

THE APPLICATION OF PHYSICO-CHEMICAL METHODS OF ANALYSIS TO THE DEVELOPMENT OF CONJUGATE VACCINES AGAINST MENINGOCOCCAL GROUP A BACTERIA



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1. INTRODUCTION

The meningococcal group A organism is responsible for 90% of the cases of endemic and epidemic meningitis caused by *Neisseria meningitidis* bacteria. Preventive immunisation should avoid a great number of deaths and be less expensive than mass immunisation campaigns performed after epidemics have begun. This is best achieved by vaccination with conjugate vaccines which, unlike polysaccharide vaccines, are immunogenic in the very young, induce immunological memory and are likely to give long-lasting protection. MVP (Meningitis Vaccine Project [1]) is developing an affordable monovalent meningococcal A conjugate vaccine for sub-Saharan Africa which is manufactured by Serum Institute of India Limited (SIIL) using **aldehyde-hydrazide condensation chemistry** (panel 2) developed at the US Center for Biologics Evaluation and Research [2]. Manufacturers and regulatory authorities have had decades of experience with meningococcal capsular polysaccharide vaccines, but the production and control of conjugate vaccines are more complex and vaccine specific. Successful development of the conjugate vaccine requires control of the production process from starting polysaccharide, to the formation of activated intermediates and their conjugation to yield the conjugate vaccine, as well as demonstration of manufacturing consistency. Since group A meningococcal conjugate vaccines are manufactured from purified components by a clearly defined chemical process, the strategy for the control of the vaccine relies heavily on determination of the molecular characterization and purity of each vaccine lot and intermediates [3]. Recent advances in bioanalytical methodology [4] permit the detailed structural characterization of conjugate vaccines and intermediates to be achieved by the use of physicochemical techniques, including **chromatography** (panel 3) and **nuclear magnetic resonance spectroscopy** (panel 4).

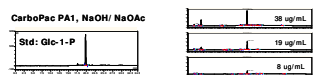
3. CHROMATOGRAPHY

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was performed on a Dionex DX-500 system fitted with a GP50 pump, ED50 detector and ASS0 autosampler, using a CarboPac PA1 column and a NaOH/Na acetate elution gradient. High performance size-exclusion chromatography (SEC-HPLC) was performed on a Waters instrument with ultra-violet (UV) or refractive index (RI) detection.

HPAEC-PAD: Mn A saccharide content

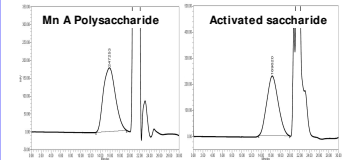
Colorimetric assays for P simple but poor selectivity and sensitivity-no int. std.

New method: Acid hydrolysis (TFA) and HPAEC-PAD analysis of Monomer 6-P-mannosamine [5]



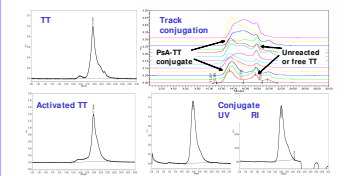
Applicable to Mn A PS, activated saccharide and Mn A-TT conjugate

SEC-HPLC-RI: Mn A saccharide size analysis



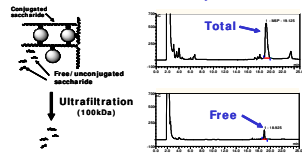
Size profiles of Mn A polysaccharide lots & activated saccharide
Track Mn A PS activation reaction

SEC-HPLC-UV/RI: TT and Mn A-TT conjugate



Size profiles of TT, activated TT and Mn A-TT conjugate
Track TT activation and conjugation reaction

Total and free saccharide by HPAEC-PAD



Conjugate bulk: ± 10% free saccharide after one year at 4°C
Integrity of Mn A-TT conjugate shown by free saccharide assay

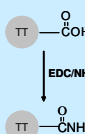
2. CONJUGATION PROCESS and CONTROL

Mn A polysaccharide

Identity -NMR
Molecular size -HPLC-RI
Moisture content
Composition (P content) -HPAEC
Protein impurity
Nucleic acid impurity
Endotoxin
O-acetyl content -NMR

Tetanus toxoid

Identity
Purity
Toxicity

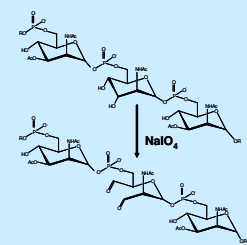


EDC/NH₂



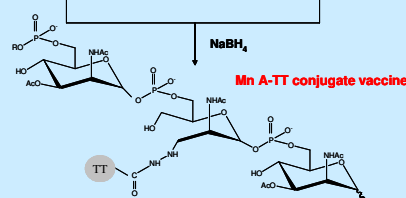
Activated TT

Number of functional groups
Molecular size distribution -HPLC-UV



Activated saccharide

Number of functional groups -NMR
Molecular size distribution -HPLC-RI



Mn A-TT conjugate vaccine

Residual reagents -NMR
Conjugation markers
Saccharide content -HPAEC
Conjugated v. free saccharide
Protein content
Saccharide protein ratio
Molecular size distribution -HPLC-UV/RI
Sterility
Specific toxicity of carrier

5. CONCLUSIONS

SEC-HPLC can be used to profile the size and heterogeneity of the polysaccharide, protein carrier, activated intermediates and conjugate vaccine, as well as to track the conjugation reaction.

The sensitive HPAEC-PAD technique can be used to quantify the saccharide throughout the process and to evaluate the integrity of the conjugate.

NMR spectroscopy provides a window into the structural integrity of the saccharide component (and O-acetylation) from polysaccharide to conjugate vaccine.

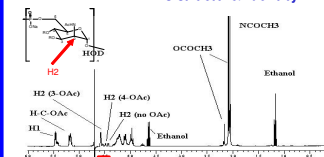
Fingerprinting by chromatography and NMR spectroscopy can be used to control the quality of conjugate vaccines and to demonstrate lot to lot manufacturing consistency

Phase I clinical trials completed (Poster P8.1.06)
Phase II in The Gambia and Mali (September 06)

4. SPECTROSCOPY

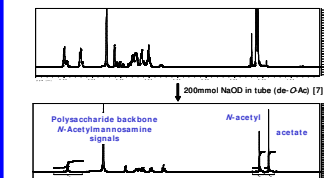
NMR spectra were acquired on a Varian Unity 400 (University of Cape Town) or 500 (NIBSC) NMR spectrometer. Samples of saccharide or conjugate (2-10mg) were freeze-dried and exchanged three times with D₂O prior to analysis, which was performed in D₂O at 300K. De-acetylated samples were prepared by addition of 200mM NaOD to the sample in the NMR tube.

¹H-NMR: Mn A PS structure/ Identity-1



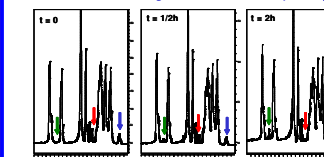
Characteristic proton profile (Identity) and residuals (ethanol)
Degree of acetylation from H1 and H2-C-OAc (H3 and H4) or H2+6 [6]
(~61.5%, calculated from > 2 mmol acetyl/g polysaccharide [7]).

¹H NMR: Mn A PS structure/ identity-2



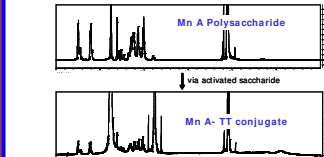
Identity of Mn A saccharide and O-acetylation -simplified

¹H NMR: Tracking the PS activation (NaIO₄)



Periodate oxidises the unacetylated residues
Follow loss of H2 (δ) and H3 (δ) signals & appearance of new H1 (δ).

¹H NMR: Tracking the conjugation process



Provides evidence for maintenance of the structural integrity of Mn A saccharide through the process

6. REFERENCES

[1] MVP is a partnership between WHO and PATH with the goal of eliminating epidemic meningitis (group A) from sub-Saharan Africa through the development, testing, licensure and widespread use of conjugate meningococcal vaccines (www.meningitis.org).
[2] C. E. Frasch, S. Kaper, S. Bari, D. M. Graybill, N. Bouveret, F. M. LaForce, P. L. C. H. R. Lee. A novel conjugation process for production of a highly immunogenic Group A meningococcal conjugate vaccine for use in Africa. Abstract, 14th International Pathogenic Neisseria Conference, Milwaukee, Wisconsin, USA, September 5-10, 2004. [3] Draft WHO Recommendations to assure the quality, safety and efficacy of group A meningococcal conjugate vaccines, to be presented to the Expert Committee on Biological Standardization (ECBS) in October, 2006. [4] Physico-Chemical Procedures for the Characterization of Vaccines, Dev. Biol. (Karger, 2000) F. Brown, M. Corbell, E. Griffiths Eds., vol. 103. [5] S. Roci, A. Bardotti, S. D'Acunzi, N. Ravenscroft, Development of a new method for the quantification of extracellular polysaccharide of *Neisseria meningitidis* serogroup A by high performance anion-exchange chromatography with pulsed amperometric detection. Vaccines, 2001, 19, 1989-1997. [6] Full ¹H NMR assignment and detailed O-acetylation patterns of capsular polysaccharides from *Neisseria meningitidis* used in vaccine production. X. Lemerle, C. Jones, Carbohydr. Res. 1995, 296, 93 - 96. [7] Use and validation of NMR assays for the identity and O-acetyl content of capsular polysaccharides from *Neisseria meningitidis* used in vaccine manufacture. C. Jones, X. Lemerle, J. Pharm. Biomed. Anal. 2002, 30, 1233 - 1247.

7. ACKNOWLEDGEMENTS

Financial support from the University of Cape Town, the National Research Foundation and the Ernest Oppenheimer Overseas Sabbatical Fellowship (for NR) is gratefully acknowledged. We thank friends and colleagues at the National Institute for Biological Standards and Control, UK (Draï on Feavers, Barbara Bolgiano and Chris Jones) for many valuable discussions and collaboration on the analysis of conjugates over the past few years. We also thank Xavier Lemerle (NIBSC) for recording some of the NMR spectra presented here.