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Application Note

Ultrafiltration Processing of Human Serum Albumin

Introduction

Human Serum Albumin (HSA) has a long history of clinical use dating back over 60 years. It is currently used in greater volume than any other biopharmaceutical solution that is available, and worldwide manufacturing is of the order of 100s of tonnes annually.[1] The serum protein content of plasma (the liquid portion of blood less erythrocytes and leukocytes) is approximately 7% (70 g/L). HSA accounts for approximately 60% of the protein found in plasma. The typical concentration is 35–50 g/L making it the most abundant plasma protein. HSA contributes over 60% of the oncotic pressure to the circulatory system. This is an osmotic pressure effect that draws fluid into the veins and arteries that keeps fluid from leaking out of the circulatory system into the surrounding tissue. HSA acts as a "transport molecule" in the body and is a highly charged entity. The clinical indications for albumin therapy in the United States include; hypovolemia, shock, burns, hypoalbuminemia, surgery or trauma, cardiopulmonary bypass, acute respiratory distress syndrome, hemodialysis, and edema. HSA therapy accounts for up to 30% of the total pharmacy budget in certain hospitals.[2] Commercial HSA product formulations are marketed as 5%, 20% & 25% solutions. The in-market price of Albumin varies but the average price is approximately $6.30–$7.00 per gm of protein.

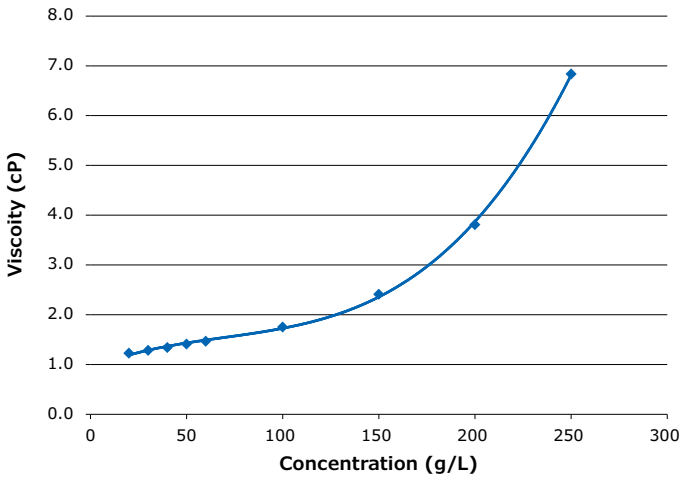
Physical Properties

The reported molecular weight of HSA ranges from 65–69 kDa. The range is due to variants in structure (isoforms) and glycosylation that can occur over time. The molecular weight by amino acid sequence is 66,437 Da with most being between 66.4–66.6 kDa.[3]

The isoelectric point of HSA is pH 4.7.

HSA viscosity is a strong function of its concentration in solution.[4]

Figure 1. Viscosity of Human Serum Albumin versus Concentration at 20 °C

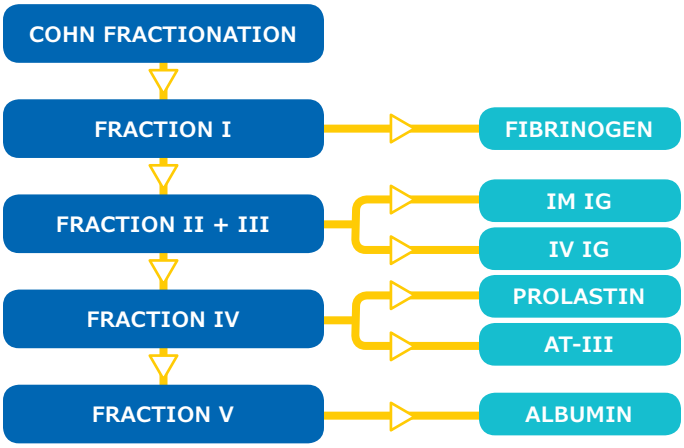


Fractionation

Plasma fractionation is the isolation of blood plasma proteins into different fractions or groups. Fractionation is largely accomplished by the manipulation of temperature, ionic & dielectric strengths (salt concentration & ethanol concentrations) and pH to cause separation by selective precipitations of proteins in the plasma. The precipitations at each fractionation step are resolubilized and further purified to obtain therapeutically useful plasma proteins. The supernatant from each fractionation step becomes the feed material for the next fractionation step. Dr. E.J. Cohn and his team at Harvard University pioneered the plasma fractionation and purification process during the early 1940s. The objective of Cohn's original process was to produce a purified HSA for blood volume replacement during World War II. The fractionation process in use today is still largely based on the Cohn Method 6 (or a variant, the Kistler & Nitschmann methodology), with modifications to recover additional therapeutically

valuable proteins such as the clotting factors (Factors VIII & IX and von Willebrands Factor) Immuno gamma Globulins (IgGs), Alpha 1 Proteinase Inhibitor (Alpha 1 anti-Trypsin), etc.

Figure 2. Therapeutic Proteins Recovered from the Fractionation Process



HSA remains largely in solution throughout the fractionation process until Fraction V. During the Fraction V operation, the ethanol concentration is raised to 40% and the pH adjusted to 4.9, at which point the HSA precipitates. This precipitate is recovered as Fraction V "Paste". The paste can be frozen and stored until a sufficient amount is accumulated for a batch. The albumin molecule is a very stable structure and the paste can be re-suspended and precipitated multiple times for improved purity.

Purification Process

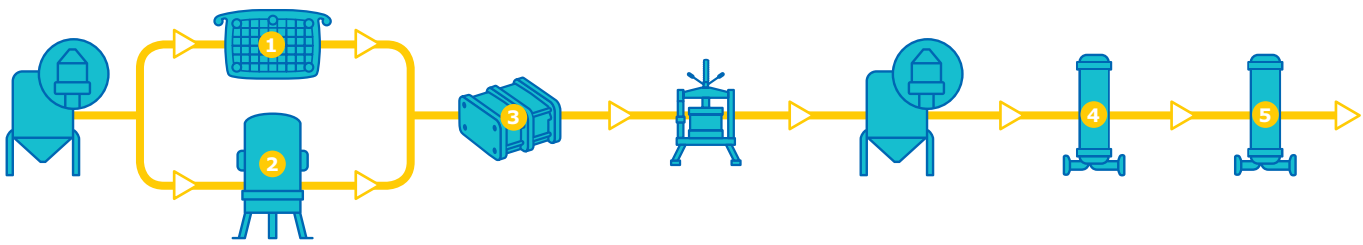
HSA was the first protein from plasma to be commercialized in the mid 1940s. Different manufacturers have made some changes and improvements to the purification process. Therefore, today albumin purification varies from manufacturer to manufacturer and the different processing schemes are proprietary. It is known that many processes are still based on cold ethanol fractionation where precipitation/re-suspension/filtration steps are employed to achieve purification. An early generation HSA product called Plasma Protein Fraction (PPF) (5%) process has fewer purification steps and contains ~88% albumin, 12% α and β globulins and <1% γ globulins.

Figure 3 presents a "typical" purification scheme for HSA. Higher purity formulations of HSA receives additional (as many as four) precipitation/re-suspension/filtration steps. One of these steps may be carried out in a non-polar solvent to remove impurities such as low level residual proteins, triglycerides and cholesterol which may impact product quality during pasteurization. The traditional clarification method for the re-suspended protein employs a body feed of filter aid into the batch and collection of this material on a plate and frame filter press. The diatomaceous earth component of filter aid can lead to aluminum ion capture by the HSA due to its highly charged nature. Evidence has been presented that infusions of HSA solutions can create aluminum toxicity in some patients.[6] Therefore a major objective of HSA purification is to achieve aluminum concentrations of <200 µg/L in the final drug product.[7] Aluminum removal is most often accomplished by an ultrafiltration (UF) based concentration/diafiltration step, utilizing a high ionic strength buffer. This UF unit operation is also frequently used to achieve a final bulk drug substance concentration in a formulation buffer. After the UF step, the HSA is sterile filtered and filled. The final step in the purification process is Pasteurization at 60 °C for 10 hrs. Derived from human plasma, HSA carries a risk of contamination by pathogenic virus. However, HSA is considered one the safest plasma products because the multiple process steps utilizing solvent have been shown to inactivate lipid enveloped virus and the pasteurization step has been demonstrated to inactivate non-enveloped virus. Nucleic acid testing (NAT) of the source plasma as an overall surveillance strategy, in addition to the clearance capability of the process, makes viral contamination of HSA unlikely. Additional viral clearance capability can be achieved by adding a viral filtration step. However, HSA batches are large thus the cost of implementing viral clearance filtration is a consideration.

Chromatographic methodologies (anion and cation exchange and size exclusion) are fairly recent developments (1980s–1990s) to improve the purity of the product, but still not widely adopted.

The purity of commercial HSA solutions is >98%. The Plasma Protein Therapeutics Association (PPTA) reports that the yield of HSA from 1 liter of plasma is 25 g. Assuming that the mean concentration of HSA in plasma is 42 g/L, the overall recovery is approximately 60%.

Figure 3. "Typical" Human Serum Albumin Purification Process



Ultrafiltration Step

Ultrafiltration Objectives

The objectives of the Ultrafiltration concentration/ diafiltration step within the purification process of albumin are:

a.) Achieve clearance of Aluminum to <200 µg/L by diafiltering with simple NaCl buffer of high ionic strength (>0.5 M NaCl).

b.) Achieve clearance of residual ethanol left over from the HSA purification steps.

c.) Adjust the batch to its final buffer concentrations.

d.) Achieve a final HSA concentration in the final buffer. Usually this is 280–300 g/L.

e.) Realize >/= 98% Step Recovery of the HSA Batch.

f.) Process the batch in 6–10 hours.

UF Process Parameters

HSA processes usually employ one UF/DF step prior to final product formulation and sterile filtration. The UF/DF processing step for HSA is optimally performed in 3 phases; first concentration/constant volume diafiltration/final concentration. This scheme is performed in addition to the other process step needed for best practice UF/DF system operation (details can be found in Technical Brief #TB032). **Figures 4 and 5** present a typical UF/DF process for HSA. The endpoint of the initial concentration for HSA solution is usually 100–140 g/L of HSA. The HSA is then diafiltered with 5–8 diavolumes for effective buffer exchange. The final UF concentration endpoint is an over concentration to >25% HSA. This allows a buffer flush recovery of the system to be added back to the batch, boosting yield thus mitigating the dilution effect.

A useful parameter in UF operations is an extrapolation of the flux vs concentration curve to the point where the filtrate flux is zero. This point is referred to as Cg. From a physical standpoint, Cg represents the membrane wall concentration of the protein against the wall of the membrane when the osmotic pressure is equivalent to the transmembrane pressure. Under this condition the filtrate flow ceases to transport across the membrane. The extrapolation of the flux versus concentration for this HSA dataset is 300–320 g/L. This parameter can be used to set an optimum diafiltration point[8] (Cg/e where e = base of the natural logarithm) and as a reference to the maximum possible concentration under the processing conditions.

Figure 4. Flux Versus time for Human Serum Albumin UF/DF

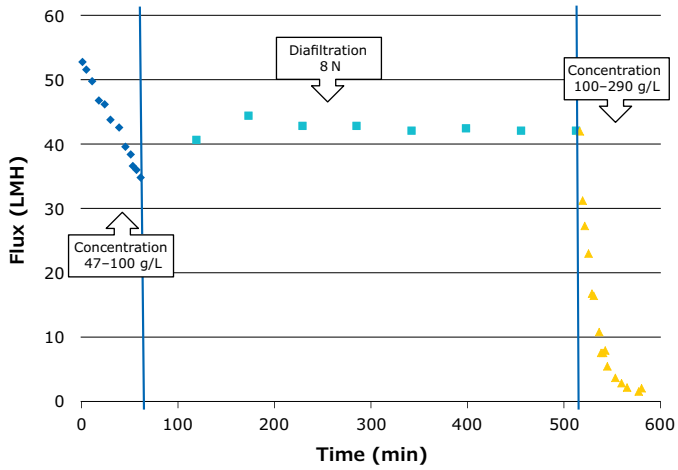
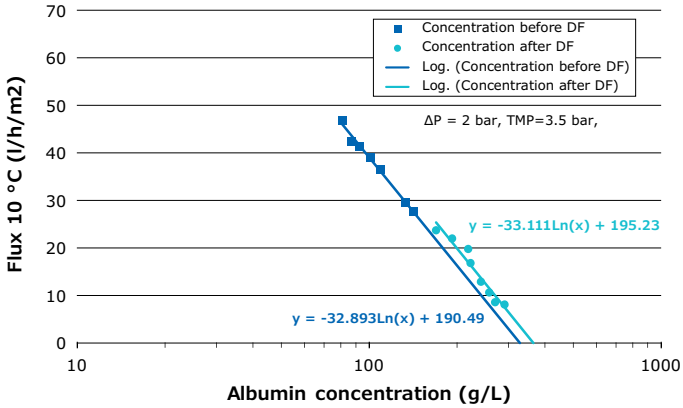


Figure 5. Flux Vs Albumin concentration



Below in **Table 1** are typical process parameters that that we have encountered based on accumulated experience working with HSA processes.

Process Parameter Considerations for UF operations on HSA

Achieving high final concentrations >250 g/L by UF concentration can be challenging. Factors that contribute to this behavior include fluid properties such as:

a.) Diffusivity of the protein

b.) Osmotic Pressure

c.) Impurities

d.) Viscosity

Table 1. Typical HSA Processing Parameters

Membrane	Loading (g/m2)	Albumin Concentration	Process Temp.	Average Flux (LMH)	Process Time (hr)	Feed Flow (L/m2/min)	TMP (bar)	Conc. Factor	N Diaf. Vol.	Yield (%)
Biomax 10 kDa A Screen	100–125	12–45 g/L (start) 100–140 g/L (diaf.) 280 g/L (final form.)	4–10 °C	50 (conc.) 30 (diaf.) 17 (final form.)	6–8	3.5–5.0	3.5	up to 23	5–8	>98%

Diffusion is a transport mechanism within a solution, where a high concentration solute diffuses throughout the solution to reach a uniform concentration. Diffusivity (diffusion coefficient) is a measure of how rapidly the solute will diffuse into the solvent. Diffusivity is a property of the solution. The Einstein-Stokes relationship shows:

$$D = \frac{Kb\ T}{6\ \pi\ \eta\ r}$$

Where:

D = Diffusivity

Kb = Boltzmann Constant

T = Temperature

η = viscosity

r = radius of a spherical particle

Diffusivity is a function of both the solution temperature and viscosity.

The UF concentration step can be described by a simplified "Gel Film Model" (assume the retention of protein solute is 100%):

$$J = k \ln(Cw/Cb)$$

Where:

J = volumetric flux

k = mass transfer coefficient = Diffusion Coefficient/ Boundary layer thickness = D/δ

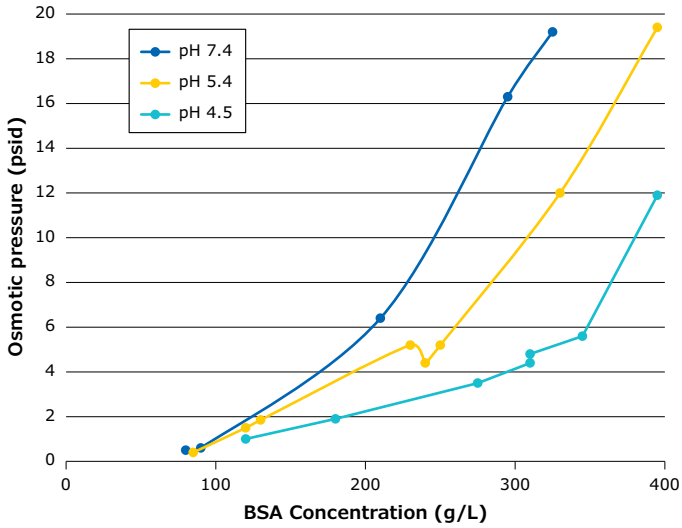
Cw = solute concentration at the membrane "wall"

Cb = solute concentration of the "bulk" solution.

A log-linear plot of J versus Cb for HSA is linear with the mass transfer coefficient being the slope. The mass transfer coefficient (k) is proportional to Diffusivity. Diffusivity is a function of both temperature and concentration. Temperature range in the HSA UF operation is limited by process considerations such as aggregate formation at higher temperatures and bioburden control. Viscosity is a function of the concentration which will increase during the UF step. The film thickness δ can be attenuated to a limited extent by crossflow (increasing mass transfer coefficient). The membrane permeability of HSA retentive membranes is low due to the pore size of the membrane. Feed flow rate (crossflow) is a variable that can be manipulated to optimize the mass transfer coefficient and maximize flux, but this variable is limited due to process scale considerations. HSA batches tend to be large (in the thousands of liters). Therefore, there are practical limits on feed flow rate (3.5–5.0 L/min/M2) because of system design considerations (pump sizing, pipe size, increased hold-up and heat input to the system).

Albumin has an osmotic pressure effect that is a strong function of concentration.

Figure 6. Osmotic Pressure of Various Aqueous Protein Solutions versus Concentration[9]



The Osmotic Pressure Model for flux is shown below:

$$J = L_p*(TMP - \sigma\pi)$$

Where:

J = volumetric flux

L_p = Membrane process permeability

TMP = transmembrane pressure.

σ = reflection coefficient =1 for fully retentive membrane.

π = Osmotic pressure = a*Cw + b*Cw2 + . . .

When Cw (protein concentration at the membrane wall) becomes high the Osmotic pressure also becomes high and counters the flux driving effect of TMP. As seen from **Figure 6**. Final formulation HSA solutions are in the pH range of 6.4–7.4[10] and ~150 mm sodium content. The osmotic pressure of albumin solutions runs from 8-18 psid over this pH range.

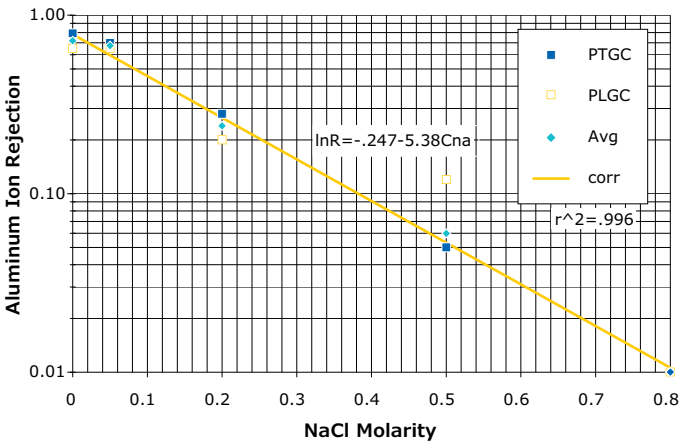
Therefore, to make the membrane as efficient as possible along the entire membrane path length and minimize installed process area the retentate pressure should be operated above the osmotic pressure of the final albumin formulation. A strategy of running at as high a transmembrane pressure as allowed by the device and solution chemistry (pressure drop due to viscosity) is employed. This is done by closing down on the retentate valve and exploits the stability of the HSA molecule to resist aggregation and denaturation under high wall concentrations while maximizing flux and minimizing the area.

Considerations for Aluminum Removal

As previously mentioned Aluminum ions from the filter aid utilized in the purification process bind to the HSA molecule and this can lead to toxicity in some patients. The electrical nature of the bond between Aluminum and HSA can be disrupted by diafiltration with a high ionic strength NaCl buffer.

Figure 7 below shows the apparent rejection coefficient of Aluminum by regenerated cellulose 10 kDa membrane and Polysufone 10 kDa membrane as a function of the ionic strength of a NaCl buffer.

Figure 7. Diafiltration of Aluminum from 10% HSA



This curve shows that the ionic strength should be at least 0.4 M for a low rejection coefficient. A common practice in the purification of HSA is to diafilter against the NaCl buffer until removal meets specification and then to diafilter out the excess salt with WFI. Typically this is done with an 8–10 diavolume wash of the batch which also removes the ethanol in addition to the salt and low molecular weight impurities. We have disclosed a method where we:

- concentrate to 10% Albumin
- add salt
- diafilter with water to remove ethanol, salt and aluminum
- concentrate to final formulation

This improved method for metal ion removal substitutes diafiltration with water in place of diafiltration with salt. This changes the diafiltration from consecutive removal of each species to simultaneous removal. It reduces the diafiltration requirements and allows a smaller system to do the same job. The diafiltration requirements are determined by the initial and final values of each component:

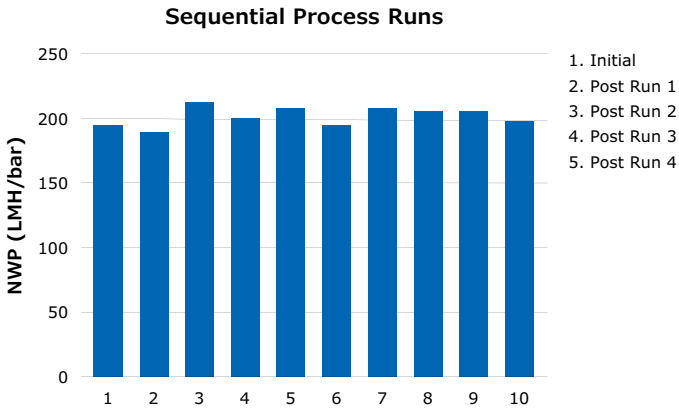
Component	Initial Content	Final Content	Log Reduction
Aluminum	200–2000 ppb	30–50 ppb	1.4–4.2
Sodium	1–5 Molar	.15 Molar	1.9–3.5
Ethanol	10–12%	.5–.05%	3.0–5.5

The values for Aluminum and Ethanol include the overall process with initial and final concentration steps. The initial Sodium values represent a range of possible concentrations after the salt addition step and before the diafiltration step. The salt concentration for the batch can be calculated from the mass balance knowing the level of impurities present in the batch and the desired levels of removal.

UF Cleaning Considerations

An effective cleaning solution used for Biomax® polyether sulfone based membranes is 0.5 M NaOH combined with up to 250 ppm NaOCl 40–45 °C, for 30–60 minutes. These cleaning parameters provide repeatability and constancy in Normalized Water Permeability (NWP) measurements as shown in the graph **Figure 8** below for the membranes that process albumin. Additional information on establishing a cleaning and monitoring program for the membrane cassettes can be found in tech brief TB1502EN00.

Figure 8. Water Permeability after Cleaning



Post UF Operations

Post UF, the batch is commonly stabilized by the addition of Sodium caprylate (sodium n- octanoate) and acetlytryptohan to a concentration 0.016 M each. Then the batch is diluted to the final required concentrations and enters pasteurization (60 °C for 10 hrs.) for viral inactivation. Sterile filtration capacity is a function of many factors, including concentration, aggregate level and buffer components. A suggested starting point without data is for sterile filtration is 450 gm/M2/bar +/- 50% for either 0.22 µm Durapore® (PVDF) or Millipore Express® SHC (PES) filters for concentration of 5–25%. Filter membrane material (PVDF/PES) may be a factor, but optimization studies are necessary to determine the best filter material.

Final Recommendations

Operating parameters and membrane performance depend on a variety of factors — feed stream composition (purity, protein concentration, etc.), membrane/device selection, target final formulation, etc. Data has been presented on concentration, diafiltration to remove the Aluminum impurity and sterile filtration. This data is "typical" but individual processes may vary depending on concentration, and buffer composition.

Please consult with our technical support for specific applications.

Additional details on TFF processes can be found in *"Protein Concentration and Diafiltration by Tangential Flow Filtration"* Technical Brief #TB032. This technical brief is available online at http://www.millipore.com/publications.nsf/docs/tb032. We have a staff of Applications and Process Engineers available to help you develop your new process or troubleshoot your existing process. Your local Applications Specialist can arrange for you to speak with this technical staff.

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10. Material Safety Data Sheet, Albumin (Human) 5% & Albumin (Human) 25%, Octapharma Pharmazeutika Produktionsges.m.b.H., Date Approved/Revised 20.10.2006

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