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Brief report

Efficient chromatographic reduction of ovalbumin for egg-based influenza virus purification

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ABSTRACT

Vaccination is the most effective prevention strategy to avoid influenza infection and for protection of large populations. The vast majority of influenza vaccines are still produced with allantoic fluid from fertilized chicken eggs. The presence of ovalbumin, which can constitute over 60% of the total protein content in allantoic fluid, can result in severe allergies. Consequently, efficient reduction of ovalbumin is critical during vaccine manufacturing. Here we present Capto Core 700, a novel core bead chromatographic flow through mode resin for removal of ovalbumin and compare it to sucrose zonal gradient ultracentrifugation, which is the industry standard for egg-based vaccine production. The results demonstrate that core bead chromatography is fully comparable to zonal centrifugation in removing ovalbumin to meet regulatory requirements. Furthermore, the scalability and the shorter process times of this method have the potential to significantly improve the productivity and economy for industrial production compared to zonal centrifugation.

1. Introduction

The emergence of new Influenza virus strains [1,2] causes seasonal influenza epidemics and may result in severe worldwide pandemics. There is thus a critical need for efficient and economical vaccine production to respond to this threat. The supply of vaccine still mainly rely on using influenza virus propagated in fertilized chicken eggs as a source, which is then processed with purification processes that have remained unchanged for decades [3–5]. One concern for egg-based vaccines is the reduction of ovalbumin, which can cause severe allergies [6]. Industrial scale processing of virus is commonly based on sucrose gradient zonal ultracentrifugation (ZUC) combined with filtration techniques. Though ZUC provides both efficient purification and concentration of influenza virus, it is time consuming, requires extensive maintenance [7] and suffers from limited scalability. Consequently, improved and more efficient and scalable technologies for purification of egg-based vaccines would be valuable. Hence, various chromatographic methods for purification of virus particles have been investigated [5,8–10]. Due to the size of the virus, flow through (FT) mode chromatography, where virions pass through the column unretained and smaller impurities are adsorbed to the resin, may be an attractive alternative.

Capto™ Core 700 is a new FT mode resin engineered for purification of viruses and other large biomolecules. It consists of beads with an inactive shell surface and an active functionalized multimodal core with octylamine ligands. The bead pores have an approximate exclusion limit of 700 kDa (Fig. 1).

This allows smaller molecules to access to the core where they can be efficiently adsorbed, while larger entities will pass in the FT. The presented work aim to evaluate Capto Core 700 as an alternative to ZUC for production of influenza vaccine and is a collaboration between GE Healthcare and the Institute for Translational Vaccinology (Intravacc).

2. Materials and methods

2.1. Virus production

Influenza strain H3N2 A/Uruguay was prepared from egg allantoic fluid produced at Intravacc. The eggs were incubated at 35 °C

Abbreviations: HA, haemagglutinin; SRID, single radial immunodiffusion; ZUC, sucrose zonal gradient ultracentrifugation; FT, flow through; kDa, kilo Dalton; UF/DF, ultrafiltration and diafiltration; DBC, dynamic binding capacity; CV, column volume; CIP, cleaning in place; GMP, good manufacturing practice.

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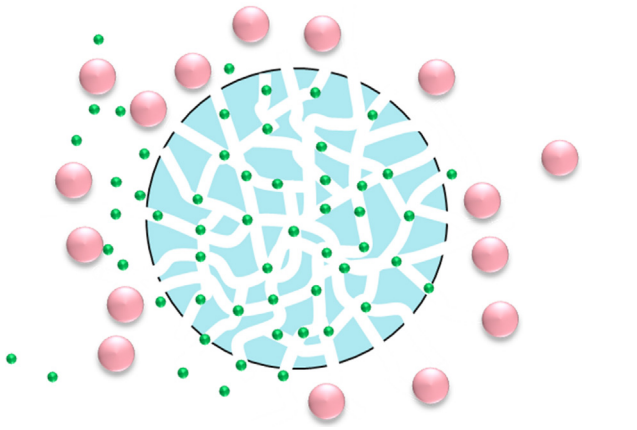

Fig. 1. Schematic cross-sectional view of a Capto Core 700 particle with an average diameter of 75 μm. Small protein impurities (green) can enter the interior of the resin particle and bind to the ligand. Larger molecular entities above the approximate exclusion limit of 700 kDa (pink), such as virus particles are hindered from entering the resin pores and will pass through the column unretained. (For interpretation of the references to color in text, the reader is referred to the web version of the article.)

and the allantoic fluid harvested 72 h post infection. Clarification was performed by continuous disk stack centrifugation followed by filtration using two parallel 10 in. 2.0 μm ULTA™ prime capsule filters. The clarified harvest was divided in two portions, of which 70 L was used for ZUC. Another 10 L intended for chromatography was subjected to additional filtration using a 1.5 in. 0.6 μm ULTA GF to protect the column from possible aggregates.

2.2. Dynamic protein binding capacity

Three duplicate runs were performed to evaluate the dynamic binding capacity (DBC) using three different Tricorn™ 5/50 Capto Core 700 columns (column volume (CV) approximately 1 mL) and an ÄKTA™ explorer 10 system. Equilibration was performed with 20 mM Tris–HCl pH 7.5 with 150 mM NaCl followed by loading of 3 mg/mL ovalbumin in 20 mM Tris–HCl pH 7.5 with 150 mM NaCl at 100 cm/h, (residence time of 3 min). The DBC was related to the UV breakthrough signal equivalent to 10% of the maximum absorbance of the pure ovalbumin solution.

2.3. Pilot scale ZUC

Clarified harvest from Section 2.1 was subjected to ZUC using a Alfa Wassermann eKII (Alfa Wassermann) with rotor K3 (3.2 L) at 20 °C and a sucrose gradient from a 0.125 M citrate buffer pH 7.8 and a 60% sucrose solution. 70 L clarified allantoic fluid was processed for 7 h, initiated at 80 mL/min with constant speed of 15,000 rpm (16,350 × *g*) and gradually increased to 18 L/h and 35,000 rpm (89,000 × *g*). The virus containing fraction at 47–35% sucrose was collected.

2.4. Pilot scale chromatography

Before chromatography, 5300 mL of the clarified allantoic fluid was concentrated 27 times and diafiltered 12 times to phosphate buffered saline (PBS) using a hollow fiber cartridge (UFP-750-E-4X2MA) with 750 kDa nominal cut-off and surface area of 0.085 m^2. Capto Core 700, packed with 10 cm bed height (CV 4.7 mL) in HiScreen™ columns was equilibrated with 11 CV of PBS followed by loading of 2 CV concentrated sample at 100 cm/h (6 min residence time) using an ÄKTA explorer 10 system. Fractions were collected throughout the run and the column washed with 5 CV of PBS, Cleaned In Place (CIP) with 5 CV of 1 M NaOH + 27% 1-propanol (contact time, 30 min). All resins, columns, chromatographic systems, and filters were supplied by GE Healthcare.

Table 1
Results from DBC determination of ovalbumin.

Experiment	DBC (mg/mL)	cv (%)
1	12.6	
2	14.8	
3	14.9	
Average	14.1	9

2.5. Analysis

2.5.1. Single radial immunodiffusion assay (SRID)

The haemagglutinin (HA) concentration was determined by SRID assay, according to the method described earlier by Wood et al. [11]. All reagents were supplied by NIBSC, Potters Bar, London, UK.

2.5.2. Ovalbumin concentration determination

Ovalbumin concentration was measured using the Serazym Ovalbumin ELISA kit (Seramun Diagnostica, Wolzig, Germany) according to the supplier's instructions.

3. Results

The dynamic binding capacity at 10% breakthrough for ovalbumin with Capto Core 700 was determined to be 14.1 mg ovalbumin/mL resin with a coefficient of variation of 9% (Table 1).

Capto Core 700 was compared to ZUC with regard to ovalbumin reduction capacity during purification of influenza from allantoic fluid at pilot scale. Harvest of 10,000 eggs with Influenza strain A/Uruguay H3N2 resulted in approximately 92 kg of clarified solution. Of this 70 L was processed by ZUC to a final volume of 471 mL, equivalent to a concentration factor of 149.

The HA concentration determined by SRID in the 47–35% sucrose centrifugation pool was ~1600 μg/mL (Table 2) and well in accordance with previously accumulated data from over 30 ZUC purification runs (data not shown) (Fig. 2). The ovalbumin was reduced from an initial concentration of ~63,500 ng/mL down to ~600 ng/mL, resulting in an ovalbumin/dose ratio of 17.1 ng/dose, based on 45 μg HA per dose. This result was compared to the use of Capto Core 700 chromatography performed with approximately

Table 2
Comparison of results from ZUC and Capto Core 700 FT chromatography.

	ZUC	UF/DF + Capto Core 700
Feed (2.0 μm)		
Volume (mL)	70,000	5300
HA[a] (μg/mL)	15	15
Ovalbumin (ng/mL)	63,545	51,165
Product		
Volume (mL)	471	9.4
Equiv. start vol[b] (mL)		249
Concentration factor	149	27
HA-SRID (μg/mL)	1600	300
HA-SRID recovery (%)	70	69
Ovalbumin (ng/mL)	608	69
Ovalbumin remain. (%)	0.00644	0.0051
Ovalbumin/dose[c] (ng)	17.1	10.3

[a] The value of HA concentration in the clarified harvest after 2 μm filtration is approximate and was estimated from the concentration from SRID after ZUC, based on the known concentration factor and expected loss of yield.
[b] The equivalent start volume is used to related the actual volume used in the chromatography step to the final recovery.
[c] Micrograms of Ovalbumin per 45 μg HA as determined by SRID.

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