

Protein Purification Techniques Vol. 1. Ionic Precipitation

Introduction

Protein precipitation achieves separation by the conversion of soluble proteins to an insoluble state, which subsequently can be removed by various means. Precipitation can be used to remove components in cell culture media that may interfere with downstream purification methods. Ideally, precipitation results in both concentration and purification. Thus, precipitation is often used early in the sequence of downstream purification, reducing the volume and increasing the purity of the protein prior to any chromatography steps.

Protein Precipitation

The main advantage of precipitation is the relative ease of use. In addition, precipitating agents can be chosen that provide a more stable product than found in the soluble form. A protein is made insoluble by changing its surface, charge characteristics or changing the solvent characteristics; the latter being preferred.¹ The greater the initial concentration of the desired protein, the greater the efficiency of precipitation.

A protein is least soluble when at its isoelectric point (pI)², therefore, selection of a buffer at or near the pI of the protein is recommended. However, some proteins may denature at their pI. Above the pI, solubility of a protein increases with the addition of salt and reaches a maximum after which there is a rapid linear decrease in solubility.³

Methods of Precipitation

There are several methods to reduce the solubility of proteins, some of which are: ionic precipitation (e.g. ammonium sulfate, sodium chloride), temperature, pH, metal ions (e.g. Cu²⁺, Zn²⁺ and Fe²⁺), nonionic polymers [e.g. polyethylene glycol (PEG)], organic solvents (e.g. ethanol, acetone), tannic acids, heparin, dextran sulfates, cationic polyelectrolytes (e.g. protamines), short chain fatty acids (e.g. caprylic acid), trichloroacetic acid (TCA), lectins (e.g. concanavalin A), group-specific dyes (e.g. Procion Blue) and ligand-antibody interaction.³

Ionic precipitation, utilizing inorganic salts, is the most common precipitation method. Precipitation by various salts or nonionic polymers is the preferred method to utilize whenever possible. These precipitations typically yield stable non-denatured products. The use of temperature, pH or organic solvents can lead to denaturation and should be performed with care to minimize any decrease in yield or activity.

Other methods are typically more specific for certain proteins or a particular class of proteins. *Protein Purification Process Engineering* by Roger G. Harrison³ contains useful information regarding these methods.

Precipitation with Ammonium Sulfate

Inorganic salts can be utilized for the precipitation of proteins, with ammonium sulfate being the most common. The advantages of ammonium sulfate are: (1) at saturation, it is of sufficiently high molarity that it causes the precipitation of most proteins; (2) it does not have a large heat of solution, allowing heat generated to be easily dissipated; (3) its saturated solution (4.04 M at 20 C) has a density (1.235g cm⁻³) that does not interfere with the sedimentation of most precipitated proteins by centrifugation; (4) its concentrated solutions are generally bacteriostatic; and (5) in solution it protects most proteins from denaturation.⁴ However, proteins in cell culture medium containing surfactants such as Pluronic F-68® cannot be precipitated with ammonium sulfate. Instead, other ionic or nonionic precipitation methods must be employed such as sodium chloride or PEG (refer to *Protein Purification Techniques, Vol. 2; Nonionic Precipitation* for further information).

The concentration of ammonium sulfate required for precipitation varies from protein to protein and should be determined empirically. Typically, ammonium sulfate is used in a series of steps performed at 2 to 8 C. For example, ammonium sulfate is added in increments to a concentration of 20% of saturation while gently stirring and allowed to

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dissolve and equilibrate between additions. Any precipitate is removed and discarded. This step typically yields macromolecules such as ribosomes, membrane fragments and even denatured proteins. This precipitation is then followed by increasing the ammonium sulfate concentration to 50% of saturation, in which the protein of interest is "salted-out" and collected via centrifugation. The remaining supernatant may contain additional "contaminating" proteins, which are then discarded. The collected precipitate can be resuspended in the minimal volume of buffer suitable for the next step in the purification process, typically via dialysis. Thus concentration, purification and buffer exchange are performed in one process.

Sodium chloride can also be utilized in a similar fashion as ammonium sulfate but with lower yield and typically an increase in denaturation of proteins. In addition, magnesium sulfate, potassium or sodium phosphate, potassium or sodium acetate and other sulfate and phosphate salts can be used with varying success.

Precipitation and purification in general are very protein specific and require a great deal of optimization. For more information about this subject or other SAFC Biosciences' products and services, please contact our Technical Services department.

References

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