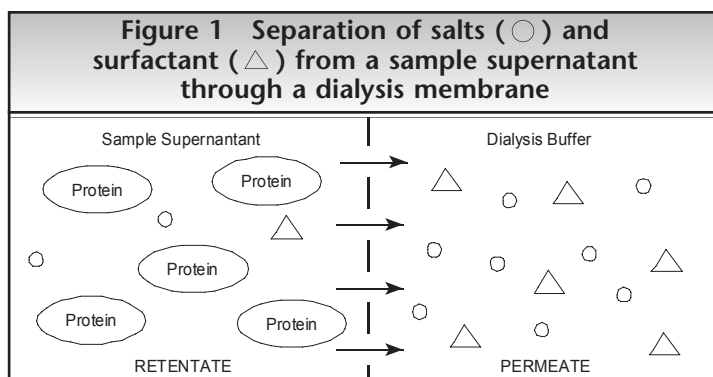


## Removing Surfactants from Serum-Free Suspension Media

Shaker flasks, spinner vessels and bioreactors are commonly used to grow cells in suspension. The continuous agitation of the media can be harmful to the cells due to shearing and foaming. Serum in suspension media protects the cells from these forces. In cultures containing low levels of serum (< 1%) or in the absence of serum, a surfactant must be added to media to protect the cells. The surfactant Pluronic® F68 is used in many of the EX-CELL™ Serum-Free Media offered by SAFC Biosciences.

Surfactants can present certain obstacles in downstream processing and protein purification. Surfactants coat proteins, which can hinder the recovery of expressed proteins, thereby making chemical labeling techniques less effective. When surfactant-containing supernatants are passed through chromatography columns, they can become clogged. To overcome these problems, cell culture supernatants can be dialyzed prior to chromatography without affecting the compound that is being isolated.

Dialysis is a method of separating smaller compounds in a solution from the larger compounds, such as proteins, through a buffer exchange.<sup>1</sup> The sample to be dialyzed is separated from a saline buffer by a porous membrane. The buffer has an osmolarity less than that of the sample solution, and therefore small solutes such as salts and surfactants diffuse through the dialysis membrane into the dialysis buffer.<sup>2</sup> By choosing a membrane with a pore size, or molecular weight cutoff, that is less than that of the protein of interest, the protein remains in the retentate, isolated from the buffer that now contains many of the unwanted smaller compounds (see Figure 1).



One of the main considerations in dialysis is the choice of membrane. Dialysis membranes are available in a wide range of molecular weight cutoffs. As a general rule, choose a membrane with a molecular weight cutoff that is at least one half the size of the compound of interest. For instance, to separate surfactants and salts from a protein that is 40 kDa in size, use a membrane with a molecular weight cutoff of 20 kDa. Additionally, the membrane pores must be large enough to allow the surfactant to easily pass through (Pluronic® F68 has an average molecular weight of 8.4 kDa)<sup>3</sup>.

The choice of dialysis buffer is also important. The buffer should be compatible with the optimal pH and osmolality ranges of the compound being isolated. This may be a simple saline solution such as Dulbecco's Phosphate Buffered Saline (DPBS) or Earle's Balanced Salt Solution (EBSS). Often it is convenient to dialyze into a buffer that will be used in subsequent separation steps, i.e. the buffer for a specific affinity chromatography column.

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Although the presence of surfactants in serum-free media for suspension cultures is a necessity, simple dialysis procedures can easily remove them from a sample before later purification steps.

For more information about this subject or other SAFC Biosciences' products and services, please call our Technical Services department.

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