oil = 188-195
(2) Castor oil = 175-183	(6) Gingelly oil = 188-193
(3) Olive oil = 185-196	(7) Cotton Seed oil = 194-196
(4) Groundnut oil = 186-194	(8) Coconut oil = 253-262

Chemicals required:

AR sodium carbonate crystals = 7 g/ 250 ml water.

Alcoholic potash (KOH) = 6 g dissolved in 200 ml rectified sprit (about 0.5N)

Phenolphthalein

HCl (about N/20) = 10 ml con. HCl in 200 ml water.

Procedure :

Titration I: Standardisation of HCl.

About 7 g of analar sodium carbonate crystals (eq. wt = 53) are weighed accurately in a chemical balance, dissolved in water, the solution is made upto 250 ml in standard flask. 20 ml is pipetted out into a clean conical flask. About 2 drops methyl orange indicator are added and titrated against hydrochloric acid taken in the burette (till the light pink colour appears). The titration is repeated to get concordant values. The strength of HCl solution is calculated.

Titration II: Determination of saponification value of an oil.

About 1-2 g of the given oil weighed accurately in a chemical balance and transferred into 250 ml round-bottomed flask. 25 ml of alcoholic potash (about N/2) is added from the burette slowly into the flask. It is then fitted with an air condenser, and heated on a water-bath for about 30 minutes. A blank is run simultaneously with the same quantity of alcoholic potash, but without the oil. Both the flask are cooled, and titrated against standard HCl solution using about 1 ml of phenolphthalein indicator. The end point is the disappearance of pink colour.

Calculation:

Calculate the strength of HCl solution (Titration I).

Let w, g be the weight of oil taken.

Let A ml be the volume of HCl required for excess alcoholic potash.

Let B ml be the blank titer value.

1000 ml of 1N HCl = 56.1 g of KOH.

1 ml 1 N HCl = 56.1 mg of KOH.

Saponification value = $\frac{56.1 * (B-A) * \text{str. Of HCl}}{w}$

Result

The Saponification value of oil=

Qualitative tests for amino acids and protein

S.No:	EXPERIMENT	OBSERVATION	INFERENCE
1.	SOLUBILITY TEST: a. Cold water b.Hot water c. Dilute potassium hydroxide d. Dilute Ammonium hydroxide e. Dilute hydrochloride 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 acids may be Arginine, Methionine, Cysteine, Tryptophan, Tyrosine or Histidine. The amino acid may be Tryptophan or Histidine. The amino acid may be Arginine, Methionine, Csteine or Tryosine.
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% hydrochloric acid and then cool in ice. Add 1mll of 5% sodium nitrite solution and leave in cold water for 3minutes. Make the solution alkaline by the addition of 2ml of 1% sodium carbonate solution and note the colour formed.	a.A red colourationwas got. b.No characteristic red colouration was got.	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 absemce of aromatic acids acid tyrosine, tryptophan or histidine.
4.	ACETIC ACID: To 2ml of amino acid solution add 1% of bromine in 33% acetic acid until an yellow colour was got. After 10	a.A dark blue or violet coloured solution was got.	This confirms the presence of Histidine.

	minutes ad 5 to 10 drops of 5% ammonium carbonate solution.	b.No characteristic blue colour was got.	This shows the absence of Histidine.
5.	HOPKINS COLE TEST: To 1ml of amino acid solution add 2ml of glacial acetic acid (exposed to sum 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. Thisconfirms the presence of tryptophan. It shows the absence of tryptophan.
6.	VOISENT RHODE TEST: Add 1ml of the 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 3-4drops 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. b.No characteristic 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. It shows the absence of tryptophan.
9.	HOFFMANN'S TEST (OR) MILLONS TEST: To 1ml of amino acid	a.A deep red	It is due to the presence of

	solution add 1 drop of Millon's reagent and place it in a water bath for 30 seconds.	colouration was got. b.No red colouration was got.	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 solution add an equal volume of Folins 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 ofamino acid solution add an equal volume of concetratd 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 the 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 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 violent 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 f cysteine. It shows the absence of cysteine.
13.	NITROPRUSSIDE TEST: Treat 2ml of amino acid solution with o.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 thol group. This indicates the presence of cysteine.
14.	LEAD ACETATE TEST: To 2ml of amino acid solution add 1ml of 10% aqueous lead acetate and 1ml of 40% sodium hydroxide. Heat in a water bath for 5 minutes.	a.Ablack precipitate was got. b.No characteristic black precipitate was got.	It is due to the presence of thiol group which indicates the presence of cysteine. It shows the absence of cysteine.
15.	BOILING MODIFICATION (OR) Mc CARTHY SULLIVAN TEST:	a.A red colouration	The methyl group is split to

	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^{0}C for 15 minutes and then added hydrochloric acid and let it stand at room temperature for 10 minutes.	was got.	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 TEST: Take a small amount of oil on a piece of paper.	A greesy spot penetrating the pape 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 2ml of dilute salt solution and few drops of unknown solution is added and shaken vigorously for 1 minute.	Minutes droplets suspended in the liquid. No droplets suspended in the liquid.	Presence of oil or lipids. Absence of oil or lipid.
4.	SAPONIFICATION TEST: Take 1ml 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	Soap formation No soap formation	Indicates the presence of fatty acids Absence of fatty acids.

	warming and shake up gently with a little distilled water.		
5.	TEST FOR UNSATURATED FATTY ACIDS: A clean test tube containing 5ml 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 to 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 5ml of conc. H_2SO_4 mix well.	Brown colour is changed to blue. No colour change.	Indicates the presence of glycerol. Absence of glycerol.