Roundtable Session 2 – Table 17: NMR in Extended Characterization of Biologics

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Abstract:

Nuclear Magnetic Resonance (NMR) spectroscopy is an advanced analytical technique that exploits the magnetic properties of specific nuclei to gain information about their chemical environment. The atomic-level resolution provided by NMR provides a wide range of applications in a pharmaceutical environment, from establishing identity through evaluation of solution behaviors and purity analysis, and strongly compliments well established mass spectrometry technology. NMR spectroscopy has long been applied in small molecule development and is considered a necessary tool for organic synthesis and material quality control. The technology has an equally long history of successful application in epitope mapping and higher order structure determination in both academia and industry settings, and has been applied to larger systems such as protein complexes and nucleic acid constructs. Recent advances in both NMR technology and pulse sequence architecture have made use of NMR in a biopharmaceutical setting amenable in terms of both increasing the sensitivity of the NMR probes, thereby decreasing the requirement for isotopic enrichment, and in leveraging non-uniform sampling techniques to decrease the experimental acquisition times, enabling higher throughput workflows. The resulting NMR fingerprint spectra of GMP materials can effectively be compared during late-stage extended characterization efforts to demonstrate DS identity and secondary/tertiary structural homology and PTM content shifts between lots and processes.

Discussion Questions:

1. In what ways are NMR used in biopharma today? Are there phase appropriate deployments?
2. Is there any experience in including NMR data sets to justify process comparability or consistency? What kind of feedback from the regulatory agencies have been received?
3. Are there specific applications for low field vs high field NMR within biologic characterization? What are the pros and cons of each?
4. How critical is it to assign spectra of large macromoleucles? Can PCA and spectral similarity be used to compare datasets?
5. How is NMR used in assessing payload/linker and conjugated ADCs?

Notes:

Best practices for sample handling:

- Keep the buffer the same as much as possible

- Analyze samples into a common matrix to ensure the same background between samples? Always a concern if the sample has changed if using dialysis or buffer exchange.
- Regarding characterization of important regions, is the Fab more relevant? However, enzymatic cleavage of the Fc and Fab regions can alter fragment behavior compared to the antibody.

Best practices for data analysis:

- Is it helpful to map the location of signal to antibody based on the assignments for the IgG1 NIST mab reference material. Is it consistent with your protein?
- Complete assignments for biologics are rare. Hence, the emphasis on similarity (same place, same intensity).
- Discussion regarding when is something comparable or not comparable
- Mestronova software has good applications but could be more user friendly

What does NMR give that the other techniques do not?

- NMR signal is average of the system, but resolution is better than more commonly used methods (e.g., near UV-CD)
- Some experience with CD not sensitive below 5% spiked sample, which limits its usefulness.
- Higher Order Structure (HOS) characterization is the expectation.
- NMR adds confidence to data packages for comparability, but NMR spectra look more complicated than near UV-CD. Discussion ensued on making the interpretation of NMR data easier to understand (analogy to complicated peptide fragments from mass spectrometry).

What kind of NMR data go into regulatory documents?

- Companies are using 1D and 2D methods - should both be included?
- Example: 2D overlays of comparison spectra (showing no additional peaks, all the expected peaks present) as a qualitative comparison but not quantitiated.
- Some questions: how would differences be interpreted? NMR is sensitive to pH and formulation - is a tiny shift meaningful if it is not detected by another method like CD?
- Discussion on the value of the NMR fingerprints. How do you take the information and convey to regulators that the information contained is better than lower resolution methods?

What are the applications for NMR?

- Innovator companies don't seem to use NMR routinely for comparability. There are more examples of comparability for biosimilar molecules comparing to the innovator molecule.
- 1D NMR for surface of nanoparticles
- Payload of ADC molecules (experience at table indicates NMR analysis for the payload but not the conjugated antibody)
- Use for formulated DS lot analysis?

Where are the struggles for NMR that are holding us back?

- Access to instrumentation (on site vs borrowing from academic institutions)
- The case studies of why NMR is better than other options is unclear - what's the case to be made?
- Table experience highlights it's useful to solve problems that other techniques can't. Recommendation to use NMR to answer difficult questions and not to be hindered by initial challenges convincing management.

- Significant up front cost investment: 3-4 million USD for a couple of instruments. (CD is $100K and relatively straightforward to train an analyst)
- Challenges with technical expertise in NMR but not necessarily biologics. And vice versa.

What NMR field strength is suitable or necessary for industry settings?

- 600-800 MHz in use
- Perhaps better to invest in cryoprobes than going to a higher field strength?

References:

Methionine oxidation:

- Li, M., et al. "Comprehensive characterization of higher order structure changes in methionine oxidized monoclonal antibodies via NMR chemometric analysis and biophysical approaches." **https://doi.org/10.1080/19420862.2023.2292688**

Impurities in biologics by NMR:

- Skidmore, S., et al. "Quantitation and characterization of process impurities and extractables in protein-containing solutions using proton NMR as a general tool." **https://doi.org/10.1002/btpr.1620**

1D-NMR profiling of antibodies:

- Poppe, L., et al. "Profiling of formulated monoclonal antibodies by (1)H NMR spectroscopy" https://doi.org/10.1021/ac401867f

1D-NMR versus multi-dimensional NMR:

- Poppe, L., et al. On the Analytical Superiority of 1D NMR for Fingerprinting the Higher Order Structure of Protein Therapeutics Compared to Multidimensional NMR Methods." https://doi.org/10.1021/acs.analchem.5b00950

2D-NMR Fingerprinting by NMR:

- Hodgson, D.J, et al. "Assessment of the higher order structure of Humira, Remicade, Avastin, Rituxan, Herceptin, and Enbrel by 2D-NMR Fingerprinting." **https://doi.org/10.1016/j.jpba.2018.09.056**

Backbone NMR assignment of the Fab fragment of the NISTmAb reference antibody:

- Solomon, T. et al. "Backbone NMR assignment of the yeast produced Fab fragment of the NISTmAb reference antibody." **https://doi.org/10.1007/s12104-023-10123-9**