Semester-IV

18CHU414B ANALYTICAL CLINICAL BIOCHEMISTRY-PRACTICAL 3H 1C

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

End Semester Exam: 3 hrs

Course outcome

The course enables the student to

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

Course outcome

The student understood

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

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

- 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.
- 6. Nelson, D. L. & Cox, M. M.(2008). *Lehninger's Principles of Bioch*emistry. 7th Ed.W. H. Freeman.
- 7. 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 Semester IV Course Code : 18CHU414B Department: CHEMISTRYYear: IIClass: B.Sc., Chemistry

PHYSICAL CHEMISTRY PRACTICAL-I

Total no. of hour's: 10 Hours

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

Suggested Materials:

- 1. T₁: Keith Wilson & John Walker.(1994). *Practical Biochemistry*. Cambridge University Press.
- 2. T₂: Alan H Gowenlock,(2005). Varley's. *Practical Clinical Biochemistry*.CBS Publisher.

ANALYTICAL CLINICAL BIOCHEMISTRY- LAB MANUAL

FOR

II B.Sc., CHEMISTRY STUDENTS

B. Prabha



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

Ex.NO	NAME OF THE EXPERIMENT
1	Carbohydrates- Qualitative method
2	Carbohydrates- Quantitative method
3	Lipids- Qualitative Test
4	Determination of the iodine number of oil.
5	Determination of the saponification number of oil.
6	Determination of cholesterol using Liebermann- Burchard reaction.
7	Proteins – qualitative,
8	Determination of protein by the Biuret reaction.
9	Determination of nucleic acids

QUALITATIVE TEST FOR CARBOHYDRATES, LIPIDS, AMINO ACIDS AND PROTIEN

Qualitative test for carbohydrates

S.	EXPERIMENT	OBSERVATION	INFERENCE	REACTION
No				
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 ₂ SO 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 D-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	a a law and a a lustice	and a set	action of the sugar
	10 2.0 ml ol sugar	coloured solution	presence of	action of the sugar.
	solution added 0.5 ml of	was got.	reducing sugar.	
	saturated picric acid and	b)No characteristic	It shows the	
	0.5 ml of 10% sodium	red coloured	absence of reducing	
	carbonate and heated in a	solution was got.	sugar and confirms	
	water bath.		the presence	
			sucrose which is a	
			non reducing sugar.	
7	BARFOED'S TEST:	a)A brick red	It indicates the	Barfoed's test is used to
	To 2.0 ml of freshly	precipitate is	presence of	detect the presence of
	prepared Barfoed's	formed and	monosaccharides.	monosaccharide(reducing)
	reagent added 1.0 ml of	settling down at		sugars in
	sugar solution and heated	the bottom or sides		solution Barfoed's
	in a boiling water bath for	of the test tube		reagent a mixture of
	3 minutes	b)No characteristic		ethanoic(acetic)acid and
	Allow to cool	red precipitate was	It shows the	copper(II) acetate is
		got	absence of	combined with the test
		500	monosaccharidas	solution and boiled A ad
			monosaccharides.	coppor(II) ovide
				copper(II) oxide
				indicates the presence of
				indicates the presence of
				reducing sugar. This test
				is specific for
			T. J. J.	monosaccharides.
8	SALIWANOFF'S TEST:	a)A cherry red	It indicates the	It indicates the presence of
	To 3.0 ml of Saliwanoff's	colour was got.	presence of keto	keto sugar sucrose and
	reagent added 4 drops of		sugar sucrose and	fructose which on
	sugar solution and heated		fructose.	treatment with HCl acid
	in a water bath for 3	b)No characteristic	It indicates the	forms 5 hydroxyl methyl
	minutes and cooled the	cherry red colour	absence of keto	furfural which then
	solution at room	was got.	sugar sucrose and	condenses with resorcinol
	temperature.		fructose.	to give cherry red
				coloured complex.
9	TOLLEN'S TEST:	a)A red coloured	It confirms the	
	To equal volume of	solution was got.	presence of	
	sugar solution and		pentose, lactose and	
	concentrated hydrochloric		galactose.	
	acid added a pinch of	b)No characteristic	It shows the	
	phloroglucinol and heated	red coloured	absence of	
	in a boiling water bath for	solution was got.	pentose, lactose and	
	2 minutes.	6	galactose.	
10	BIAL'S TEST	a)A greenish blue	It indicates the	Bial's test is used to
	To 2 0ml of Bial's	coloured solution	presence of	distinguish between
	reagent added 0.5 ml of	was got	pentose	pentoses and
	the test solution and	h)No characteristic	It indicates the	hexoses They react with
	heated in a hoiling water	greenish hlue	nresence of	Bial's reagent and are
	neated in a boining water	greemsn blue	presence of	Dial S leagent and are

product.Appe	ed
green colour p	earance of
indicates the p	precipitate
pentoses and	presence of
muddy brown	formation of
shows the pre	n precipitate
hexoses.	esence of
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. 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. d)A small sunflower shaped crystals were observed. d)A small sunflower shaped crystals were observed. d)A rod shaped	

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 K₂Cr₂O₇

 N_1

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

----- N

 $=\frac{W\times 10}{Eq.mass of K_2 Cr_2 O_7}$

Calculation:

Burette Solution: KMnO₄

Pipette Solution: K₂Cr₂O₇

Reagents: dil.H₂SO₄

Indicator: Self (KMnO₄)

S.No.	Volume of K ₂ Cr ₂ O ₇ solution	Burette Reading (ml)		Volume of KMnO ₄	Concordant Value (ml)
		Initial	Final	Solution (ml)	
	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 (N₂) = V_1N_1 / V_2

$$= \mathbf{x} \mathbf{X} \mathbf{y} / \mathbf{z}$$

= 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 FeSO₄ is titrated against standard KMnO₄.

$-C_{22} \cap +E_{22} (S \cap) + U S \cap -$	$\sim 2C_{11}$ S C_{-1} $2C_{2}$ S C_{-1} U_{-1} C_{-1} C_{-1} U_{-1} U_{-1} C_{-1} U_{-1} U_{-1} C_{-1} U_{-1} U_{-1} C_{-1} U_{-1} $U_{$
$-C_{12}O_{+}F_{22}O_{12}O_{12}+D_{22}O_{12}O_{12}$	^ Zしれるしれキンドせいしれキロ?し

 $10FeSO_4 + 8H_2SO_4 + 2KMnO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 5Fe_2(SO_4)_3 + 8H_2O_4$

$2Cu \equiv 2FeSO_4$	\equiv 2 liters of 1N KMnO ₄
1 atom of copper (63.6g)	\equiv 1liter of 1N KMnO ₄
1000ml of 1N KMnO ₄	\equiv 63.6g of copper
1ml of N/10 KMnO ₄	\equiv 0.00636g of copper
	\equiv 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: KMnO₄

Pipette Solution: Unknown Solution

Reagents: dil.H₂SO₄

Indicator: Self (KMnO₄)

S.No.	Volume of K ₂ Cr ₂ O ₇ solution	Burette Reading (ml)		Volume of KMnO ₄	Concordant Value (ml)
		Initial	Final	Solution (ml)	
	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
$$\left. \begin{array}{c} \text{(Fehling B)} \\ \text{Ferric alum - 24 g} \\ \text{Con. H}_2\text{SO}_4 - 20 \text{ ml} \end{array} \right\}$$
 $\left. = \text{dissolved in 200ml} \right.$

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° C. The hot solution is then titrated against KMnO₄ 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 KMnO₄ 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 KMnO₄ 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 K₂Cr₂O₇

 N_1

= y N

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

----- N

 $=\frac{W\times 10}{Eq.mass of K_2 Cr_2 O_7}$

Calculation:

Burette Solution: Sodium thiosulphate

Pipette Solution: K₂Cr₂O₇

Reagents: dil.H₂SO₄

Indicator: Starch

S.No.	Volume of K ₂ Cr ₂ O ₇ solution	Burette Reading (ml)		Volume of thio Solution	Concordant Value (ml)
		Initial	Final	(ml)	
	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 $(N_2) = V_1N_1 / V_2$

$$= \mathbf{x} \mathbf{X} \mathbf{y} / \mathbf{z}$$

= 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 CCl₄, 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: K₂Cr₂O₇

Reagents: dil.H₂SO₄

Indicator: Starch

S.No.	Volume of K ₂ Cr ₂ O ₇ solution	Burette Reading (ml)		Volume of thio Solution	Concordant Value (ml)
		Initial	Final	(ml)	
	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 oi	1 = 194-196
(4) Groundnut of	1 = 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	= <u>56.1 * (B-A) * str. Of HCl</u>

W

Result

The Saponification value of oil=

	S.No:	EXPERIMENT	OBSERVATION	INFERENCE
ſ	1.	SOLUBILITY TEST:		
		a. Cold water	a.Insoluble	The amino acid may be
			b.Soluble	The amino acid may be Arginine, Methionine,
		b.Hot water	Soluble	Cysteine, Tryptophan or Histidine.
		a Diluta potassium	Solubla	The amino acids may be
		hydroxide	Soluble	Cysteine Tryptophan
		d. Dilute Ammonium	Soluble	Tyrosine or Histidine.
		hydroxide	a.Soluble	The amino acid may be
		e. Dilute hydrochloride acid	b.Insoluble	Tryptophan or Histidine.
		f. Alcohol		The amino acid may be
				Arginine, Methionine,
		, i i i i i i i i i i i i i i i i i i i		Csteine or Tryosine.
-	2.	NINHYDRIN TEST:		
		To 5ml of amino acid	A violet colouration	This is due to the formation
		solution taken in a test tube,	was got	of Rheumann's purple which
		add 0.5ml of 1% ninhydrin		indicates the presence of
		reagent. Place the test tube in a		amino acid.
		boiling water bath for 1-2		
		minutes and cool to room		
ŀ	3.	PAULY'S DIAZO TEST:		
		To 2ml of amino acid	a.A red	The amino acid with
		solution add 1ml of 1%	colourationwas got.	diazotized sulphanilic acid in
		sulphanilic acid in 10%		alkaline medium forms the
		hydrochloric acid and then		highly coloured azo
		cool in ice. Add Imll of 5%		compound. This indicates the
		leave in cold water for		acid tyrosine, tryptophan or
		3 minutes. Make the solution	b.No characteristic red	histidine.
		alkaline by the addition of 2ml	colouration was got.	It shows the absemce of
		of 1% sodium carbonate		aromatic acids acid tyrosine,
		solution and note the colour		tryptophan or histidine.
-		formed.		
	4.	ACETIC ACID:		
		10 2ml of amino acid	a.A dark blue or violet	Inis confirms the presence of
		33% acetic acid until an vellow	got	
		colour was got. After 10	500	

Qualitative tests for amino acids and protein

	minutes ad 5 to 10 drops of 5%	b.No characteristic	This shows the absence of
_	ammonium carbonate solution.	blue colour was got.	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 	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.
		violet coloured ring	
		was got.	
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.	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.
		b.No characteristic red violet coloured ring was got.	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 	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.
		was got.	
8.	EHRLICH'S TEST: Add 2ml of ehrlich's reagent to 2ml of the amino acid solution.	a.A red colouredsolution was got.b.No characteristic red	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
		coloured solution was	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	colouration was got.	phenolic hydroxyl group. This test confirms the
	bath for 30 seconds.	b.No red colouration was got.	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 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 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 precipitatewas got.b.No characteristicblack precipitate wasgot.	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 amine acid	was got	form homogystaing which
To this of the annuo acid	was got.	form nonocysteme which
solution, add the following		gives a red colour with
reagents in order and mix after		sodium nitroprusside
each addition. Add 1.5ml of		solution. It confirms the
0.5N sodium hydroxide, 1.5ml		presence of methionine.
of 1% glycine, 0.3ml of 10%		
sodium nitro prusside solution		
and place it in a incubator at		
$37-40^{\circ}$ C for 15 minutes and		
then added hydrochloric acid		
and let it stand at room		
temperature for 10 minutes.		

Qualitative tests for lipids

S.No:	EXPERIMENT	OBSERVATION	INFERENCE
1.	GREESE TEST:		
	Take a small amount of oil	A greesy spot	Indicates the presence of
	on a piece of paper.	penetrating the pape	lipids
		will be formed.	
		No greesy spot was	
		formed.	Absence of lipids.
2.	TEST FOR FREE FATTY		
	ACIDS:	Disappearance of	Presence of fatty acids since
	Take a few drops of	colour.	the alkali is neutralized.
	phenolphthalein solution in a	No disappearance of	Absence of fatty acids.
	test tube and in it one to two	colour.	
	drops of very dilute alkaline		
	solution just sufficient to give		
	the solution a pink colour. Now		
	add a few drops of oil and		
	shake.		
3.	EMULSIFICATION:		
	Take a clean dry test tube	Minutes droplets	Presence of oil or lipids.
	add 2ml of dilute salt solution	suspended in the	
	and few drops of unknown	liquid.	Absence of oil or lipid.
	solution is added and shaken	No droplets suspended	
	vigorously for 1 minute.	in the liquid.	
4.	SAPONIFICATION TEST:		
	Take 1ml of unknown	Soap formation	Indicates the presence of fatty
	solution in a test tube and add		acids
	an equal amount of alcoholic		
	KOH solution, mix them	No soap formation	Absence of fatty acids.
	thoroughly and keep the		
	mixture during the colour of		

		•	
	warming and shake up gently		
	with a little distilled water.		
5.	TEST FOR UNSATURATED		
	FATTY ACIDS:		
	A clean test tube containing	Appearance of	Indicates the presence of
	5ml of CCl_4 and add a drop of	permanent yellowish	unsaturated fatty acids.
	lipid or oil, now bromine water	red colour.	
	is added drop by drop and	No characteristic	Absence of unsaturated fatty
	number of drops added is	colour formation.	acids.
	recorded.		
6.	ACROLEIN TEST:		
	For a few drops of oil or	A pungent colour of	Indicates the presence of
	lipid, a few crystals of	acrolein is produced.	glycerol.
	potassium hydrogen sulphate	No pungent colour is	Absence of glycerol.
	warm gently to mix and then	produced.	
	heated strongly.		
7.	DICHROMATE TEST:		
	Take a dry test tube, 3 or 4	Brown colour is	Indicates the presence of
	ml of oil or lipid, a few drops	changed to blue.	glycerol.
	of 5% potassium dichromate	No colour change.	Absence of glycerol.
	solution and 5ml of conc.		
	H_2SO_4 mix well.		