

# Technical Bulletin

## Protein Purification Techniques Vol. 2. Nonionic Precipitation

### Introduction

Nonionic 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; therefore it is often used early in the sequence of downstream purification.

### Nonionic Polymers

The use of nonionic polymers for the precipitation is a method that can help prevent protein denaturation and assist in removal of detergents. Typically, larger proteins precipitate at lower concentrations of nonionic polymers. Several water-soluble uncharged polymers used for precipitation include dextrans, polyvinyl pyrrolidone, polypropylene glycols and polyethylene glycols.<sup>1</sup> Polyethylene glycols (PEG) are the preferred non-ionic polymers for protein precipitation because the viscosity of concentrated solutions is lower than other nonionic polymers.<sup>1</sup>

### Polyethylene Glycols

Polyethylene glycols are polymers of ethylene oxide typically ranging in size from 200 Da to 20 kDa. PEG is very soluble in water due to the ether oxygens spread along the length of the polymer, which are strong Lewis bases and form hydrogen bonds with water molecules.<sup>1</sup> In addition, the formation and equilibration of precipitates take significantly less time with PEG as the precipitating agent than with ammonium sulfate or ethanol.<sup>2,3</sup>

One drawback to PEG is the difficulty of removal when used at high concentrations. The most common means of removal of PEG are: ion exchange (PEG is not retained), addition of salt to form a two-phased system with the protein partitioning to the PEG-depleted phase, ultrafiltration or by the addition of ethanol (PEG being soluble in aqueous ethanol but not the protein).<sup>2,3,4</sup> The removal of PEG can be improved by the use of PEG 6000 with little decrease in yield or activity.

### Precipitation with Polyethylene Glycols

Polyethylene glycols can be used in sequential additions (rather than continuous addition), similar to the use of ammonium sulfate for protein precipitation. Precipitate is removed at each stage of the sequence and can be assayed for the target protein. This can perform a similar function as a "salt-cut" but with differing precipitation results. However, it is unlikely that the use of ionic and nonionic precipitation in the same purification scheme will be of benefit.

Another benefit of PEG precipitation is the removal of nonionic detergents (Triton™ X-100 & Tween™ series) from the proteins. Often nonionic detergents improve the solubility of proteins, especially membrane proteins, but they can interfere with downstream purification. Precipitation with PEG can separate the proteins from these nonionic detergents.

### Concentration with Polyethylene Glycol

Polyethylene glycol can also be used to concentrate small-scale samples, however, it can be cumbersome. This is done by placing the samples in dialysis tubing of 7 kDa cutoff, or smaller, at 2 to 8 C placing the tubing onto crystalline PEG (10 kDa or greater), for a period of 5 - 60 minutes, depending on the degree of concentration desired and removing the remaining PEG by rinsing in buffer or distilled water. Typically, this is followed by a short dialysis step prior to any further purification.

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.

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