Capto Core 700 in vaccine processing

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Capto™ Core 700 in vaccine processing

A cost-effective alternative to size exclusion chromatography

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The aim with this white paper is to discuss the advantages of using Capto Core 700 in virus purification compared to size exclusion chromatography (SEC), a technique commonly used in several vaccine processes.

Capto Core 700 represents a new generation of chromatography media (resins) where the beads are designed and optimized for purification of viruses and other large biomolecules. The core bead technology allows for dual functionality combining size separation with binding chromatography. Viruses and other large biomolecules that are too large to penetrate the core beads of the chromatography medium are collected in the column flowthrough (FT mode). Contaminants (< M_r 700 000) on the other hand pass through the inert outer shell and bind to the ligands in the inner core.

The performance in terms of hemagglutinin (HA) yield (%) and protein removal of Capto Core 700 was compared with SEC using Sepharose™ 4 Fast Flow, a technique traditionally used in vaccine production. Influenza virus produced in Madine-Darby canine kidney (MDCK) cells were used as the model system and the experiment resulted in comparable results. A process economy model was made in order to compare productivity and operational costs for the novel core bead chromatography and traditional SEC. Capto Core 700 presented a more cost-effective production in all investigated scenarios.

Size exclusion separation in the vaccine industry

Recently, a trend has started in the vaccine industry to modernize legacy processes. The drivers for this are increased regulatory requirements as well as cost pressure on manufacturing processes. Obsolete process steps are being replaced by modern purification techniques, aiming for reduced process time, increased yield, purity, and robustness with an overall more efficient vaccine production.

Many of the legacy virus vaccine processes contain SEC for purification of, for example polio (1), and several other vaccines. Depending on the size and shape of the vaccine entities, different chromatography media can be used. For instance, Sepharose 4 Fast Flow is an SEC medium often used for reduction of contaminant levels in several processes. Compared to many other purification options, SEC has the advantage of scalability. However, due to the limiting load volume, SEC has been considered as a challenging option for processing of large sample volumes even though alternative purification options have previously also been limited.

The new gear in the vaccine tool box

For more efficient size separation of biomolecules, a new generation of bioprocessing chromatography medium has been developed called core beads. The first launched core bead product is Capto Core 700 and the design consists of an inactive shell and a ligand-containing core. The inert outer layer of cross-linked agarose prevents viruses and other large entities with a molecular mass (M_r) greater than approximately 700 000 from entering the core of the bead while small molecules penetrate the beads where they are captured. The excluded target molecules are collected in the flowthrough. The octylamine ligand in the core of the bead is multimodal in nature, being both hydrophobic and positively charged. This internalized ligand strongly binds various contaminants over a wide range of pH and salt concentrations. Thus, sample from the previous step can be loaded directly on Capto Core 700 without any extra sample adjustments, see data file 28-9983-07. The core bead binds to a variety of substances including, proteins, peptides, and nucleic acid fragments and is therefore suitable for purification of virus and other large entities originating from both eggs (2, 3) and cell culture (4).

Capto Core 700 is a possible candidate for replacing an SEC step in vaccine purification processes. As mentioned above, Sepharose 4 Fast Flow is used for SEC in several

approved vaccine processes and is therefore a natural choice of chromatography medium for a comparison with Capto Core 700. Influenza virus produced in MDCK cells was chosen as the model system.

The most obvious advantage when comparing Capto Core 700 with SEC is that Capto Core 700 allows for significantly higher load volumes with minimal sample dilution. The time for the separation is also significantly lower as the required residence time is only a few minutes. The sample load and the size of the Capto Core 700 column are determined by the quantity of contaminants present while the load on SEC is volume based. The high protein binding capacity (approximately 12 to 18 mg/mL of chromatography medium) for a range of different proteins reduces column volume and thereby buffer consumption significantly.

Materials and methods

All chromatography media, columns, chromatographic systems, Biacore™ system, normal flow filters and hollow fiber filters, Cytodex™ 3 microcarrier, WAVE™ Bioreactor 20/50, and WAVEPOD™ II controller were supplied by GE Healthcare, Uppsala, Sweden.

Cell culture of MDCK, infection, and clarification

MDCK cells (inoculation concentration of 500 000 cells/mL) were grown on Cytodex 3 microcarriers for 48 h in an Applikon™ Bioreactor (Applikon Biotechnology). The final cell density was approximately 2 500 000 cells/mL at which point the cells were infected with influenza A/Solomon Islands/3/2006 (H1N1) and harvested at 96 h post-infection. Two × 2" ULTA™ Prime GF capsules (2 and 0.6 µm) were connected in series for clarification.

The clarified harvest was concentrated 20 fold on an M_r 500 000 hollow fiber filter (GE Healthcare, UFP500C4X2MA) and diafiltrated 10 fold to buffer (20 mM Tris, 150 mM NaCl, pH 7.5).

HA quantitation

Virus was quantitated by measuring virus hemagglutinin (HA) using a method developed for the Biacore T200 instrument, with recombinant HA protein immobilized on the dextran matrix. Virus-containing samples were mixed with a fixed concentration of a monoclonal antibody (virus-strain specific) and injected on the chip surface. Free antibodies (not bound to virus at equilibrium) were bound to the surface HA. More detailed descriptions are given in references 5 and 6.

Host cell protein quantitation

Host cell protein (HCP) was quantitated with the Biacore T200 instrument using an in-house produced polyclonal antibody raised against MDCK cell lysate. The antibodies were immobilized on the dextran matrix surface of the sensor chip. Samples were injected and HCP were allowed to bind to the immobilized surface antibodies. A more detailed description is given in reference 6.

Benchmarking study

The performance of Capto Core 700 in terms of virus yield and impurity removal was compared to that of Sepharose 4 Fast Flow in a laboratory-scale setup. Capto Core 700 was packed in Tricorn™ 5/50 column (column volume [CV], 1 mL) and 10 CV of clarified and concentrated virus material was loaded. Sepharose 4 Fast Flow was packed in Tricorn 10/600 (CV, 47 mL) and 0.1 CV of concentrated virus feed was loaded. Recovery of virus was measured by the quantitation of HA and reduction of HCP was compared between the two media.

Columns:	Tricorn 10/600 packed with Sepharose 4 Fast Flow, CV 47 mL Tricorn 5/50 packed with Capto Core 700, CV 1 mL
Sample:	Influenza H1N1 cultivated in MDCK cells, concentrated 20 fold and diafiltrated 10 fold on an M_r 500 000 hollow fiber filter (GE Healthcare, UFP500C4X2MA) to 20 mM Tris, 150 mM sodium chloride pH 7.5
Sample load:	Capto Core 700, 10 CV (10 mL feed) Sepharose 4 Fast Flow, 0.1 CV (4.7 mL feed)
Buffer:	20 mM Tris, 150 mM sodium chloride, pH 7.5
Flow velocities:	Capto Core 700, 100 cm/h, residence time: 3 min Sepharose 4 Fast Flow, 30 cm/h
Cleaning in place (CIP):	Capto Core 700, 30% 2-propanol in 1 M sodium hydroxide
System:	ÄKTA system

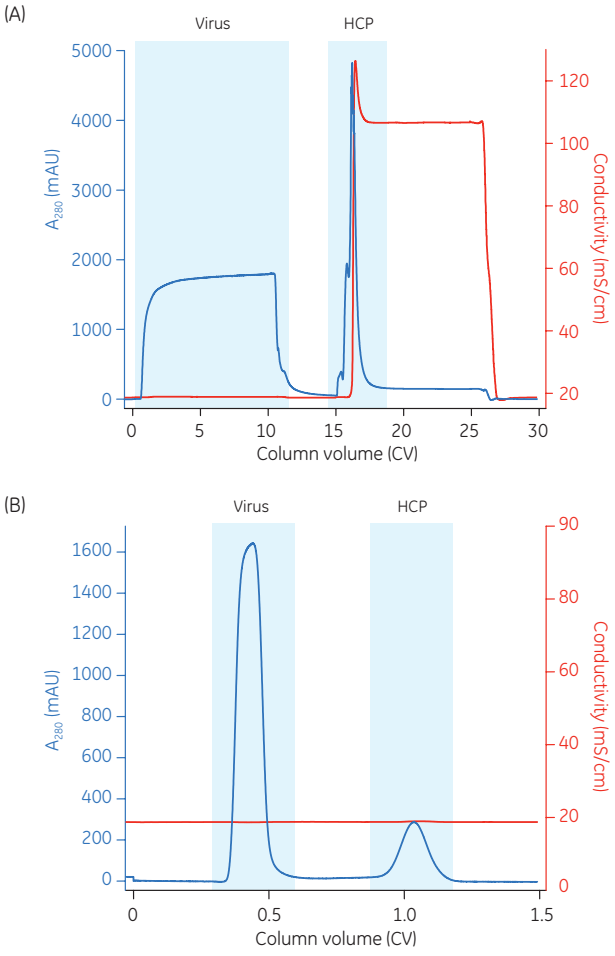

Fig 1. Chromatograms from purification of MDCK-produced influenza virus using (A) Capto Core 700 and (B) Sepharose 4 Fast Flow. Shaded areas represent virus and HCP peaks.

Table 1. Analytical results for HA and HCP after clarification and cross flow filtration as well as purification with either Capto Core 700 or Sepharose 4 Fast Flow

	Step HA yield (%)	Step HCP reduction (%)	Process HCP reduction (%)
Raw harvest	100	0	0
Normal flow filtration (NFF)	n.d.	67	67
Ultrafiltration/diafiltration (UF/DF)	n.d.	79	93
(NFF + UF/DF)	60	-	93
Either:			
Capto Core 700	85	32	95
Or:			
Sepharose 4 Fast Flow	86	31	95

n.d. = not determined

The purification performance of Capto Core 700 was compared to that of Sepharose 4 Fast Flow. Both chromatography methods provided a similar yield of virus HA and reduction of HCP.

In the example in Table 1, Capto Core 700 and Sepharose 4 Fast Flow were used as a polishing step; 93% of the HCP was removed in the clarification and cross flow filtration steps and an additional 30% was removed using one of the chromatographic methods.

Another option would be to load clarified harvest directly on the core beads. In our experience, the HCP removal is typically 85% to 90% when loading cell-based clarified influenza harvest on Capto Core 700 at a load of 10 mg/mL of chromatographic medium (data not shown). That can be beneficial for example in a situation where small fragments of cell debris remain after the clarification. If those fragments are concentrated during the cross flow filtration as well as the target virus, aggregates can be formed which could subsequently disturb the chromatographic purification.

In process development when evaluating Capto Core 700, it is advantageous to test the chromatography medium both with and without a previous concentration step. The outcome in terms of purity and process time must be carefully balanced to the cost of equipment for one or two purification steps, respectively. That is in reality not an option for traditional SEC where the load is based on the column volume, typically 0.1 to 0.25 of the column volume.

Process economy

The experimental data above suggest that the performance of the different purification techniques in terms of HA recovery and HCP reduction is comparable for MDCK feed. To further compare the different chromatographic purification techniques, a comparison of the process costs was made. The calculations compare hardware investments, process time, cost for disposables, labor cost, and buffer cost for Capto Core 700 chromatography vs SEC with Sepharose 4 Fast Flow at different pilot and process scales (100, 1000, and 3000 L). A schematic illustration of a typical purification process for cell-based influenza vaccine can be seen in reference 3. For calculations of productivity, the HA yield for normal flow filtration was assumed to be 60%. The Capto Core 700 yield was set to 90% and the yield for the UF/DF process steps for sample concentration and formulation was estimated to be 80%.

Equipment and consumables, including buffer holding bags and chromatography media, columns, and chromatography skids, all came from GE Healthcare.

This model gives indications about advantages and disadvantages with the different purification techniques but does not include facility-related costs, QC/QA costs, and system-validation costs.

Table 2. Summary of the assumptions made for the process economy model

General assumptions

Annual production	40 batches/yr
Buffer storage	Single use < 500 L
	Stainless steel > 500 L
Material	Influenza virus from MDCK cells, 3 µg HA/mL
	Clarified harvest containing 120 µg total protein/mL
	20 × UF/10 × DF with 80% total protein removal
Dose	15 µg HA/dose, pandemic situation, i.e., only one strain of influenza
Buffers	Buffers and cleaning solutions as well as their respective hold tanks or bags required for chromatography and sanitation were included. Single-use bags were used for sample and buffer storage up to 500 L. For larger volumes, stainless steel vessels were used (internal price data)
Buffer cost	$2/L
Labor	Hands-on preparations and process monitoring was included, $100/h and person
Depreciation	10 yr
Interest rate	10%

Capto Core 700

Investigated scales	100 L	1000 L	3000 L
Capto Core 700 volumes	0.2 L	2 L	6 L
Columns	AxiChrom™ 50/300*	AxiChrom 100/300	AxiChrom 200 /300
System	ÄKTApilot™	ÄKTAprocess™	ÄKTAprocess
Equilibrium	7 CV		
Sample load	12 mg total protein/mL of medium (30 CV)		
Wash	5 CV		
CIP	10 CV†		
Reuse of chromatographic medium	20 times‡		

Sepharose 4 Fast Flow

Investigated scales	100 L	1000 L	3000 L
Sepharose 4 Fast Flow volumes	60 L	330 L (2 cycles)	330 L (6 cycles)
Columns	Chromaflow™ 400/500	Chromaflow 1000/500	Chromaflow 1000/500
System	ÄKTApilot	ÄKTAprocess	ÄKTAprocess
Equilibrium	3 CV		
Sample load	0.1 CV		
Wash	1 CV		
CIP	1 CV		
Reuse of chromatographic medium	200 times‡		

* Packing of Capto chromatography media is primarily recommended in AxiChrom columns for maximal performance.

† Required volumes of equilibration buffer and CIP solution depend on buffers/solution type and must be investigated for each specific case and feed. Volumes can be significantly reduced following an optimization of the process.

‡ Number of cycles for reuse of the chromatographic medium must be investigated for each specific case and feed. A modest approach to number of cycles for Capto Core 700 was chosen in this case study. To challenge the economic model, a significantly higher number of cycles was used for Sepharose 4 Fast Flow.

Process economic outcome

The operational cost was lower for Capto Core 700 for all three scales investigated in this model (Fig 2). At the 100 L scale, the cost was 1.5 fold lower and 3 fold lower than at the 3000 L scale even though it was assumed that the Sepharose 4 Fast Flow was used for 10 fold more cycles than Capto Core 700. The number of cycles a chromatographic medium can be used for must be tested in each case.

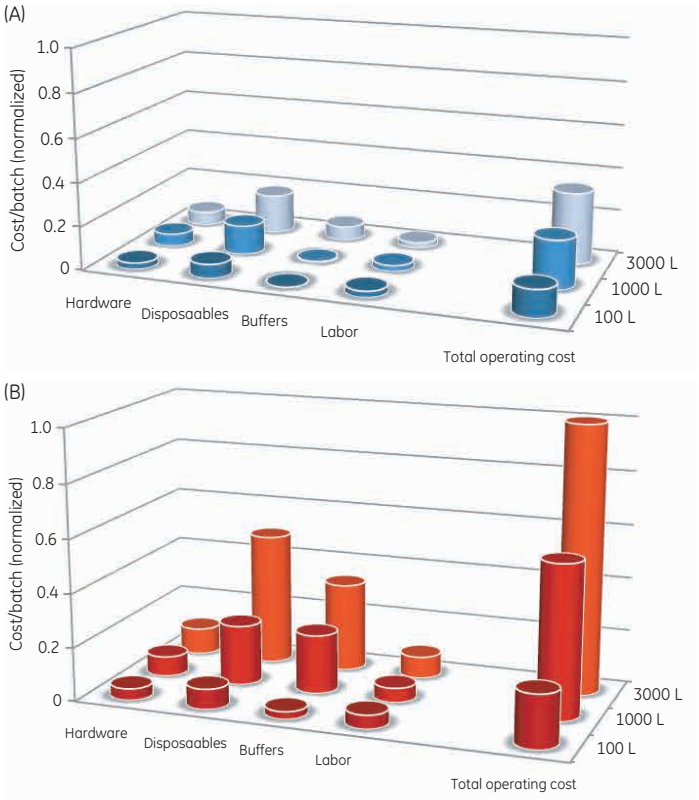

Fig 2. The cylinders illustrate normalized operational costs at different scales for (A) Capto Core 700 chromatography and (B) SEC using Sepharose 4 Fast Flow. The costs are separated into hardware, consumables, buffers, labor (and the total operating cost is also shown).

The hardware cost was comparable for the different techniques but the buffer cost and the cost of disposables were substantially higher for SEC with Sepharose 4 Fast Flow as compared to Capto Core 700 at all scales.

The productivity for Capto Core 700 (doses/process time) was 1.5 fold higher at the 100 and 1000 L scales and 2.6 fold higher at the 3000 L scale (Fig 3). The same relation can be seen for the process time. The doses/process time ratio starts to diverge when the maximum of standard column size is reached for the Sepharose 4 Fast Flow option and the column must be cycled multiple times to process the whole sample volume. The column volume for Capto Core 700 was 6 L (one cycle) and 330 L for Sepharose 4 Fast Flow chromatography (6 cycles) at the 3000 L scale.

When looking at total operation cost at different scales for the two different chromatographic techniques, the difference was considerable in this model. The operational cost/batch was only 2.7 fold higher when scaling up 30 fold, from 100 L to the 3000 L scale for Capto Core 700 and 5 fold higher for Sepharose 4 Fast Flow.

The outcome of the process economy model showed that the Capto Core 700 alternative offers notable advantages both in terms of scalability and productivity compared with SEC using Sepharose 4 Fast Flow. In this example, that means that at the 3000 L scale, 27 000 doses more can be processed per hour for a third of the cost when using Capto Core 700 instead of Sepharose 4 Fast Flow.

This case study has been developed to display how the choice of separation technique impacts the manufacturing costs. Regional cost differences will impact the process economy model.

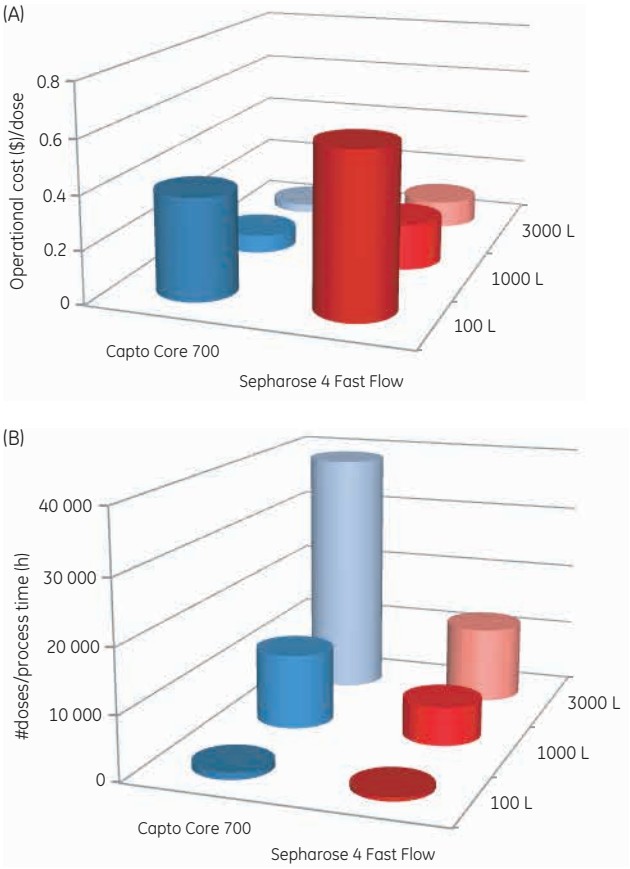

Fig 3. The cylinders illustrate (A) the operational cost ($)/dose and (B) productivity for the two compared purification techniques at 100, 1000, and 3000 L scale.

Discussion and conclusions

The focus of this study was to investigate the use of Capto Core 700 instead of size exclusion chromatography using Sepharose 4 Fast Flow for purification of a cell-based influenza vaccine. The experimental outcome showed comparable results in terms of HCP removal and HA yield.

The total operational cost for a 1000 L batch is a one third the cost using Capto Core 700 compared with a traditional SEC (Sepharose 4 Fast Flow) step. The most important benefit is that the productivity (doses/h) is approximately 2.6 fold higher for the core bead alternative compared to the SEC alternative. Prepacked Capto Core 700 ReadyToProcess™ columns are available and as many vaccine production processes are suitable for single-use equipment, benefits could be gained from increased flexibility, increased productivity, and the possibility of optimized utilization of the facility (7, 8).

The main advantage of using Capto Core 700 as an alternative to traditional SEC is the more than 100 fold larger loading capacity with minimal dilution of the sample. Contaminant concentration rather than volume will limit the capacity of Capto Core 700 and the purification process can be performed at substantially higher flow rates compared to SEC. The combination of these factors will have a strong influence on process economy, which was also shown in the comparison of the process costs. The calculations compare hardware investments, process time, cost for disposables, labor cost, and buffer cost for chromatography using Capto Core 700 vs SEC at different scales. In the process economic model, 12 mg of protein/mL Capto Core 700 medium was loaded, which corresponds to over 30 CV loads of the 20 fold concentrated influenza harvest. Accordingly, the volume is equivalent to 600 CV of unconcentrated, clarified harvest. In SEC where the load volume is a limiting factor, 0.1 to 0.25 CV is typically the maximum load regardless of whether the sample is concentrated or not. Due to the limiting load volume, SEC has not been considered as a practical option for the processing of large sample volumes. However, the core bead technology brings separation based on molecular size to a new level allowing large sample loads and high purity resulting in efficient bioprocessing with low operational cost.

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