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Efficient purification of pneumococcal polysaccharides in a chromatography workflow

This application note demonstrates the purification of pneumococcal capsular polysaccharides using chromatography. The described process constitutes a viable alternative to the traditional process for purification of pneumococcal polysaccharides to eliminate the need for phenol extraction, while increasing process recovery and yield.

Introduction

Pneumococcal infection is caused by the bacterium *Streptococcus pneumoniae* and can lead to many different health conditions depending of type of infection. Of the more than 90 different serotypes, classified according to the structure of their polysaccharide capsule, only a few number of serotypes leads to diseases in humans. Bacteria are present in the respiratory tracts without causing any illness. Invasive infections, however, can range from minor ear or sinus infections to more severe bloodstream infections or pneumonia. Some people are more susceptible to infection, such as small children (under 2 years), elderly (over 65 years), and people with existing chronic diseases such as lung disease.

Available vaccines are either polysaccharide vaccines or conjugate vaccines, where the capsular polysaccharide is covalently coupled to a carrier antigen. Purification of capsular polysaccharides for vaccine production is traditionally performed by phenol extractions and dialysis to remove protein, followed by ethanol precipitation and centrifugation to remove endotoxins. In addition to giving low recoveries, this purification strategy is time-consuming. Removal of phenol from the antigen by dialysis can take 10 to 15 days. Phenol handling also constitutes environmental and health risks and the process is difficult to scale to meet market demands.

In this work, we propose an alternative strategy for purification of pneumococcal capsular polysaccharides (Fig 1).

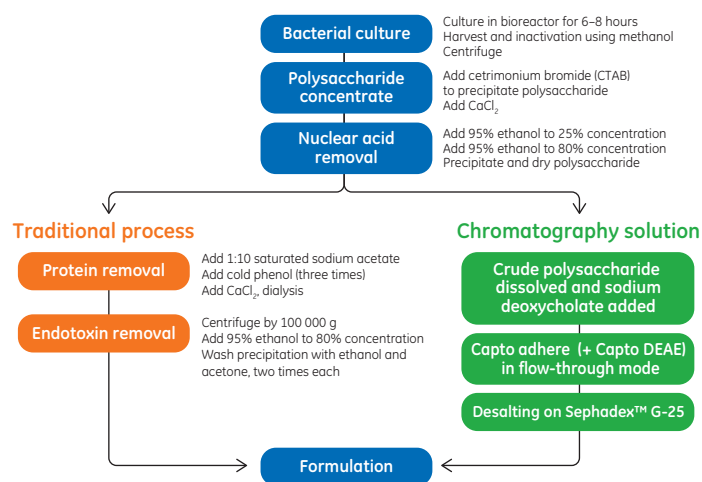


Fig 1. Traditional versus proposed pneumococcal polysaccharide purification process.

In the described processes, phenol extraction is replaced by a chromatography workflow to meet challenges such as low recoveries in time-consuming procedures. For most serotypes, the alternative purification procedure constitutes a one-step chromatography process using Capto™ adhere resin. Capto adhere is a multimodal anion exchanger designed to remove key contaminants such as DNA, host cell proteins, larger aggregates, and viruses in a single step. For some serotypes, remaining impurities were removed by including a second chromatography step using Capto DEAE resin. Capto DEAE is a weak anion exchanger designed for capture of protein from large feed volumes to meet industry demands for fast, efficient, and cost-effective processes.

The described processes were modified from the process developed by Ji *et al.* for the purification of the pneumococcal capsular polysaccharide of serotype 23F (1). The presented work was conducted by GE Healthcare's Fast Trak team in Shanghai, CN.

Materials and methods

Sample preparation

Crude precipitate of pneumococcal polysaccharides after one step of traditional ethanol precipitation was dissolved in ultrapure water to a concentration of 10 g/L. The polysaccharide solution was diluted 1:10 v/v in saturated sodium acetate solution and pH was adjusted to 7.0. The polysaccharide-containing solution was added 10% sodium deoxycholate to a final concentration of 0.5% and incubated on ice for 5–10 min. Samples were adjusted to pH 6.1–6.2 with 1 M phosphoric acid and incubated on ice for 3–6 h. Samples were centrifuged at 12 000 × g for 30 min before subjected to active charcoal depth filtration to clarify the sample and remove part of the DNA.

Chromatography

Depending on serotype, four different chromatography process designs were used (Table 1). Chromatography steps were run in flow-through mode.

Desalting

Desalting of the collected polysaccharide-containing samples was conducted on a Sephadex G-25 resin.

Analysis

Quantification of pneumococcal polysaccharide was performed by the Anthrone method for determination of carbohydrates. Detection of various impurities was conducted according to the methods and standards in European Pharmacopoeia 7.0 (2). Nucleic acids were measured at a wavelength of 260 nm and the DNA concentration was calculated as 1.0 AU (A_{260}) = 50 µg/mL DNA. Protein content was determined by the Modified Lowry Protein Assay Kit (Thermo Fisher Scientific).

Results

The chromatographic processes run in simple flow-through mode offered good separation of polysaccharides from impurities. Compared with what can be expected from the traditional purification process, the described process designs improved recovery by 30%–50% and only required one week from sample preparation to desalted product. Results are listed in Tables 2 to 5. As shown, impurity levels met the requirements of European Pharmacopoeia (2).

The described purification process can successfully be applied also to other polysaccharides (3).

Table 1. Chromatography conditions for purification of pneumococcal capsular polysaccharides

Process design	Serotype	Sample pH	Sample conductivity	Sample load	Column(s)	Loading buffer*	Equilibration buffer*
1	2, 9N, 9V, 10A, 12F, 15B, 20, 22F, 23F, and 33F	7.6	23 mS/cm	1 column volumes (CV)	Capto adhere in XK16/10	Buffer A, pH 7.6	80% Buffer A, pH 7.6 + 20% Buffer B, pH 7.6
2	1, 5, 7F, 14, 17F, and 18C	7.6	23 mS/cm	0.5 CV	Capto adhere in XK16/10 + Capto DEAE in XK16/10	Buffer A, pH 7.6	80% Buffer A, pH 7.6 + 20% Buffer B, pH 7.6
3	3, 4, 6B, 8, 11A, and 19F	7.6	54 mS/cm (type 4: 45 mS/cm) (type 8: 35 mS/cm)	1 CV (type 8: 0.8 CV)	Capto adhere in XK16/20	Buffer A, pH 7.6	40% Buffer A, pH 7.6 (type 4: 60%) (type 8: 65%) + 60% Buffer B, pH 7.6 (40% for type 4) (35% for type 8)
4	Serotype 19A	6.0	54 mS/cm	0.2 CV	Capto adhere in XK16/20	Buffer A, pH 6.0	40% Buffer A, pH 6.0 + 60% Buffer B, pH 6.0

* Buffer A: 50 mM sodium phosphate, pH 7.6 or 6.0, Buffer B: 50 mM sodium phosphate + 1.0 M NaCl, pH 7.6 or 6.0

Table 2. Process results for process design 1

Polysaccharide (PS) serotype	PS conc. (µg/mL)	Recovery (%)	Protein (µg/mL)	DNA (µg/mL)	Protein/PS (%)	Protein required (%)*	DNA/PS (%)	DNA required (%)*
2	285.4	84	3.8	2.15	1.3	2	0.75	2
9N	281.5	86	5.25	0.3	1.8	2	0.11	1
9V	290.8	70	5.88	0.35	1.9	2	0.12	2
10A	163.8	72	9.8	0.35	5.6	7	0.21	2
12F	107.8	39	2.91	0.2	2.6	3	0.19	2
15B	275	88	7.12	0.45	2.5	3	0.16	2
20	731	90	9.22	0.45	1.2	2	0.06	2
22F	292	90.9	4.23	0.3	1.4	2	0.10	2
23F	256	40	4.45	1.4	1.7	2	0.55	2
33F	299	76	1.60	0.3	0.5	2.5	0.10	2

Note! Recovery is for the total process including pretreatment and chromatography.

* Max. level as required by European Pharmacopoeia (2)

Table 3. Process results for process design 2

Polysaccharide (PS) serotype	PS conc. (µg/mL)	Recovery (%)	Protein (µg/mL)	DNA (µg/mL)	Protein/PS (%)	Protein required (%)*	DNA/PS (%)	DNA required (%)*
1	329	40	4.4	4.4	1.33	2	1.3	≤ 2
5	381	54	18.5	5.4	4.85	7.5	1.4	2
7F	716.8	70	28.7	2.9	4.0	5	0.4	2
14	320.1	50	8.54	2.5	2.67	5	0.78	2
17F	933.7	66	11.83	1.7	1.27	2	0.18	2
18C	650.4	58	13.7	5.9	2.11	3	0.9	2

Note! Recovery is for the total process including pretreatment and chromatography.

* Max. level as required by European Pharmacopoeia (2)

Table 4. Process results for process design 3

Polysaccharide (PS) serotype	PS conc. (µg/mL)	Recovery (%)	Protein (µg/mL)	DNA (µg/mL)	Protein/PS (%)	Protein required (%)*	DNA/PS (%)	DNA required (%)*
3	841.0	56	3.89	1.6	0.46	5	0.19	2
4	498	60	5.28	1.3	1.06	3	0.78	2
6B	867.3	55	4.36	1.4	0.50	2	0.16	2
8	318.4	50	4.36	3.9	1.37	2	1.22	2
11A	2063.4	70	12.78	2.1	0.62	3	0.10	2
19F	1922.5	75	12.3	1.9	0.64	3	0.1	2

Note! Recovery is for the total process including pretreatment and chromatography.

* Max. level as required by European Pharmacopoeia (2)

Table 5. Process results for process design 4

Polysaccharide (PS) serotype	PS conc. (µg/mL)	Recovery (%)	Protein (µg/mL)	DNA (µg/mL)	Protein/PS (%)	Protein required (%)*	DNA/PS (%)	DNA required (%)*
19A	130.4	32	2.52	1.1	1.93	2	0.84	2

Note! Recovery is for the total process including pretreatment and chromatography.

* Max. level as required by European Pharmacopoeia (2)

Conclusions

Here, we describe purification of pneumococcal capsular polysaccharides in easily scalable processes based on modern chromatography resins. Compared with the traditional purification process, the recovery could be increased by more than 30% using the described process designs, while meeting the quality requirements of European Pharmacopoeia (2). Eliminating the need for time-consuming, multi-step phenol and ethanol extractions, the suggested processes allowed pneumococcal capsular polysaccharides to be successfully purified with high recovery and purity in a way that benefits the environment and operator health.

Reference

1. Ji *et al.* Design and optimization of a chromatographic purification process for *Streptococcus pneumoniae* serotype 23F capsular polysaccharide by a design of experiments approach. *Journal of Chromatography A* **1348**, 137–149 (2014).
2. European Pharmacopoeia 7.0 (8th Ed), **1**, 813-815 (2013).
3. Application note: Efficient purification of meningococcal polysaccharides in a two-step chromatography workflow. GE Healthcare 29216880, Edition AA (2016).

Ordering information

Product	Description	Product code
Capto adhere	Multimodal anion exchange resin, 10 L	17544405
Capto DEAE	Anion exchange resin, 10 L	17544305
XK16/20 column	Empty column, 20 L	28988937
Sephadex G-25 resin	Size exclusion chromatography resin, 5 kg	17003303

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