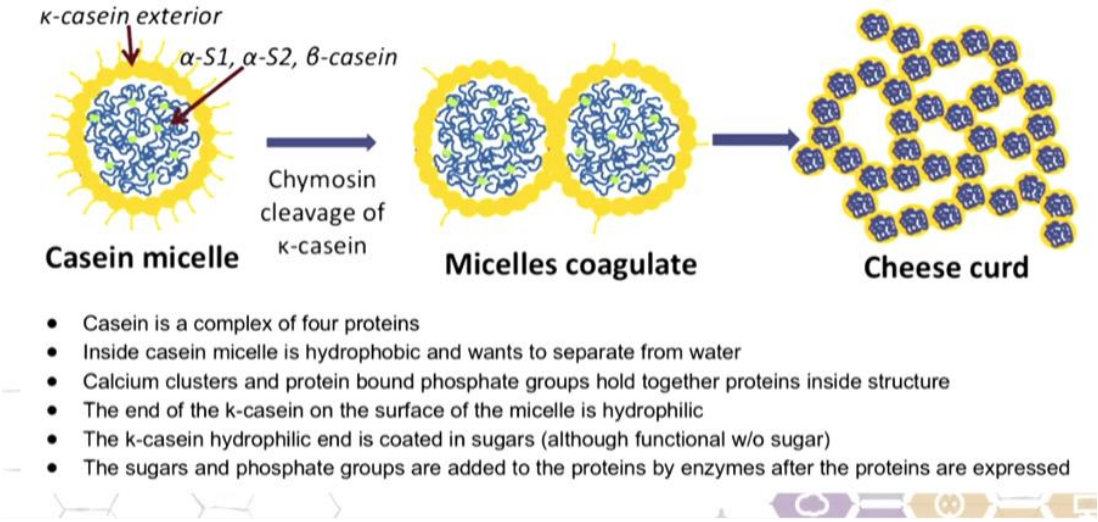
Experiment 3: Isoelectric Precipitation Casein Protein present in milk.

Objective:

- To perform the isoelectric precipitation of casein present in milk.
- Qualitative estimation of casein present in milk

Principle:

Milk is a mixture of many types of proteins, most of them present in very small amounts. Milk proteins are classified into three main groups of proteins on the basis of their widely different behaviors and forms of existence. They are caseins (80%), whey proteins and minor proteins. Casein is a heterogeneous mixture of phosphorous containing proteins in milk. Casein is present in milk as calcium salt and calcium caseinate. It is a mixture of alpha, beta and kappa caseins to form a cluster called micelle. These micelles are responsible for the white opaque appearance of milk. Casein, like proteins, is made up of many hundreds of individual amino acids. Each may have a positive or a negative charge, depending on the pH of the milk system. At some pH value, all the positive charges and all the negative charges on the casein protein will be in balance, so that the net charge on the protein will be zero. That pH value is known as the isoelectric point (IEP) of the protein and is generally the pH at which the protein is least soluble. For casein, the IEP is approximately 4.6 and it is the pH value at which acid casein is precipitated. In milk, which has a pH of about 6.6, the casein micelles have a net negative charge and are quite stable. During the addition of acid to milk, the negative charges on the outer surface of the micelle are neutralized (the phosphate groups are protonated), and the neutral protein precipitates. The same principle applies when milk is fermented to curd. The lactic acid bacillus produces lactic acid as the major metabolic end-product of carbohydrate [lactose in milk] fermentation. The lactic acid production lowers the pH of milk to the IEP of casein. At this pH, casein precipitates.



Sample: Milk

Materials:

1) Raw milk - 100ml
2) 0.2N HCl - 50ml
3) Diethyl ether - 50ml
4) 50% Ethanol - 50ml
5) Whatman No 1 filter paper strip (Size 25×50mm) - 2 no

Procedures:

i. Measure 100ml of milk in a measuring cylinder and transfer 25ml of milk to four Oakridge centrifuge tubes each.

ii. Centrifuge the milk in a centrifuge at 4000rpm at room temperature (25- 30o C) for 20 minutes. This is done to remove the fats and lipids from the mixture.

iii. After centrifugation, carefully remove the fats and lipids from the surface of the milk with a spatula.

iv. Then transfer the milk from all the tubes into a beaker and add equal volume of distilled water and stir well. Now check the pH.

v. Start adding 0.2N HCl drop by drop into the milk mixture and stir well. Note the PH at which precipitation (white curdy substances) appears. The pH should be 4.6.

vi. Take the curdy precipitate and allow it to sediment.

vii. Now decant the supernatant using a filter paper and funnel and wash the precipitate with distilled water to remove the salts, and then wash with diethyl ether and ethanol.

viii. Dry the precipitate and take the weight of the casein and record it