VAC.06 - Study of a Critical Process Parameter in the meningococcal C conjugate vaccine manufacturing developed by Bio-Manguinhos

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Introduction:

Neisseria meningitidis is one of the most important pathogens as causes of meningitis and other clinical manifestations worldwide. Bio-Manguinhos has developed all steps to produce, purify and control an effective Brazilian meningococcal C conjugate vaccine by modified reductive amination where oxidized polysaccharide (PSC) was coupled to hydrazide-activated monomeric tetanus toxoid used as carrier protein. Nowadays this vaccine is under Phase II/III clinical trials. The first step involves a mild oxidation of native PSC by sodium periodate, leading to terminal aldehyde moieties. Currenlty the reaction is carried out at a range for the oxidation reaction that allows a gain of flexibility in obtainging the oxidized polysaccharide. Therefore, several reaction times must be studied, considering the critical process parameters (CPPs), to avoid the obtention of a molecule with different physico-chemical characteristics from the one established and minimize the risks of loosing intermediate product.

Objective:

Determine the range of reaction time that allows oxidized polysaccharide obtention with similar critical quality attributes (CQAs) capable to be used in conjugation reaction.

Methodology:

In this study three oxidized PSC batches were produced, in pilot scale, using three different reaction times for quenching by glycerol addition. After purification using ultrafiltration, these batches were analyzed following the WHO recommendations as sialic acid content, SEC-HPLC profile, 1HNMR spectra for identity and qNMR for aldehyde moiety content, molecular weight by SEC-MALLS and residual glycerol content.

Results:

Oxidation reaction was very reproducible since all batches showed similar results in all quality control tests. Sialic acid content was 97.5 mg/mL, 97.67 mg/mL and 101.04 mg/mL for batches 1, 2 and 3, respectively. SEC-HPLC profiles were similar showing Kavacceptable (0.40-0.55) and residual glycerol was lower than 0.5 g/L according to stablished specifications for all batches. 1HNMR spectra showed typical assignments confirming the identity of PSC and the aldehyde moiety relative content (CHO) was around 8% as measured in the range of 5.0 a 5.3 ppm by qNMR. Finally the preliminary molecular weight data obtained by SEC-MALLS were =12.26 KDa, =10.19 KDa and =12.59 KDa for batches 1, 2 and 3, respectively, without significant differences (Mann Whitney test, $p \geq 0.05$). These results were comparable to a previous one when a batch produced and quenched with the estabilished reaction time was studied (=10.09 KDa).

Conclusion:

All results showed that oxidized PSC batches have similar physico-chemical properties and consequently similar CQAs. However, these oxidized PSC must be used to produce conjugates in order to evaluate if they will have appropriate polysaccharide-protein ratios and similar immunogenicity in mice as observed previously. This further improvement in this study is essential to assume a range of oxidation reaction time that could promote flexibility in the production process of meningococcal C conjugate vaccine.

Keywords: Meningococcal conjugate vaccines; Critical Process Parameters (CPPs); Critical Quality Attributes (CQAs)