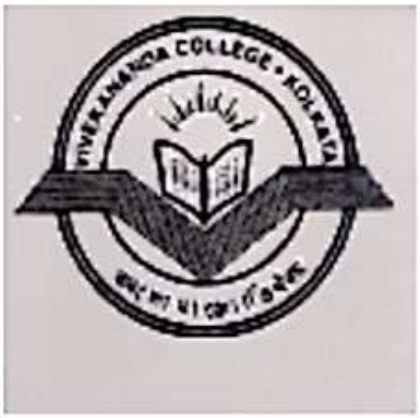

VIVEKANANDA COLLEGE,THAKURPUKUR

TOPIC: AMMONIUM SULPHATE FRACTIONATION AND LYOPHILISATION

COURSE:CC2

SEMESTER:2(HONOURS)

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DEPARTMENT:BIOCHEMISTRY

Pre-treatment — Pre-treatment can be in the form of additives that stabilize the products, bulking agent, that add mass to the product, or filtration that reduce volume and concentrate the product. It determines the quality of lyophilisation.

Freezing — The principal function of freezing process is to separate the solvent from the solutes. For an aqueous system, water forms ice crystals and solute gets confined to the interstitial region between the ice crystals.

Drying — Once the sample reaches complete frozen state, the pressure is reduced and heat is applied to initiate sublimation of ice crystals.

Primary drying — 90% of water is removed.

Secondary drying — The remaining 10% is removed.

Factors affecting lyophilisation

Factors that affect the efficiency of lyophilisation are, —

1) Sample size.
2) Area of the sample
3) Thickness of sample
4) Solute concentration.
5) Type of instrument and
6) Temperature of vacuum
7) Temperature of condenser and
8) Eutectic temperature.

Solvent fractionation — Solvent fractionation or extraction is a process of separating various metabolites of an organism (plant, microbes, animal tissue) based on the

differential solubilities in different solvents such as water and organic solvents like acetone or hexane.

Addition of water miscible neutral organic solvent particularly ether or acetone decreases the solubility of most globular proteins in water such that they precipitate out.

Protein solubility at a fixed pH and ionic strength is a function of the dielectric constant of the medium. Low dielectric constant lower the solvating power of their aqueous solution of dissolved ions such as proteins.

Water has a relatively high dielectric constant whereas C_6H_6 has a low value. The large class of substrates that are non ionic, but polar compounds, like sugar readily dissolves in water.

Since ethanol has a lower dielectric constant than water, its addition to an aqueous protein solution increases the attractive forces between opposite charges, thus decrease in degree of ionisation of R groups of proteins. As a result, the protein molecules tend to aggregate and precipitate.

Application

Liquid column fractionation used for

1) coal liquefaction and petroleum products used in palm oil manufacture and obtaining confectionary fat from palm oil.
2) It is an useful tool of separation in food and biotechnology industry.
3) In oil industry, it is used to determine the triglyceride composition of palm oil.

Salting in

The solubility of a protein at low ionic strength generally increases with the salt concentration. The explanation of this salting in phenomenon is that as the salt concentration of the protein solution increases, the additional counter ions more effectively shield the protein molecules' ~~multiple~~ multiple ionic charges and thereby increase protein solubility.

Salting out

At high ionic strength, the solubilities of proteins as well as those of most other substances decreases. This effect is known as salting out, which results from the competition between the added salt ions and the other dissolved solutes for molecules of solvation. Salting out is the basis of one of the most commonly used protein purification procedures. Ammonium sulphate is the most commonly used reagent for salting out proteins, because its high solubility permits the achievement of solutions with high ionic strength.

Ammonium sulphate fractionation

Proteins are soluble in aqueous solution because the charged and polar side chain groups of amino acids interact with water. In other words, proteins become hydrated. If the protein - solvent interaction is prevented, proteins will interact with one another and form aggregates that precipitate out of solution. Different proteins will ~~to~~ behave differently and hence possible to separate different proteins based on different solubility properties.

Example: For example, as the salt concentration of a solution is increased, the amount of water that is available to interact with proteins is decreased. This will result in more hydrophobic domains of proteins interacting with hydrophobic domains of other proteins. Therefore it is possible to selectively precipitate some proteins under conditions where

20/3/19

$(3ml + x) \times 20\% = x \times 100\%$ 1 ml

$\Rightarrow 60 + 20x = 100x$

$\Rightarrow 60 = 80x$

$\Rightarrow x = \frac{60}{80} = \frac{3}{4}$

$\therefore x = \frac{3}{4} = 0.75ml$

76.68g $(NH_4)_2SO_4$ → 100%, 100ml

0.75ml is added to 3ml.

Ammonium sulphate fractionation — Procedure

1) The ammonium sulphate is slowly added to a protein of interest will have to be initially determined empirically. The concentration of ammonium sulphate needed to precipitate a particular protein is usually expressed as percentage of saturated ammonium sulphate. Either solid or saturated solution can be added. In this way it is possible to concentrate a specific protein by adding a specific amount of ammonium sulphate to precipitate out non-desirable protein and then recovering the supernatant.

2) Once dissolved, ammonium sulphate-protein suspension/solution is allowed to slowly reach equilibrium (kept cold). Some proteins