



Diafiltration and Concentration of Human Serum Albumin



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Application
Note

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Purification by
Crossflow Filtration
PESUmax
(Albumin Membrane)

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Abstract

Human Serum Albumin (HSA) is usually obtained from plasma (Picture 1) according to Cohn or a variation of the Cohn procedure from the Kistler-Nitschmann process. In this process, various plasma proteins are removed by precipitation. The alcohol, pH, and temperature are varied to cause individual proteins to precipitate so that they can be separated (see Figure 1).

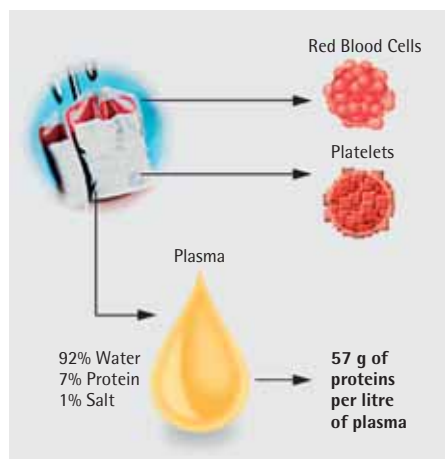
The production process uses established cold ethanol fractionation technology in the purification of clinical useful proteins from blood plasma. During the Human Serum Albumin manufacturing process, one integral step is the diafiltration and final product concentration. The ethanol added during the fractionation process must be removed during the final stages of processing, as well as citrate, which is added during blood donation to act as an anti-coagulant. Citrate in the albumin solution is known to chelate aluminium from the glass container and must be carefully controlled in order to maximize product shelf-life.¹

To meet the design brief, Sartorius Stedim Biotech application engineers worked to optimize the diafiltration, concentration, and cleaning processes.

The diafiltration method used a concentrated sodium chloride solution, diluted with water for injection (WFI). This facilitated removal of both citrate and ethanol from the product. The salt solution was then removed by diafiltering against more WFI, resulting in an albumin solution free of ethanol and salts and with citrate at levels well below specified limits. Sartorius Stedim Biotech also developed a new ultrafiltration (UF) membrane – PESUmax, specifically for this application. The new membrane features higher flux rates compared to membranes traditionally used for albumin processing and low protein binding.

Principles of Crossflow Filtration

In crossflow filtration, the 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. In an optimization procedure the cross-flow efficiency is maximized, thus allowing the use of higher transmembrane pressures which result in increased flux, translating 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 (P_i), retentate back pressure (P_o), and permeate back pressure (P_f).



Picture 1: Plasma Fractionation

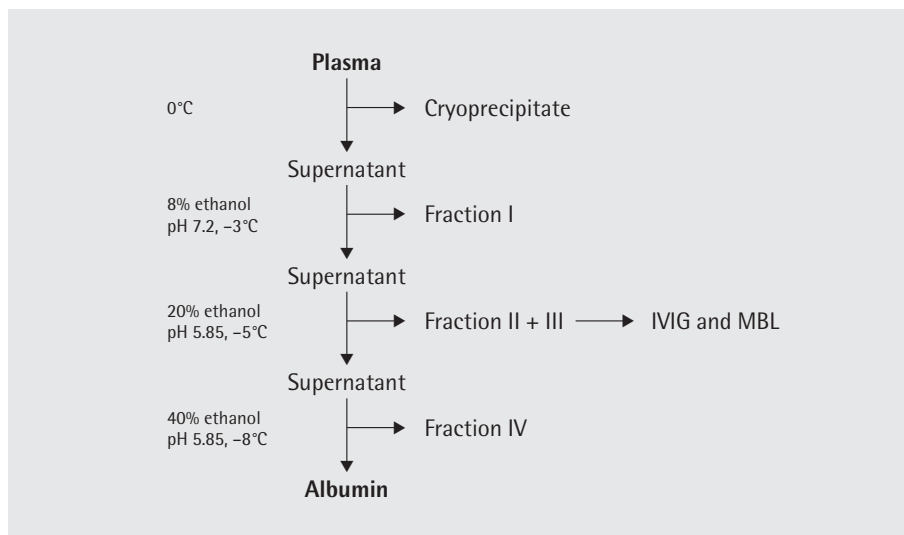


Fig. 1: Cold Ethanol Plasma Fractionation according to Kistler-Nitschmann process³

Fluid flow through a membrane is best described by the Hagen-Poiseuille equation for streamline flow through channels:

$$J = [e \cdot r \cdot (TMP)] / [8 \cdot u \cdot x]$$

where

- J = flux
- e = membrane porosity
- r = mean pore radius
- TMP = transmembrane pressure
- u = viscosity of fluid
- x = thickness of the membrane

Based on this mathematical model, the ability to influence the flux for a given membrane is limited to using the maximum possible transmembrane pressure.

During filtration the solution will build up on the membrane surface a gel layer, despite the cross-flow sweeping action, by a phenomenon known as concentration polarization.

Once the gel forms, the flux declines and no longer depends on transmembrane pressure as described by the Hagen-Poiseuille equation independent from TMP.

Objectives

During manufacturing of HSA, one important step is the diafiltration followed by the final product concentration. Crossflow systems equipped with ultrafilter membrane cassettes retain the target product (HSA) and release unwanted substances into the filtrate. Initial concentration of the feed volume leads to volume reduction and eases fluid handling.

This new cassette design incorporates an albumin-tight membrane. The rejection efficiency with albumin is higher than 99.9%. The new PESUmax Sartocoon Cassette features higher flux rates compared to membranes traditionally used for albumin applications. PESUmax can also withstand high chemical cleaning treatments and storage procedures. The new cassette development has resulted in projects that have achieved complete process and cleaning validation of Sartoflow systems within three consecutive validation runs with a significant yield increase.

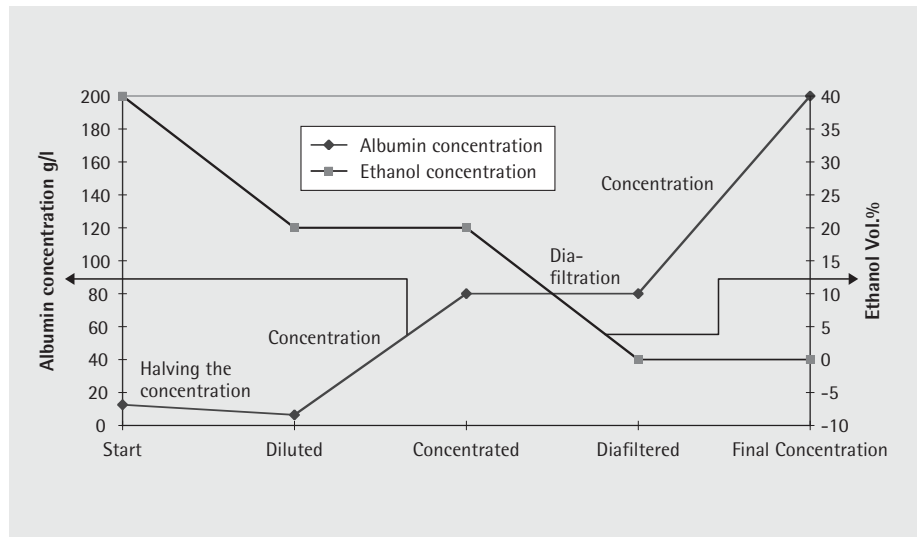


Fig. 2: Process steps for albumin filtration

Description of Trial Procedure

In one of the later plasma fractionation steps the supernatant is separated into Fraction IV and albumin. The albumin concentration in this step is around 13 g/l. The alcohol content of this solution is 40 vol. %. This solution is then further processed in 4 steps:

1st step:

Dilution of the solution with water to an alcohol concentration of 20 vol %. This step has been necessary until now because polysulfone ultrafiltration cassettes would have been irreversibly negatively influenced in performance when exposed to an alcohol concentration of 40 vol %.

2nd step:

Initial concentration for volume reduction of the albumin concentrate to around 60–80 g/l. The alcohol concentration remains constant during this step. (This step is the longest of the entire process according to M.Y.Jaffrin²)

3rd step:

Diafiltration with water to remove the alcohol. Volume and process parameters remain constant.

4th step:

Concentration to an albumin content (28–30% HSA) for final formulation of 25% albumin.

This application note contains the results of a HSA concentration step.

Materials and Methods

Properties medium:

2000 ml of Human Serum Albumin at an initial protein concentration of 6.5% HSA. Final volume is 500 ml with 25.5% Albumin for final formulation.

Crossflow system:

Sartoflow Alpha System in ultrafiltration setup. 1 m³/hr rotary lobe pump, jacketed feed vessel cooled with chilled WFI (maintained temperatures below 10°C).

Membrane:

Sartocon Slice PESUmax Cassette, Catalog Number: 305146AL01K--SW, membrane area 0.1 m².

Flushing the Membrane, Determining the Water Flux Rate

After assembling the system and cassette installation, the system was rinsed with 10 l WFI at $p_{in} = 2$ bar, $p_{out} = 0.5$ bar, and $p_{per} = 0$ to flush and to remove the preservative (20% ethanol) from the new cassette.

A fresh 2 l WFI was then circulated for five minutes. The water flux rate was determined ($p_{in} = 2$ bar, $p_{out} = 0.5$ bar, and $p_{per} = 0$).

The initial clean water flux rate was determined with 430 l/hm².

This water was drained out of the system.

Concentration

As the protein-containing solution is introduced to the system, the secondary boundary layer must be developed slowly. This prevents fouling effects and reduces cleaning in place issues after filtration. This prevents fouling effects and reduces cleaning in place issues after filtration. Slowly increasing inlet pressure or using an optimization procedure (under total recirculation condition – meaning that the permeate channel is connected back to the feed tank) prevents increased deposition of substances on the membrane surface.

The permeate volume was measured by weight balance.

The crossflow rates were kept constant throughout the experiment, resulting in an average Trans Membrane Pressure (TMP) of 1.6 bar.

The average permeate flow was performed with 61.8 LMH (l/hm²).

Results

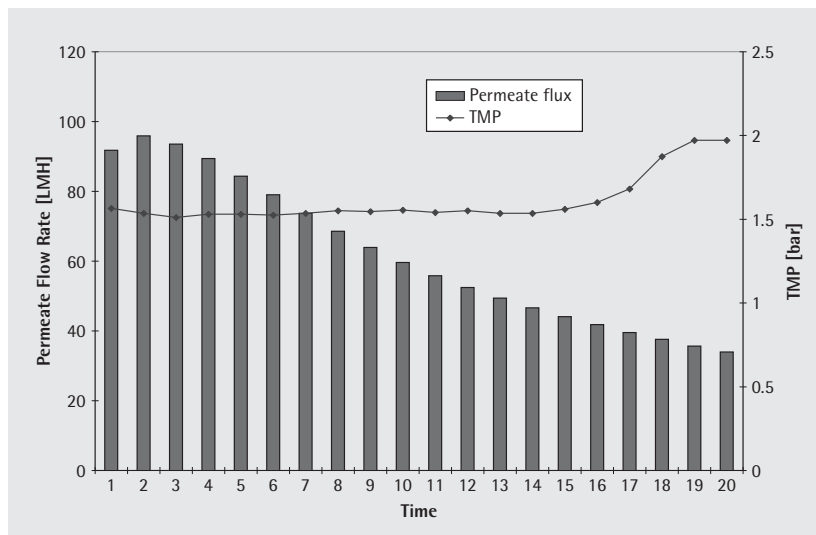


Fig. 3: Albumin Concentration

Albumin Filtration with Sartocoon Slice Cassette | Type PesuMax

Membrane area = 0.1 m² | Sartocoon Slice Equipment – Sartoflow Alpha system.

Initial albumin concentration = 6.96 g/L

Start volume = 2.0 L

Permeate pressure = 0 bar

Elapsed Time min	Permeate [g]	Permeate flux (l/hm ²)	TMP (bar)
1	214	92	1.6
2	447	96	1.5
3	655	94	1.5
4	834	89	1.5
5	984	84	1.5
6	1106	79	1.5
7	1209	74	1.5
8	1279	69	1.6
9	1341	64	1.5
10	1391	60	1.6
11	1433	56	1.5
12	1467	52	1.6
13	1496	49	1.5
14	1522	47	1.5
15	1543	44	1.6
16	1560	42	1.6
17	1567	39	1.7
18	1576	38	1.9
19	1579	36	2.0
20	1582	34	2.0
Average flux		61.8	

Conclusion

The permeate flow rate during Human Serum Albumin (HSA) concentration has been measured.

PESUmax Albumin Cassettes are developed for albumin diafiltration and product concentration. Additional experiments in HSA concentration indicate a possibility to achieve albumin concentration of above 28%, resulting in a final concentration of 25% Albumin formulation. Sufficient scale-up concepts allow for customized process automatization¹, cleaning in place integration, and process performance documentation for large scale Albumin processing.

References

1. Karen Todd. Ultrafiltration for Plasma Fractionation (Helix March 2001), Sartorius Stedim UK Limited
2. Jaffrin MY, Charrier JPH, Optimization of ultrafiltration and diafiltration processes for albumin production. Journal of Membrane Science 97, 1994: 71–81
3. Web graphic download source: www.biochemsoctrans.org

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