Minimizing Product Loss on Ultrafiltration Membranes

by Pete Gagnon, Validated Biosystems

We've received almost two dozen inquiries concerning loss of protein on ultrafiltration membranes; either in diafiltration or endpoint filtration applications. These are not concerns with the usual 5-10% loss, but with 15-25% losses, some over 40%. In the case of the 40%, the direct loss from the membrane step was about 25%. The balance was lost because that step also induced heavy aggregate formation, and the aggregates had to be removed downstream. The magnitude of this particular problem was unusual but the pattern is common.

We're not going to try to solve all these problems in this article. Several are product or situation specific. Instead, we are going to discuss a couple of fundamental issues that lie at the bottom of most serious filtration losses. Nineteen of the 23 inquiries we received were victim to either one or both of two factors: either the original sample was in a high concentration of salt, and/or the sample was equilibrated to a very low ionic strength.

High feedstream salt concentrations are always a concern in filtration applications. Membranes are not inert, despite the best efforts of their manufacturers. They are hydrophobic. Strong precipitating salts (such as sulfates, phosphates, and citrates) promote hydrophobic interactions between proteins and membranes. These can result in either adsorption or aggregation. Even sodium chloride can be a problem if it is concentrated enough. If there really is no alternative to exposing a high-salt sample to a filtration membrane, you may be able to bring losses within tolerable bounds simply by diluting the feedstream. This sounds counterintuitive, since it will increase process time, but reducing the initial salt concentration will decrease promotion of hydrophobic interactions. Practical experience indicates that this approach can cut losses by a factor of 2-5. The process time increase is unattractive, but it compares very favorably with direct product loss or induction of aggregates.

Regardless of the initial feedstream composition, targeting very low salt concentrations can be as bad a problem — or worse. Such conditions are often targeted to prepare a sample for a subsequent ion exchange chromatography step. One of the most frequent problematical situations occurs with antibodies being prepared for a cation exchange step. Under low pH, low conductivity conditions, many antibodies aggregate or precipitate spontaneously from solution. The tendency toward permanent aggregate formation is typically worse than encountered with high salt conditions. This is because most precipitating salts are protein-stabilizing at the same time they promote secondary hydrophobic associations. Ionic deficiencies, on the other hand, are typically protein destabilizing. Even though there is no direct promotion of hydrophobic interactions from the solvent composition, the insoluble protein interacts spontaneously with the hydrophobic membrane in the same way that it interacts spontaneously with other insoluble proteins. In the absence of a compensatory stabilizing force, the probability of permanent denaturation is highly elevated. The longer the process time and the more concentrated the protein, the more likely you are to encounter a problem.

Although antibodies are a known hazard group for this type of problem, they are not the only victims. Any time you expose proteins to very low conductivity — especially if the exposure occurs at extreme pH or close to your product's pI — you need to be aware of the possibility. The trick, in all these cases,

is to avoid situations where protein solubility becomes a limiting factor. (A simple technique for evaluating relative protein solubility is downloadable from our electronic library. Title: An adaptation of hydrophobic interaction chromatography for estimation of protein solubility optima). The downside of altering conditions to avoid protein solubility limitations is that you may be left unable to equilibrate your feedstream to the target conditions you seek.

Fortunately, if you can get your sample part or most of the way to a targeted set of chromatography conditions, there is a simple and broadly applicable method for getting it the rest of the way. In many cases, it's possible to use this technique to avoid filtration altogether, and thereby avoid encumbering your overall purification procedure with the extra equipment, expendable supplies, SOPs, labor, and validation. The technique is called on-line dilution, and it involves mixing your sample with a diluent buffer on-line in the chromatograph. If for example, it is necessary to keep 0.05M NaCl in your sample to maintain solubility, but the ionic strength must be no greater than 0.01M to support binding to an ion exchanger, then simply dilute it 5-fold on the chromatograph. The reason you can get away with the low ionic strength here, but not on a filtration system, is that precipitation reactions always have a kinetic component — precipitation takes time. With on-line dilution, the pre-column residence time of the protein in aggregating or precipitating conditions is simply too short for a problem to occur. For more information on this technique, download: Validated Biosystems, 2(1) 1-14 (1997) from our electronic library.

No matter how you choose to deal with membrane-associated protein losses (or aggregation), it is important that you not get ambushed by it. Most of the people who have written to us on this issue were caught by surprise, usually during scale-up. There are a couple of reasons for this. One, during process development, small process volumes make it relatively simple to proceed quickly from process step to process step. Solubility problems may not have time to become readily apparent. Two, it's a lot easier to see an aggregation or precipitation problem in a large vessel, than it is in a small vessel. For either or both these reason, aggregation and precipitation problems are often overlooked — or at least underestimated. Avoiding these problems requires that process developers accurately simulate manufacturing process times to their best of their ability, and also that they apply analytical methods capable of detecting even low level aggregation. This is easily done with most protein solutions by evaluating spectrophotometric "absorbance" at 600nm. Proteins don't actually absorb light at this wavelength. If there is any positive signal, it is due to turbidimetric scatter by aggregates and/or precipitates. If the equipment is readily available, low angle laser light scattering analysis can be very helpful as well.