Purification of Capsular Polysaccharides of *Streptococcus pneumoniae* Serotype 19F by Cellufine™

Introduction

Streptococcus pneumoniae is one of the major pathogens causing high level morbidity and mortality worldwide, especially children and the elderly populations. Pneumococcal vaccines based on the capsular polysaccharide (CPS) on the bacteria surface, which is one of the most important virulence factors, have been used to prevent these infectious diseases. Traditional ethanol precipitations are general processes for purifying CPSs, which are suitable for most serotypes. However, they are complex and time consuming. As a result, pneumococcal vaccines are quite expensive [1]. To overcome these disadvantages, a lot of improved processes are proposed. Some of these processes include chromatography processes. Here we would like to propose simplified two step chromatography purification process of CPS without ethanol precipitations (Figure 1). This process includes hydrophobic interaction chromatography (HIC) and anion exchange chromatography (AEX). We introduce two optimized resins, Cellufine MAX Butyl HS (HIC) and Cellufine MAX Q-hv (AEX), for this process.

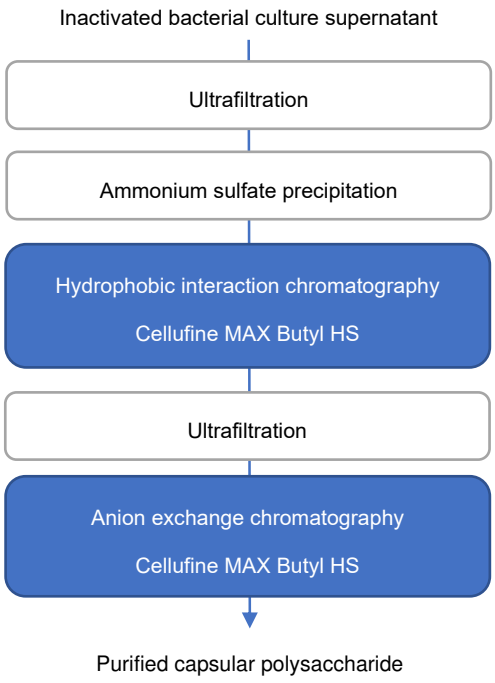


Figure 1. Proposed CPS purification process

Method and materials

Crude polysaccharide sample preparation

Streptococcus pneumoniae 19F (ATCC 49619) was grown on Tryptic soy agar with 5% defibrinated sheep blood at 37 °C for 24 h. The culture was prepared by inoculating a colony into 2,000 mL of Brain heart infusion broth and incubating at 37 °C for 24 h. Then the cells were lysed by the addition of 10% (w/v) sodium deoxycholate for 24 h at 37 ℃. The supernatant was collected by centrifugation (12,000 rpm, 15 min) and membrane filtration (Cellulose acetate 0.45 μm) and concentrated by the cross-flow ultrafiltration device (PES, 100,000 MWCO) with MilliQ water. 50% saturation of ammonium sulfate was added to the polysaccharide solution and incubated for 24 h at 4 °C. The supernatant was collected by centrifugation (12,000rpm, 15min) and membrane filtration (Cellulose acetate 0.45 μ m). This supernatant was used for next HIC step.

Chromatography steps

The HIC and AEX resins used in this work were packed into Super Edge Empty Mini Columns (6.7 mmID×30 mmH, 1.06 mL = 1 column volume (CV)). All chromatography experiments were performed using an AKTA

avant25 system under the following conditions. The HIC step was run in flow-through mode at 0.212 mL/min (residence time 5.0 min). The resins were equilibrated with 50 mM sodium phosphate, 2.0 M ammonium sulfate, pH6.8. The 40CV crude polysaccharide sample which contains 726 µg/mL polysaccharide, 73.4 µg/mL protein and 772 µg/mL nucleic acid was loaded and washed by the 10 CV same buffer after loading. Non adsorbed fractions were collected as FT and wash pool. The pool was concentrated and diafiltrated by the cross-flow ultrafiltration device (PES, 100,000 MWCO) with MilliQ water. The AEX step was run in bind and elute mode at 0.424 mL/min (residence time 2.5 min). The resins were equilibrated with 50 mM sodium phosphate, pH 6.0. The buffer exchanged sample after Cellufine MAX Butyl was loaded until 10% breakthrough and washed the 15CV same buffer after loading. The binding sample was eluted with 50 mM sodium phosphate, 1.0 M NaCl, pH 6.0 by the stepwise elution (32%, 20CV→100%, 20CV). The first stepwise were collected as final CPS sample.

Analysis

Quantities of polysaccharide (Ps) were measured by anthron-sulfuric acid method, and amounts of impurities (protein and nucleic acid) were measured according to WHO Technical Report Series; proteins (Pr) were determined method of Bradford with protein assay kit (Bio-Rad Laboratories) and nucleic acids (NA) were measured 260nm absorption spectroscopy and calculated amount of nucleic acid by 1AU = 50 µg/mL.

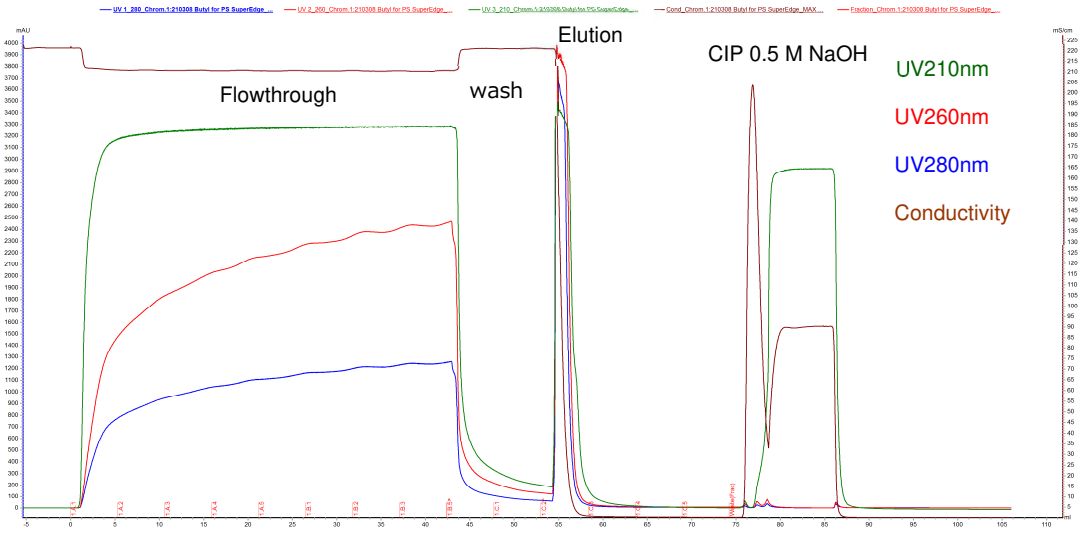
Results and discussion

The results Cellufine MAX Butyl HS was shown in Figure 2, and the comparison among HIC were shown in Table 1. In this process, most of polysaccharide was collected in the FT and wash pool and impurities were partially eliminated. Compared among resins, Cellufine MAX Butyl HS which has optimal ligand density for CPS purification showed good polysaccharide recovery and elimination of impurities.

	Ps µg/mL	Ps Recovery%	Ps Purity%	Pr µg/mL	NA µg/mL	Pr/Ps %	NA/Ps%
Requirement	-	-	-	-	-	3	2
Loading sample	726	-	46.2	73.4	772	10	106
Cellufine MAX Butyl HS	518	89	54.3	N.D.	436	0	84
Cellufine MAX Butyl	500	86	52.6	7.91	444	2	89
Commercial resin A	510	88	51.9	7.24	466	1	91
Commercial resin B	450	77	48.5	3.97	474	1	105

Table 1. Comparison among the results of HIC resins.

(a)



(b)

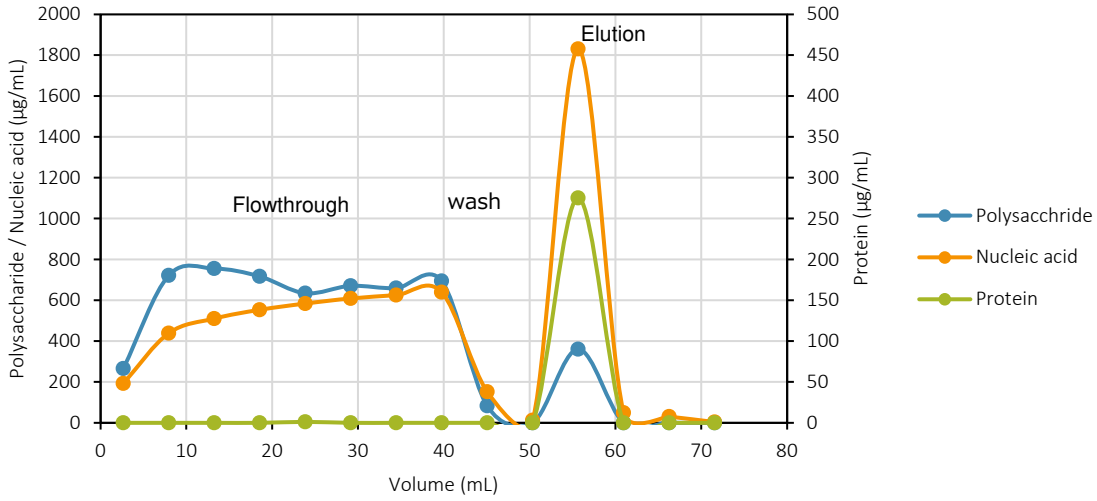


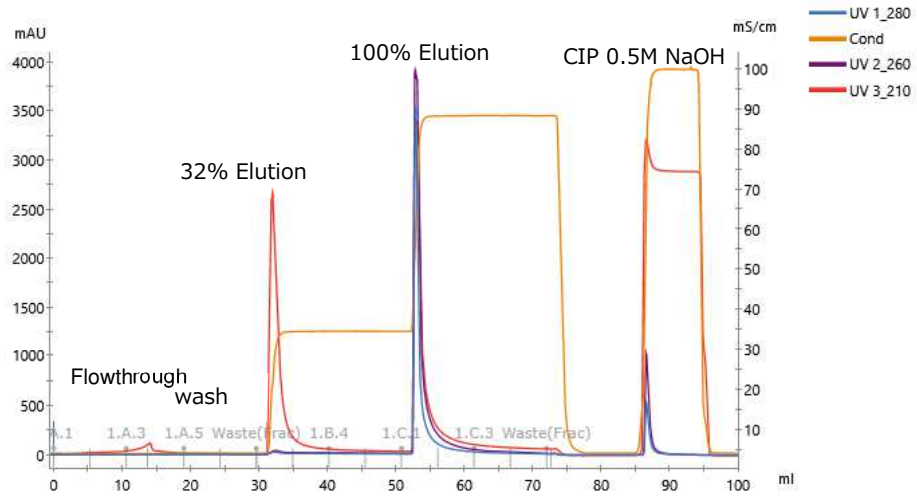
Figure 2. Experimentally obtained chromatogram of Cellufine MAX Butyl HS (a) and the results of fraction analysis (b).

The results Cellufine MAX Q-hv was shown in Figure 3, and the comparison among AEX were shown in Table 2. In this process, polysaccharide eluted at the first step (32% elution buffer) and impurities were eluted at the second step (100% elution buffer). Cellufine MAX Q-hv showed excellent separation polysaccharide recovery and elimination of impurities.

	10% DBC mg/mL-resin	Ps Recovery%	Ps Purity%	Pr µg/mL	NA µg/mL	Pr/Ps %	NA/Ps%
Requirement	-	-	-	-	-	3	2
Loading sample	-	-	75.6	5.13	162	1	31
Cellufine MAX Q-hv	6.39	97.8	98.7	N.D.	4.25	0	1
Cellufine MAX Q-h	6.56	63.1	99.3	N.D.	1.48	0	1
Cellufine MAX Q-r	5.01	83.2	96.3	N.D.	8.23	0	4
Commercial resin C	1.87	92.2	94.2	N.D.	5.30	0	6
Commercial resin D	4.46	94.9	96.8	N.D.	6.95	0	3
Commercial resin E	5.86	91.1	98.0	1.55	4.15	1	2

Table 2. Comparison among the results of AEX

(a)



(b)

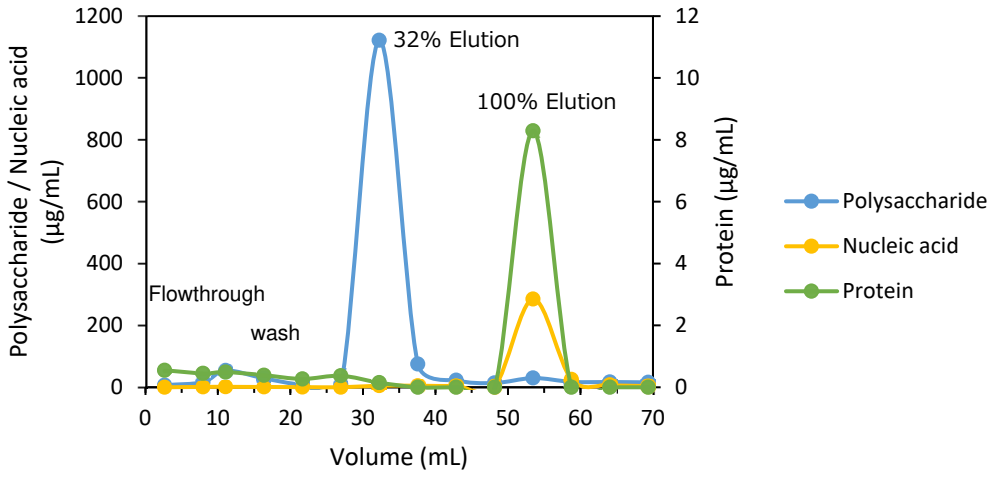


Figure 3. Experimentally obtained chromatogram of Cellufine MAX Q-hv (a) and the results of fraction analysis (b).

Conclusions

We showed the simplified CPS production process with two chromatography steps. This process does not include any ethanol precipitation so it could be more efficient. We introduced optimized HIC (Cellufine MAX Butyl HS) and AEX (Cellufine MAX Q-hv) resin and achieved excellent separation and high recovery.

Reference

[1] Norma Suárez et al., Purification of Capsular Polysaccharides of *Streptococcus pneumoniae*: Traditional and New
Methods, Frontiers in Bioengineering and Biotechnology, 6 (2018), 145.

[2] WHO (2009). Recommendations to Assure the Quality, Safety and Efficacy of Pneumococcal Conjugate Vaccines.
http://www.who.int/biologicals/areas/vaccines/pneumo/Pneumo_final_23APRIL_2010.pdf?ua=1

Product information

Cellufine MAX Butyl HS
https://www.jnc-corp.co.jp/fine/en/cellufine/guide/pdf/ion_max/TD_MAX_IEX_N1_V5_E.pdf
Cellufine MAX Q-hv
https://www.jnc-corp.co.jp/fine/en/cellufine/guide/pdf/affinity/TD_Phosphate_N1_V2_E.pdf
Operating instructions manual and technical data can be downloaded as pdf from the following website.https://www.jnc-corp.co.jp/fine/en/cellufine/guide/index.html

Purchase information

Product	Quantity	Catalogue No.
Cellufine MAX Butyl HS	1ml x 5 (Mini-Column)	22200-51
	5ml x 1 (Mini-Column)	22200-55
	100ml	22200
	500ml	22201
	5 lt	22202
	10 lt	22203
Cellufine MAX Q-hv	1ml x 5 (Mini-Column)	22100-51
	5ml x 1 (Mini-Column)	22100-15
	100ml	22100
	500ml	22101

	5 lt	22102
	10 lt	22103

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