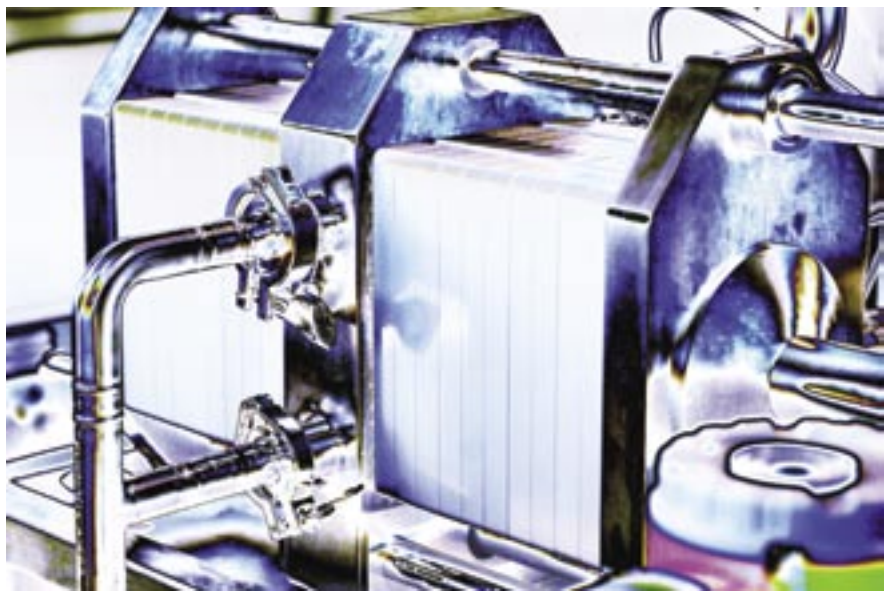
Downstream Ultrafiltration for Human Serum Albumin

Purification By Crossflow Concentration of Human Serum Albumin with Ultrafiltration Membranes

Frank Meyeroltmanns and Matthias Grabosch

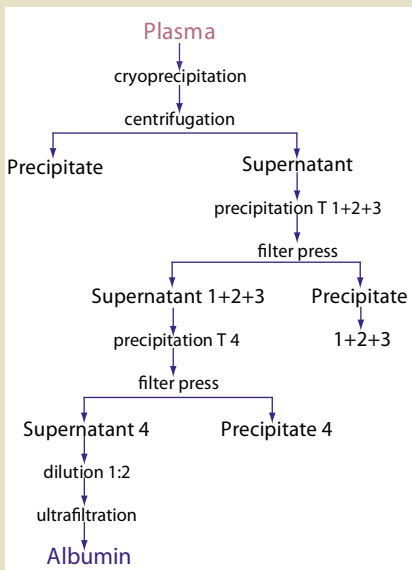
Human serum albumin (HSA) is usually obtained from plasma using the Cohn fractionation procedure or a variation of it from the Kistler-Nitschmann process in which various plasma proteins are removed by precipitation (Figure 1). Alcohol, pH, and temperature are varied to cause individual proteins to precipitate out for separation. The production process uses established cold ethanol fractionation technology to purify clinically useful proteins from blood plasma. A diafiltration and final product concentration step is integral to that manufacturing process. The ethanol must be removed during the final processing stages, as does citrate, which is added during blood donation to act as an anticoagulant. Citrate in albumin solutions is known to chelate aluminium from glass containers and must therefore be carefully controlled to maximize product shelf-life (**1**).

In our development laboratories, application engineers optimized the diafiltration, concentration, and cleaning processes. Our diafiltration method uses a concentrated sodium chloride solution, diluted on-line with water for injection (WFI) to remove both citrate and ethanol from the product. The salt solution is then removed by diafiltering against more WFI, resulting in an albumin solution that is free of ethanol and salts and with citrate at levels well below specified limits. We developed a new ultrafiltration (UF) crossflow cassette (PESUmax) specifically for this application. It features low protein binding and higher flux rates than those of membranes traditionally used for albumin processing. It can also withstand high chemical cleaning treatment and storage procedures.



WWW.SARTORIUS.COM

Figure 1: Cold ethanol plasma fractionation according to the Kistler-Nitschmann process



Product Focus: Human serum albumin (HSA)

Process Focus: Downstream processing (optimization)

Who Should Read: Process engineers, separations scientists

Keywords: Human serum albumin, ultrafiltration, crossflow filtration, diafiltration

Level: Intermediate

Principles of Crossflow Filtration

In crossflow filtration, an influent stream (feed) is divided into two effluent streams, defined as the *retentate* and the *permeate*. The concentrate or nonfiltered portion "crossflows" over the membrane at high linear velocities. This flow creates a continuous self-cleaning sweeping action. Optimizing the procedure involves maximizing crossflow efficiency. This allows use of higher transmembrane pressures, resulting in increased flux that translates to higher permeate rates. The goal is to reach an optimal flux with a minimal decline during filtration. Variables that can be manipulated include inlet pressure (*Pi*), retentate back pressure (*Po*), and permeate back pressure (*Pf*). Fluid flow through a membrane is best described by the Hagen-Poiseuille equation for streamline flow through channels:

$$J = (\pi \cdot r^2 \cdot TMP)/(8 \cdot \mu \cdot x)$$

where
J = flux
r = mean pore radius
TMP = transmembrane pressure
μ = viscosity of fluid
x = thickness of the membrane.

Transmembrane Independent Relationship: Based on that mathematical model, the ability to influence the flux for a given membrane is limited to using the maximum possible transmembrane pressure. During filtration, a solution will build a gel layer on the membrane surface despite the crossflow sweeping action, through a phenomenon known as concentration polarization. Once that gel forms, flux declines and no longer depends on transmembrane pressure, as described by the Hagen Poiseuille equation independent from TMP. This phenomenon is expressed best by the following relationship:

$$J = k \cdot \ln (Cg/Cb)$$

where
J = flux
k = mass-transfer coefficient
Cg = concentration of retained species forming gel layer
Cb = concentration of retained species of the bulk fluid.

An optimization process balances crossflow dynamics (ΔP, or differential pressure) with filtration flux (TMP) to generate the highest sustained flux. This goal is realized by disrupting the laminar-flow fluid boundary at the membrane surface to minimize the gel layer.

The first stage of this procedure requires optimization of crossflow rates by controlling ΔP. To limit the influence of other variables, TMP and feed concentration remain constant whereas crossflow rates are varied. The feed concentration is held constant by recycling both the permeate and retentate back to the feed vessel (Figure 2).

To establish proper profiles, the following formulas are used to describe the relationship of ΔP and TMP.

$$\Delta P = Pi - Po$$

and

$$TMP = [(Pi + Po)/2] - Pp$$

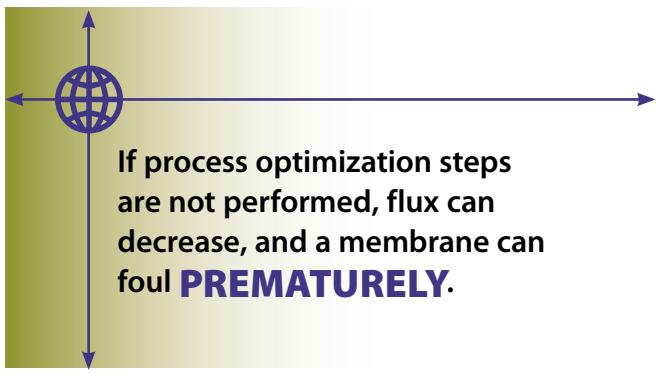
where
Pi = Inlet pressure (feed pressure)
Po = Outlet pressure (retentate pressure)
Pp = Permeate pressure (filtrate pressure).

Plotting the resulting flux values against corresponding crossflow rates can establish a profile to determine optimum crossflow. An optimum rate should correspond with the point that gives the maximum flux (or the point on the upper end of the plot).

The next stage entails optimizing the transmembrane pressure. In this stage, TMP is manipulated while the crossflow rate and feed concentration are held constant. Another profile is established by plotting the resulting flux values against the corresponding TMPs (Figure 3). In this case the optimum rate is chosen as the uppermost point on the linear portion of the plot. If these process optimization steps are not performed, flux can decrease, and the membrane can foul prematurely.

Diafiltration and Concentration

One step in manufacturing human albumin is diafiltration, which is followed by final product concentration. During diafiltration, crossflow systems equipped with ultrafilter membrane cassettes retain the target product (human albumin) and release unwanted substances into the filtrate. Initial concentration reduces the feed volume to ease fluid handling (Figure 4).



In a later step, the albumin concentration in supernatant IV, called fraction IV, is about 13 g/L, and its alcohol content is 40 vol %. This solution is than further processed in four steps:

Dilution: The solution is diluted with water to an alcohol concentration of
20 vol %. This step is necessary because polysulfone ultrafiltration cassettes react negatively and irreversibly when exposed to 40 vol % alcohol.

Initial concentration: The volume of albumin concentrate is reduced to
60–80 g/L. The alcohol concentration remains constant here. This is the longest step of the entire process, according to Jaffrin — two to three hours, depending on batch volume and system size (2). Diafiltration with water removes the alcohol. The volume and process parameters remain constant. Concentration of the albumin content to (28–30% HSA) follows, for a final formulation of 25% albumin.

Figure 2: Typical crossflow system configuration

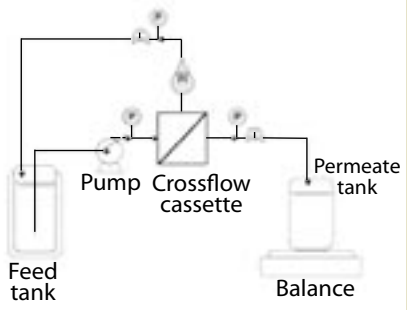
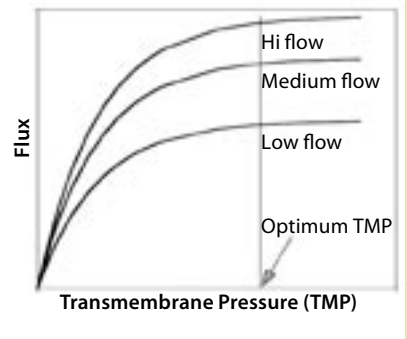


Figure 3: Effect of transmembrane pressure (TMP) and crossflow velocity on flux rates



Sartoflow 20 system with hydraulic closure technique for HSA processing
(WWW.SARTORIUS.COM)

Figure 4: Process steps for albumin filtration

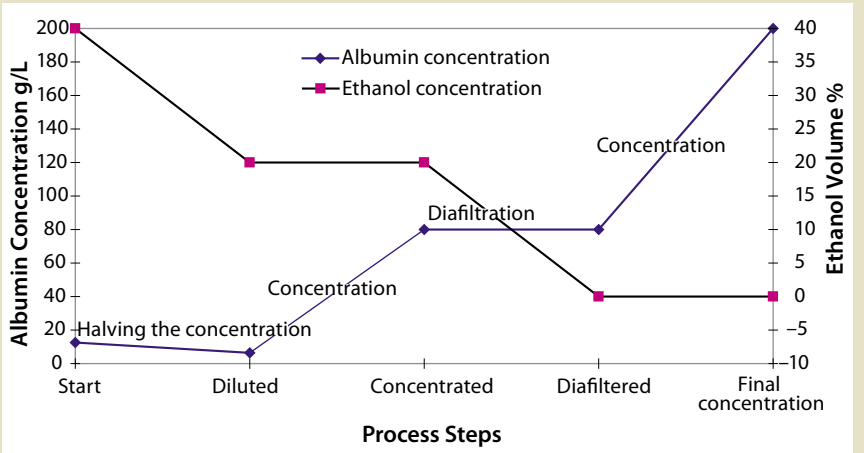
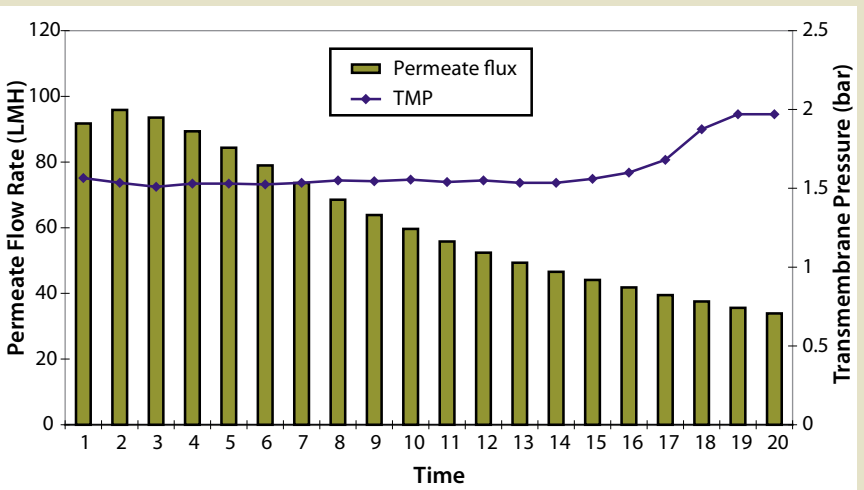


Figure 5: Albumin concentration (PESUmax)



HSA Concentration

The following describes the results of that HSA concentration step.

Materials and Methods: HSA (2,000 mL) was used at an initial concentration of 6.5%. The final volume was 500 mL, with 25.5% albumin in the final formulation. The Sartoflow Alpha crossflow system was used for ultrafiltration. The rotary lobe pump operated at 1 m^3/hr, connected to a double-jacketed feed vessel cooled with cold WFI to ensure temperatures below 10 °C. The temperature was carefully monitored during the experiment. The membrane used was the Sartocon Slice PESUmax cassette, membrane area 0.1 m^2 (material number 305146AL01K–SW).

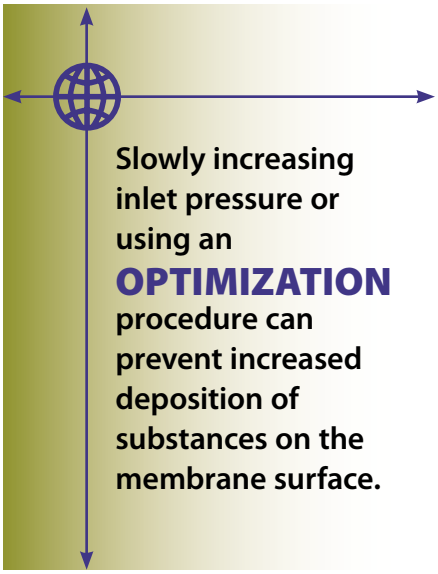
Flushing the Membrane, Determining the Water Flux Rate: After assembling the system and installing the cassette, we rinsed 10 L WFI at p_{in} = 2 bar, p_{out} = 0.5 bar, and p_{per} = 0 through the entire system to flush it and to remove the preservative (20% ethanol) from the cassette. The rinsed liquid was replaced by 2 L fresh WFI, which was circulated for five minutes in the system. The permeate water flux rate was also set at p_{in} = 2 bar, p_{out} = 0.5 bar, and p_{per} = 0. The initial clean water flux rate was determined with 430 L/hm^2. This water was then rinsed from the system.

The Concentration Step: During start-up with the protein solution, the secondary boundary layer must be developed slowly to prevent unwanted fouling and reduce the need for cleaning-in-place after filtration. Slowly increasing inlet pressure or using an optimization procedure can prevent increased deposition of substances on the membrane surface (under a total recirculation condition in which permeate is connected to the feed tank).

The permeate volume was counted by balance control. Crossflow rates were kept constant throughout the experiment, resulting in an average TMP of 1.6 bar. The average permeate flow was performed with 61.8 LMH. The results are shown in Figure 5.

Large-Scale Processing

Demonstrations of permeate flow performance during HSA concentration have shown that an albumin concentration of above 28% can be finalized in a 25% albumin formulation. The method allows for customized process automation (1), clean-in-place integration, and process performance documentation for large-scale albumin processing.



References

1 Todd K. Ultrafiltration for Plasma Fractionation (*Helix* March 2001).

2 Jaffrin MY, Charrier JP. Optimization of Ultrafiltration and Diafiltration Processes for Albumin Production. *J. Membrane Sci.* 97, 1994: 71–81.

For Further Reading

Dosmar M, Meyeroltmanns F, Gohs M. Factors Influencing Ultrafiltration Scale-Up. *BioProcess Int.* 3(8) 2005: 40–50.

Corresponding author ***Frank Meyeroltmanns,*** *Dipl.-Ing., is head of product management, Crossflow, and* ***Matthias Grabosch*** *is senior scientist in application development at Sartorius AG, Goettingen, Germany; 011-49-551-308-3954; frank.meyeroltmanns@sartorius.com.*