March 2024 NMR Topic of the Month: ACS NMR

What is an acceptable ^{1}H NMR for an ACS journal?

According to the *NMR Guidelines for ACS Journals*, which is available on the ACS' *Manuscript Publication and Submission* website, and the specific guidelines from individual journals, the common spectroscopic requirements are:

1. The largest peak in the ^{1}H NMR spectrum should normally arise from the compound, not the solvent. (Rule 2.3)
2. All peaks should be visible on the spectrum… At minimum, the spectral window should be -1 ppm to 9 ppm for ^{1}H NMR… (Rule 2.6)
3. A copy of a spectrum with a signal-to-noise ratio sufficient to permit seeing peaks with 5% of the intensity of the strongest peak should be included in the supporting information. (Rule from *J. Org. Chem.* and similar)

There are many other formatting and labeling rules, but these are the spectroscopic rules.

What does the first guideline mean for sample concentration?

Assuming all the signals relax the same and that the sample is in 99.96% chloroform-d (CIL standard).

$$1.5 g/mL \times \frac{mol}{120.384 g} \times \frac{1000 mL}{L} \times \frac{1000 mM}{M} \times 0.04\% = 5.0 mM$$

In other words, your target's largest signal must represent a proton concentration of at least $5.0 mM$. To properly calculate this requires you to know the target's concentration, the formulaic number of protons, and the signal's splitting pattern. If you do not have a suitable proton concentration no amount of acquisition time will ever produce an acceptable spectrum.

This $5.0 mM$ threshold really is a minimum for samples in standard NMR grade chloroform-d because the first assumption about the relaxation is rarely ever true. Chloroform relaxes at a very slow rate, so its peak will be artificially tall/narrow in most spectra. A better real-world minimum spin concentration for the target's tallest peak would be $10.0 mM$. See our Training Center - Documentation - Techniques - Dilution Series: Concentration Effects poster for a real spectrum example.

How about a concrete concentration example?

NMR system sensitivity checks are run on a 0.1% ethylbenzene in chloroform-d sample using the methylene quartet. The ethylbenzene molar concentration of that sample is:

$$0.866 \frac{g\ EtBz}{mL\ EtBz} \times \left(\frac{mol\ EtBz}{106.167 g\ EtBz} \times \frac{1000 mmol\ EtBz}{mol\ EtBz} \right) \times \left(\frac{1000 mL\ EtBz}{L\ EtBz} \times 0.1\% \frac{L\ EtBz}{L\ of\ sample} \right) = 8.16 mM\ EtBz$$

The strongest signal from ethylbenzene comes from the methyl group because it has three protons contributing to it (spin concentration of $24.48 mM = 8.16 mM \times 3$), which is spread out between three peaks in a ratio of 1:2:1 due to the splitting by the J-coupling to the methylene protons. Therefore, the tallest peak of the methyl triplet represents $12.24 mM = 24.48 mM \times 2/(1 + 2 + 1)$ of spin concentration, which is plenty.

The methylene signal from ethylbenzene has two protons contributing to it (spin concentration of $16.32 mM = 8.16 mM \times 2$), which is spread out between four peaks in a ratio of 1:3:3:1 due to the splitting by the J-coupling to the methyl protons. Therefore, the tallest peaks of the methylene quartet each represent $6.12 mM = 16.32 mM \times 3/(1 + 3 + 3 + 1)$ of spin concentration, which still should show nicely.

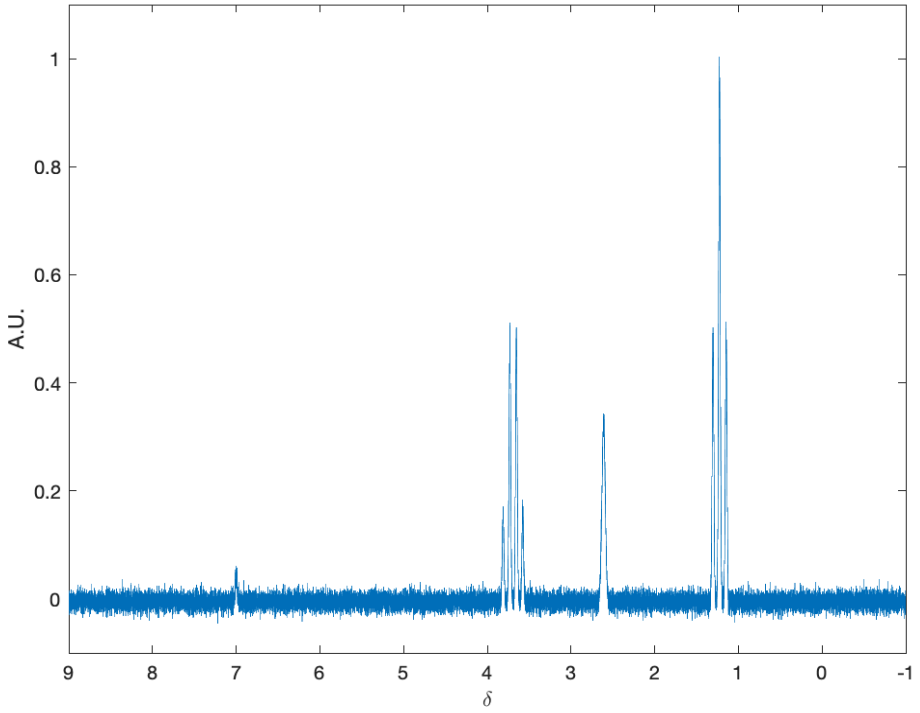
That same sample also contains 0.01% tetramethylsilane, which is to say TMS has a molar concentration of:

$$0.648 \frac{g\ TMS}{mL\ TMS} \times \left(\frac{mol\ TMS}{88.22 g\ TMS} \times \frac{1000 mmol\ TMS}{mol\ TMS} \right) \times \left(\frac{1000 mL\ TMS}{L\ TMS} \times 0.01\% \frac{L\ TMS}{L\ of\ sample} \right) = 0.735 mM\ TMS$$

But the ^{1}H methyl spin concentration is twelve times that of the TMS (or $8.81 mM = 0.735 mM \times 12$), and concentrated in (essentially) a singlet. So it is also prominent in the spectrum.

What does a minimally acceptable ^{1}H spectrum look like?

Below is a (mock) spectrum of ethanol with an identifiable 5% contaminate at 7 ppm. No, the baseline isn't smooth as glass, but there's no need to acquire more.



In case you're wondering, the picture above has a signal-to-noise ratio of 40:1 using the highest methyl peak as the signal. Modern NMR systems usually have signal-to-noise ratio benchmarks that are greater than ten times this for the methylene peak in the ethylbenzene sample above - using a single transient/scan. In other words, a proper sample should have no trouble meeting all three ACS guidelines in a short amount of acquisition time.

What's the point?

There are two main takeaways.

1. Too many people are running samples that are too dilute. Occasionally, there may be issues that mandate such low concentrations, but whenever possible you should produce a proper sample. The amount of target you need in the sample is calculable, from there you should know your reaction/purification yields well enough to determine appropriate amounts of starting materials.
2. Too many people are running samples for too long, presumably because they like very smooth baselines. This really needs to stop, it's wasteful and asinine. Especially for spectra that will not be included in a paper/patent, such acquisitions are a waste of instrument time and research funds. Remember, signal-to-noise improves as the square root of the number of scans, so you reach a point of diminishing returns very quickly. Besides, as detailed above, even publication quality spectra are not required to have immaculate baselines.

References

1. The American Chemical Society (www.acs.org).
2. Cambridge Isotope Labs (www.isotope.com).
3. Details regarding the ^{1}H sensitivity test can be found in the TopSpin Basic NMR Experiments User Manual, Version 005, Section 10.5, p.104-106, Bruker Corporation 2023.
4. Concentration Effects and Sensitivity Options posters on our website and in the labs (https://nmr.tamu.edu/Documentation.php).
5. November 2021's Topic of the Month: Signal-to-Noise on our website (https://nmr.tamu.edu/Tidbits.php).