Estimation of Cholesterol Liberman – Burchard Reaction

Principle:

Cholesterol is readily soluble in acetone, while most complex lipids are insoluble in this solvent.
Blood or serum is extracted with an alcohol-acetone mixture which removes cholesterol and other lipids and precipitates protein. The organic solvent is removed by evapotation on a boiling water bath and dry residue dissolved in chloroform. The cholesteror is then determined colorimetrically using the Liebermann-Burchard reaction.

Acetic anhydride reacts with cholesterol in a chloroform solution to produce a characteristic blue-green color. The exact nature of the chromophore is not known but the reaction probably includes esterification of the hydroxyl group in the 3 position as well as other rearrangement in the molecule.The cholesterol is determined colorimetrically using the Libermann – Burchard reaction.

Materials :

1. Serum or blood.	25 ml
2. Alcohol-acetone mixture (1:1).	21
3. Chloroform.	500 ml
4. Acetic anhydride-sulphuric acid mixture (30:1 mix just before use, Care!).	930 ml
5. Stock cholesterol solution (2 mg/ml in chloroform).	250 ml
6. Working cholesterol solution. (Dilute the above solution one in five with chloroform to give a solution of 0.4 mg/ml.)	11

Procedure:

1- Place 10 ml of the alcohol-acetone solvent in a centrifuge tube and 0.2 ml of serum or blood.
2- Immerse the tube in a boiling water bath with shaking until the solvent begins to boil.
3- Remove the tube and continue shaking the mixture for a further 5 min.
4- Cool to room temperature and centrifuge.
5- Decant the supernatant fluid into a test tube and evaporate to dryness on a boiling water bath.
6- Cool and dissolve the residue in 2 ml of chloroform.
7- Add 2 ml of acetic anhydride-sulphuric acid mixture to all tube and thoroughly mix.
8- Leave the tubes in the dark at room temperature and read the extinction at 680 nm.
9- Carry the experiment in a test tube one for the standard:
a-0.2 ml of standard then 2 ml of chloroform.
b-Add 2 ml of acetic anhydride-sulfuric acid mixture and thoroughly mix.
c- Leave the tube in the dark at room temperature and read the extinction at 680 nm.

Calculation:

Determine the concentration of the unknown sample according to the following equation:

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

Name: ***No.***

Experiment 11:

Results Sheet

Calculate the concentration of cholesterol in your sample.