Nuclear Magnetic Resonance Laboratory course
Assignment 3

The Signal-to-noise ratio and Quantitative NMR

Dr. rer. nat. habil. Thomas Meier, HPSTAR

September 12, 2024

Abstract

Signal intensities in Nuclear Magnetic Resonance (NMR) spectroscopy are highly sensitive observables that correlate directly with the absolute concentrations of species in a sample, making NMR an invaluable technique for quantitative studies in both chemistry and biology. The ability of NMR to provide quantitative information with high precision and specificity makes it a powerful tool for analyzing complex mixtures, determining reaction yields, and assessing molecular dynamics or metabolic pathways. However, achieving accurate quantitative results in NMR, often referred to as Quantitative NMR (QNMR), presents several intrinsic challenges. These challenges arise primarily due to the necessity of maintaining strict control over a wide array of experimental parameters, including pulse calibration, relaxation times, receiver gain, temperature stability, and sample homogeneity.

Quantitative NMR demands precise calibration of RF pulses to ensure uniform excitation across the entire sample, as well as accurate determination of the relaxation properties (such as T1 and T2 relaxation times) of the nuclei under investigation. These relaxation times must be accounted for because incomplete relaxation can lead to signal attenuation, distorting the apparent concentrations of species. Furthermore, external conditions such as temperature and magnetic field homogeneity must be carefully regulated to minimize variations in signal response, which could otherwise result in inaccurate quantification.

This series of experiments is designed to provide a comprehensive introduction to the fundamental principles of Quantitative NMR. Throughout the course of these experiments, you will develop a deeper understanding of the practical considerations that are essential for obtaining reliable and reproducible quantitative data. You will also acquire the necessary skill set to perform QNMR experiments successfully, enabling you to apply these techniques to a variety of sy

1 Theoretical background

For these experiments, you will need the following theoretical background. Please prepare necessary mathematical expressions and derivations:

- The intensity of an NMR signal is either given by the integral over the spectral line in frequency domain or by the zero-time intensity of the free induction decay in time domain. Explain why and under which circumstances both approaches are equivalent.
- The Signal-to-noise ratio after a single scan after a 90 degree excitation pulse generated in a solenoidal coil is given by:

$$SNR = \frac{\eta N_{coil} A_{coil}}{\sqrt{4 k_B T R_{coil}}} \cdot \frac{\omega_0}{\sqrt{\Delta f}} \cdot \frac{N}{V} \cdot \frac{\mu_0 \gamma_n^2 \hbar^2 B_0}{3 k_B T} \cdot I(I+1) \qquad (1)$$

  with all parameters and constants defined similar to *Meier 2016*. Calculate the ^1H SNR achievable in a coil (5 turns, 3 mm diameter, 0.5 mm copper wire) using a glass vial of water as a sample (coil's filling factor is about 75 %). The magnetic field shall be 9.4 T. Assume room temperature.

- Rationalise why the signal to noise ratio is proportional to $\sqrt{N_{scans}}$ rather than N_{scans}. N_{scans} being the number of accumulations in an NMR experiment.
- describe three ways to improve SNR
- Judging from eq. 1 one would assume that quadrupolar nuclei (e.g. ^{27}Al) would result in more intense signals than spin-1/2 nuclei like ^{1}H. Why is this not the case in general?
- In order to estimate the number density N/V of a certain nucleus or a specific molecular subunit in a sample, one needs to carefully estimate SNR for an internal reference of known concentration and correlate it with acquired signal intensity of the unknown nucleus while keeping experimental parameters unchanged. Derive an expression correlating the number density of nucleus A, $(N/V)_A$ to the number density of nucleus B, $(N/V)_B$.

2 Tasks

Please work on the tasks step-by-step and summarize your observations thoroughly and logically when you hand in the assignment. Please provide data plots and calculations to underline your conclusions. We recommend the use of ONMR running in Origin7 or later, for data analysis.

1. Prepare the sample: Synthesise or obtain a high-purity sample of lanthanum trihydride (LaH_3) for use in the NMR experiments, ensuring that the sample meets the required physical and chemical specifications for optimal spectroscopic analysis.
2. Fabricate a custom NMR coil: Design and construct an inductive coil tailored to your experimental setup. The coil should be optimized to achieve maximal sensitivity for the nuclei of interest, taking into account the dimensions of the sample, coil geometry, and desired resonant frequencies.
3. Tune and match the NMR circuit: Precisely tune and impedance-match the RF circuit for both ^{1}H and ^{139}La resonance frequencies at the given magnetic field strength. This ensures efficient energy transfer and minimizes signal loss, crucial for high-quality data acquisition.
4. Optimize experimental parameters: Perform systematic parameter optimization for both ^{1}H and ^{139}La to ensure the accurate digitization of NMR signals. This includes adjusting pulse widths, receiver gain, dwell time, and other acquisition parameters to maximize signal fidelity and minimize noise.
5. Perform relaxation and nutation experiments: Conduct nutation experiments to determine the optimal RF pulse lengths for coherent spin manipulation. If required, measure the longitudinal (T_1) and transverse (T_2) relaxation times for both nuclei, as these are critical for understanding the dynamics of the spin system and for accurate quantitative analysis.
6. Verify the signal-to-noise ratio (SNR): Confirm that the signal-to-noise ratio (SNR) scales with the square root of the number of scans, i.e., $SNR \propto \sqrt{N_{\text{scans}}}$. This relationship is fundamental in NMR signal averaging and should be experimentally validated.
7. Calculate and compare SNR values: Calculate the theoretical SNR for ^{1}H and ^{139}La nuclei using the appropriate equation (equation 1) and compare these theoretical values with the experimentally measured SNR. Discrepancies should be analyzed and accounted for, considering factors such as coil efficiency, sample homogeneity, and noise sources.
8. Calculate hydrogen atom density: Determine the number density of hydrogen atoms in the LaH_3 lattice by using NMR data and relevant physical parameters. Compare this value with the expected stoichiometry of the compound. If deviations are observed, investigate potential sources of error, such as sample impurities, defects in the crystal structure, or inaccuracies in the experimental setup.

3 Literature

- Eiichi Fukushima, *Experimental pulse NMR - a nuts and bolts approach*
- Dustin Wheeler and Mark Conradi, *Practical Exercises for Learning to Construct NMR/MRI Probe Circuits*
- Noel Mispelter, *NMR probeheads for biophysical and Biomedical Experiments*
- Reyes, A. P., Ahrens, E. T., Heffner, R. H., Hammel, P. C., and Thompson, J. D. (1992). Cuprous oxide manometer for high-pressure magnetic resonance experiments. Review of Scientific Instruments, 63(5), 3120–3122. https://doi.org/10.1063/1.1142564
- Meier, T. (2016). High Sensitivity Nuclear Magnetic Resonance at Extreme Pressures [Leipzig University], $http : //www.qucosa.de/recherche/frontdoor/?tx_slubopus4frontend[id] = 20390$