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Rapid processes for purification of capsular polysaccharides from *Neisseria meningitidis* serogroups A and C

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ABSTRACT

The glycoconjugate vaccines against *Neisseria meningitidis* are highly effective, however most of these vaccines are expensive and still out of reach in the developing world as well as the technical know-how and the set-up required for the consistent production of pure polysaccharide is limited. Our laboratory has developed rapid, efficient and scalable processes for the downstream purification of *N. meningitidis* serogroup A (MenA) and serogroup C (MenC) capsular polysaccharides (PS). The MenC-PS was purified with a novel 2-step procedure including de-*O*-acetylation and hydrophobic interaction chromatography whereas, MenA-PS was purified using a rapid method as compared to the prior art. The purified PSs were analyzed by various analytical tests including nuclear magnetic resonance, molecular weight, composition and purity analyses to meet desired specifications. Our results provide a proof of principle for the purification of MenA-PS and MenC-PS with reduced timelines.

1. Introduction

Meningitis due to *Neisseria meningitidis* is one of the major infectious diseases affecting 500,000 to 1.2 million people and killing between 50,000 and 135,000 people per year globally [1]. The polysaccharide capsules found in different serogroups of *N. meningitidis* are structurally different and are responsible for their virulence and disease [2]. Purified polysaccharide vaccines have been ineffective or poorly immunogenic in infants. However, in the last three decades, a new paradigm of success was achieved with the development of glycoconjugate vaccines and was based on the concept of Avery and Goebel [3]. The first glycoconjugate vaccine was developed against *Haemophilus influenzae* in the late 1980's [4,5]. Since then, various glycoconjugate vaccines including meningococcal conjugate vaccines have been developed and have considerably reduced the scourge of infectious diseases.

Glycoconjugate vaccines are highly effective vaccines exhibiting T-dependent immune responses. A T-cell dependent protein carrier conjugated to the polysaccharide leads to the construction of a glycoconjugate. There is large body of evidence in the literature defining the immunogenic aspects of polysaccharide conjugate vaccines but details explaining the technical know-how for production and purification of the capsular polysaccharides (PSs) are limited. The production of purified PS of desired quality is one of the key requirements for effective conjugation with the carrier protein. The cost for cultivation and the purification of polysaccharides is generally high and involves a series of production and purification steps. Furthermore, the purified polysaccharide is required to meet the desired specifications e.g. as per World Health Organization technical report series (WHO-TRS) [6,7].

Various monovalent as well as combination meningococcal conjugate vaccines have been licensed and are being successfully used in reducing meningococcal disease burden in the areas of introduction. However, most of them except MenAfriVac (a monovalent serogroup A conjugate vaccine) cannot be afforded by developing countries, where the disease burden is significant [3,8]. Out of the 13 serogroups of *N. meningitidis*, serogroup A and C are the most prevalent in terms of conjugate vaccine preventable meningococcal diseases [9]. Hence, there is a need for cost effective multivalent meningococcal conjugate vaccines preferably containing serogroup A and C conjugates and which can be afforded by low income country settings.

As an attempt in this direction, the objective of this study defines innovative “Rapid-Process” protocols for the purification of

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capsular polysaccharides of *N. meningitidis* serogroup A (MenA-PS) and serogroup C (MenC-PS) which are simple, less labor-intensive with reduced timelines and yield highly purified polysaccharides, which comply with the desired specifications [6,7].

2. Materials and methods

2.1. Bacterial strains

N. meningitidis serogroup A (Albrecht and Ghon) Murray (ATCC® 13077™) and *N. meningitidis* Serogroup C (Albrecht and Ghon) Murray (ATCC® 13102™) strains were procured from ATCC (American Type Culture Collection), USA.

2.2. Production process for MenA-PS and MenC-PS

The media components for MenA and MenC shake flask culture and fermentation were adapted from published literature [10,11] and optimized medium was achieved by various shake flask experiments to get highest polysaccharide yields (results not shown) before taking the fermentation run. The common optimized medium composition for both MenA-PS and MenC-PS production was as follows: mono sodium glutamate at 1 g/L (Sigma), di-sodium hydrogen phosphate at 3.25 g/L (Merck), potassium chloride at 0.09 g/L(Sigma), casamino acid at 10 g/L (Difco), yeast extract at 3.75 g/L (Fluka), dextrose at 5 g/L (Sigma), L-cystine at 0.03 g/L (Sigma), magnesium sulphate at 0.60 g/L (Sigma), and nicotinamide adenine dinucleotide (NAD) at 0.25 g/L (Sigma). A loopful of the working cell bank was inoculated on GC agar plates (GC agar base at 36 g/L (Difco), agar at 5 g/L (Sigma), haemoglobin powder at 10 g/L (Sigma), isovitaleX at 10 ml/L (BBL)) for overnight growth and thereafter 12–15 colonies were inoculated in flask containing 100 ml culture broth till the OD_{590} reached 0.7–0.9. The fermentations of the two organisms were carried out separately in Biostat B plus Sartorius glass bioreactors with 3 L working volume. The bioreactor was seeded with the flask culture. The bioreactor working conditions were: temperature at 36 °C; pH maintained at 7.2 with 1 M NaOH; airflow at 0.2 L/min till 1.1 L/min; dissolved oxygen (pO_2) at 20% and the rate of agitation was between 100 and 500 rpm. The culture was fed with 500 ml of feed containing dextrose (40 g/L, Sigma) and L-glutamic acid (24 g/L, Sigma), feed starting when the culture OD_{590} reached to 1.0.

After the completion of growth phase as observed with the increase in pH and decrease in optical density (OD_{590}), fermentation was terminated by the addition of 0.1% (v/v) formaldehyde (HCHO) and the temperature of fermenter was maintained at 37 °C for 30 min. Fermentation broth (FB) was centrifuged at 4690 × g for 30 min at 4 °C (Sorvall, RC6+). After centrifugation, the supernatant containing crude MenA-PS or MenC-PS was concentrated up to 1/10th of starting volume by using 100 kDa molecular weight cut off polyether sulfone (PES) slice membrane (0.1 m^2) (Millipore). The concentrated supernatant for MenA-PS and MenC-PS was diafiltered with 8–10 volumes of MilliQ water (MQW). After diafiltration, the material was further processed for the purification of PSs as described below.

2.3. MenA-PS purification procedure

The purification process for MenA-PS as described by Costantino [12] has been modified with the following details. The 100 kDa diafiltered and concentrated FB containing the crude PS is treated with cetyl trimethyl ammonium bromide (CTAB) at a final concentration of 1.2% (w/v) for 1 h (h) at room temperature (RT) after few optimization experiments to find out minimum time required to get maximum CTAB pellet weight. Subsequently, the mixture was centrifuged at 4690 × g for 30 min and the pellet obtained was dissolved in minimal volume of 96% (v/v) ethanol and mixed for 20 min using a mixer (IKA RW20) at 700 rpm to get a homogenous suspension. Afterwards, 96% (v/v) ethanol was again added so as the final volume corresponds to the volume of the diafiltered concentrate. The dissolution was further continued for 40 min with continuous stirring at RT. Later on, centrifugation was performed at 4690 × g for 30 min. The supernatant obtained was clarified using zetacarbon filter until the OD_{260} reached ≤0.2. The clarified supernatant was subsequently filtered through 0.22 μm filters. Afterwards, calcium chloride ($CaCl_2$) was added into the filtered supernatant at a final concentration of 0.15 M which was then kept for 30 min at RT. Centrifugation was performed at 4690 × g for 30 min to obtain pellet. Finally the pellet was dissolved in MQW, followed by 300 kDa diafiltration against 10 ± 2 volumes of 50 mM $CaCl_2$ and then with 10 ± 2 volumes of MQW. The purified MenA-PS thus obtained was filtered with 0.22 μm millipak 20 (Millipore), and stored at or below −20 °C for further use.

2.4. MenC-PS purification procedure

The diafiltered and concentrated FB containing the crude PS was de-*O*-acetylated by treating with 1 N NaOH for 2 h at 75 ± 5 °C. The de-*O*-acetylated polysaccharide was then cooled to a temperature below 40 °C. Concentration and diafiltration of the crude de-*O*-acetylated PS was performed through 100 kDa PES membrane (0.1 m^2) with 20 ± 2 volumes of MQW, followed by 9 ± 1 volumes of 20 mM Tris HCl buffer (pH 7.4). Subsequently, sterile filtration was done with 0.22 μm PES membrane (0.1 m^2) (Corning).

De-*O*-acetylated polysaccharide was further purified by hydrophobic interaction chromatography (HIC) using XK-16 column (GE Healthcare) packed with 20 ml phenyl sepharose 6FF (GE Healthcare) on an AKTA Avant chromatography system (GE Healthcare). The column was equilibrated with 5–10 column volume (CV) of 20 mM Tris HCl buffer, pH 7.4 ± 0.1 containing 20% ammonium sulphate at a flow rate of 60 cm/h. The 200 ml material was loaded at a flow rate of 60 cm/h, and the flow through containing the purified polysaccharide was collected. Finally, column was regenerated with 20 mM Tris HCl buffer pH 7.4 ± 0.1 (3–5 CV), sanitized with 0.5 M NaOH (5 CV) followed by washing with MQW (5–8 CV) and stored in 20% ethanol (5 CV) for further use. Concentration and diafiltration of the purified polysaccharide was performed with 100 kDa PES membrane (0.1 m^2), with 7 ± 1 volumes of MQW followed by sterile filtration with 0.22 μm millipak 20 (Millipore), and the purified PS was stored at or below −20 °C for further use.

2.5. Analytical procedures for MenA-PS and MenC-PS

Phosphorus concentration for MenA-PS was determined by Ames' method using D-ribose-5-phosphate disodium dihydrate (Sigma, 83875) as a standard [13]. O-acetyl content of MenA-PS was determined by Hestrin's method using acetyl choline chloride (Sigma, A6625) as standard [14]. Total sialic acid of MenC-PS was determined by resorcinol assay using a series of sialic acid standards (Sigma, A0812) [15]. Protein impurity for MenA-PS and MenC-PS were determined by Lowry's method using bovine serum albumin (Sigma, P0834) as a standard [16]. Nucleic acid (NA) content in MenA-PS and MenC-PS was estimated by spectrophotometric reading at 260 nm and the amount was calculated assuming an absorbance of 1.0 A_{260} = 50 μgNA/mL [17]. Lipopolysaccharide (LPS) for MenA-PS and MenC-PS was determined using compact and simple Endosafe®-PTS™ apparatus as per the manufacturer's instructions. This is a rapid, point-of-use test system that provides quantitative results within 15 min. The PTS™ utilizes Limulus Amebocyte Lysate (LAL) reagents in an FDA-licensed disposable test

cartridge with a handheld reader for a completely contained, real-time endotoxin testing system. Kinetic chromogenic reagents and endotoxin controls are dried in the channels of the polystyrene cartridge. Diluted sample is pumped into the channels where it is mixed with reagents and is monitored for changes in optical density and results are interpolated against an archived endotoxin standard curve.

Relative average molecular size of the PSs was determined using High-performance liquid chromatography (HPLC) (Alliance, Waters). The PWXL-5000 and PWXL-4000 columns (Tosoh Bioscience) were used in series for MenA-PS or MenC-PS analyses. A range of 5 kD to 800 kD Pullulans (Shodex) were used as molecular weight standards. The total column volume was determined with deuterium oxide (D_2O, Merck). Elution time for 800 kDa Pullulan marker was 12.7 min and total column volume (Vo) was 23.0 min. The HPLC was performed using 0.1 M sodium nitrate, pH 7.2 ± 0.1 with a run time of 30 min at a flow rate of 1 ml/min.

The identity of both the polysaccharides was verified by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The NMR's were recorded over Bruker-500 MHz NMR instrument. For both the PSs, 10–15 mg of sample was dissolved in 600 μl of D_2O and transferred in an NMR tube and the spectra were recorded. The peak for D_2O was considered as a reference peak. MenA-PS and MenC-PS were also identified serologically by combining with the specific antisera against each polysaccharide using the Pastorex meningitis kit (BioRad).

As the WHO specifications [6,7] to determine the purity and to characterize the polysaccharide for composition is based on dry weight basis, the polysaccharides were first lyophilized and then tested for O-acetyl, sialic acid and phosphorus content as applicable. Moisture content of lyophilized cake was determined by Thermo Gravimetric Analyzer (TGA) from Perkin Elmer and was subtracted to get the exact dry weight of a PS.

Determination of MenA-PS and MenC-PS in fermentation harvest: The harvest yields of MenA-PS and MenC-PS were calculated using competitive enzyme-linked immunosorbent assay (Inhibition-ELISA). In this assay, eight thousand fold diluted rabbit antiserum against *N. meningitidis* serogroup A (222281; BD) or C (222301; BD) was incubated for 1 h at 37 °C with seven 2-fold dilutions of the test antigens (MenA or MenC fermentation harvest). The standard consisted of respective purified PS at various concentrations in duplicate that is 2.5, 5, 10, 20, 40, 80 and 160 μg/ml diluted in phosphate-buffered saline (PBS) containing 0.1% v/v Brij35 (Sigma) and 5% fetal bovine serum (FBS, Gibco Lifesciences) in 96 well micro titer plate (Plate A). A separate plate (plate B) was coated with a mixture of methylated human serum albumin (m-HSA) with MenA-PS or MenC-PS each at 5 μg/ml and subsequently blocked with 5% FBS after overnight incubation at 2–8 °C. To this plate B, antitoxin-antigen mix from plate A was added and incubated at 37 °C for 1 h. The plate was washed with phosphate-buffered saline, pH 7.4 containing 0.1% Brij35. The plate was incubated for 60 min at RT with peroxidase labeled anti-rabbit IgG antibodies (Sigma, A6154) in PBS, 0.1% Brij35 and 5% FBS. Plate was washed again and incubated for 10 min at RT with the 100 μl peroxidase substrate, 3,3′,5,5′-tetramethylbenzidine-H_2O_2 in sodium acetate buffer. The reaction was stopped by adding 50 μl of 2 M H_2SO_4. The plate absorbance was recorded at 450 nm (A_{450}) on an ELISA reader (Tecan micro plate reader) with a reference to A_{630}. The control consisted of coated wells incubated without the inhibitors (serum + diluent buffer only) and had the maximum optical density, referred as optical density for no antigen control. The A_{450} of standard purified PS dilutions was used to prepare standard curve using CombiStat software. This standard curve was used to extrapolate the PS concentration in harvest test samples. The same assay could be successfully used for estimation of purified PS samples whenever required.

3. Results

3.1. Fermentation and production of MenA-PS and MenC-PS

The optimized fermentation medium composition could help the growth of both the organisms i.e. *N. meningitidis* serogroup A and C. In order to substantiate the reproducibility of bacterial growth, several fermentation batches for each of the two organisms were run. All the batches for MenA and MenC achieved optical density (OD_{590}) with a similar trend in growth curves over time and peaking at OD_{590} of 11.0 and 9.0 in approximately 9 and 10 h, respectively. The various fermentation parameters and bacterial growth patterns for MenA and MenC have been elaborated in Fig. 1a and b, respectively. The cultures were harvested for downstream purification in the late stationary phase as soon as the OD_{590} started declining. The MenA and MenC PS yields in the crude harvest were estimated by inhibition ELISA and were found to be 950 (±35%) mg MenA-PS and 664 (±26%) mg for MenC-PS per liter of clarified fermentation harvest.

3.2. Purification and characterization of MenA-PS

The MenA-PS was purified with the optimized purification scheme as described in Fig. 2. The diafiltration of the crude polysaccharide (fermentation harvest) with 100 kDa PES membrane enabled the removal of small sized impurities. The subsequent use of 1.2% CTAB facilitated the precipitation of crude polysaccharide within 1 h at RT. Resolubilization of the pellet in high concentration of ethanol was achieved within 1 h by use of a homogenization step. The removal of other contaminants such as nucleic acids was facilitated by carbon filtration [12]. Further concentration of the polysaccharide was performed by using 0.15 M calcium chloride precipitation. This was followed by redissolution of pellet in MQW and diafiltration with 10 cycles each of 50 mM $CaCl_2$ and MQW to get the purified polysaccharide. The purified MenA-PS had an average relative molecular weight of 699 kDa. The purification procedure of MenA-PS polysaccharide resulted in a yield of about 375 mg purified MenA-PS/L of fermentation broth (approximately 40% of the harvest yield). The MenA-PS was analyzed by a series of analytical tests and the results met the quality specifications as per WHO-TRS [6] and in-house set criteria and are presented in Table 1. The ^{1}H-NMR spectra had desired peaks which reveals the identity of specific PS (Fig. 4a) when compared with spectra reported earlier [18] and the PS reacted positively with specific serum in agglutination test confirming its identity. The HPLC profile of purified PS indicates desired size distribution and purity of the MenA-PS as presented in Fig. 4c.

3.3. Purification and characterization of MenC-PS

The novel purification scheme for MenC-PS is described in Fig. 3 whereas Table 1 describes the identity, polysaccharide content, protein, nucleic acid, endotoxin content and average relative molecular weight. The de-*O*-acetylation of the fermentation harvest, further processing and purification of the crude MenC-PS with HIC (as described in material and methods) resulted in purified de-*O*-acetylated MenC-PS with an yield of about 210 mg/L (approximately 32% of the harvest yield). The analytical results for purified MenC-PS were in compliance with the WHO specifications [7] or in-house set criteria and are presented in Table 1. The PS reacted positively with specific serum in agglutination test indicating its identity. The ^{1}H-NMR spectra matched with desired peaks and

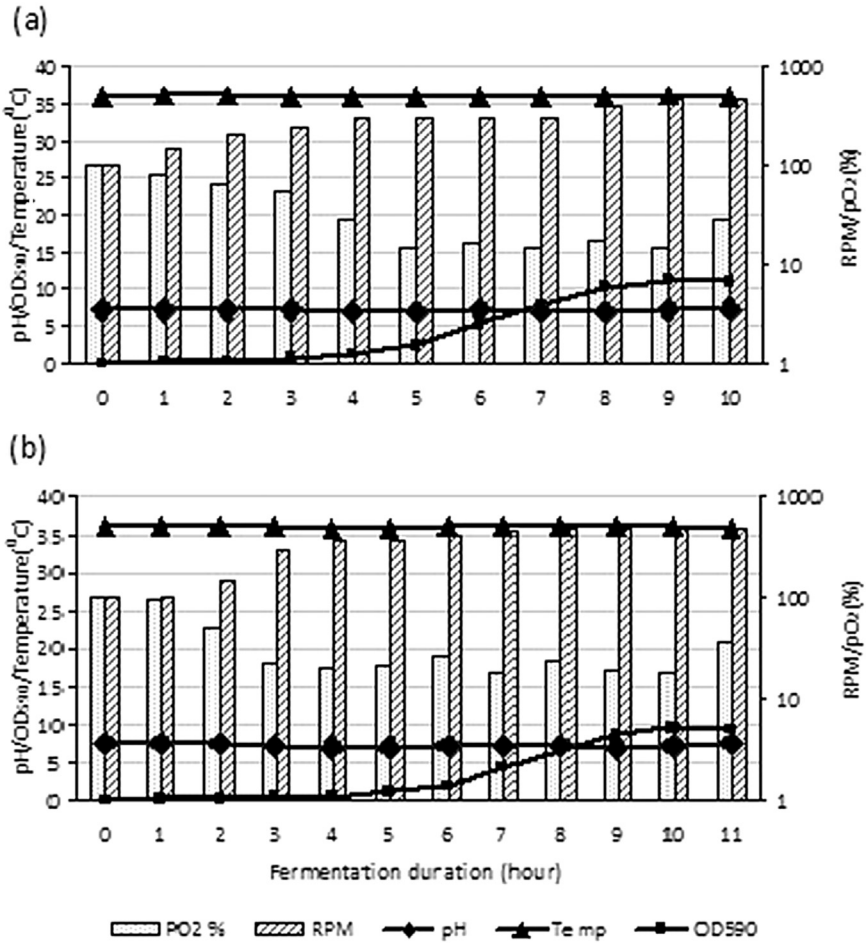

Fig. 1. Fermentation parameters and bacterial growth curves for *N. meningitidis* serogroup A (a) and serogroup C (b); OD_{590}: Optical density at 590 nm; RPM: Revolutions per minute; pO_2: Dissolved oxygen; Temp: Temperature.

related well with that reported earlier [18] further revealing the identity (Fig. 4b) and the HPLC profile indicates size distribution and purity of MenC-PS, as shown in Fig. 4d. The elution profile shows one broad peak of MenC-PS having an average relative molecular weight of 331 kDa.

4. Discussion

Development of polysaccharide conjugate vaccines have been one of the major successes in field of vaccinology. The burden of conjugate vaccine preventable diseases including meningococcal diseases is significantly high in developing countries. However, the set-up required for the consistent production of highly pure bacterial polysaccharides and its validation by analytical techniques are found in established laboratories, well equipped with the relevant equipment and optimized process protocols [19]. To have this kind of laboratory set-up in the developing world is not easy to accomplish. This can be partially attributed to the lack of sufficient literature in public domain as not many reports are available on purification of bacterial capsular polysaccharide especially for *N. meningitidis* serogroups. The other challenge is the costly and labor intensive production and purification processes of the polysaccharides including use of costly enzymes [20–23]. Improvement in one or more of the steps of polysaccharide production or purification could reduce the cost of overall conjugate vaccine manufacturing to certain extent. We made a step forward in this direction and attempted to simplify and develop rapid processes for *N. meningitidis* serogroup A and C capsular polysaccharide purification. Furthermore, the process for MenC-PS purification is a novel process developed in our laboratory.

It is well known that the bacterial PSs are precipitated with the use of CTAB. This is a common and preferred method to precipitate the capsular polysaccharides of *N. meningitidis* which is reported to take an overnight incubation [12]. Macha et al. have reported a rapid polysaccharide purification protocols for *H. influenzae* type b [24]. We attempted to find out the minimum time to get optimum MenA-PS precipitation with a defined concentration of CTAB. The purification process mentioned for MenA-PS in our laboratory describes final CTAB concentration as 1.2% (w/v) and we observed that maximum precipitation happens within 1 h. Consequently, the pellet obtained after CTAB precipitation is mixed with high concentration of ethanol using a mixer to get a homogenous solution and thereafter addition of more ethanol under continuous stirring facilitates the proper mixing of the CTAB pellet. The CTAB pellet, which is rubbery in nature and highly viscous, loses its viscosity to a greater extent during homogenization thereby allowing the proper and rapid mixing of rubbery CTAB pellet within an hour [25]. Also of particular relevance is the fact that, moderate homogenization does not affect the polysaccharide structure of MenA-PS and is evidenced by ^{1}H-NMR and even the PS size was also not affected apparently as evidenced by the HPLC characterization and relative molecular weight analysis. Further, the purified MenA-PS matched the desired analytical specifications set in accordance to WHO-TRS [6]. The overall purification process for MenA-PS in our laboratory

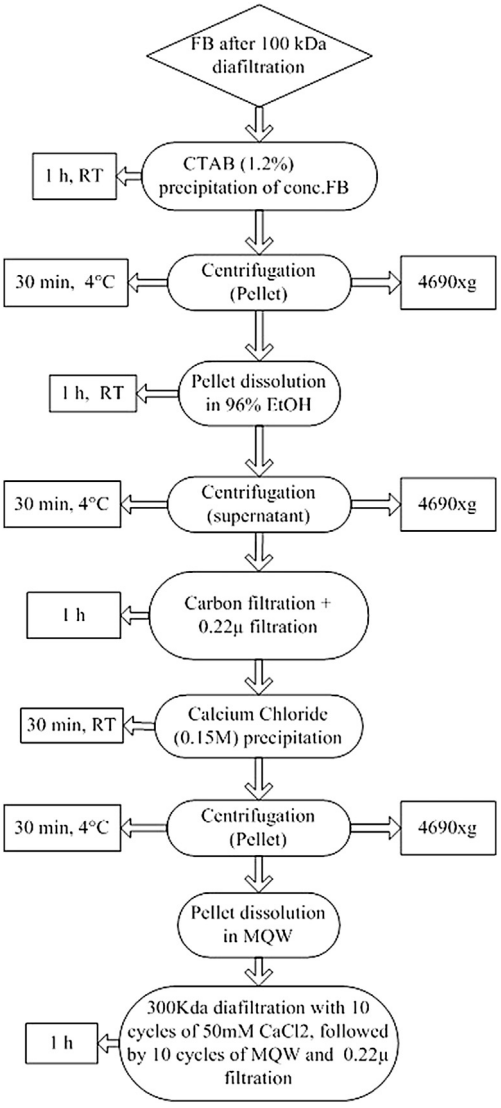

Fig. 2. Purification process flow for *N. meningitidis* serogroup A polysaccharide; FB: Fermentation broth; h: Hour; RT: Room temperature; MQW: MilliQ water; Conc.: Concentrated; min: Minutes; EtOH: Ethanol; $CaCl_2$: Calcium chloride.

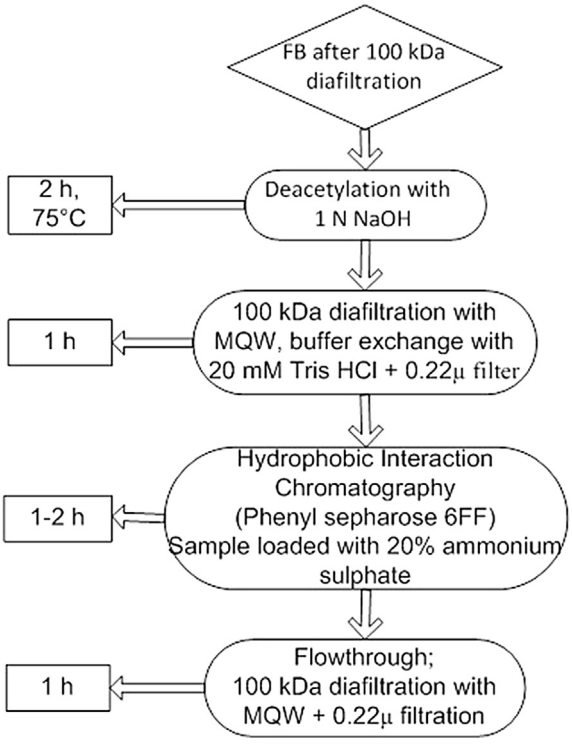

Fig. 3. Purification process flow for *N. meningitidis* serogroup C polysaccharide FB: Fermentation broth; h: Hour; MQW: MilliQ water; NaOH: Sodium hydroxide.

takes 8 ± 2 h, while, the purification of MenA-PS as mentioned in the prior art requires more than 30 h [12]. Therefore, the time reduction of more than 20 h in the purification of MenA-PS in our laboratory can be considered as a factor which may contribute to an extent towards the reduction of the overall production cost of the vaccine. Furthermore, the process is well defined, simple, reproducible and scalable.

MenC-PS in its natural form is an *O*-acetylated (*O*-Ac) PS, however, there are reports that *O*-Ac may not be essential for its immunogenicity [26], hence the WHO-TRS suggests use of either *O*-acetylated or de-*O*-acetylated PS in the MenC-PS conjugate vaccines [7]. The de-*O*-acetylated PS of MenC in our laboratory was produced using a 1 N NaOH treatment of the fermentation broth.

Table 1
Analytical characterization of purified MenA-PS and MenC-PS.

Tests (unit)	MenA-PS	MenC-PS	Desired specifications
Polysaccharide content (mg/ml)	1.7 ± 1.2	2.1 ± 1.4	Actual value[a]
Phosphorous (% w/w)	10.1 ± 1.4	Not applicable	Not less than 8% for MenA-PS [6]
O-acetyl content (mmol/g PS)	2.6 ± 0.2	de-*O*-acetylated	Not less than 2 mmol/g for MenA-PS [6]; de-*O*-acetylated or not less than 1.5 mmol/g MenC-PS [7]
Sialic acid content (% w/w)	Not applicable	99.8 ± 0.5	Not less than 80% of the dry weight for MenC-PS [7]
Endotoxin (IU/µg PS)	5.7 ± 6.3	0.6 ± 1.1	Less than 100 IU/µg [6,7]
Protein (% w/w)	0.4 ± 0.2	0.3 ± 0.1	Not more than 1% [6,7]
Nucleic Acid (% w/w)	0.1 ± 0.1	0.4 ± 0.3	Not more than 1% [6,7]
Average relative Molecular Weight (kDa)	699 ± 102	331 ± 23	Actual value from HPLC[a]
Identity	1. Complies 2. Complies	1. Complies 2. Complies	1. Positive agglutination reaction with specific serum [6,7] 2. ^{1}H-NMR spectra meeting desired peaks [6,7]

[a] In-house specifications; MenA-PS: *N. meningitidis* serogroup A capsular polysaccharide MenC-PS: *N. meningitidis* serogroup C capsular polysaccharide; HPLC: High performance liquid chromatography; kDa: kilo-dalton; ^{1}H-NMR: proton nuclear magnetic resonance spectroscopy.

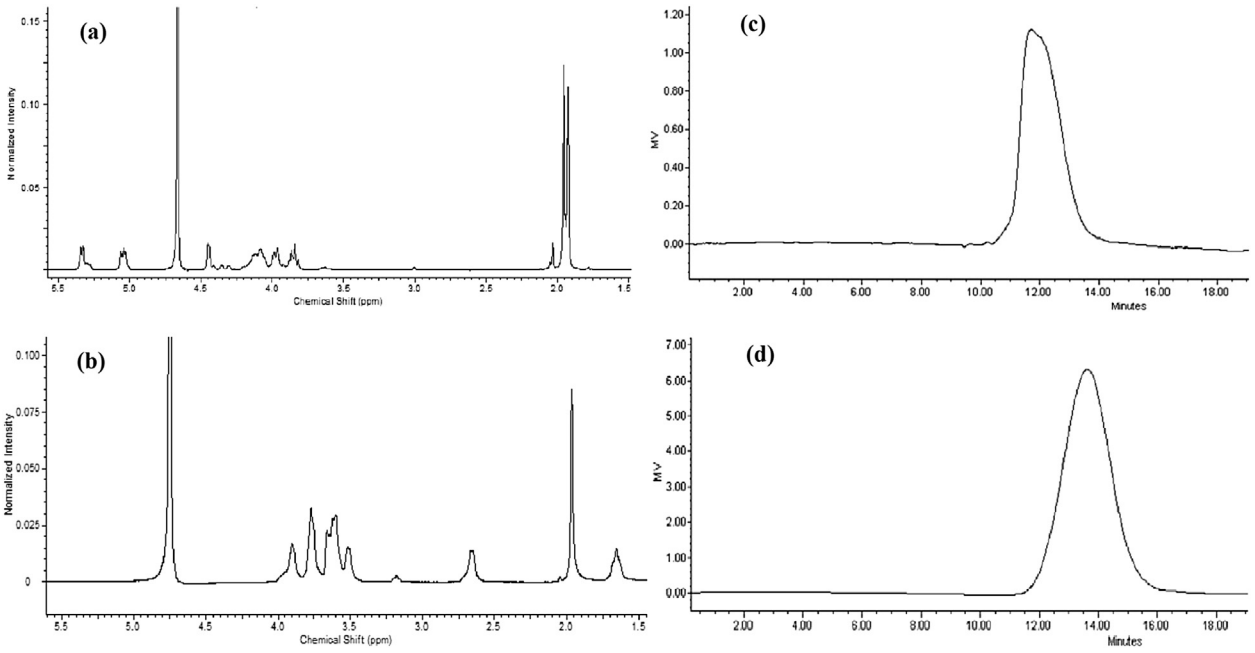

Fig. 4. 500 MHz ^{1}H-NMR spectrum of *N. meningitidis* polysaccharide recorded in D_2O for serogroup A (a) and serogroup C (b). HPLC profile of *N. meningitidis* polysaccharide eluted with 0.1 M sodium nitrate buffer on TSK 5000-4000 PWXL columns for serogroup A (c) and serogroup C (d), data recorded using RI detector.

The reaction time of about 2 h was quite less and moreover the concentration of NaOH and temperature combination used to achieve the de-*O*-acetylation did not affect the identity and chemical structure of the PS evident through its quality analyses. Though Michon and coworkers also describe the use of NaOH (with a range of 0.01 till 0.5 N) for de-*O*-acetylation but the time described is significantly high with an average of approximately 16 h [27]. Previous research findings suggest the use of anion exchange and size exclusion chromatography to purify the MenC polysaccharide [28]. The MenC-PS purification in this study was accomplished by utilizing hydrophobic interaction chromatography (HIC) as a technique. The studies presented in this paper are the first to show that the MenC-PS can be successfully purified using phenyl sepharose HIC. Overall, MenC-PS purification process including de-*O*-acetylation and HIC can be finished within 7 ± 1 h and the product qualifies as per the WHO-TRS specifications [7].

Despite all the advancements in the vaccine manufacturing technologies, the conjugate vaccines needed for the developing countries are emerging far more slowly than we could wish. One of the factors for the inequity is the high-priced polysaccharide production and the cost further inflates with the other steps of conjugation. Plausible experimental methodologies are urgently required to make the process (more) cost effective or rather affordable to the low cost vaccine manufacturers. The hallmark of the present study are the "rapid and scalable purification processes" for the isolation of purified bacterial PSs from two major serogroups (A and C) of *N. meningitidis*. In conclusion, the analytical results from this study validate the findings and provide a proof of concept for the rapid downstream purification of MenA and MenC polysaccharides. These processes are robust, affordable, and industrially scalable and can be accomplished with reduced time lines.

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