Estimation of total lipids by colorimetric method.

Principle:

Lipids react with sulfuric acid to form carbonium ions which subsequently react with the vanillin phosphate ester to yield a purple complex that is measured photometrically at 540 nm. The intensity of the colour is proportional to the Total lipids concentration.

Materials:

1. Vanillin reagent, 0.04M. Dissolve 6.1 g of vanillin in water and dilute to 1 liter. This solution is stable for about 2 months in a brown bottle at room temperature.

2. . Phosphovanillin reagent. Add 350 ml of the vanillin reagent and 50 ml of water to a flask. Add with constant stirring, 600 ml of concentrated (85%) phosphoric acid. This solution is also stable for about 2 months in a brown bottle at room temperature.

3. Sulfuric acid, concentrated, reagent grade.

4. Standard solution. A good U.S.P. grade of olive oil may be used as a standard. In two tarred 100 ml volumetric flasks add approximately 0.5 and 1 ml of the olive oil and weigh again to obtain the exact weight of oil added. (It is time consuming to try to weigh out exactly 500 mg , or any other definite weight, of the oil; the approximate amounts are added. and the exact weight determined.) The above standards should be about 500 and 1,000 mg/dl. Dissolve the oil in absolute ethanol and dilute to the mark with the ethanol. This solution is stable for about month in the refrigerator.

5. Standard solution of cholesterol (1g/100 ml acetone)

Procedure:

In separate tubes add 20 µl of water (blank), 20 µl of samples, and 20 µl of standards. To each tube add 0.2 ml of concentrated sulfuric acid. Mix well, preferably on a vortex mixer. Place all tubes in boiling water bath for 10 min, remove, and cool in water to room temperature. To each tube add 10 ml of the phosphovanillin reagent and mix well. Incubate at 37^0C in a water bath for 15 min. Cool and read standards and samples against blank at 540 nm.

Calculations:

$$\text{Concentration of unknown} = \frac{\text{Absorbance of unk}}{\text{Absorbance of std}} \times \text{Conc of std}$$

Name: ***No.***

Experiment 8:

Results Sheet

Concentration of standard cholesterol solution : mg/ml

Concentration of standard olive oil solution : mg/ml

1)

Ast. Cholesterol =
Aun cholesterol =

2)

Ast. Olive Oil =
Aun Olive Oil =