Sensitivity in NMR

cg fry: last updated 26 Oct 2009

Sensitivity is a hallmark problem for NMR. It is apparent, however, that many users of the facility are unaware of the progress made in this area over the last five years.

50 mg of material is *not* required for ^{13}C spectra; 10 mg can be easily observed < 1 mg is sufficient (1 μmol / 5 mM)
1 mg is *much* more than required for ^{1}H acquisitions; 0.1 mg can be easily observed < 15 μg is often enough (30 nmol / 0.05 mM).

This note will summarize the current limits of detection in NMR, and provide estimates of how much material is required to obtain data within the constraints of the particular equipment available in our facility.

The fundamental relationships involved in estimating the amount of material required for an experiment are expressed starting with the signal-to-noise ratio, *S/N*:

$$S/N \propto n\gamma_e \sqrt{\gamma_d^3 B_o^3 t} \qquad (1)$$

where n is the number of nuclear spins being observed, γ_e is the gyromagnetic ratio of the spin being excited, γ_d is the gyromagnetic ratio of the spin being detected, B_o is the magnetic field strength, and t is the experiment acquisition time. Other factors that are involved in *S/N* are the probe filling factor (e.g., the fraction of the coil detection volume filled with sample), and various other probe and receiver factors that are typically approximately equivalent for equipment built in the same period of time.

It is obvious to users that the highest field instrument available provides the best sensitivity. For fixed t, we would need 4× as much material with our 300s as on NMRFAM's 750 to obtain spectra with identical *S/N*: $\frac{N_{300}}{N_{750}} = \left(\frac{750}{300}\right)^{3/2} = 4$. So for very limited sample amounts, it usually (but not always! see below) is best to go to the highest field available.

What is usually less apparent, however, is that the probe availability and tube configuration can have even larger effects on the amount of material required than the size of the magnet. For limited sample amounts, users should always use the smallest diameter probe available. A 3mm probe will offer ~50% improvement in *S/N* for identical amounts of sample over a 5mm probe.[1] Thus, the combination of a 500 with a 3mm probe has nearly the same *S/N* for identical sample amount as a 750 with a 5mm probe.

The advent of susceptibility plugs[2] from Shegimi brings an immediate 2.5 to 3× decrease in the amount of sample required for any NMR experiment. To obtain usable line shapes in NMR, the solvent volume normally must extend the length of the coil above and below the coil: if the coil is 16mm in length, the total solvent length should be 48mm. Susceptibility plugs allow the solvent volume to be limited to just the rf coil length.

Now the combination of a 500 with a 3mm probe and susceptibility plugs is 10× improved over a 300 with a standard 5mm tube. It turns out our 500s are even better than this factor of 10; the Varian equipment is generationally improved over the Bruker ACs. The INOVA-500 can provide reasonable ^{1}H spectra of 60 nmol of material in a few hours acquisition time.

Last, but certainly not least, the acquisition of cryogenically-cooled probes by NMRFAM (the NMR facility in Biochemistry) has significantly decreased sample amount requirements for ^{1}H and ^{13}C NMR. Thermal noise generation in the probe and the first-stage receiver electronics dominates noise in NMR experiments. These new probes have built-in first-stage receivers and rf coils that are cryogenically cooled (~20K), and have *S/N* improvements of 3 to 4× standard probes (decreasing to ~2× improvements for buffered samples). There is a new direct-X—direct ^{13}C and ^{15}N detect—cryogenically cooled probe available at 500 MHz at NMRFAM (as of Oct 2009); see below for more. NMRFAM has ^{1}H probes at 500, 600 750, 800 and 900 MHz.

A summary of sample amount requirements for ^{1}H detection on equipment available on the UW–Madison campus is provided in the following table; these numbers are quite conservative. The longer experiment times available on the higher field instruments are factored into the table.

Instrument	**Sample/probe configuration**	**Solvent volume**	**Min sample amount: ^{1}H 1d**	**Min sample wgt MW=500: ^{1}H 1d**
AC-300	5 mm standard tube	500 µl	2000 nmol	1 mg
Unity-500	5 mm + susc plugs	170 µl	200 nmol	100 µg
Inova-500	3 mm + susc plugs	60 µl	60 nmol	30 µg
Avance-500 (Biochem)	5 mm cryoprobe + susc plugs (no salt)	170 µl	50 nmol	25 µg
Chem-600	3 mm + susc plugs	60 µl	50 nmol	25 µg
Inova-500	nanoprobe	50 µl	30 nmol	15 µg
Avance-750 (Biochem)	2.5mm + susc plugs	45 µl	30 nmol	15 µg
600 MHz (Biochem)	5 mm cryoprobe + susc plugs (no salt)	170 µl	25 nmol	12.5 µg
800 MHz (Biochem)[a]	5 mm cryoprobe + susc plugs (no salt)	170 µl	16 nmol	8 µg
800 MHz (Biochem)[a]	5 mm cryoprobe + susc plugs (w salt)	170 µl	25 nmol	12.5 µg

[a]The 900 is fairly similar in S/N to the 800; its primary utility is providing maximum dispersion.

Estimates for ^{13}C direct observe experiments can be made in a similar fashion: on our 500 MHz spectrometers, ~ 2 μmol of material can be observed in a few hours.

Only 5mm probes are available for these experiments, but susceptibility plugs provide the same factor of 2.5 decrease in sample amount needed. This reduction is factored in for the 2 μmol number.

Making use of ^{1}H for excitation and detection in a ^{1}H–^{13}C correlation experiment (e.g., HSQC), S/N can be improved by $4^{5/2} = 32$. The 3mm probe can be used for these experiments, and ~40 nmol of material would be needed. Quaternary carbons can be detected in a similar manner, but this experiment (gHMBC) is less efficient: ~200 nmol of material would be required. These values are conservative, so less material may be feasible. Inverse heterocorrelation experiments become increasingly valuable as γ decreases: for ^{15}N at natural abundance, and for most nuclei having smaller γ values, inverse excitation and detection via ^{1}H or ^{31}P become a requirement for performing a successful experiment.

Optimization of the rf coil to specialized sample conditions (usually requiring that the probe be removed from the magnet for sample changing; but Pharmacy's microflow probe is an interesting exception) can lead to large improvements in sensitivity. Our nanoprobe is capable of obtaining similar sensitivity for limited sample amounts as optimal conditions on the 750. ^{1}H data has been obtained on nanoprobes with just 400 ng of sucrose, but on a "routine" basis ^{1}H 1d data can be obtained on 25 μg with just a few minutes acquisition time.

The current state-of-the-art in detection is being performed at U. IL at Urbana using microcoil probes.[3] ^{1}H NMR can be done with pmol (ng) quantities of common organic compounds.[4] One of these probes is available at the Pharmacy NMR facility at the UW–Madison. Direct observe ^{13}C and 2D ^{1}H-^{13}C experiments can be done with ~50 nmol (~20μg) of material at natural abundance.[5]

NMRFAM has upgraded one of the Bruker AVANCE-500 spectrometers with a direct–^{13}C, ^{15}N observe cryoprobe. Sensitivities for both nuclei are ca. 6× better than for our probes. See Charlie for more information about this new capability available to campus researchers.

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[1] One might expect larger improvements based on reduction in solvent volume, but filling factors reduce with probe diameter.

[2] For more information on susceptibility plugs and vendors, see
http://cic.chem.wisc.edu/nmr/Guides/Other/solvent_tube_suscplug.pdf

[3] Olson DL, Peck TL, Webb AG, Magin RL, Sweedler JV, "High-resolution microcoil H-1-NMR for mass-limited, nanoliter-volume samples," Science **270**(5244), 1967-1970 (1995).

[4] Behnia B, Webb AG, "Limited-sample NMR using solenoidal microcoils perfluoucarbon plugs, and capillary spinning," Anal. Chem. **70**(24), 5326-5331 (1998). Subramanian R, Lam MM, Webb AG, "Rf microcoil design for practical NMR of mass-limited samples," JMR **133**(1), 227-231 (1998).

[5] Subramanian R, Webb AG, "Design of solenoidal microcoils for high-resolution C-13 NMR spectroscopy," Anal. Chem. **70**(13), 2454-2458 (1998). Raju Subramanian, Jonathan V. Sweedler, and Andrew G. Webb, "Rapid Two-Dimensional Inverse Detected Heteronuclear Correlation Experiments with <100 nmol Samples with Solenoidal Microcoil NMR Probes," J. Am. Chem. Soc., 121 (10), 2333 -2334, 1999.