

QUALITATIVE TEST OF LIPIDS

- A large number of heterogeneous compounds are referred to as lipids including fats, steroids, waxes, and related compounds, which are related more by their physical than their chemical properties.
- They have the common property of being: 1. relatively insoluble in water 2. soluble in polar solvents such as ether and chloroform.

1. Solubility Test

- The test is based on the property of solubility of lipids in organic solvents and insolubility in water.
- Principle: The oil will float on water because of lesser specific gravity.
- Test: Take 3ml of solvents in each test tube and add 5 drops of sample. For water and ethanol, it is insoluble and for chloroform and ether, it is soluble and hence the given sample is lipid.

2. Spotting Effect

- All the lipids are greasy in nature. Therefore the test may be taken as group test for lipids.
- Principle: The oil does not wet the paper.
- Test: Take 3ml of ether in a test tube and dissolve 5 drops of oil in it. Put a drop of the solution on the filter paper and let it dry. A translucent spot on the filter paper was observed and this indicates the greasy character of the lipid.

3. Acrolein Test

- Used to detect the presence of glycerol or fat.
- When fat is treated strongly in the presence of a dehydrating agent like potassium bisulphate (KHSO_4), the glycerol portion of the molecule is dehydrated to form an unsaturated aldehyde acrolein that has a pungent irritating odour.

4. Saponification-

- Esters can be hydrolysed by alkali to yield the parent alcohol and salt. When the fatty acid possesses a long chain the salt formed is a soap which we commonly use. This process is called saponification. Oils and fats usually contain long chain fatty acids and are, therefore, the starting materials for the preparation of soap.
- Take approximately 100 mg of oil or fat in a test tube. Add 3 mL of alcoholic-KOH and mix well. Place the tube in a boiling water bath for 15-20 min. Appearance of foamy solution Indicates the presence of oil or fat

5. Test for cholesterol**A. Liebermann-Burchard Test (Test for Sterol)**

- Take approximately 100 mg of fat or 1 mL of oil in a test tube, dissolve by adding 1 mL of chloroform add equal volumes of acetic anhydride followed by drop wise addition (along walls) of concentrated sulphuric acid.
- Formation of an emerald green colour Confirms the presence of cholesterol

B. Salkowski Test:

- Take 1 mL of oil or fat sample in a test tube and dissolve in 1 mL chloroform to this add equal volume of concentrated sulphuric acid along the walls of the tube. (Do not mix the contents).
- Observes formation of blue-green colour at the junction of two liquids, this Indicates presence of cholesterol

6. Test for free fatty acids:

- Take a few drops of phenolphthalein solution in a test tube and add to it one or two drops of very dilute alkali solution, just sufficient to give the solution a pink colour.
- Now add a few drops of the oil and shake. The colour will disappear as the alkali is neutralized by the free fatty acids present in the oil.

7. Tests for unsaturation of fatty acids:**A. Bromine water**

- Take 1 mL of oil or 100 mg of fat in a test tube add 2 mL of nonpolar organic solvent and dissolve completely. To this add potassium permanganate solution or bromine water drop by drop.
- If decolourisation of potassium permanganate or bromine water is observed, it indicates presence unsaturated fats or fatty acids
- If no decolorisation observed it Indicates presence of saturated fats and fatty acids

B. Hubl's iodine

- Take 1 mL of oil or 100 mg of fat in a test tube add 2 mL of nonpolar organic solvent and dissolve completely. Add Hubl's reagent drop wise mixing the contents.
- If decolourisation of iodine solution is observed it Indicates presence of unsaturated fats
- If no depolarization of Iodine is observed it indicates absence of unsaturated fats or oils or presence of saturated fats or oils.

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