Efficient purification of meningococcal polysaccharides in a two-step chromatography workflow

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This application note demonstrates the purification of meningococcal capsular polysaccharides using chromatography. The described process constitutes a viable alternative to the traditional process for purification of meningococcal polysaccharides to eliminate the need for time-consuming phenol extraction steps. In the suggested process, high recoveries could be achieved.

Introduction

Meningococcal disease is a life-threatening illness caused by the bacterium *Neisseria meningitidis* (also called meningococcus) that infects the blood, brain, and spinal cord. From the 13 clinically significant serotypes, classified according to the structure of their polysaccharide capsule, the serotypes A, C, W135, and Y are responsible for 90% of all global cases.

Available vaccines are either polysaccharides 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. This purification strategy is time-consuming and the phenol handling constitutes environmental and health risks. In addition, the process is difficult to scale to meet market demands.

In this work, we propose an alternative strategy for purification of meningococcal polysaccharides (Fig 1). In the described process, phenol extraction is replaced by chromatography steps to meet challenges such as low recoveries in time-consuming procedures. In the initial step, Capto™ adhere resin was used. 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. In the second step, Capto DEAE resin was used. Capto DEAE is a weak anion exchanger designed for capture of proteins from large feed volumes to meet industry demands for fast, efficient, and cost-effective processes.

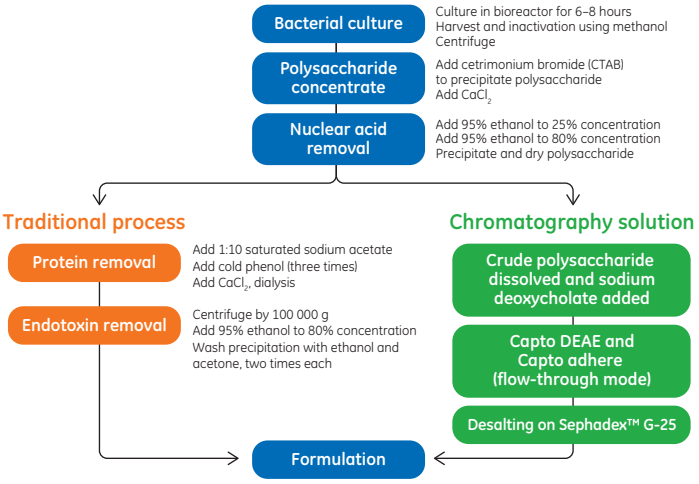

Fig 1. Traditional versus proposed meningococcal polysaccharide purification process.

This two-step process for purification of meningococcal polysaccharides was previously published by Tian *et al*. (1). The presented work was conducted by GE Healthcare's Fast Trak team in Shanghai, CN.

Materials and methods

Sample preparation

Crude extracts containing meningococcal polysaccharides were treated with cetyl trimethylammonium bromide (CTAB) and subjected to fractional precipitation using ethanol. Precipitates containing polysaccharides of groups A, C, Y, and W135 were each dissolved o/n in 20 mmol/L Tris-HCl, pH 8.0 and adjusted to 5 mg/mL stock solutions. The stock solutions were diluted 1:1 in 20 mmol/L Tris-HCl, pH 8.0 containing 2% sodium deoxycholate (SDC) to a final concentration of 2.5 mg/mL. The diluted samples were filtered using a 0.45 µm pore size filter.

Purification process

An XK 16/20 column packed with Capto adhere resin was serially connected to an XK 16/10 column packed with Capto DEAE resin. The columns were equilibrated with 20 mmol/L Tris-HCl, pH 8.0 containing 1% SDC. Polysaccharide-containing sample (12 mL) was injected onto the columns and polysaccharides, proteins, and nucleic acids were monitored at 206, 260, and 280 nm, respectively. While proteins and nucleic acids are more specifically detected at 260 and 280 nm, respectively, compounds such as carboxyl groups, ester links, amide, peptide bonds, proteins, peptides, amino acids, steroids, nucleotides, and fatty acids will be detected along with with polysaccharides at 206 nm, giving rise to a plurality of peaks later in the chromatogram at this wavelength (not shown). Flowthrough, containing the polysaccharides, was collected. Columns were operated on an ÄKTA™ avant 150 chromatography system.

Desalting of polysaccharide-containing sample was conducted on a HiPrep™ 26/10 Desalting Column using deionized water as mobile phase.

More details about this purification process can be found from the publication by Tian and colleagues (1).

Analysis

Quantification of meningococcal polysaccharide was performed by the Anthrone method for determination of carbohydrates. Determinations of phosphorus, acetoxy, protein, sialic acid, and nucleic acid content as well as molecule size distribution, as analyzed by size exclusion chromatography (SEC), were conducted according to the methods and standards in European Pharmacopoeia 7.0 (2).

Results

The Capto adhere and Capto DEAE chromatography steps offered good separation of all polysaccharides from impurities in simple flow-through mode (Fig 2–5).

Columns: Serially connected Capto adhere XK16/20 and Capto DEAE XK 16/10 columns
Sample: Ethanol precipitated polysaccharide A (2.5 mg/mL)
Buffer: 20 mmol/L Tris-HCl, pH 8.0 + 1% SDC
System: ÄKTA avant 150

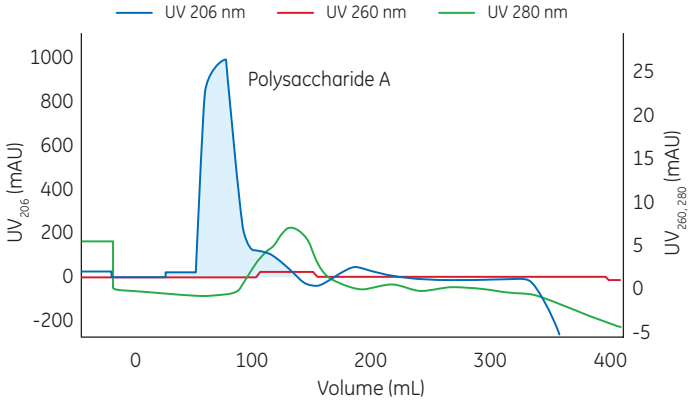

Fig 2. Chromatogram of polysaccharide of serogroup A after purification on Capto Adhere and Capto DEAE resins.

Columns: Serially connected Capto adhere XK16/20 and Capto DEAE XK 16/10 columns
Sample: Ethanol precipitated polysaccharide C (2.5 mg/mL)
Buffer: 20 mmol/L Tris-HCl, pH 8.0 + 1% SDC
System: ÄKTA avant 150

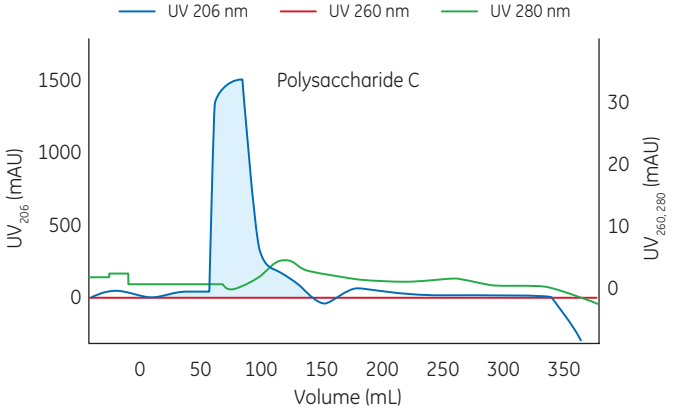

Fig 3. Chromatogram of polysaccharide of serogroup C after purification on Capto Adhere and Capto DEAE resins.

Columns: Serially connected Capto adhere XK16/20 and Capto DEAE XK 16/10 columns
Sample: Ethanol precipitated polysaccharide W135 (2.5 mg/mL)
Buffer: 20 mmol/L Tris-HCl, pH 8.0 + 1% SDC
System: ÄKTA avant 150

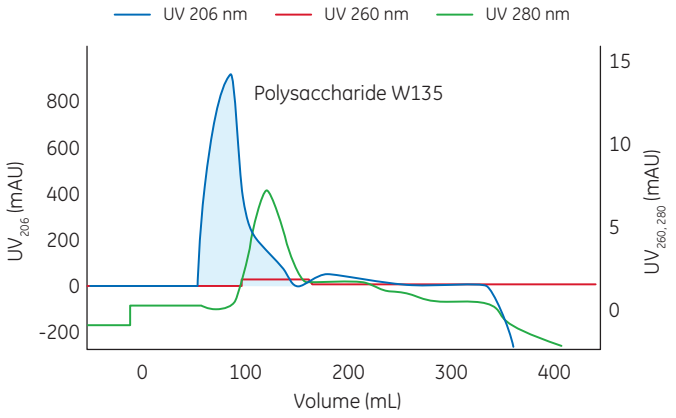

Fig 4. Chromatogram of polysaccharide of serogroup W135 after purification on Capto Adhere and Capto DEAE resins.

Columns: Serially connected Capto adhere XK16/20 and Capto DEAE XK 16/10 columns
Sample: Ethanol precipitated polysaccharide Y (2.5 mg/mL)
Buffer: 20 mmol/L Tris-HCl, pH 8.0 + 1% SDC
System: ÄKTA avant 150

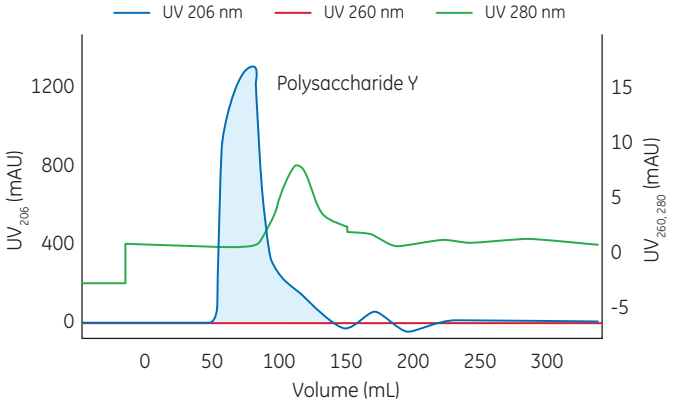

Fig 5. Chromatogram of polysaccharide of serogroup Y after purification on Capto Adhere and Capto DEAE resins.

Table 1. Control tests of final polysaccharide preparations

Polysaccharide serogroup	Polysaccharide content (µg/mL)	Phosphorus content (mg/g)	Acetoxy (mmol/g)	Protein content (mg/g)	Sialic acid content (mg/g)	Nucleic acid content (mg/g)	Molecule size distribution (%)	Endotoxin content (Eu/µg)	Recovery (%)*
A	789	83 (> 80)†	2.29	BDL (< 10)†	-	5 (< 10)†	75.1 (> 65)†	< 25 (< 100)†	77
C	613	-	2.22	BDL (< 10)†	847 (> 800)†	7 (< 10)†	90.3 (> 75)†	< 25 (< 100)†	73
W135	716	-	0.36	BDL (< 10)†	523 (> 560)†	1 (< 10)†	94.7 (> 80)†	< 25 (< 100)†	69
Y	523	-	0.82	BDL (< 10)†	587 (> 560)†	4 (< 10)†	89.0 (> 80)†	< 25 (< 100)†	58

BDL = below detection limit.

* Total recovery, including pretreatment and chromatography.

† Level as required by European Pharmacopoeia 7.0 (2).

Overall purification recovery after the chromatography steps was determined to 77%, 73%, 68%, and 58% for the A, C, W135, and Y serogroups, respectively. Levels of biological indicators of impurity met the requirements of European Pharmacopoeia (2). Results are listed in Table 1.

The described chromatography workflow can easily be scaled and successfully applied to other polysaccharides (3).

Conclusions

Here, we describe the purification of meningococcal polysaccharides in an easily scalable process based on modern chromatography resins. In the described process, high recoveries of the A, C, W135, and Y serogroups could be achieved, while meeting the quality requirements of European Pharmacopoeia (2). Eliminating the need for time-consuming, multi-step phenol extractions, the suggested process allowed all four meningococcal A, C, W135, and Y polysaccharide serogroups to be successfully purified with high recovery in a way that benefits the environment and operator health.

Acknowledgement

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Reference

1. Tian *et al*. Development of procedure for purification of meningococcal polysaccharide of groups A, C, Y and W135 by chromatography. *Chin J Biologicals* **9** (2013)
2. European Pharmacopoeia 7.0 (8th Ed), **1**, 803-805 (2013).
3. Application note: Efficient purification of a pneumococcal polysaccharide in a chromatography workflow. GE Healthcare 29216881, 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
XK 16/20	Empty column, 20 mL	28988937
HiPrep 26/10 Desalting Column	Prepacked with Sephadex G-25 resin	17508701

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