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Diploma in Pharmacy 2nd Year
Biochemistry & Clinical Pathology
Experiment
To perform qualitative analysis of lipids.

Aim:

To perform qualitative analysis of lipids.

Reference :

‘ Dr. Gupta G.D. , Dr. Sharma Shailesh, Kaur Manpreet, “Practical Manual of Biochemistry & Clinical Pathology” Published by Nirali Prakashan, Page no 10 – 14

Materials Required

Filter paper, phenolphthalein solution, dilute alkali solution, test tube, water, etc

Theory :

1. **Test for Free Fatty Acids:** Titrating oil against KOH in the presence of phenolphthalein indicator yields an estimate of the amount of free fatty acids in the oil. The amount of KOH needed to neutralise the free fatty acids in 1gm of sample is known as the acid number.

2. **Emulsification :** When oil or liquid fat is dispersed in water and shaken, it becomes finely divided to form an emulsion; this process is emulsification which is permanent and complete in the presence of emulsifying agents (e.g., bile salts, proteins, soaps, mono- and diglycerides). Emulsification aids in the processes of fat digestion in intestines. Emulsifying agents lower the liquid surface tension.

3. **Saponification Test :** Hydrolysis of esters by alkali yields the parent alcohol and salt. The salt yielded by long chain fatty acids is soap; this process is saponification. Since oils and fats contain long chain fatty acids, they are the starting materials for soap preparation.

Procedure

The following qualitative tests are performed for identification of lipids :

1. **Test for Free Fatty Acids:** A few drops of phenolphthalein solution should be taken in a test tube and 1-2 drops of very dilute alkali solution should be added to it to develop a pink colour. This solution should be shaken with a few drops of oil. The pink colour disappears due to neutralisation of the alkali by the free fatty acids present in the oil.
2. **Emulsification:** In a clean and dry test tube, 2ml water should be added, and in another test tube, 2ml dilute bile salt solution should be added. Each test tube should be added with 2 drops of mustard oil and vigorously shaken for a minute. On leaving the tubes undisturbed for 2 minutes, the oil in the first tube breaks down in small pieces and floats on the water surface; while the oil in the second tube suspends in the bile salt liquid in the form of minute droplets (i.e., permanent emulsification
3. **Saponification Test:** Iml of oil should be thoroughly mixed with an equal amount of alcoholic KOH solution in a test tube. The mixture should be kept during the course of warming and shaken gently with small amount of distilled water. Some oil drops appear indicating incomplete saponification; while no oil drops indicate complete saponification.

Result :

Qualitative analysis of lipids was performed.

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