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Communication

Reducing t_1 noise through rapid scanning

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ABSTRACT

The so-called t_1 noise, which arises due to random instabilities in the spectrometer hardware, remains the primary source of noise that limits the sensitivity of most 2D NMR experiments, particularly in the expanding group of solid-state NMR methods that utilize dipolar-recoupling. In this communication we revisit the relationship between the signal intensity and the t_1 noise produced. It is shown that since the latter scales linearly with the signal strength, the use of a conventional relaxation delay of $1.3T_1$ may prove far from optimal. In cases where the fluctuations occur on a shorter timescale than the recycle delay, a considerably faster repetition rate should be used to maximize the time sensitivity in a 2D experiment than what is used to maximize the sensitivity in 1D. This is demonstrated with the acquisition of $^1H\{^{13}C\}$ Dipolar-mediated Heteronuclear Multiple-Quantum Correlation (D-HMQC) type spectra in which the sensitivity could be nearly doubled by choosing a very short relaxation delay corresponding to $0.2T_1$.

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1. Introduction

One of the main factors limiting the application of advanced two-dimensional (2D) nuclear magnetic resonance (NMR) methods is the prevalence of t_1 noise. This noise, generally, occurs due to various instrumental instabilities, which lead to random fluctuations of NMR intensities between the scans during the acquisition of the 2D dataset [1]. As a result, the Fourier-transformed 2D spectrum exhibits ridges of intense noise along the indirect dimension, wherever a peak appears in the directly-detected dimension. Common sources of t_1 noise include temperature changes, magnetic field fluctuations, as well as instabilities of radiofrequency (rf) power and phase [2]. Despite the dramatically improved stability of modern NMR spectrometers, this artifact can significantly reduce the overall signal-to-noise ratio (*SNR*) of an otherwise sensitive experiment.

A number of solutions have been reported for reducing t_1 noise, including various post processing methods [3–5] and spreading the t_1 noise more thinly along F1 through oversampling [6]. The t_1 noise from particularly strong resonances, such as those originating from solvents, can be further suppressed through presaturation [7]. Recently, Mo et al. have also demonstrated that t_1 noise in lengthy 2D NOESY spectra could be significantly reduced if the experiment were cut into k shorter experiments, each consisting of 1/k scans [8]. This strategy succeeds in reducing the t_1 noise resulting from slow changes in the magnetic field and temperature, which carry a smaller weight when the experiment is concluded quickly. The t_1 noise may also be minimized by carefully managing the overall stability of the spectrometer performance and maintaining constant sample and ambient temperatures [9].

In the case of magic-angle-spinning (MAS) solid-state NMR (SSNMR), by far the most important source of t_1 noise is the instability in the rotor spinning [10]. The long-established methods relying on cross-polarization (CP) and spin-diffusion-based approaches are reasonably insensitive to the MAS frequency and the rotor phase [9,10]. However, the rotation frequency can have a large impact on the signal amplitude when a recoupling experiment is used to acquire dipolar heteronuclear multiple-quantum correlation (D-HMQC) [11,12] or double-quantum single-quantum (DQ/SQ) [7] correlation experiments. The so-called non-"γ-encoded" sequences are particularly sensitive, as these require a precise control of the MAS rotor's phase with respect to the timing of recoupling sequences. Changes in the MAS frequency (albeit not phase) can also alter the efficiency of these sequences as they depend on the ratio of the rf power and the rotation frequency being preserved [13]. As a result of these instabilities, t_1 noise of this type is typically seen to increase as a function of the t_1 evolution period. Lastly, secondary motional modes of the rotor, such as the in-out mode, affect the tuning of the probe and lead to changes in the rf power and phase from scan to scan. These sources of instabilities occur on a timescale that is shorter than the recycle delay

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and cannot, thus, be eliminated by the approach of Mo et al. [8]. Recently, Nagashima et al. have attempted to address one of these sources of t_1 noise, namely the sensitivity to the rotor phase, with their dipolar-heteronuclear universal-quantum correlation (D-HUQC) which makes use of γ-encoded dipolar recoupling [14].

The abovementioned D-HMQC techniques have been recently gaining remarkable popularity as they are far more robust with respect to quadrupolar interactions and offset than CP-based approaches since only two 90° pulses are required on the indirect channel. For instance, D-HMQC experiments allow for the measurement of correlation spectra with quadrupolar nuclei [15–18], and spin-1/2 nuclei with large chemical shift anisotropies [19,20]. The t_1 noise is therefore, once again, an important limitation for the application of 2D SSNMR spectroscopy.

The t_1 noise is perhaps best described with the synonym: multiplicative noise (N_{mult}) [1]. Namely, unlike conventional, additive, noise (N_{add}), which is constant and independent of the NMR response, t_1 noise depends directly on the intensity of the NMR signal. This is an important distinction since most 2D SSNMR experiments are performed in such a way that maximizes the sensitivity in F2 and thus also increases the t_1 noise along F1. For example, the intensity of the NMR signal in a single scan (S) following saturation is given by:

$$S = S_{\mathrm{eq.}}\left(1 - \exp\left(\frac{-d_1}{T_1}\right)\right), \tag{1}$$

where d_1 is the recycling delay, T_1 is the spin-lattice relaxation time and $S_{\mathrm{eq.}}$ is the amplitude of signal at equilibrium, per scan. When accounting for the fact that additive noise scales only as the square root of the number of scans while the signal scales linearly, the time averaged SNR is given by:

$$SNR = \frac{S_{\mathrm{eq.}}\left(1 - \exp\left(\frac{-d_1}{T_1}\right)\right)}{\langle N_{\mathrm{add}}\rangle\sqrt{d_1}}, \tag{2}$$

where N_{add} corresponds to the noise level in a single scan, and is defined as a variance of the noise over all data points

$$\langle N_{\mathrm{add}}\rangle = \frac{1}{n}\sqrt{\sum_{i=1}^{n} N_i^2} \tag{3}$$

with n denoting the number of data points and N_i the noise contribution to the ith data point. Independently of $S_{\mathrm{eq.}}$ and N_{add}, expression 2 has one maximum at d_1/T_1 of ca. 1.3 [21]. In the absence of t_1 noise, the best choice for d_1 is thus $1.3T_1$, which is commonly used to maximize the sensitivity per unit of time.

In the presence of multiplicative noise Eq. (2) is no longer valid and its use can lead to important sensitivity losses. The noise level in F1 can be determined as follows:

$$\langle N_{\mathrm{F1}}\rangle = \sqrt{\langle N_{\mathrm{add}}\rangle^2 + \langle N_{\mathrm{mult}}\rangle^2}, \tag{4}$$

with $\langle N_{\mathrm{mult}}\rangle$ expressed as:

$$\langle N_{\mathrm{mult}}\rangle = x\langle N_{\mathrm{add}}\rangle\left(1 - \exp\left(\frac{-d_1}{T_1}\right)\right), \tag{5}$$

where x is the ratio between the t_1 and t_2 noise levels at equilibrium (i.e. with a recycle delay $> 5T_1$) and corresponds to the hypothetical multiplicative noise level from a single scan. This choice of this parameter was made as it renders the solution of the subsequent equations independent of $S_{\mathrm{eq.}}$ and the exact noise levels. In direct analogy to Eq. (2), we can then express the signal-to-noise ratio in the F1 dimension of a 2D NMR experiment as:

$$SNR = \frac{S_{\mathrm{eq.}}\left(1 - \exp\left(\frac{-d_1}{T_1}\right)\right)}{\langle N_{\mathrm{add}}\rangle\sqrt{d_1}\sqrt{1 + x^2\left(1 - \exp\left(\frac{-d_1}{T_1}\right)\right)^2}}. \tag{6}$$

If we then take the derivative of Eq. (6) over d_1 we can determine the function's maximum and determine the following relationship between x and the relaxation delay that maximizes sensitivity per unit of time:

$$x = \frac{\exp\left(\frac{d_1}{T_1}\right)\sqrt{\left(\exp\left(\frac{d_1}{T_1}\right) - 1\right)\left(-\exp\left(\frac{d_1}{T_1}\right) + 2\frac{d_1}{T_1} + 1\right)}}{\left(\exp\left(\frac{d_1}{T_1}\right) - 1\right)^2}. \tag{7}$$

This relationship, of course, reproduces the expected d_1/T_1 ratio of c.a. 1.3 when x is equal to 0 and also suggests the use of shorter relaxation delays when $x > 0$. Unfortunately, it is not possible to isolate d_1 in order to have a convenient, and exact, closed-form solution to Eq. (7). If we assume that x is large (and thus d_1/T_1 is short) it is possible to considerably simplify Eq. (7) using a Taylor expansion with a first term equal to:

$$x = \frac{T_1}{d_1}. \tag{8}$$

This relationship nearly perfectly reproduces Eq. (7) when $x > 2$ (see Fig. 1). Accordingly, in cases where t_1 noise is significant, the relaxation delay should be set to the following value in order to maximize the SNR in the F1 dimension:

$$d_1 = \frac{T_1}{x} = \frac{\langle N_{\mathrm{add}}\rangle}{\langle N_{\mathrm{mult}}\rangle_{\mathrm{eq.}}}T_1. \tag{9}$$

The factor $\langle N_{\mathrm{add}}\rangle/\langle N_{\mathrm{mult}}\rangle_{\mathrm{eq.}}$ includes the noise levels in F2 and F1 dimensions corresponding to a relaxation delay of $5T_1$, which can be easily measured. Note that our conclusion is counter to earlier suggestions that recycle delays of $5T_1$ should be applied to minimize t_1 noise, which seems to be unnecessary if proper presaturation is performed [1]. In cases when x is relatively small the sensitivity gains available by shortening the relaxation delay are minor and thus a relaxation delay of $1.3T_1$ can be used. Eqs. (7)–(9) are plotted in Fig. 1, where the relationship between the optimal relaxation delay and t_1 noise level can be more clearly visualized.

To demonstrate the improvements in SNR from rapid scanning, we have acquired ^{1}H{^{13}C} D-HUQC spectra of ^{13}C,^{15}N-enriched glycine, using a 1.3-mm MAS probe with poor spinning stability (*vide infra*), a spinning frequency of ~40 kHz and relaxation delays

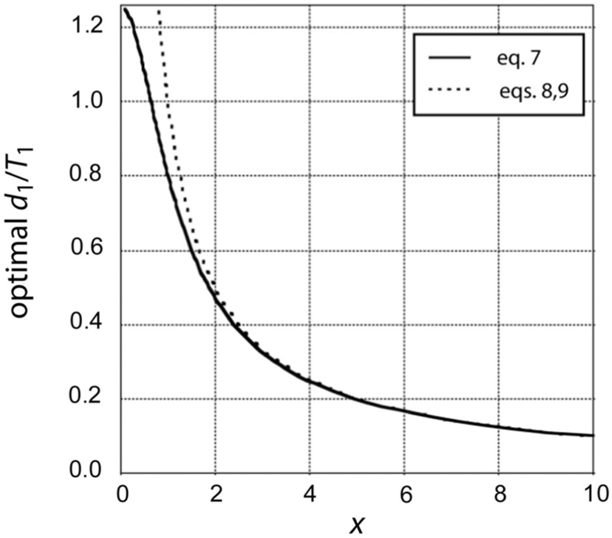

Fig. 1. The relationship between the optimal relaxation delay, expressed as a fraction of the T_1 relaxation time, and the importance of t_1 noise (x) is plotted. The exact relationship (Eq. (7), solid line) and our analytical approximation (Eqs. (8) and (9), dotted line) are compared.

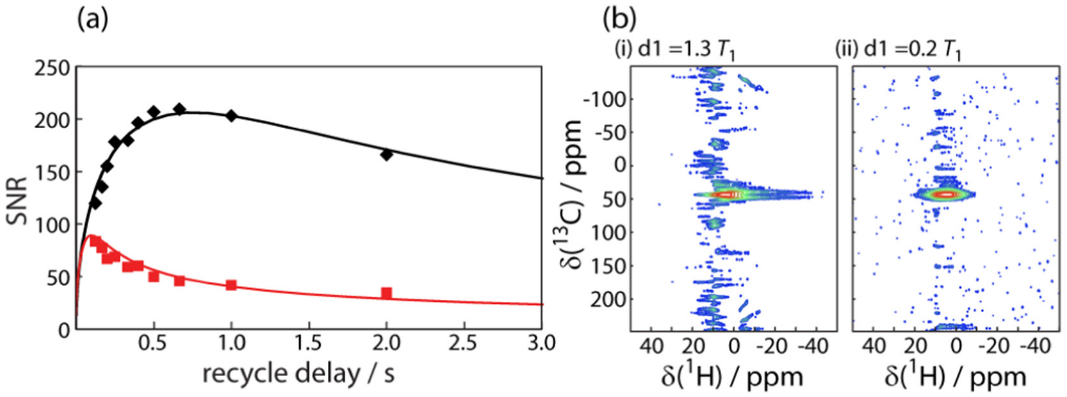

Fig. 2. The signal-to-noise ratios measured along the F2 (black diamonds) and F1 (red squares) dimensions of $^1H\{^{13}C\}$ D-HUQC experiments lasting 17 min are plotted as a function of the recycling delay (a). The data are fit using Eqs. (2) and (6) with values of $S_{eq}/\langle N_{add}\rangle$ = 250, x = 6.0, and T_1 = 0.6 s. Example $^1H\{^{13}C\}$ D-HUQC spectra are shown in (b) with recycling delays of 1.3T_1 (i) and 0.2T_1 (ii). The 'floor' is set to the same relative level in each case. As can be seen, although the use of a recycling delay lasting 1.3T_1 yields the highest sensitivity in F2, a recycling delay of 0.2T_1 yields the highest overall sensitivity.

varied between 0.125 s and 2 s. The T_1 relaxation time was measured to be 0.6 s using a saturation recovery experiment. Presaturation was used to ensure that each scan initiated at the same steady-state. In all cases, the experiment time was maintained constant at 17 min. Unlike the seminal paper of Nagashima,[14] we chose to use an $m = 2$ heteronuclear dipolar recoupling sequence ($R18_2^5$) [22] in order to decouple the ^1H homonuclear dipolar coupling interactions during the heteronuclear recoupling. We have plotted the *SNR* that were obtained along F1 and F2 in Fig. 2a and fit them using Eqs. (2) and (6) with $S_{eq}/\langle N_{add}\rangle$ = 250 and $x = 6$. As can be seen from the data in Fig. 2, the relaxation delay which provides the highest signal-to-noise ratio for this 2D experiment is of only 0.2T_1 rather than 1.3T_1. In this case, the use of the more conventional relaxation delay of 1.3T_1 would have led to a 50% reduction in *SNR*. This can be clearly seen in Fig. 2b where we compare the 2D spectra acquired with relaxation delays of 1.3T_1 and 0.2T_1. Here, it is also worth mentioning that in experiments with severe t_1 noise the optimal recycling delay may become shorter than the shortest allowable duty cycle. Of course, in those situations the shortest safe recycling delay should be used, which was the case in our example.

In summary, we have demonstrated that that in case of 2D NMR measurements with strong t_1 noise originating from fluctuations that are faster than the recycle delay, far shorter relaxation delays than the typically used $d_1 = 1.3T_1$ are required to maximize the sensitivity per unit of time. A simple expression is given in order to determine the appropriate value.

2. Experimental

All experiments were performed on a Bruker AVANCE III 400 MHz SSNMR spectrometer using a 1.3-mm MAS probe. This probe, which was designed for rapid sample changing at low-temperatures, suffers from additional instabilities caused by the VT and Venturi gas flows that increase the t_1 noise. For the $^1H\{^{13}C\}$ D-HUQC experiments a $^{13}C,^{15}N$-enriched glycine sample, purchased from ISOTEC, was used. The sample spinning frequency was set to 40 kHz and the rf magnetic fields for the ^1H and ^{13}C pulses were set to 100 kHz while those for the $R18_2^5$ recoupling were of 180 kHz (4.5ν_R). The evolution in the indirect dimension was synchronized with the rotation frequency and a total of 128 t_1 increments were acquired for each spectrum. A minimum of 4 scans were acquired with a recycling delay of 2 s and this number was increased to 64 when the recycling delay was decreased to 0.125 s. Purely absorptive phase 2D lineshapes were obtained using the States-TPPI method.

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