These are water insoluble organic substances, physically oily and greasy that can be extracted from cells by organic solvents such as ether and chloroform. Lipids provide insulation to the body against variation in temperature of weather. Lipids are also reserve source of energy during starvation. Structurally, lipids cover a diversify group of compounds like steroids, fatty acids and some vitamins.

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Lipids

Classification

Simple Lipids

These are esters of fatty acids with alcohol.

- i. Neutral: Ester of fatty acids with glycerol.
 - Contain saturated fatty acids, e.g. stearic acid and palmitic acids, etc.
 - Contain unsaturated or essential fatty acids, e.g. linoleic acid and oleic acid, etc.
 - Triglycerides—containing three fatty acid molecules esterified with three-hydroxyl group of glycerol, e.g. tristerin.
- ii. *Waxes:* True wax., e.g. bee's wax.
- iii. *Vitamins:* Vitamin A, vitamin D.

Compound Lipids

These are esters of fatty acids with alcohol which contain other element functional group, e.g. phospholipids, glycerides, etc. (Glycerides when react with methanol in the presence of acid or basic catalyst, produce glycerols.)

Other

Like carotenoids, steroids (e.g. cholesterol), vitamin D, ketone bodies, etc.

Stepwise Details for Identification of Simple Lipids

Physical Tests

Step 1

Solubility test: Lipids are insoluble in water but soluble in organic solvents like chloroform, ether, etc. Take in separate test tubes 5 ml of water, 5 ml of ether and 5 ml of chloroform. Add 2 to 3 drops of sample of lipid to each test tube and observe. In chloroform and ether sample it miscible while it is immiscible with water and floats on water.

Step 2

Grease spot test: This is oily and greasy to touch sensation. Due to greasy nature lipids make a spot on filter paper. Dissolve sample in organic solvents like ether or chloroform and place a drop of the solution on a filter paper and wait for drying. A translucent stain appears on filter paper.

Step 3

Litmus test: Due to neutral nature so no change in colour of red litmus paper.

Step 4

Specific gravity test: Because specific gravity of fat is less than one so on addition of water to lipids, fats or fatty acids float on water.

Chemical Tests

Step 1

Emulsification test: In this process dispersed phase (lipid/fatty material) is divided into very small droplets and distributed uniformly in water (dispersion medium) to form an emulsion. A suitable emulsifying agent can be added to form a stable emulsion. To alcoholic solution of sample, add double quantity of water, shake vigorously. A white emulsion is formed which on standing separates in the form of oil droplets of dispersed phase (lipid/fatty material). A stabilizing or emulsifying agent like bile salt is added, an emulsion is produced which remains stable on standing.

Step 2

Saponification test: In this process fats are hydrolyzed by alcoholic solution of alkali hydroxide (potassium hydroxide, sod. hydroxide or calcium hydroxide) to produce soap. Fatty acids can produce soap directly with alkali. To 1 ml of alcoholic sample, add 2 ml of 10% sodium hydroxide and boil very carefully on water bath till mixture do not separate when one drop of oil is added. A soft mass is produced which soon redissolves. To this add 15 ml water, divide the solution in three parts and perform following tests:

- (a) To 3 ml of sol., add NaCl to saturate it. A white ppt. of soap is produced. Then add 5 ml of water, ppt. dissolves because sodium soap is soluble in water.
- (b) To 3 ml of sol., add equal quantity of conc. HCl or H_2SO_4 . Small drops of oil will be separated which float on top of water because soap salt of fatty acid is converted into insoluble fatty acid by addition of HCl or H_2SO_4 .
- (c) To 3 ml of sol., after neutralizing it, add 2% CaCl₂ solution. A white ppt. of soap is produced because calcium soap is insoluble in water.

Step 3

Iodine absorption test: Unsaturated fatty acids react with iodine due to presence of double bond. To 5 ml solution of sample in chloroform, add Hubl 's iodine (iodine + mercuric chloride) drop-wise with continuous shaking and observe. If solution decolorizes means unsaturated fatty acids are present.

Specific Test for Glycerol

Step 1

Formation of acrolein test: Glycerol can be dehydrated from fat with the help of solid potassium bi-sulphate and acrylic aldehyde or acrolein is obtained which possesses irritating odour. To 2 drops of glycerol, add little NaHSO₄ in dry test tube, on heating irritating odour is produced.

Stepwise Details for Identification of other Lipids

Specific Test for Cholesterol

Step 1

Physical form of cholesterol: Cholesterol is a non-saponifiable lipid because it does not contain any fatty acid, so cannot produce soap. It contains basic steroidal nucleus, fat soluble and is found only in animals. It appears in the form of white crystals.

Step 2

Solubility test: Lipids are insoluble in water but soluble in organic solvents like chloroform, ether, etc. Take in separate test tubes 5 ml of water, 5 ml of ether and 5 ml of chloroform. Add 2 to 3 drops of sample of cholesterol to each test tube and observe. In chloroform and ether, cholesterol is miscible while it is immiscible with water and floats on water.

Step 3

Salkowski's test: To 2 ml sol. of cholesterol in chloroform, add 2 ml conc. sulphuric acid very slowly and mix carefully. The upper layer of chloroform becomes red and lower layer of sulphuric acid changes to yellow with green fluorescence.

Step 4

Liebermamm-Burchard test: To 2 ml sol. of cholesterol in chloroform, add 10 drops of acetic anhydride and 2 drops of conc. sulphuric acid very slowly and mix carefully. The rose red colour is obtained which rapidly changes to blue and finally to bluish green.

Experiment 9

OBJECT

To identify the given sample of lipid (fatty acid).

S. no.	Test	Observation	Inference
А.	Physical test		
1.	Colour		
2.	Smell		
3.	<i>Solubility test:</i> Take separately 5 ml water, 5 ml ether and 5 ml chloroform in test tubes and to each test tube, add 3–3 drops of sample.		
4.	<i>Grease spot test:</i> A drop of solution of sample in chloroform or ether is placed on filter paper and is dried.		
5.	<i>Litmus test:</i> A drop of solution of sample, in chloroform or ether is placed on litmus paper and is dried.		
6.	<i>Specific gravity test:</i> To 4 ml of sample, add 5 ml of water in a test tube and allow to stand.		
B.	Chemical test		
1.	<i>Emulsification test:</i> To alcoholic solution of sample, add double quantity of water, shake vigorously and allow to stand.		
2.	 Saponification test: To 1 ml alcoholic sol. of sample, add 2 ml 10% sod. hydroxide, boil, add 15 ml water and perform following tests: (a) 3 ml sol., add NaCl to saturate (b) 3 ml sol., add 3 ml conc. HCl (c) 3 ml neutralized sol., add calcium chloride sol. 		

S. no.	Test	Observation	Inference
3.	<i>Iodine absorption test:</i> To 5 ml solution of sample in chloroform, add Hubl's iodine drop-wise with continuous shaking.		
4.	Confirmation test:		

Conclusion

The given sample is _____.

Remarks

Experiment 10

OBJECT

To identify the given sample of lipid (cholesterol).

S. no.	Test	Observation	Inference
A .	Physical test		
1.	Colour		
2.	Smell		
3.	<i>Solubility test:</i> Take separately 5 ml water, 5 ml ether and 5 ml chloroform in test tubes and to each test tube, add 3–3 drops of sample.		
4.	<i>Grease spot test:</i> A drop of solution of sample in chloroform or ether is placed on filter paper and is dried.		
5.	<i>Litmus test:</i> A drop of solution of sample in chloroform or ether is placed on litmus paper and is dried.		
6.	<i>Specific gravity test:</i> To 4 ml of sample, add 5 ml of water in a test tube and allow to stand.		
В.	Chemical test		
1.	<i>Emulsification test:</i> To alcoholic solution of sample, add double quantity of water, shake vigorously and allow to stand.		
2.	 Saponification test: To 1 ml alcoholic sol. of sample, add 2 ml 10% sod. hydroxide, boil, add 15 ml water and perform following tests: (a) 3 ml sol., add NaCl to saturate (b) 3 ml sol., add 3 ml conc. HCl (c) 3 ml neutralized sol., add calcium chloride sol. 		

S. no.	Test	Observation	Inference
3.	<i>Iodine absorption test:</i> To 5 ml solution of sample in chloroform, add Hubl's iodine drop-wise with continuous shaking.		
4.	<i>Salkowshki's test:</i> To 2 ml sol. of cholesterol in chloroform, add 2 ml conc. sulphuric acid very slowly and mix carefully.		
5.	<i>Liebermann-Burchard test:</i> To 2 ml sol. of cholesterol in chloroform, add 10 drops of acetic anhydride and 2 drops of conc. sulphuric acid very slowly and mix carefully.		

Conclusion

The given sample is _____.

Remarks

VIVA VOCE/SYNOPSIS

- Q1. Classify lipids.
- **Q2.** Enlist the name of compound lipids.
- **Q3.** Write biochemical role of lipids in the body.
- Q4. What is emulsification test?
- Q5. What is saponification test?
- Q6. What is grease spot test?
- Q7. What is Salkowski's test?
- **Q8.** Write specific test for glycerol.
- **Q9.** Write specific test for cholesterol.

Q2. (4)

MCQs

Q1.	Q1. Which of the following does not belong to disaccharide?				
	(1) Stearic acid	(2) Oleic acid	(3) Palmitic acid	(4) Cholesterol	
Q2.	2. Which of the following does not contain any fatty acid?				
	(1) Stearic acid	(2) Oleic acid	(3) Palmitic acid	(4) Cholesterol	
Q3.	3. Which of the lipid does not show saponification test?				
	(1) Stearic acid	(2) Oleic acid	(3) Palmitic acid	(4) Cholesterol	
Q4.	4. Which test is the confirmatory test for cholesterol?				
	(1) Salkowski's test		(3) Both		
	(2) Liebermann-Burchard test		(4) None		
Q5.	5. Which vitamin belongs to lipids class?				
	(1) Fehling A	(2) Benedict's reagents	(3) Fehling B	(4) Molisch reagent	

Ans

Q1. (4)

Q3. (4)

Q4. (3) **Q5.** (2)