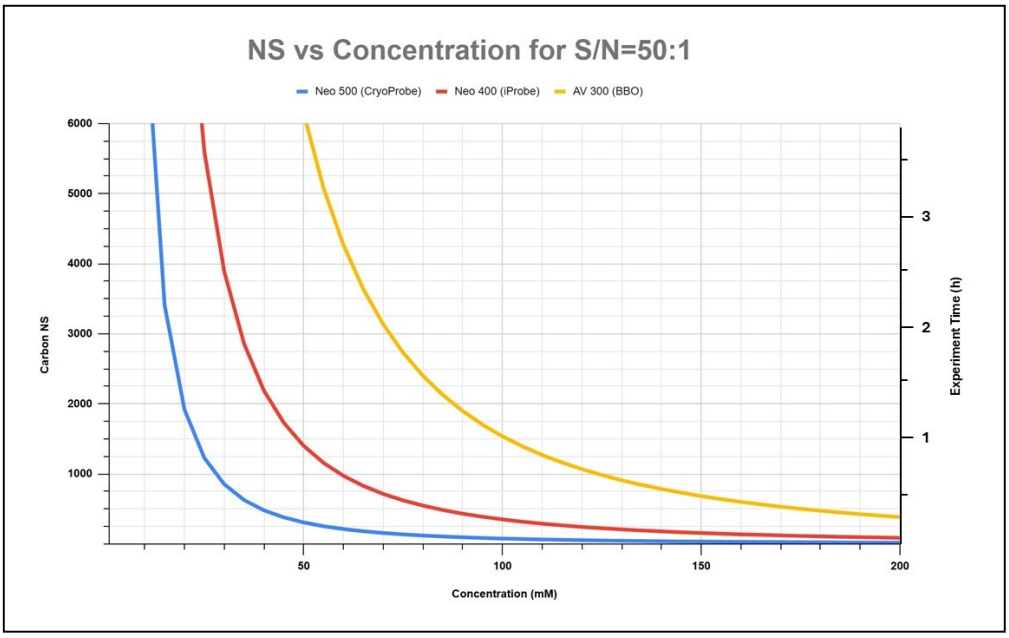
Dr. Mike Lumsden
Coordinator, NMR3
September 25, 2025

"How Much Sample Do I Need for ^{13}C NMR?"

Based solely on magnetic moment considerations, the ^{13}C nucleus is ~60x less receptive to NMR spectroscopy relative to the ^{1}H nucleus. However, upon factoring in the low ^{13}C natural abundance (1.1%), the receptivity drops to ~5800x less. Despite this sensitivity challenge, ^{13}C NMR data remains an important component in the characterization of new synthetic targets. Consequently, researchers must become familiar with sample amounts and time requirements for successful ^{13}C NMR spectroscopy. This inevitably leads to the popular question surely asked of every NMR facility manager multiple times – "How much sample do I need for ^{13}C NMR?". A variation of this question shifts the focus to the time requirement and goes something like - "How many scans do I need for ^{13}C NMR?". These questions are important ones but unfortunately cannot be answered as presented. There are parallels with being asked – "How long is a piece of string?" - as an answer can't be provided without additional information. To address the NMR question, details such as the spectrometer and probe choice, amount of sample available, minimum acceptable S/N, maximum acceptable experiment duration, and the compound molar mass are needed (moles matter, not mass). This is not an exhaustive list. Typically, it has been my experience that at least some of the necessary details are missing for a complete answer, and so NMR Managers will necessarily resort to providing "rules of thumb". Historically, I have responded with the suggestion that "25-30 mg of pure compound" should be a comfortable amount along with encouraging the researcher to use our 500 MHz instrument equipped with a cryoprobe. This was never a satisfying response in my mind but seemed the best I could do with the information available.

Recently, I stumbled upon a blog post by Brendan Duggan, an NMR manager at the University of California San Diego, where he attempts to address this question more completely. His approach was to recognize that the S/N from a routine ^{13}C experiment can be expressed as $\mathbf{S/N = k \cdot C \cdot \sqrt{NS}}$, where C is the sample concentration, NS is the number of scans, and k is a scalar which combines all the many other variables that contribute to the sensitivity. However, for a given field and probe combination and for a given set of acquisition parameters, k can be measured from the S/N obtained from a sample of known concentration. With a value for k available, one can at least estimate ^{13}C S/N as a function of sample amount and/or time investment with some confidence. Proceeding in this fashion, I used the readily available ^{31}P sensitivity standard sample (48.5 mM triphenylphosphine in acetone-d_6) and acquired ^{13}C NMR spectra on each facility spectrometer using default acquisition parameters (NS = 128, DS=8, AQ=1.75 s, D1=0.5 s, total time = 5 min 14 s).



S/N was measured using the *para*-aromatic carbon signal from which a determination of k was made on each of the three instruments (remaining mindful that [^{13}C nuclei] contributing to this signal is 3 x 48.5 mM). Shown in the figure above are plots of required NS as a function of sample concentration to achieve a 50:1 S/N on the three facility instruments. These curves are of a $y = 1/x^2$ format; as concentrations tends towards zero, NS tends towards infinity. From this exercise, we glean the following three important facts:

1. $S/N_{500} = (57.1) \times C \times \sqrt{NS}$, where C is concentration in units of moles L^{-1}. Alternatively, $S/N_{500} = (9.52 \times 10^4) \times n \times \sqrt{NS}$, where *n* is the moles of solute in 600 µL of solvent.

2. The ^{13}C S/N on the 500 is 2.14x larger than the Neo-400 with iProbe (note that one would expect only a 40% increase due solely to the increase in field strength).
3. The ^{13}C S/N on the 500 is 4.48x larger than the AV-300 with BBO probe.

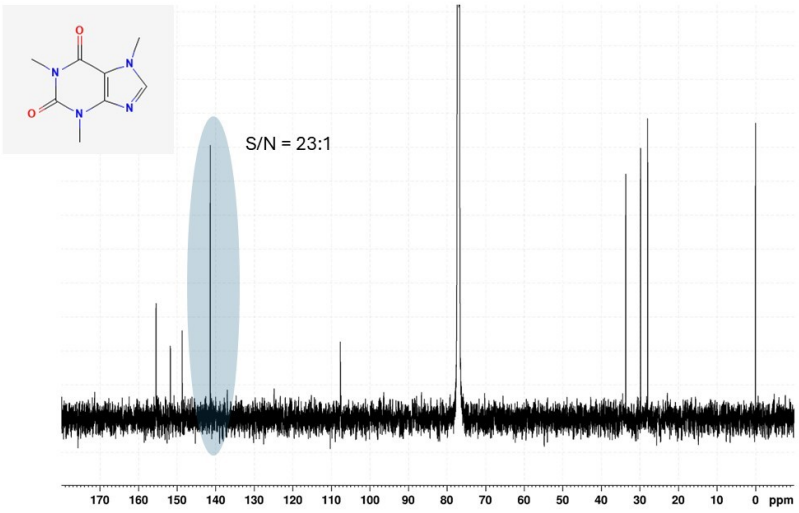
We are now positioned to address the title question in a more thorough manner. To proceed, I will assume a target S/N of 50:1 and that the goal is to obtain a daytime ^{13}C in automation (20-min maximum duration) on the 500 using the cryoprobe. Under these assumptions, we predict the following:

To obtain a ^{13}C Spectrum with S/N 50:1 on the 500 in 20-minutes, prepare a 38 mM sample.

Note that 38 mM corresponds to 23 µmol in 600 µL. From this single result and knowledge of the relative spectrometer sensitivities, we are equipped to assess other sample quantities, acceptable time investments, or S/N goals on any of the three spectrometers within NMR[3]. To be clear, the goal here is NOT meant to accurately predict experimental S/N values but rather to provide useful estimates. Variations in T_1, T_2 broadening, nOe enhancements, J-coupling to heteronuclei, and molecular symmetry are just some of the factors that make this approach semi-quantitative at best.

Example 1

I have a pure sample of caffeine with a mass of 1.25 mg which I use to prepare a 10.7 mM sample in $CDCl_3$ and need a ^{13}C spectrum. Our sample quantity is fixed so our variables are time and target S/N. I am sample limited so will aim to use the 500 MHz instrument. Using the above findings, we can predict the following:



- Our caffeine sample is 38/10.7 or 3.5x less concentrated than the reference. Thus, a 20-min experiment should yield a S/N ~ 14:1. Is this enough sensitivity?
- If 14:1 is deemed inadequate, we can predict that for a 50:1 S/N, we will need to increase the experiment length beyond 20-min by $(50/14)^2$ meaning ~4-h of NMR time.
- If achieving 50:1 is too costly in time and 14:1 is inadequate, perhaps 25:1 is an acceptable compromise? We can estimate that a S/N of 25:1 will require $(25/14)^2$ x 20 min or ~ 1-h of 500 MHz NMR time.

The spectrum above was obtained on a 10.7 mM caffeine sample in 1-h (NS = 1680) on the 500. Our semi-quantitative approach has performed admirably; all protonated ^{13}C-nuclei appear within a S/N range of 20 - 25:1! Due to longer T_1 values, non-protonated ^{13}C nuclei have much smaller k values (observed S/N reductions of ~ 60-75% in caffeine).

Example 2

I have prepared 15 mg of a new compound which mass spec shows to have MW=275 g mol^{-1}. I need a ^{13}C spectrum with 30:1 S/N but want to use as little material as possible as it is only soluble in DMSO-d6. I plan to use the 500 and our very kind NMR facility manager has approved a one-time overnight reservation of 12-h. How much material should I use to prepare my NMR sample?

- Since we have loosened our S/N requirements to 30:1, we prepare a 38 mM x 3/5 = 23 mM sample for a 20-min carbon experiment.
- Running the experiment for 12-h is a 36x increase in length which, for an equivalent amount of sample, is a 6x increase in S/N. Thus, we can reduce our sample concentration 6x leading to a 3.8 mM sample requirement.
- Using 600 µL of DMSO-d6, we need to dissolve 2.3 µmol of material. For MW=275, this corresponds to a mass of only 625 µg of material!