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Capture of Human Serum Albumin from Plasma Using HyperCel™ STAR AX Salt Tolerant Anion Exchange Chromatography Sorbent

Impact on Process Economics in a Two-Step Streamlined Process

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1. Summary

▶ This study describes the development of a model two-step purification process for serum albumin from human plasma using HyperCel™ STAR AX "salt tolerant" anion-exchange sorbent as capture step.

▶ Comparative assessment of HyperCel STAR AX sorbent with a conventional rigid DEAE agarose sorbent, typically used in plasma fractionation, was performed.

▶ Design of Experiment (DoE) / high throughput screening optimization of purification conditions was performed for both sorbents.

▶ A process economics analysis was performed to evaluate the impact of the elimination of feedstock dilution on process cost.

2. Introduction

Capture of neutral to acidic proteins generally uses anion exchange (AEX) sorbents. Conventional anion exchangers requires feed dilution to decrease ionic strength, or needs diafiltration to achieve sufficient protein binding capacity. These operations, however, increase buffer consumption and processing time, and increase overall process cost. Using a "salt tolerant" anion exchanger such as HyperCel STAR AX sorbent would allow direct capture from undiluted feedstocks and result in significant process economics benefits at production scale.

HyperCel STAR AX sorbent was evaluated in a two-step purification process without dilution, taking the purification of Human Serum Albumin (HSA) from cryo-poor plasma as a model (Figure 1).

Table 1
*Properties of HyperCel STAR AX Sorbent*

Average particle size	80 µm
Ion exchange ligand	Primary amine
Dynamic binding capacity[1] at conductivity 15 mS/cm	>100 mg BSA/mL within pH range 7.5 – 8.0
Recommended operating range of feedstock conductivity	2 – 15 mS/cm
Recommended cleaning conditions[2]	1 M NaOH

[1] Determined using 5 mg/mL BSA in 25 mM Tris-HCl , 0.14 M NaCl at 2 minute residence time
[2] Injection of 5 column volumes (CV) of 0.5 – 1 M NaOH, 1 hour contact time

Figure 1
Overview of Process Development for Purification of HSA from Cryo-Poor Plasma

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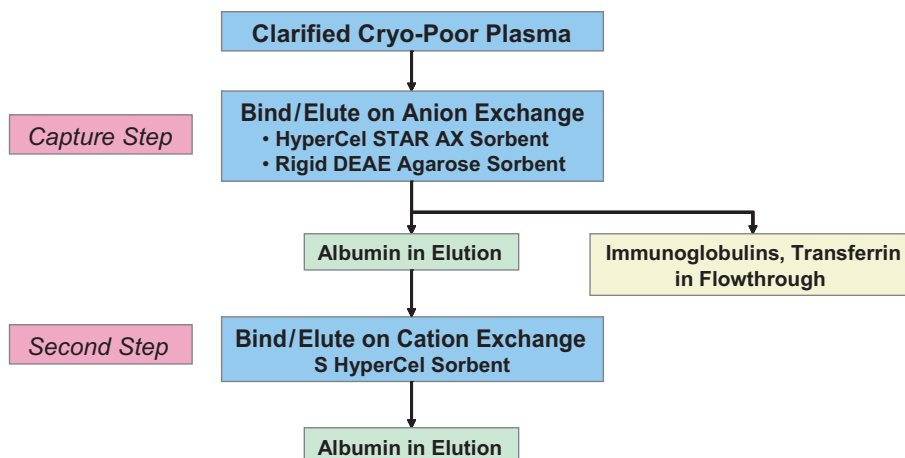
3. Materials and Methods

3.1. Plasma Preparation and Analytical Methods

Frozen human plasma was thawed at 4 °C for one day and centrifuged at 4500 x g for 10 minutes at 4 °C to remove small aggregates, and then filtered through a Pall 0.2 µm Supor® 200 membrane.

SDS-PAGE in non-reducing conditions	Nupage◆ 4-12% Bis-Tris precast gels, staining with Coomassie SimplyBlue◆ SafeStain (Life Technologies)
Immunoglobulin (Ig) quantification	Protein A HPLC column (Applied Biosystems)
Transferrin (Trf) quantification	ELISA assay kit (Cygnus Technologies)
Albumin quantification	Bromocresol green colorimetric assay (Fisher Diagnostics)

Recovery and purity were calculated as shown below. Purity was measured as the ratio between the amount of albumin and the total amount of major plasma proteins (albumin, immunoglobulins and transferrin) measured in the samples.

▸ $\text{Albumin recovery (\% of load)} = \dfrac{\text{Albumin in elution (mg)} \times 100}{\text{Albumin in load (mg)}}$

▸ $\text{Albumin purity (\%)} = \dfrac{\text{Albumin (mg)} \times 100}{\text{Ig (mg)} + \text{Trf (mg)} + \text{Albumin (mg)}}$

3.2. Chromatography Runs

3.2.1. DBC vs. Plasma Dilution on HyperCel STAR AX Sorbent and Rigid DEAE Agarose Sorbent

DBC was determined in 0.5 cm I.D. columns packed with 1 mL of HyperCel STAR AX sorbent or rigid DEAE agarose sorbent. DBC at 10% breakthrough ($\text{DBC}_{10\%BT}$) was evaluated by quantification of albumin in the flowthrough fractions after loading undiluted and 1.6- and 3-fold diluted plasma (respectively 11, 7 and 4 mS/cm).

Flow rate	0.5 mL/min (2 minute residence time)
Equilibration buffer	50 mM Tris-HCl, pH 7.6 at 11, 7 or 4 mS/cm (10 CV)
Sample load	Plasma (undiluted or 1.6-fold or 3-fold diluted)
Wash	Equilibration Buffer (10 CV)
Strip	50 mM Na acetate, pH 4.0 + 1 M NaCl (10 CV)

3.2.2. HSA Capture Runs on Column

The optimal conditions determined by 96-well plate / high-throughput screening for HyperCel STAR AX sorbent and rigid DEAE agarose sorbent (see 3.3) were transferred to chromatography runs on columns with load at 60% of $\text{DBC}_{10\%BT}$ of undiluted plasma on HyperCel STAR AX sorbent, and 3-fold diluted plasma on rigid DEAE agarose sorbent.

Flow rate	0.5 mL/min
Equilibration buffer	50 mM Tris-HCl, pH 7.6, adjusted to optimal conductivity for each sorbent
Sample load	Plasma at optimal dilution
Wash	Determined by 96-well plates optimization (10 CV)
Elution	
Strip	50 mM Na acetate, pH 4.0 + 1 M NaCl (5 CV)
CIP	1 M NaOH (2 CV)

3.3. DoE Optimization of Wash and Elution Conditions on 96-Well Plates

High throughput screening optimization of pH and conductivity conditions based on DoE was performed. An extended central composite design was established using the Minitab◆ software, as described in Table 2, for the four parameters *Wash pH, Wash conductivity, Elution pH, Elution Conductivity*.

Table 2
Test Conditions for High Throughput Screening Optimization of Wash and Elution Conditions of HSA Capture Step on AEX Sorbents

Sorbent	Load	Wash pH	Wash Conductivity (mS/cm)	Elution pH	Elution Conductivity (mS/cm)
HyperCel STAR AX	Cryo-poor plasma	5.5 to 7.5	2 to 20	3.5 to 5.5	2 to 50
Rigid DEAE Agarose	3-fold diluted cryo-poor plasma				

Based on this DoE, 27 combinations were tested for their impact on albumin yield and purity. The screening of pH / conductivity combinations was done using AcroPrep™ Advance 96-well filter plate containing 100 µL of sorbent following this sequence:

	Equilibration		Load	Wash		Elution	
Volume (µL)	200	200	150	200	200	200	200
Incubation time (min)	5	5	60	5	5	10	10

HSA yield and purity obtained for each condition were recorded, data modelled and models validity checked. Contour plots were generated in order to analyze the impact of each factor on albumin recovery yield and purity, and optimal conditions were determined.

3.4. Process Economics Analysis

The process economics analysis was conducted using the BioSolve Cost of Goods (CoG) analysis software from Biopharm Services Ltd. A concentration of 50 g/L HSA in plasma was fixed. CoG analysis was performed considering 2,000 L batches, with an average number of 200 batches per year, corresponding to the processing of 400,000 L of plasma per year, meeting the average capacity of a fractionation plant. The HSA recovery for dilution steps was considered to be 100%. The flow rate, column bed height, and number of cycles were kept constant, respectively 200 cm/hr, 20 cm and 200 cycles.

4. Results and Discussion

4.1. Optimization of a Two-Step Process for HSA Purification

4.1.1. Effect of Feed Conductivity on Dynamic Binding Capacity (DBC) of Salt-Tolerant vs. Conventional AEX Sorbent

In order to address the effect of feed conductivity on DBC, evaluation of DBC on HyperCel STAR AX sorbent and rigid DEAE agarose sorbent was performed using plasma at different dilutions (Figure 2).

DBC values obtained on HyperCel STAR AX sorbent were maintained around 30 mg/mL with load at conductivities ranging from 4 mS/cm (3-fold diluted) to 11 mS/cm (undiluted). In contrast, an increase of load conductivity resulted in a significant decrease of DBC of the rigid DEAE agarose sorbent.

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This data confirmed the salt tolerant behaviour of HyperCel STAR AX sorbent and indicated that this sorbent could be used for high capacity capture of HSA directly from non diluted plasma. In this condition, DBC was more than 2-fold higher on HyperCel STAR AX sorbent than on the standard AEX sorbent.

Additionally, the DBC of HyperCel STAR AX sorbent was maintained in a broad range of conductivities, bringing more process robustness and flexibility, by accommodating potential feedstock ionic strengths variations during the process.

Figure 2

Dynamic Binding Capacity of HyperCel STAR AX Sorbent and Rigid DEAE Agarose Sorbent for Human Serum Albumin Using Undiluted (11 mS/cm), 1.6-fold Diluted (7 mS/cm) and 3-fold Diluted (4 mS/cm) Plasma
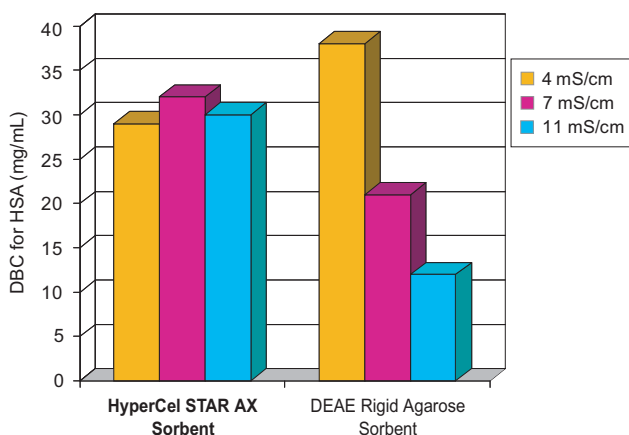
4.1.2. Optimization of Process Conditions for HSA Capture

Optimization of wash and elution conditions was performed using DoE / high-throughput screening on AcroPrep Advance 96-well filter plates (Figure 3 and Table 2). Optimal wash and elution conditions allowing best elution yield combined to lowest contaminants content (Immunoglobulins + Transferrin) in elution were determined by response surface modelling analysis.

Figure 3
*96-Well Plate / High Throughput Screening for Optimization of HSA Capture*

1. Design of Experiment (DoE)
- Critical parameters (wash and elution pH and conductivity)
- Quality attributes (elution yield, purity)

2. Screening on 96-Well Plates
- AcroPrep™ Advance Filter Plates

3. Analytical Testing
- HSA quantification (Bromocresol Green)
- Quantification of Transferrin (ELISA)
- Quantification of IgG (Protein A-HPLC)

4. Result Analysis
- Design space for optimum performances

5. Transfer to Column

Response surface modelling of HyperCel STAR AX sorbent data revealed that HSA purity was significantly impacted by wash conditions (Figure 4A). Increase in wash conductivity led to higher HSA purity, with an optimal zone for wash conductivity >15 mS/cm. Optimal elution conditions zone was determined as pH 3.5 to 4.2 at conductivities of 2 to 27 mS/cm (Figure 4B).

Finally, optimal combinations of wash and elution conditions providing estimated yield and purity ≥ 90% were chosen as follows according to the model predictions:

– Wash: pH 7.5, 20 mS/cm, allowing maximal purity.
– Elution: pH 4.0, 2 mS/cm, providing high elution yield and the possibility to directly load elution from HyperCel STAR AX sorbent on an orthogonal cation exchange sorbent (see process in Figure 1).

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Figure 4

Optimization of Wash and Elution Conditions for HSA Purification on HyperCel STAR AX Sorbent. (A) Effect of Wash Conditions on Purity. (B) Effect of Elution Conditions on Elution Yield
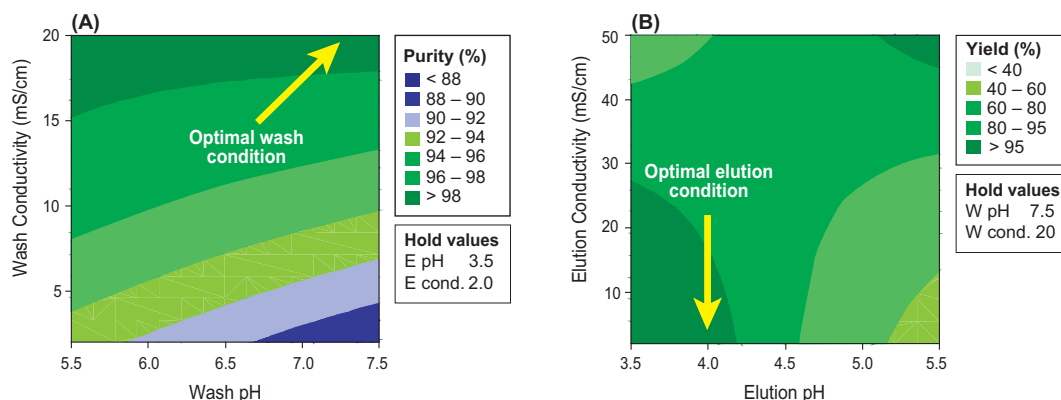
*Fixed conditions: (A) Elution at pH 4, conductivity 2 mS/cm; (B) Wash pH 7.5, conductivity 20 mS/cm.*

A similar optimization carried out with the rigid DEAE agarose sorbent yielded the following optimal combinations of wash and elution conditions providing yield and purity ≥ 90%:
– Wash: pH 7.5, 9 mS/cm
– Elution: pH 4.0, 25 mS/cm

4.1.3. Transfer of Conditions to Column Chromatography

Conditions optimized on 96-well plates for both sorbents were next applied to column runs (Figure 5). For HyperCel STAR AX sorbent, elimination of contaminants with high conductivity wash as predicted by DoE optimization was efficient, yielding 99% pure HSA. Elution driven by pH drop also resulted in a satisfying elution yield (90%). Application of optimized conditions including a high conductivity wash on rigid DEAE agarose sorbent also allowed to obtain pure HSA (98%), whereas a combination of low pH and high conductivity was required to obtain satisfying recovery at elution (yield 90%). On rigid DEAE agarose sorbent, using alternative elution condition (low pH and conductivity, as for HyperCel STAR AX sorbent) preparing a direct load to the following cation exchanger resulted in good purity but only partial elution (73% yield, see Table 3).

Figure 5

HSA Capture on HyperCel STAR AX Sorbent and Rigid DEAE Agarose Sorbent

(A) HyperCel STAR AX Sorbent

(B) Rigid DEAE Agarose Sorbent
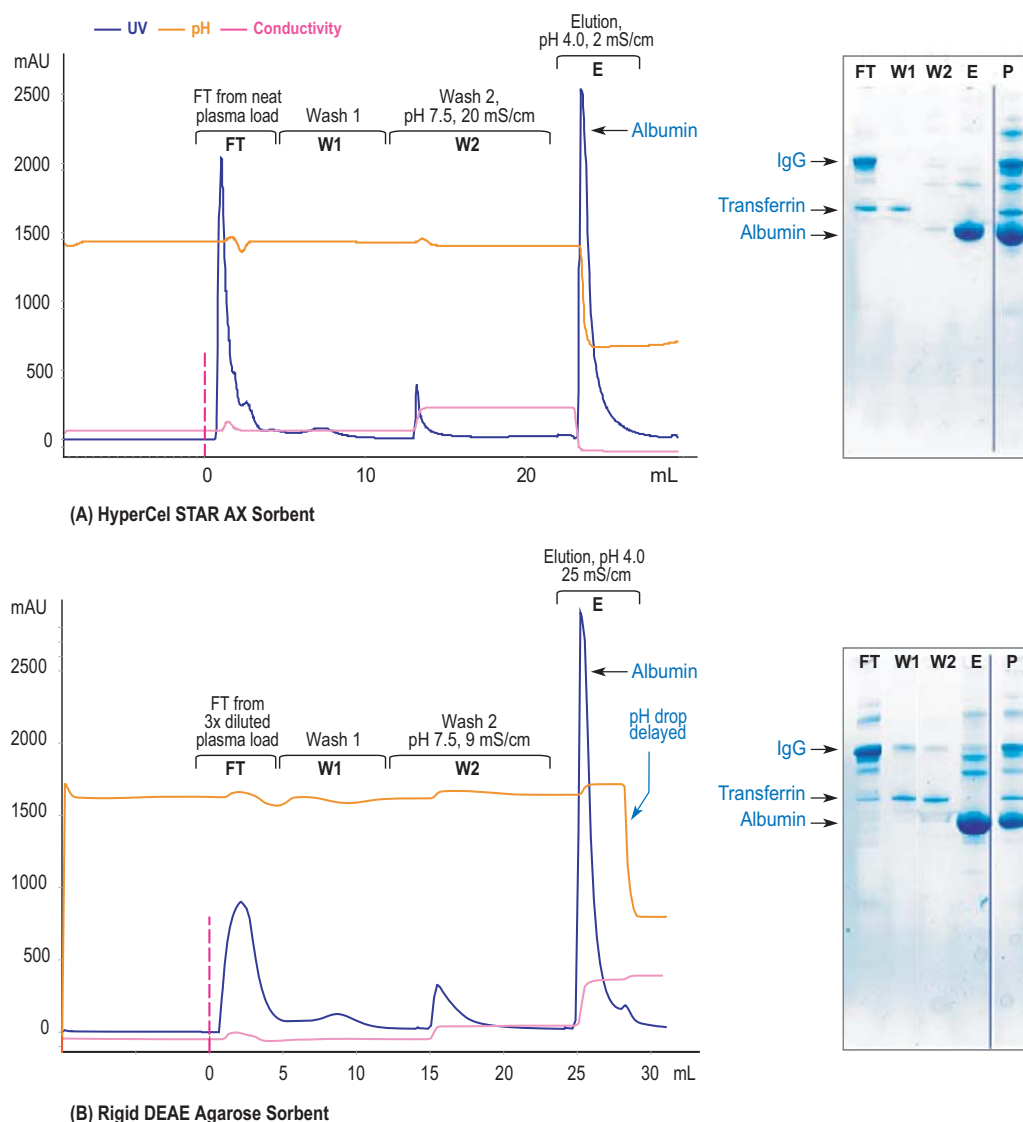
Runs were performed in "optimal" conditions depicted in Table 3. Chromatograms (left) and SDS-PAGE gels (right). FT: Flowthrough; W1: Wash 1; W2: Wash 2; E: Elution, P: Plasma Load, non-diluted for (A) and 3-fold diluted for (B).

Table 3

Performance of HyperCel STAR AX Sorbent and Rigid DEAE Agarose Sorbent for Capture Purification of HSA on Column in Optimal or Alternative Conditions (Load = 60% of $DBC_{10\%BT}$)

Sorbent / Conditions	Load	Wash Conditions	Elution Conditions	Recovery Yield (%)	HSA Purity (%)
HyperCel STAR AX / Optimal	Cryo-poor plasma	pH 7.5, 20 mS/cm	pH 4.0, 2 mS/cm	89	99
Rigid DEAE Agarose / Optimal	3-fold diluted cryo-poor plasma	pH 7.5, 9 mS/cm	pH 4.0, 25 mS/cm	89	98
Rigid DEAE Agarose / Alternative	3-fold diluted cryo-poor plasma	pH 7.5, 9 mS/cm	pH 4.0, 2 mS/cm	73	98

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4.1.4. Second HSA Purification Step on Cation Exchange Sorbent

HSA purified on HyperCel STAR AX sorbent and rigid DEAE agarose sorbent was next used as loading feed to optimize second purification step of HSA on S HyperCel cation exchanger. To obtain conductivity compatible with CEX run, the elution from rigid DEAE agarose sorbent in optimal conditions had to be diluted 5 times (conductivity 6 mS/cm). HSA fractions eluted at low conductivity were loaded without modification for optimization of the second step. After optimization of S HyperCel chromatography on 96-well plates (not shown), HSA was eluted from this second column at pH 7, 15 mS/cm with 95% recovery and more than 99% purity.

4.2. Process Economics Analysis

4.2.1. Process Scenarios

Different scenarios derived from the present study were compared in a process economics analysis (Figure 6). The process using HyperCel STAR AX sorbent (Scenario 1) is the easiest since optimal performance is obtained without plasma dilution. In contrast, different options exist when using rigid DEAE Agarose sorbent depending on dilution. Using different dilution steps before capture and second chromatography step leads to different performance as summarized in Figure 6.

Figure 6

Process Scenarios Used for Process Economics Analysis
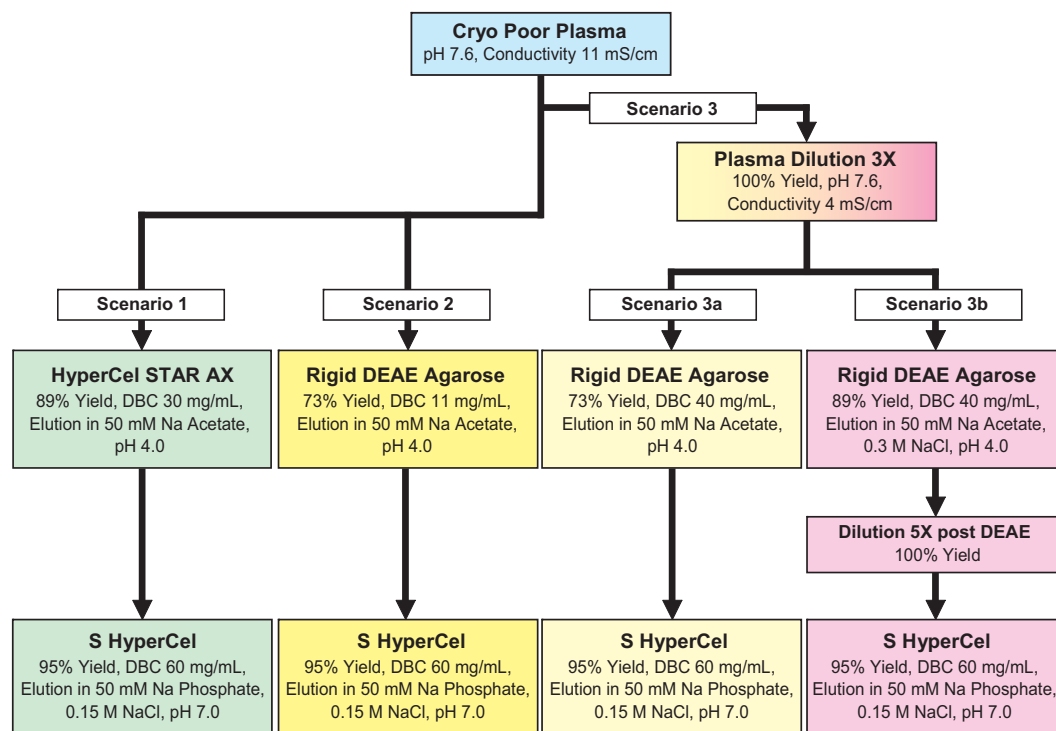
4.2.2. Cost of Goods (CoG) Comparison

Process 1: CoG corresponding to the HyperCel STAR AX sorbent process (Scenario 1) was compared to each of the scenarios using rigid DEAE agarose sorbent (Figure 7). In all cases, the total cost of goods in US$/g of HSA was lower with HyperCel STAR AX sorbent, allowing 14 to 67% savings compared to processes including rigid DEAE agarose sorbent.

Process 2 corresponds to the least favorable conditions, with an increase in costs of 67% compared to Scenario 1. For one part, this is due to the lower DBC obtained at capture step with undiluted plasma, which leads to a higher number of runs to process the same amount of HSA. This results in a higher buffer consumption, water usage and material / tankage costs. In addition, lower throughput in kg/year (-22%) was obtained due to the poorer elution yield from rigid DEAE agarose sorbent with low salt pH.

Process 3a showed less extra-costs compared to process 1, although the savings using HyperCel STAR AX sorbent were close to 20%. In this case, the higher cost/g of HSA was mainly driven by the decreased throughput, due to the low elution yield from rigid agarose DEAE as in process 2.

Process 3b was the most cost-effective process based on the use of rigid DEAE agarose sorbent. In this case, cost increase compared to scenario 1 is due to the two dilution steps applied before first and second purification runs. Since these dilutions allow optimal conditions for all chromatography steps, the throughput is preserved and a lowest cost increase is anticipated. However, there is still a 14% cost difference, and several unit operation steps are added due to dilution requirements. This leads still to a significant advantage of the HyperCel STAR AX sorbent based process. To estimate the impact of the 5-fold dilution between first and second step on the cost advantage of process 1 vs. process 3b, simulation of a process employing a low (2-fold) dilution factor was done. This scenario could be applied if a salt-tolerant cation exchange sorbent was used for the second step. In this case, the process cost using HyperCel STAR AX sorbent was still reduced vs. a process based on rigid DEAE agarose sorbent (4%).

Figure 7

Cost Comparison Between Scenario 1 (HyperCel STAR AX sorbent) with Scenarios 2, 3a and 3b (Rigid DEAE Agarose Sorbent)
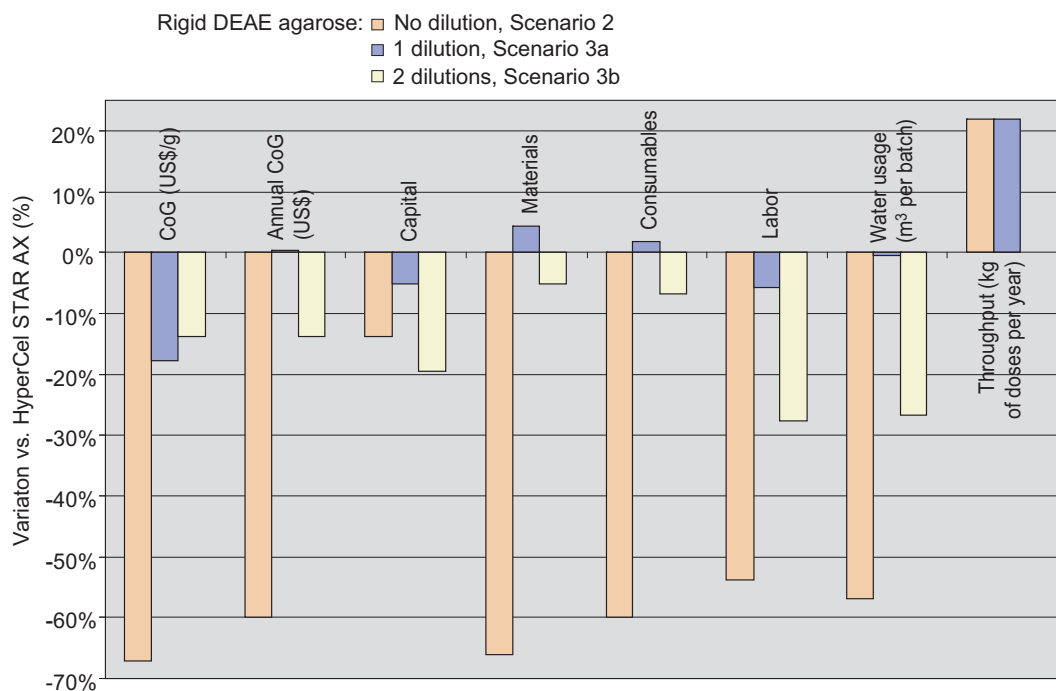
Percentage variations from scenarios using rigid DEAE agarose sorbent to scenario 1 are presented for global cost of goods in US$/g and US$/year. Savings on capital, materials, consumables and labor are expressed as the percentage of cost variation; variation in water usage and throughput are expressed as percentage variation in volume (m³) used per year and kg of HSA produced per year respectively.

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5. Conclusion

▸ HyperCel STAR AX sorbent can efficiently capture HSA from plasma without dilution, allowing good elimination of major plasma contaminants such as immunoglobulins and transferrin.

▸ DBC of HyperCel STAR AX sorbent is maintained in a wide range of conductivities, bringing process flexibility and robustness.

▸ DBC of HyperCel STAR AX sorbent with crude plasma is more than 2-fold higher than that of a conventional rigid DEAE agarose sorbent.

▸ The use of HyperCel STAR AX sorbent allows developing a streamlined two-step process for HSA purification, avoiding any dilution requirement.

▸ Process economics analysis indicates that elimination of dilution steps thanks to the use of HyperCel STAR AX sorbent provides strong economical benefits (reduced capital and consumable cost, reduced water usage) compared to conventional anion exchange sorbents.
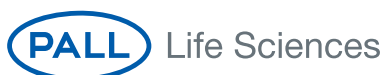
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