

Table 6: Best Practices in NMR Data Acquisition and Analysis

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Scope:

The higher-order structure (HOS) of protein therapeutics is currently characterized by methods with low to medium structural resolution, such as Fourier transform infrared (FTIR), circular dichroism (CD), intrinsic fluorescence spectroscopy (FLD), and differential scanning calorimetry (DSC). Several high-resolution nuclear magnetic resonance (NMR) methods now represent powerful approaches for HOS characterization.

This roundtable will discuss best practices in NMR data Acquisition and analysis.

Questions for Discussion:

1. What NMR methods are currently employed for Product Characterization and comparability/similarity assessments?
2. What analysis software is used for analysis?
3. Establishment of criteria to determine comparability/similarity are crucial to apply NMR to support regulatory filings. Is it possible to create universal criteria for the various NMR methods or will we need to generate specific criteria for each new product?
4. How extensively are NMR data used in regulatory filings for biopharmaceuticals?
5. What other modalities outside of protein therapeutics are attractive for NMR?

Discussion Notes:

Q: What kind of methods are your primary focus for protein characterization or comparability/similarity?

- One member stated that 1D methods are preferred, with PROFILE technique (Mats Wikstrom)
- PCA approach seems to be gaining traction (especially Profound method)
 - With original PROFILE, limitation - signal/noise gets pretty low - need to be at 60 s/n to get proper comparison
 - With PROFOUND, with PCA different components, first dimension is original spectra, second is like the PROFILE - can use the spectrum as is, can use original s/n in the 1D. Would recommend the PROFOUND technique
- General affinity for using 1D profile method due to simplicity and ease of data collection

Q: - What do people use for 2D work?

- 2D ¹³C Methyl is common
- Used with RNA therapeutics – specifically PMO oligomer - strong signals that come from dimethylamine group - used SIERRA filter to knock signal down so it doesn't interfere with other signals - good for removing excipient signals as well
- For IND filing, need to show that the product retains structural conformity - 2D ¹³C methyl very strong tool for that application – can use ¹³C 2D and 1D H w/ profile
- ¹H-¹³C useful to structural integrity
- 2D ¹³C spectra not just for methyl, also aromatic carbons
- Typical 4 hours for data collection of 2D ¹³C experiment (SOFAST/ALSOFAST)
- 2D ¹⁵N - not commonly used for mAbs, too long experiment, low natural abundance of ¹⁵N
- INEPT sequence for useful for HN correlations - s/n was really bad - on monoclonals

Q: Multiple questions about isotopic labelling of mAbs

- Not generally done. Some tried, but focus is on real products, and with so many products in parallel, very difficult to produce materials in timely fashion
- Did in a couple of cases for specific information about certain target/epitope mapping, etc. Not routine
- Note that there aren't many if any publications for labelled mAbs - Merck may be working on this?

Q: Use of NMR in nucleic acid systems?

- siRNAs - leveraging fluorines - 2'fluoro - none of the bio buffers have fluorine, so no background in the spectra.
- If you detect on F for 1D instead of proton, get rid of all background proton signals
- Phosphorous measurements H/P, or 1D P experiment also worked well. P is less resolved in a nucleic acid, but some have phosphothioates, resonate in their own chemical shift region.
- If you have only a few, can be well resolved. Phosphothioates also have stereoisomers that can be resolved
- Q: how big are the siRNAs? 40mer, some are 10-15mer, but mostly small relative to mRNA vaccine. Antisense 17mer in queue

- Any challenges for overlapped signals in nucleic acids?
 - Anything in the aliphatic region, everything is piled on top of each other. W/ chemical modifications, it helps disperse the resonances well

- Sofast HMQC on RNAs, get signals, a day or so to run. Get artifacts with chemometric analysis - natural abundance NH HSQC, looks bad, reports of four days to acquire

Q: Types of methods used for establishing comparability?

- Echos – issue - only looks at intensity
- CCSD - Had some issues, can become a huge time sink, getting assignments is a problem
- 2D - PCA makes most sense, requires sufficient replicate lots

Q: How do you define similarity or difference?

- Is it possible to establish universal qualification criteria for NMR, or does it have to be established on a program basis?
 - If you can correlate your chemometric data to potency, perhaps
 - How do you get enough data to set criteria without having the criteria be too tight due to sampling bias?
 - NMR is such a fine method, can see lots of differences, how to tell what's a significant difference or not, need to correlate with impact to binding/immune response/PK
 - 1D proton and 2D methyl doesn't provide site specific information, generally - it's not trivial to get that site specific information...
 - Biosimilars as a guide? Filgrastim CCSD ranges - Williamson et al -
 - Spectral similarity scoring in general – reference to insulin biosimilars using m-distances - anything below 3.3 is considered similar/comparable

Q: What kind of software - Topspin, Sparky, NMRPipe, Mnova, NMRFX, JMP?

- Most seemed to use Mnova for analysis, some interest in leveraging JMP
- Some discussion about software compliance and phase appropriate NMR method deployment – Is it necessary for the software to be compliant if the method will be used for extended characterization?

Q: Anyone use Chemical shift prediction?

- Some have tried, specifically with ShiftX2, not very good outcomes, the predictions are a little variable
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C: Don't underestimate the usefulness of the proton 1D for impurities and evaluation of complex formulations

- Confirmed it's often used in vaccine development