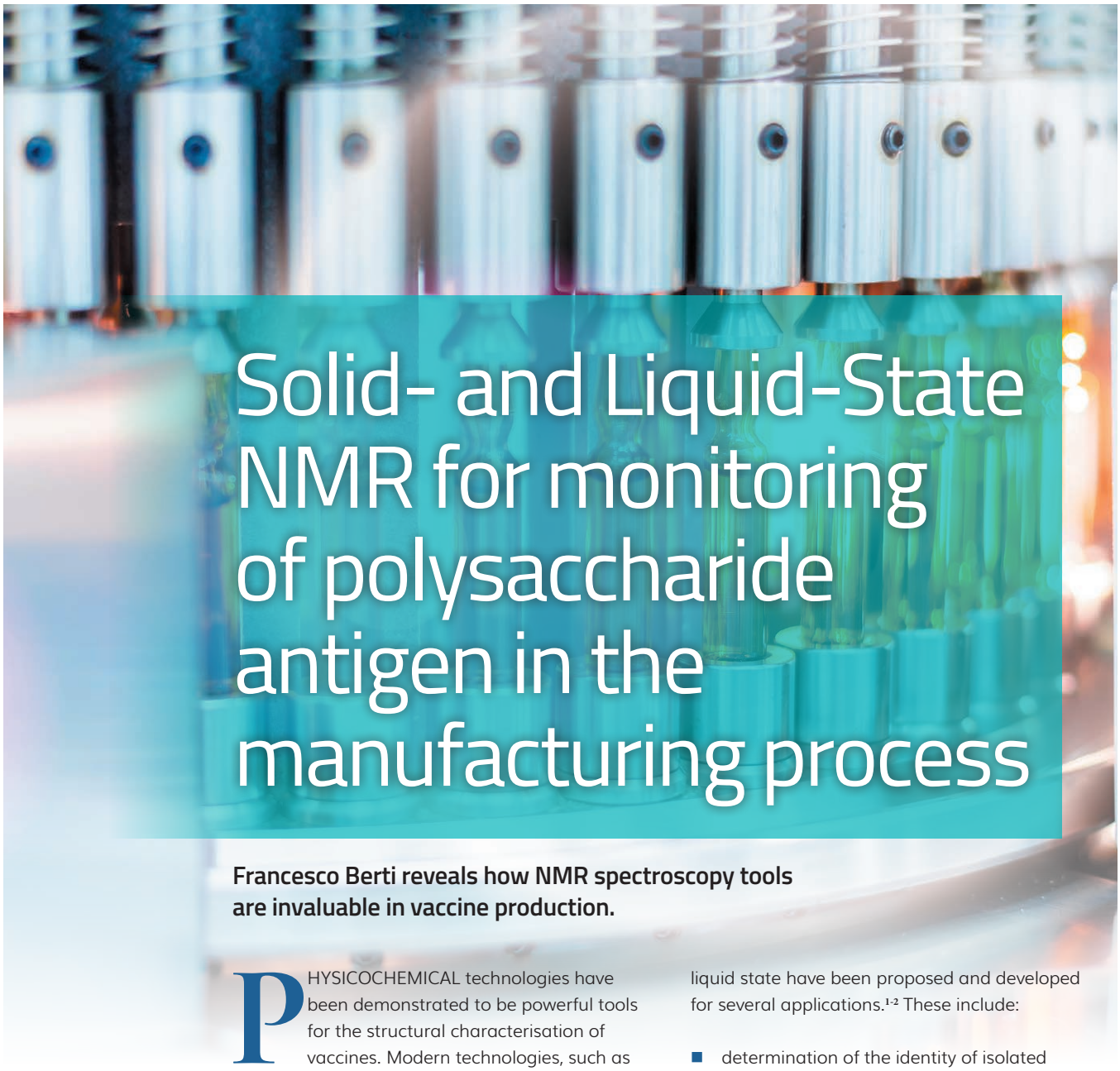


Solid- and Liquid-State NMR for monitoring of polysaccharide antigen in the manufacturing process

Francesco Berti reveals how NMR spectroscopy tools are invaluable in vaccine production.

"Modern technologies, such as Nuclear Magnetic Resonance (NMR) spectroscopy, are today crucial to vaccine characterisation"

PHYSICOCHEMICAL technologies have been demonstrated to be powerful tools for the structural characterisation of vaccines. Modern technologies, such as Nuclear Magnetic Resonance (NMR) spectroscopy, are today crucial to vaccine characterisation. High-field NMR spectroscopy has been found to be an extremely powerful and robust tool for tracking the industrial process manufacturing of carbohydrate-based vaccines. A combination of High Resolution (HR) solid- (Magic Angle Spinning, MAS) and liquid-state ^{1}H NMR has been used for the identity and structural testing of a bacterial polysaccharide antigen along the entire manufacturing process, starting from the bacterial cells up to the final formulation vaccine.

The need for characterisation of bacterial polysaccharide antigen along the entire manufacturing process

For analysis of polysaccharide-based vaccines, qualitative and quantitative HR NMR methods in liquid state have been proposed and developed for several applications.[1-2] These include:

- determination of the identity of isolated polysaccharides and their combination vaccines
- quantification of labile groups, which might be crucial for immunogenicity (eg, O-Acetyl content)
- identification of end groups as markers of depolymerisation of the carbohydrate chains
- polysaccharide identification and monitoring of the conjugation process to assess the consistency of the production process
- determination of polysaccharide-protein ratio
- quantification of NMR-sensitive residual process contaminants.

HR-MAS solid-state applications have also been developed for the deep characterisation of capsular polysaccharide produced by different *Neisseria meningitidis* serogroup A strains directly on whole cells.[3,4] This methodology

represents an *in vivo* determination of capsular polysaccharide expressed on the surface of bacterial cells. It confirms how HR-MAS NMR can distinguish between different detailed complex carbohydrate structures expressed on bacteria.

Comparison of ^{1}H NMR profiles collected in liquid-state has been used for confirming the identity and structural integrity (eg, O-Acetylation pattern) of *Neisseria meningitidis* serogroup A polysaccharide-protein conjugate antigen, in the manufacturing process intermediates, from the purified bacterial polysaccharide up to bulk conjugate (drug substance).[5]

A liquid-state NMR methodology has been applied to quadrivalent conjugate vaccine formulation (Menveo®, GSK Vaccines) containing the meningococcal serogroup A drug substance, in addition to three other conjugate antigens against serogroup C, W, Y.[6]

As shown in **Figure 1**, the manufacturing process for producing the CRM_{197} protein-meningococcal serogroup A conjugate, as one of the four components constituting the quadrivalent Menveo® vaccine, includes the following steps: »

Figure 1

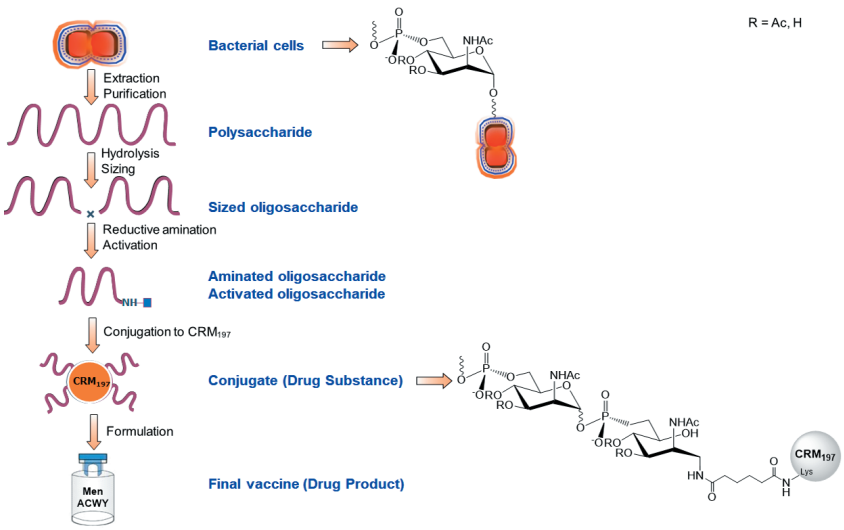
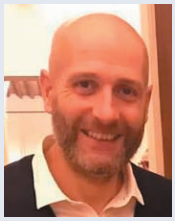


Illustration of the manufacturing process steps from bacterial growth to the final quadrivalent vaccine formulation, containing the relative CRM_{197} protein-polysaccharide conjugate. Figure adapted from scheme and chemical structures [N-Acetyl-Mannosamine-6-Phosphate partially O-Acetylated at position C_3 or C_4, →6)-α-D-Man*p*NAc(3/4OAc)-(1→OPO_3→, constituting the repeating unit of *Neisseria meningitidis* serogroup A capsular polysaccharide] published in [5, 6].

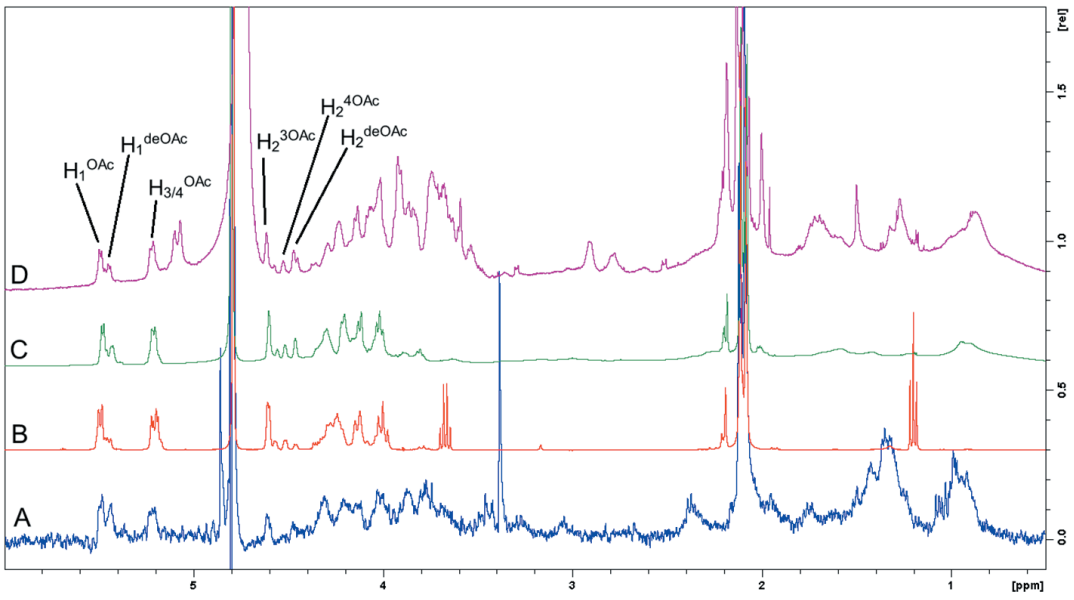
"NMR spectroscopy is a very powerful tool for control of identity and structural integrity of meningococcal serogroup A polysaccharide antigen along the entire production process of a quadrivalent vaccine"



Francesco Berti

Francesco is currently Scientific Director at GSK Vaccines – Siena, Italy. He is an expert, with 20 years of experience, in the R&D of vaccines containing carbohydrate- and protein-based vaccines (e.g. *Neisseria meningitidis, Streptococcus pneumoniae*, group B and A *Streptococcus, Staphylococcus aureus*). He has authored and co-authored over 95 published scientific papers and reviews, 4 book chapters and more than 20 patents. In 2017 he was awarded of Derek Horton Award in Industrial Carbohydrate Chemistry by the American Chemical Society. Disclosure: Francesco is an employee of GSK group of companies and an inventor of patents related to this topic. This work was undertaken at the request of and sponsored by GlaxoSmithKline Biologicals SA.

Figure 2



Comparison of ^{1}H 400 MHz NMR spectra collected at 25 ± 0.1°C in solid-state (A) bacterial cells strain and in liquid-state (B) purified polysaccharide, (C) protein-polysaccharide conjugate (drug substance), (D) quadrivalent vaccine (drug product). Some labels are reported for peaks assignment: H_1^{OAc}, proton at position C_1 of O-Acetylated unit; H_1^{deOAc}, proton at position C_1 of not O-Acetylated unit; $H_{3/4}^{OAc}$, protons at position C_3 and C_4 of O-Acetylated unit; H_2^{3OAc}, protons at position C_2 of 3-O-Acetylated unit; H_2^{4OAc}, protons at position C_2 of 4-O-Acetylated unit; H_2^{deOAc}, protons at position C_2 of not O-Acetylated unit of N-Acetyl-Mannosamine-6-Phosphate constituting the repeating unit of *Neisseria meningitidis* serogroup A capsular polysaccharide. Figure adapted from NMR profiles published in [4, 5, 6].

- bacterial growth to produce the polysaccharide antigen
- extraction and purification of the polysaccharide antigen
- size reduction by chemical hydrolysis
- chemical modification (reductive amination and activation) to insert the functionality for protein coupling
- conjugation to CRM_{197} protein
- vaccine formulation.

The tracking of identity and structural integrity of antigens along the entire manufacturing process is important for excluding unexpected modification which might impact the quality of the vaccines. In this case, for meningococcal serogroup A conjugate antigens, a combination of ^{1}H solid-state HR-MAS NMR (spectrum collected on bacterial cells) and ^{1}H liquid-state NMR (spectra collected on all the other intermediates) methodologies confirms the integrity of the polysaccharide antigen along the entire manufacturing process, starting from the bacterial growth up to final vaccine formulation. The ^{1}H HR-MAS NMR spectral pattern correlated with all the other ^{1}H NMR profiles (**Figure 2**). In addition, the quantification of both O-Acetyl groups as a labile group, crucial for immunogenicity, as well as NMR-sensitive residual process contaminants (eg, ethanol) is also feasible.

Conclusion

NMR spectroscopy is a very powerful tool for control of identity and structural integrity of meningococcal serogroup A polysaccharide antigen along the entire production process of a quadrivalent vaccine. A combination of ^{1}H solid-state HR-MAS and ^{1}H liquid-state NMR methods allows for a very rapid quality tracking, which might be implemented as a routine analytical tool for vaccine control.

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