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NMR quantitation: influence of RF inhomogeneity

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Abstract

The NMR peak integral is ideally linearly dependent on the sine of excitation angle (θ), which has provided unsurpassed flexibility in quantitative NMR by allowing the use of a signal of any concentration as the internal concentration reference. Controlling the excitation angle is particularly critical for solvent proton concentration referencing to minimize the negative impact of radiation damping, and to reduce the risk of receiver gain compression. In practice, due to the influence of RF inhomogeneity for any given probe, the observed peak integral is not exactly proportional to $\sin \theta$. To evaluate the impact quantitatively, we introduce a RF inhomogeneity factor $I(\theta)$ as a function of the nominal pulse excitation angle and propose a simple calibration procedure. Alternatively, $I(\theta)$ can be calculated from the probe's RF profile, which can be readily obtained as a gradient image of an aqueous sample. Our results show that without consideration of $I(\theta)$, even for a probe with good RF homogeneity, up to 5% error can be introduced due to different excitation pulse angles used for the analyte and the reference. Hence, a simple calibration of $I(\theta)$ can eliminate such errors and allow an accurate description of the observed NMR signal's dependence on the excitation angle in quantitative analysis.

Keywords

quantitative NMR; RF inhomogeneity; excitation angle; solvent concentration reference

Introduction

The NMR signal is proportional to the population difference between the excited state and ground state. In a simple one-pulse experiment, the observed signal is expected to be proportional to the sine of the excitation angle as well as the total number of nuclei. A universal concentration determination is possible, and the choice of solvent as the concentration reference becomes natural.^[1] While the residual solvent signal or its satellites in a deuterated solvent can be readily observed along with a typical analyte, it may need stringent calibration as a change in the manufacturer's deuterium content, e.g. from 99.9 to 99.8% may lead to the residual proton concentration change by 100%!^[2,3] On the other hand,

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a protonated solvent, with its stable concentration and low or no cost, has many advantages as a native concentration standard, especially for aqueous samples in biological and metabolomic NMR. However, the strong solvent signal frequently has to be excited by a very small excitation angle to overcome the detrimental effect of radiation damping for quantitation,^[1,4] while the dilute analyte is preferably observed by a 70–90° excitation pulse.^[5]

The NMR observable is the sum of signals over the volume of a sample, and the RF field is not completely homogeneous within any NMR probe.^[6] The detection efficiency is not the same throughout the volume of a sample, especially for the ‘faraway’ regions (at the outer edges of the RF coil).^[7] Strictly speaking, the excitation angle should be treated as a spatially dependent function.

While mapping out the complete excitation profile for a given probe is possible,^[8,9] this process is not always convenient due to potential gradient nonlinearity^[10] or does not work well with the ‘faraway’ region where the RF attenuates significantly. On the other hand, for a canonical description of a probe’s RF inhomogeneity one can use a simple ratio of signal intensity ($A_{810^\circ}/A_{90^\circ}$) after 810° and 90° excitations.^[6]

For quantitation purposes, it is convenient to introduce a RF inhomogeneity factor $I(\theta)$, which depends on the nominal excitation angle θ , so that the observed signal’s peak integration can be expressed as^[1,11,12]:

$$A \propto \sin\theta \times I(\theta) \quad (1)$$

For any given sample, θ can be easily calculated from a given pulse length versus that of the nominal 360° pulse, which is defined as the second null peak integral (the first null occurs near 180° pulse excitation).^[6] We prefer the 360° calibration method as it is less dependent on relaxation delays between scans (since most magnetizations return to their starting positions).

Apparently, for an ideal probe with very high RF homogeneity or a sample that is restricted to the homogeneous region of the probe, $I(\theta)$ is expected to be largely independent of θ . However, for most probes, especially cryoprobes, the influence of $I(\theta)$ in Eqn (1) cannot be ignored and needs to be corrected for accurate quantitation when significantly different excitation angles are used for the reference and analyte.^[1] A simple example of RF inhomogeneity’s impact is the fact that the observed NMR signal intensity after a 450° or 810° pulse excitation is typically less than that after a 90° excitation.

In this paper, we propose $I(\theta)$ be calibrated as a function of nominal excitation angle for quantitative analysis. We also describe a simple procedure to calculate $I(\theta)$ based on a gradient image of an aqueous sample in the probe under similar operating conditions. The calculated $I(\theta)$ is in good agreement with our experimental calibration.

Experimental

All NMR spectra were acquired at 298 K for a sample of 99% D₂O doped with 0.1 mg/ml GdCl₃ on a Bruker Avance 500 MHz NMR spectrometers equipped with either a 5 mm TXI room-temperature or cryoprobe. The nominal 360° pulse length (about 65 μs; with a modest, however, constant transmitter attenuation) was determined by seeking the second null water resonance integral. The excitation pulse length was then incremented from 6 μs so that the corresponding pulse angle changes from about 8.4° to 90°. In our set-up, the shortest pulse length was 6 μs so that the impact of finite pulse rise and fall time could be ignored. The acquisition time was 1.4 s with a sweep width of 12 ppm, and the receiver gain was held constant at 64 throughout.

All data were Fourier transformed, phased and base-line corrected. NMR signal amplitude was treated as the water proton resonance integral.

Results and Discussions

In quantitative analysis when two excitation angles are used, we are primarily concerned with the change of $I(\theta)$ for those two angles. For convenience, we can define $I(90^\circ)$ as 1. From Eqn (1), $I(\theta)$ for any other θ can experimentally determined as

$$I(\theta) = A_\theta / (A_{90^\circ} \times \sin\theta) \quad (2)$$

where θ varies from a very small angle (for strong solvent signals) to 90°. For $0 < \theta < 10^\circ$, $\sin \theta$ approaches θ (in radians; the difference is no more than 0.5%) and it is expected that $I(\theta)$ becomes a constant for a small θ (vide infra). In this equation, both A_θ and A_{90° can be experimentally determined as resonance peak integral. θ is a nominal pulse angle, which can be calculated from the corresponding pulse length, once the nominal 360° pulse length is calibrated.

Figure 1 shows the experimentally determined $I(\theta)$ plotted against excitation angle θ for a room temperature probe (solid line) and a cryoprobe (dashed line). For the room temperature probe, the dependence of $I(\theta)$ on θ is smaller, as the probe is considered to be of higher homogeneity (measured $A_{810^\circ}/A_{90^\circ} \sim 0.68$; vide infra). This is frequently the case for modern conventional inverse probes. The impact of $I(\theta)$ is not expected to contribute more than 3% error even if we assume it is a constant for all excitation angles smaller than 90°.

The dashed line in Fig. 1 shows that the dependence of $I(\theta)$ on θ is more significant as the homogeneity for a cryoprobe is typically lower than its conventional counterpart. In this case, failing to recognize $I(\theta)$'s dependence on θ alone may potentially lead to errors in quantitation as large as 5%.

When $I(90^\circ)$ is defined as 1, $I(\theta)$ is generally smaller than 1 for both probes for excitation angles smaller than 90°. In other words, RF inhomogeneity will lead to a lower signal integral than its corresponding nominal excitation angle suggests. This results from the fact that $\sin \theta$ is smaller than θ (in radians; $0 < \theta < \pi/2$). Because the difference between them

becomes smaller when θ approaches 0° , it is expected that the curves in Fig. 1 can be extrapolated almost horizontally for excitation angles less than 10° .

To help understand the general property of $I(\theta)$ for a typical probe, we can conduct its calculation with the help of a gradient image of the RF profile. In Fig. 2, the solid line shows a gradient image along the z -axis of a long aqueous sample in the room temperature probe used for Fig. 1. This profile was obtained by a simple gradient echo image (TR = 10 s and TE = 0.008s) after a small-angle pulse excitation. Under ideal conditions (without the consideration of relaxation or static magnetic field inhomogeneity), the NMR signal at position z is proportional to the sine of the excitation angle at that position θ_z , the receiving function $R(z)$ and apparent spin density $\rho(z)$:

$$A_z \propto \sin(\theta_z) R(z) \rho(z) = \sin\left(\frac{\theta}{360^\circ} \alpha_{\max} E(z)\right) R(z) \rho(z) \quad (3)$$

where θ is the nominal excitation angle, $E(z)$ is the normalized excitation function (with its max value set to 1 for the max RF strength within the sample) and $\alpha_{\max}$ is an $E(z)$ -dependent pulse angle that is slightly larger than 360° to allow observed $A_{360^\circ} = 0$ (*vide infra*).

The observed total signal after a nominal θ angle pulse excitation is

$$A_\theta \propto \int_z \sin\left(\frac{\theta}{360^\circ} \alpha_{\max} E(z)\right) R(z) \rho(z) dz \quad (4)$$

Combining Eqns (1) and (4), we can express $I(\theta)$ ($0^\circ < \theta < 90^\circ$) as

$$I(\theta) = \frac{1}{\sin(\theta)} \int_z \sin\left(\frac{\theta}{360^\circ} \alpha_{\max} E(z)\right) R(z) \rho(z) dz / \int_z \sin\left(\frac{90^\circ}{360^\circ} \alpha_{\max} E(z)\right) R(z) \rho(z) dz \quad (5)$$

which suggests $I(\theta)$ is a constant for small excitation angles as θ can move out of the sine term and the integral to cancel with $\sin(\theta)$.

In the current study, z can be approximated as a Cartesian z coordinate if we assume the imaging z gradient is linear and thus $\rho(z)$ is a constant for a uniform sample. Additionally, for a single coil probe that shares the excitation and detection RF circuit, the excitation function $E(z)$ is the same as the receiving function $R(z)$ due to the principle of NMR reciprocity.^[11] Under the condition of small angle excitation ($<10^\circ$), Eqn (3) can be simplified:

$$A_z \propto E(z) R(z) = E(z)^2 \quad (6)$$

Hence, the probe's excitation function $E(z)$ can be easily derived as the square root of the observed gradient image, which is also the profile for the receiving function $R(z)$ (Fig. 2 dashed line).

Then we can seek the maximal pulse angle $\alpha_{\max}$ by the nominal 360° pulse excitation as A_{360° becomes zero in Eqn (4). Figure 3 gives the plot of the calculated peak integral as a function of the maximal pulse angle $\alpha_{\max}$ experienced within the sample (at -2 mm) for a probe characterized in Fig. 2. Overall, the peak integral appears sinusoidally dependent on $\alpha_{\max}$. However, close examination near 360° reveals that $\alpha_{\max}$ is actually 379° to result in a null peak integral (Fig. 3, inset). In this particular case, we define the pulse length corresponding to $\alpha_{\max} = 379^\circ$ as the nominal 360° pulse length for the whole sample. Our calculation is consistent with the common knowledge that the nominal 360° length for the whole sample is slightly (about 5%) longer than that of the bulk region for a typical NMR sample in a probe with good RF homogeneity. For the probe in Fig. 2, $A_{810^\circ}/A_{90^\circ}$ and $A_{450^\circ}/A_{90^\circ}$ (all with nominal angles) are calculated as 0.68 and 0.77, respectively. Both are consistent with our experimental observation of 0.67 and 0.78.

Equation (5) can be utilized to calculate the theoretical $I(\theta)$ once the excitation function is known. For the room temperature probe (data illustrated in Figs. 1 and 2), the calculated $I(\theta)$ is plotted in Fig. 4, which is in very good agreement with the experimentally determined curve (Fig. 1, solid line), especially for excitation angles larger than 40° . The difference between Fig. 1 solid line and Fig. 4 is slightly more significant ($\sim 1\%$) for pulse angles smaller than 30° . We attribute this difference mainly to the limited pulse length and off-resonance effects.

Finally, we want to note that $I(\theta)$ determined by our experimental calibration is applicable to other samples under the same geometric conditions (i.e. a standard NMR sample within an NMR probe) and similar one-pulse data acquisitions. $I(\theta)$'s dependence on θ is expected to be smaller for a volume-restricted sample (e.g. in a Shigemi tube). For the quantitation based on a spectrum acquired after multiple pulses, the 1D signal in principle can still be used as the concentration reference; however, considerations for corrections should include not only RF inhomogeneity and the active volume but also relaxation and transfer efficiency, which can be obtained through calculation or time-zero HSQC.^[13,14]

Conclusions

We show that RF inhomogeneity can cause a slight deviation for the observed NMR signal's dependence on the sine of the excitation angle. In accurate quantitative analysis, the negative impact of RF inhomogeneity for any given NMR probe cannot be ignored. A RF inhomogeneity factor $I(\theta)$ can be introduced, which can be easily calibrated experimentally. Alternatively, $I(\theta)$ can be calculated from a simple gradient image of a typical sample in the probe.

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Figure 1.

Correlation of the RF inhomogeneity factor $I(\theta)$ with nominal excitation angle for a room temperature probe (solid line) and a cryoprobe (dashed line). As a room temperature probe in general has a higher RF homogeneity factor than its cryoprobe counterpart, the $I(\theta)$ as defined in Eqn (2) for a room temperature probe is less dependent on θ .

Figure 2.

A gradient image along the z -axis (solid line) of an aqueous sample in the room temperature probe for Fig. 1. The units on both axes are arbitrary; however, for ease of understanding, the vertical axis is normalized to 1 at the maximum intensity and the horizontal axis is converted to mm corresponding roughly to the z displacement of the sample. The RF excitation profile can be easily obtained as the dashed line under the condition of small excitation angle (less than 10°) and a linear gradient.

Figure 3. Peak integral as a function of the maximal pulse angle (α_{max}) in the sample. The inset shows that the nominal 360° pulse length, which is defined to have a null peak integral, corresponds to $\alpha_{\text{max}} = 379^\circ$ (at location $z \sim -3$ mm) in Fig. 2.

Figure 4. Calculated $I(\theta)$ for the room temperature probe used in Figs. 2 and 1. It is in good agreement with the RF inhomogeneity factor determined experimentally (Fig. 1, solid line).