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RESEARCH ARTICLE

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Common problems and artifacts encountered in solution-state NMR experiments

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Abstract

NMR experiments contain many parameters that need to be set properly to produce spectra free from errors and artifacts. Modern NMR spectrometers nowadays incorporate standard experimental templates with ideal parameters that suit many common samples. These address many of the problems associated with most parameters but because of the variety of samples and various experiments that can be performed, the obtained spectra can be less than ideal displaying unwanted artifacts and inaccurate spectral properties. In this paper, some of the most common problems and artifacts in NMR experiments are presented and solutions discussed.

KEYWORDS

artifact suppression, artifacts, NMR parameter optimization, NMR spectroscopy

1 | INTRODUCTION

Nuclear magnetic resonance spectroscopy (NMR) is one the most versatile and useful analytical techniques for investigating the structure and dynamics of various molecular systems. This non-destructive technique covers a wide range of applications from basic sciences such as biology, chemistry and physics to applied sciences such as medicine, health and pharmacy and also to industry. Although often seen as almost independent subdisciplines, (Nuclear) Magnetic Resonance Imaging (MRI) and traditional non-imaging NMR experiments are of course inherently related. In recent years, MRI has become not only increasingly used in the clinical context but also its sphere of application has been expanding in any context the sample is not heterogeneous. Although the information sought from an NMR (incl. MRI) experiment can vary, each experiment is nevertheless based on the application of a pulse sequence. A pulse sequence is the application of a series of radio frequency pulses interspersed with time delays and perhaps magnetic gradient pulses followed by signal acquisition. A particular sequence is designed to interrogate the NMR-sensitive nuclei in a sample in a particular way to acquire the desired information. Through the years an enormous number of such sequences have been and continue to be devised.

NMR experiments are traditionally loosely classified into solution-state or solid-state NMR experiments effectively according to the rate of reorientational motion of the species being probed. If the reorientational motion is sufficiently slow that the observed characteristic is not completely motionally averaged the spectral characteristics are spread over a larger frequency range. This requires modified NMR spectrometer hardware and there is one enormously important distinction compared with solution-state NMR – that is that the spectrometer limitations influence the acquired data. In contrast, in solution-state NMR experiments the behavior of the spectrometer in generating the pulse sequence can, to a first approximation, be considered ideal. This paper is primarily concerned with solution-state NMR. The behavior of most MRI spectrometers is closer to that of a solution-state spectrometer than a solid-state spectrometer.

A typical NMR experiment can be considered as a single volume of interest (voxel) experiment – that is the signal acquired is normally an average over the entire sample volume although some parts of the sample may be more heavily weighted depending on the geometry and performance of the radiofrequency (RF) coils. In contrast, an MRI experiment is an extension of an NMR experiment in which localization is achieved by dividing the sample into numerous voxels with each voxel giving rise to an

independently accessible NMR signal. Most simply the signal from each voxel is an average over all species present (i.e., no chemical shift information), in closer analogy with traditional NMR, the chemical shift information is retained and each voxel gives rise to a spectrum. Importantly, many artifacts are common to both MRI and traditional NMR.

In the ideal case, the acquired NMR data would be acquired without noise and without artifacts. In practice, however, the performance of NMR experiments depends on many external factors and to obtain valid and as error "free" NMR spectra as possible, many experimental parameters generally need to be set carefully. There are many things that can go wrong in NMR experiments and this is the reason why in setting-up experiments for new samples, experienced NMR users do not start from scratch but rather seek and use tried and tested datasets that worked previously.

With the advent of modern NMR instruments and automation, NMR users nowadays are often not too concerned about, or even oblivious to, the intricacies of setting-up NMR experiments and optimizing various experimental parameters. This is because many of the common NMR experiments nowadays are carried out with preloaded dataset parameters with some being optimized automatically before the start of data acquisition. Indeed, the increased levels of automation has allowed a new class of "non-NMR specialist" users. Many problems that lead to artifacts in NMR experiments that were causes of concern a few years ago have now been addressed through the use of "optimized" datasets.

Nothing simplifies NMR data analysis more than starting with a good dataset. Given the increased automation and reliance on standard parameter sets, it is not uncommon that when problems and artifacts occur that many new users are either bewildered by, or even worse, totally unware of their presence. With the increasing application of NMR in various fields, many current users of NMR spectrometers are not NMR specialists so that the emergence of these common problems presents a big challenge for them. The presence of spectrometer problems and artifacts may lead to serious consequences such as incorrect analysis and missing information. It could also lead to mediocre experiences with NMR and detracts from the usefulness of this technique as an important analytical tool.

There are many NMR publications that deal with some of the common practical problems in NMR[1–6] but none provides a comprehensive account. The purpose of this paper is to present these common problems and artifacts so that NMR users can identify them, understand their origin and lastly, be able to eliminate or at least minimize them. The problems encountered in NMR experiments which often lead to artifacts can be classified according to their effect on the obtained spectra. These problems with likely causes and solutions are presented comprehensively below.

2 | DISTORTED PEAKS

This is perhaps the most common problem encountered in any NMR experiment. There are various causes why spectra may contain distorted peaks.

2.1 | Shims not optimized

In a perfectly homogeneous magnetic field all spins of the same type and environment would resonate at exactly the same frequency and the linewidth would be determined solely by the inherent relaxation mechanisms of the spin and thus by its true spin-spin relaxation time, T_2. In such a case, the resonance of a single spin would appear as a Lorentzian lineshape (i.e., the Fourier Transform of a single decaying exponential with time constant T_2) with a linewidth at half-height of $\upsilon_{\frac{1}{2}} = 1/(\pi T_2)$. In reality, the magnetic field has some degree of heterogeneity because of the limitations of magnet construction which is further degraded by the probe and the sample perturbing the field as well as other factors (e.g., a thermocouple being positioned too close to the sample). This means that the measured linewidth is now determined not only by the inherent relaxation properties but by the inhomogeneous static field. Simplistically this leads to the concept of an inhomogeneously broadened Lorentzian line described by T_2*. In general, however, the degree of heterogeneity will not be constant through the sample and the observed lineshape may not even be Lorentzian.

The static magnetic field across the effective volume of the sample should be as homogeneous as possible to achieve the narrowest peak lineshape and thus the best sensitivity in any NMR experiment. Normally, a sufficiently long sample (typically 4 cm or more) is used and centered within the RF coils to ensure that the sample/air interfaces that would generate background magnetic gradients because of the magnetic susceptibility differences are well outside the RF coils. Considerable effort is normally spent to correct ("shim") the sample before performing any NMR experiments. In this process, the static magnetic field throughout the sample is made more homogenous by adjusting the currents through various solenoids in various axes or directions or shims around the NMR probe to produce small corrective magnetic fields. Nevertheless, the shims have limitations and cannot correct for all sources of magnetic field inhomogeneity – especially those that result from perturbations with different geometries than the shim coils. Short samples being particularly problematic since the volume of the sample detected by the RF coils is now subject to background gradients produced by the ends of the sample (e.g., see Fig. 2 in Price WS, Stilbs P, Jönsson B, Söderman O. Macroscopic background gradient and radiation damping effects on high-field PGSE NMR

diffusion measurements[7]). Similarly, the presence of undissolved solid materials and bubbles can lead to magnetic field inhomogeneity in the sample which cannot be minimized by shimming and thus will lead to distorted peaks.

Depending on the nature of the sample and its condition, proper sample preparation steps need to be considered and implemented accordingly to obtain a homogenous sample that is shimmable. For most cases, a sample volume of 0.5 mL or more is recommended to ensure a sample length of at least 4 cm. Centrifuging and filtering the sample before the NMR experiment is common practice to remove undissolved solids while degassing is also performed in some instances to remove paramagnetic oxygen which can broaden peaks and interfere with relaxation measurements.

The shims can be grouped into spinning and non-spinning shims. The meaning is that the spinning shims have rotational symmetry along the static field (e.g., if $\mathbf{B}_0$ is directed along the Z-axis, then the Z shim is a spinning shim whereas XZ is not). Various spectra of 2-butanol where shims were set to certain values to demonstrate their effect on peak lineshapes are shown in Figure 1.

Clearly, the spectra from the fully shimmed sample gave the sharpest symmetrical peak lineshapes which are desirable. As the peaks adopt a distinct lineshape depending on which shim parameter is not optimized, one can have some idea which particular shim needs to be optimized to obtain narrow and symmetrical peaks.

The theoretical background to sample shimming has been considered in detail by Hoult and Chmurny.[8] There are three ways to shim the sample: (a) manually by looking at the free induction decay (FID) or spectrum of a single resonance while adjusting the values of each individual shim coil, (b) as for (a) but optimizing the signal from a field-frequency locking compound (typically the ^{2}H signal from a deuterated solvent) as the shims are adjusted and (c) automated shimming based on an imaging variation of (b). Option (b) and increasingly, since almost all modern spectrometers have the ability to generate magnetic field gradients through a sample, (c) are the most common techniques on high-resolution spectrometers. In contrast option (a) is always available and will work when options (b) and (c) fail – but more skill and background knowledge is required. Option (a) is the starting point for a new probe with no shim set to start from.

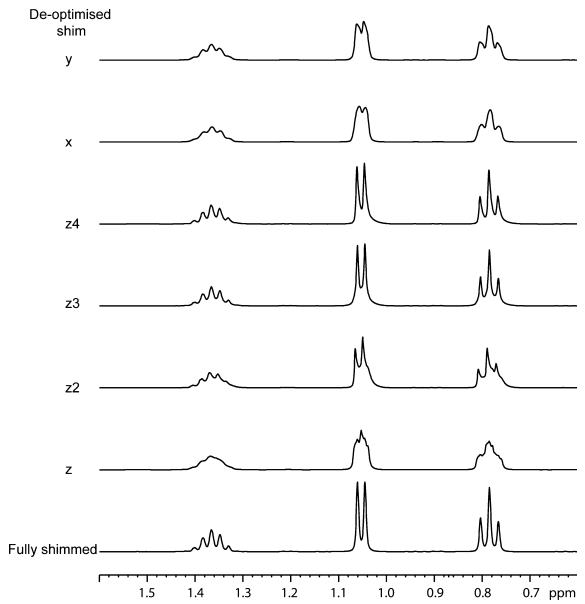

FIGURE 1 Effect of various shim values on peak lineshape in 400 MHz 1D ^{1}H NMR spectra of 10% (v/v) 2-butanol in D_2O at 25°C. The spectra were obtained after all the relevant shims had been optimized ("fully" shimmed) and then the one specific shim was deliberately de-optimized

For most users option (b) or (c) will be the normal procedure and will work satisfactorily for "standard" samples with similar volumes and positions in the probe– but neither is fool proof. Option (b) has the implicit assumption that the shimming is sufficiently close to the optimal value whereas option (c) is generally even more stringent requiring that the sample being shimmed has a "standard" shape – that is close to that of a standard sample (e.g., a normal NMR tube containing 0.5 mL of sample. As many standard probes only include z-magnetic gradient coil (no x- and y-magnetic gradient coils), manual checking and adjustment of the non-spinning shims are strongly recommended from time to time. Thus, all of the shimming might be performed manually or, instead, performed manually until the shims are sufficiently "in the ball park" such that options (b) or (c) can be attempted.

As noted above, in addition to the inherent homogeneity of the static magnetic, the magnetic field homogeneity in the sample depends on the shape and magnetic susceptibility properties of the sample and its containment (i.e., of the solvent, NMR sample tube and sample volume). Any change in these properties will result in the sample needing to be reshimmed. It is recommended that a good shim parameter file for a given probe and solvent is loaded before shimming as the previous user may have ran a sample with different solvent.

2.1.1 | Solution

The most common approach in manually shimming a sample with a deuterium lock is to maximize the lock level by adjusting the relevant shim parameters beside the lock power, lock gain and lock phase. Lock power refers to the strength of continuous RF field used to excite the deuterium signal, lock gain to the amplification of the detected lock signal and lock phase as the lock signal has to be in pure dispersion for optimum stable lock. Care has to be taken to balance the lock power and lock gain to obtain

sufficient lock level as applying too much lock power can "saturate" the lock signal (the excited deuterium spins do not have sufficient time to relax) leading to an unstable lock level that drifts up and down. There are three ways to adjust shims to achieve maximum lock level: (a) zero-order process where shims are adjusted independently of other shims in one step, (b) first-order process where two or more shims are adjusted in a given order and iteratively to yield an optimum level and (c) second-order process where one shim is moved to a certain value after which other shims are adjusted using iteratively (by a first-order process).

Initially, the sample is spun at 10-20 Hz after which the Z and Z2 shims are adjusted iteratively (first-order process) to yield a maximum lock level. The spinning is then halted and then the non-spinning X and Y shims are adjusted by a first-order process to increase the lock level. Note that if X and Y shims are already close to optimum values the lock level will not drop significantly when the spinning is halted. Second-order optimization is then performed on the X and XZ shims and afterward on Y and YZ. Lastly, the non-spinning shims XY and X2-Y2 are first-order optimized. After this point, the sample can be spun (or not) for further first-order optimization of the Z and Z2 shims followed by the Z3 (and possibly Z4) shim and then second-order optimization of the Z1 and Z2 shims.

Spinning the sample at 10-20 Hz improves the peak lineshape to some extent and has the advantage of making shimming easier as non-spinning shims (those that do not include Z-component shims) need not be optimized while spinning. However, "spinning side-bands," which are two small additional peaks located symmetrically about the true peak, can occur if the non-spinning shim components are considerably far from the optimized values or if the sample tube is unsymmetrical (see Figure 2). Optimizing the X and Y shims and shims with X and Y components can alleviate this problem. This is also the reason why distorted, non-cylindrically symmetric, NMR tubes should not be used.

Having a sealed standard sample containing an easily shimmable compound (or mixture) is a must in the NMR laboratory. Normally, the manufacturer provides such a sample and when a sample proves difficult to shim, you can switch to the standard sample to determine if it is the probe that is less than optimal (e.g., a broken insert) or whether the real sample has some problem (e.g., unexpectedly containing paramagnetics so that the natural linewidth is unusually broad and that this is being confused with poor shimming).

Because the shape and magnetic susceptibility of the sample, shimming of subsequent samples becomes a minor issue if all subsequent samples have the same shape/volume and magnetic susceptibility. This would be a particularly important consideration if a series of samples is being set to run with a sample changer.

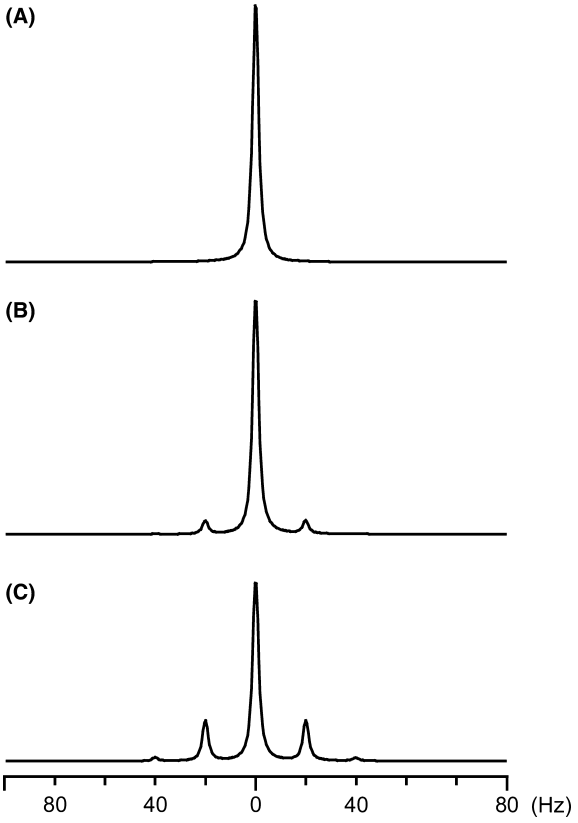

FIGURE 2 Spinning side-bands appearing around the HOD peak of 10%(v/v) 2-butanol in D_2O. Each ^1H 1D spectrum was obtained at 400 MHz with the sample spinning at 20 Hz. (A) reference spectrum showing no visible sideband (fully optimized); (B) X-shim deliberately set away from the optimum value by ~2000 arbitrary units (typical range of values spans ~10 000 arbitrary units) and (C) X-shim set to a much larger value of ~3000 arbitrary units from the optimum

Sometimes, it is necessary to use a short sample because of limited sample availability, require especially good RF homogeneity, or radiation damping (see Section 6). This can be performed by using a physically short sample dispensed into a normal tube or special NMR tube setups which will be discussed later. A cogent approach to shimming a physically short sample is to first shim on a regular length sample, as described above, and then iteratively slightly reduce the volume then re-shim. At each volume reduction, it is important to center the sample in the RF coils. It will be mainly the Z and Z2 shims which will need changing with each iteration. If the sample is not in short-supply it may, depending upon the pulse sequence, to approximate a short sample via changing some of the RF pulses to slice selective pulses.

2.2 | Receiver gain set too high

If the receiver gain is set too high the large signals will get "clipped" meaning that the initial most intense part of the FID will be truncated as it is beyond the capability of the

analog to digital converter (ADC). Thus, the FID will have a flat horizontal signal profile at the start (rectangular like) instead of the usual exponential decay. The FIDs and the resulting spectra when the receiver gain is increased until the FID is clipped is shown in Figure 3. The resulting Fourier transformed NMR spectrum of the clipped FID would show sinc function-like peaks (NB sinc(x)=sin(x)/x) with baseline distortions manifested as "wiggles" instead of the usual smooth Lorentzian like peaks.

2.2.1 | Solution

The remedy to avoid clipping the FID is to set the receiver gain one to two dB lower from the receiver gain settings when clipping is observed. It is possible that subsequent scans could be much larger than the first scan especially in 2D experiments where several FIDs are acquired. In this case, it is recommended that a short preliminary experiment with less scans is first performed to determine the optimum value for the receiver gain or alternatively the FIDs can be checked and processed during the early part of the experiment.

2.3 | Acquisition time set too short

Similar clipping of the FID, but in the temporal sense instead of amplitude, results in sinc function-like peaks as shown in Figure 4. This occurs when the acquisition time is too short compared with the T_2* of the peaks such that the signals have not decayed sufficiently by the end of the acquisition. In general, in multidimensional datasets there is never sufficient acquisition time for the complete FID to be acquired.

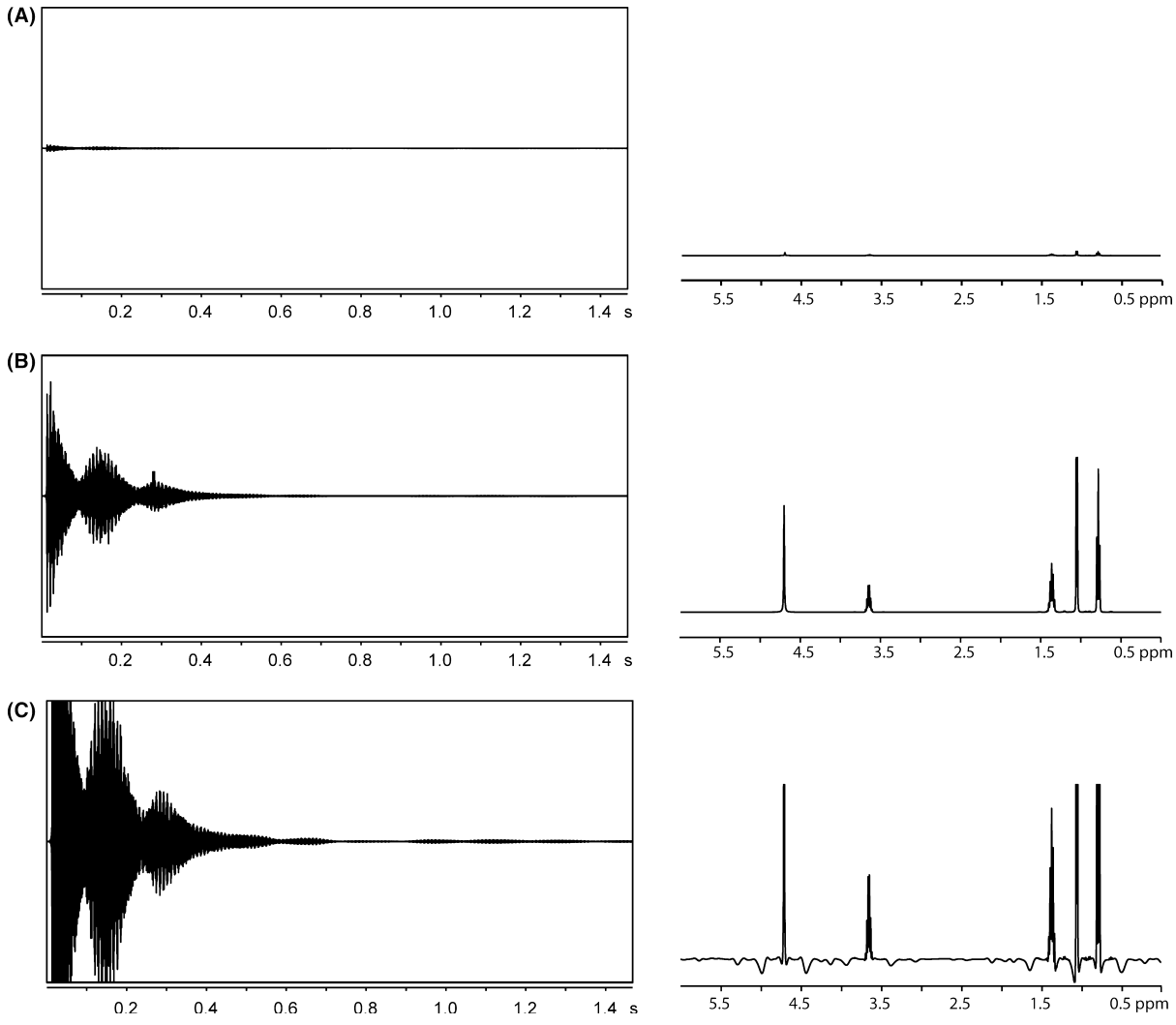

FIGURE 3 Effect of receiver gain setting on the FID and the resulting 1D ^{1}H spectrum of 10%(v/v) 2-butanol in D_2O at 400 MHz. The FIDs (left column) and their corresponding NMR spectra (right column) are displayed with the same scaling. (A) receiver gain set too low leading to a very small (poorly digitized) FID and small NMR peaks, (B) receiver gain set to optimum leading to a large well digitized FID and large undistorted NMR peaks, and (C) receiver set too high leading to a "clipped" FID and with distorted NMR peaks and baseline

2.3.1 | Solution

The application of exponential decaying function before Fourier transformation is a common practice in NMR data processing to "smoothed" the FID so that it is no longer truncated and smoothly decays to zero and the resulting spectrum is devoid of sinc function wiggles. This window function is a characterized by linebroadening parameter expressed in Hz which determines the rate of decay of the smoothen (apodized) FID and the broadening of the peaks in the spectrum. As shown in Figure 4C, the resulting spectrum contains no visible wiggles however the resulting peaks are broader. There appears to be "increase in S/N" (less visible noise) because of exponential multiplication but it is only an apparent increase. In addition, the above problem can be remedied by multiplying (convoluting) the FID with a "matched" decaying exponential broadening function (commonly referred to as a matched filter) with a line broadening value equal to the linewidth of the peak. Alternatively, the acquisition time can be extended to accommodate more signals until they decay substantially – but especially in multidimensional experiments this is rarely a practical solution because of the overall experimental time limitations. Note, however, that setting the acquisition time too long leads to the recording of wasteful data points with no significant signal. At worst, in dilute samples when the magnitude of signal and noise are comparable, extending the acquisition time could lead to a deleterious increase in the proportion of noise over signal being included in the FID, which in turn, leads to a significant decrease in the signal-to-noise ratio (S/N) of the spectrum. This is clearly exemplified in Figure 5. Thus, if a longer recycle time is required it should be added to the delay before the first RF pulse and not by extension of the

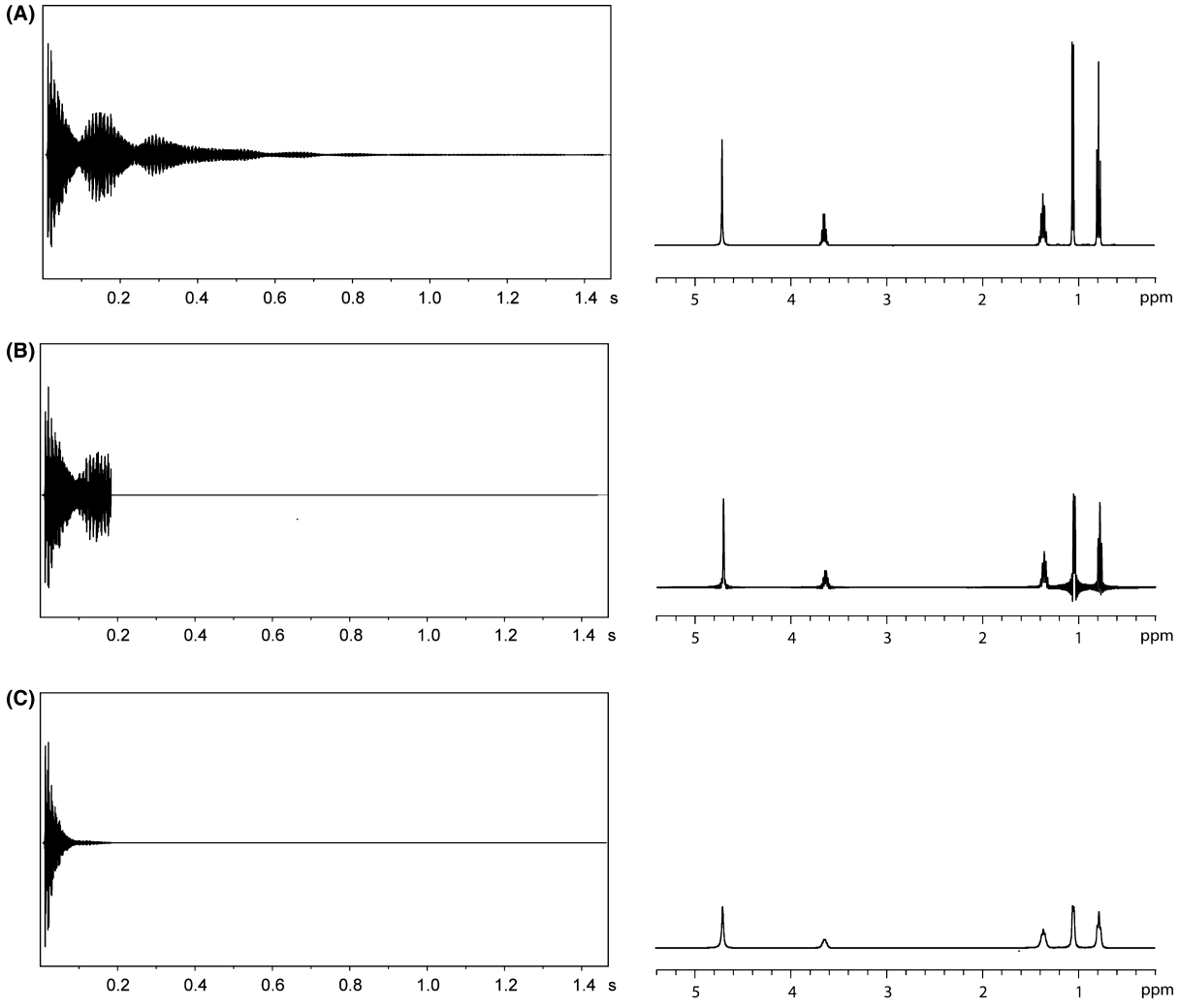

FIGURE 4 Effect of short acquisition time on the ^{1}H FID (left) and spectrum (right) of 10% (v/v) 2-butanol in D_2O obtained at 400 MHz. (A) Acquisition time set sufficiently long to allow the FID to decay completely leads to undistorted peaks, (B) acquisition time set too short leads to a clipped FID and distorted peaks and (C) application of exponential broadening function with a line broadening parameter of 5 Hz to the FID in (B) eliminates distortion wiggles but creates broad peaks

acquisition time. One could also make use of "zero-filling" during processing instead of increasing the acquisition time. Here, zero amplitude data points are appended to the end of the FID to increase the total number data points. A two-fold increase in the number of data points by zero-filling has the beneficial effect of increasing the resolution of peaks (to a limited extent), especially for the definition of multiplet structures in ^{1}H spectra.

3 | LOW SENSITIVITY

3.1 | Low sample concentration

The sensitivity of an NMR experiment depends on numerous factors. The most obvious being the concentration of the components of interest in the NMR sample. More accurately NMR sensitivity is proportional to the amount of sample in the effective detection region of the probe which corresponds to ~1.6 cm in length for a 5 mm probe. In a typical experiment, a sample volume of 0.5-0.6 mL in a standard 5 mm NMR tube is normally prepared and this yields a sample length of ~4-5 cm which is much greater than the effective detection length (i.e., the NMR sample extends beyond the RF coil). This large volume is required to ensure magnetic homogeneity in the sample for ease of shimming (see Figure 6A). As noted above. The use of a shorter sample would introduce inhomogeneity caused by the liquid to air interface which would lead to shimming problems and thus distorted lineshapes. Note the sample edges perpendicular to the field cause no distortion.

3.1.1 | Solution

If the availability of the solute is limited, a more concentrated sample with considerably less volume can, assuming no deleterious concentration effects like molecular association, be used in two special NMR sample tube set-ups shown in Figure 6B,C. In both cases, the short liquid sample is physically constrained by solid structures (below and above the liquid) which are magnetically susceptibility "matched" to the liquid solvent. The first set-up shown in Figure 6B uses standard NMR tube with Doty® susceptibility plugs which are threaded and can be positioned or moved inside the NMR tube using a long detachable screw. The second set-up (Figure 6C) uses a special NMR tube from the Japanese NMR tube manufacturer Shigemi. This unique NMR tube incorporates a plunger and a base which are both made from special glass that matches the magnetic

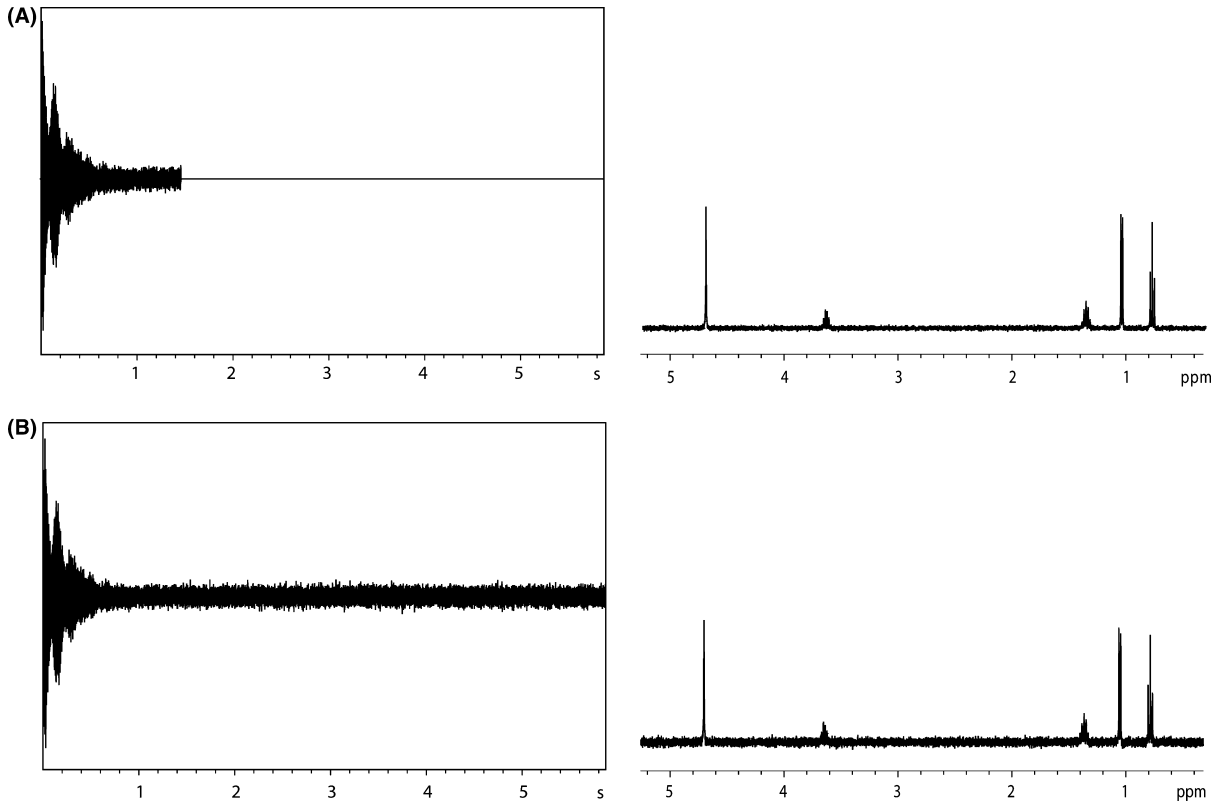

FIGURE 5 Effect of long acquisition time on the ^{1}H FID (left) and spectrum (right) of 10% (v/v) 2-butanol in D_2O obtained at 400 MHz. The RF pulse duration was set deliberately short with a low power level so that the solute signals obtained were comparable with the noise. (A) Acquisition time set sufficiently long to allow the FID to completely decay yields a spectrum with visible noise (B) acquisition time set too long leads to more intense noise and thus a lower S/N

susceptibility of the solvent allowing a small amount of concentrated sample to be physically constrained to a limited length and be positioned within the effective detection region of the probe. As the glass plug and base are susceptibility matched to the solvent, the sample appears magnetically like a normal sample thereby facilitating shimming and yielding peaks with acceptable lineshape. The beauty of these two sample set-ups is that the shimming procedure is still identical to that of shimming a magnetically long sample.

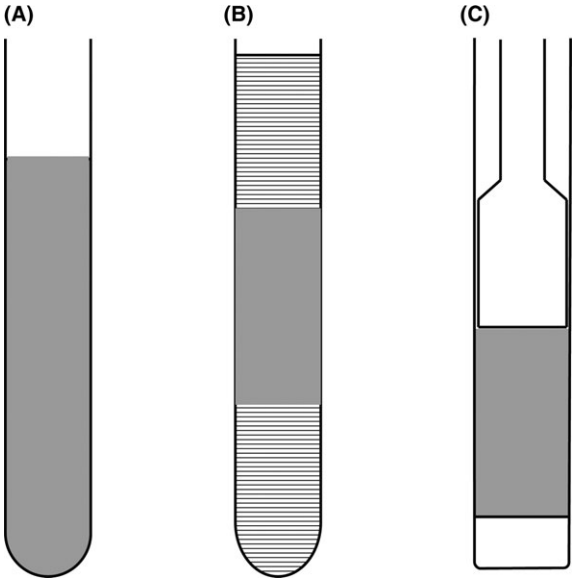

FIGURE 6 Three types of NMR sample tube set-up. (A) Standard 0.5 mm o.d. NMR tube with ~0.5 mL liquid (shown in gray) (B), standard NMR tube with Doty® susceptibility plugs (indicated region with horizontal lines) and with <0.5 mL liquid and (C) susceptibility "matched" Shigemi® NMR tube with typically <0.2 mL liquid. The Doty plugs and two parts of the Shigemi tube are magnetically susceptibility matched to the sample so that although the real sample is physically short (i.e., the gray cylindrical volume on the right), the entire tube assembly and sample appear magnetically long, like the standard tube shown in (A), which facilitates shimming

It is important to note that the length of an NMR sample can have a substantial effect on 90° pulse duration. This is because of the RF field inhomogeneity in the sample as the RF coil is relatively short and is centered at a fixed "optimum" position in the probe. Shorter samples centered appropriate in the probe is situated in a relatively more homogeneous region of the RF field than that in the longer samples. It is therefore expected that the 90° (and 180°) pulse duration will be shorter for shorter samples. Pulse calibration profiles for 2.4 and 1.0 cm D_2O samples in Shigemi tubes focusing on the residual HOD signal are shown in Figure 7. Pulse duration of 18 μs yields an almost negligibly small peak for the relatively long 2.4 cm sample and this therefore corresponds to the 180° pulse length for this sample. By comparison, the corresponding HDO signal for the short 1.0 cm sample is larger but inverted meaning that the 180° pulse duration for this shorter sample is shorter than 18 μs. The overall trends of pulse durations for the short and long samples are also reflected by the rest of the HOD peak amplitudes.

3.2 | Improper setting of number of scans, repetition time and pulse duration

The sensitivity which is measured as a signal-to-noise ratio (S/N) is proportional to the square root of the number of scans ($\sqrt{n}$). Performing more scans increases sensitivity, however, the number of scans is recommended to be in multiples of at least four or eight so that the minimum phase cycle steps of a particular NMR pulse sequence (which are designed to remove unwanted artifacts and also,

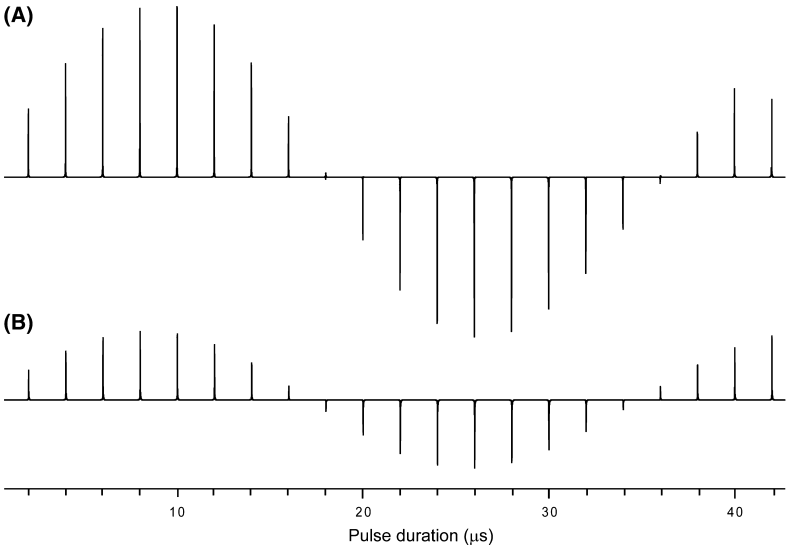

FIGURE 7 ^1H NMR pulse calibration profiles of different length D_2O samples in Shigemi tubes at 600 MHz. The sample lengths are (A) 2.4 cm and (B) 1.0 cm. The HOD peaks in the spectra are plotted with increasing pulse duration

perhaps, coherence selection) can be completed. The folding peaks artifacts ("quadrature images") that could appear if fewer than four scans are acquired in a 1D experiment are illustrated in Figure 8.

This type of artifact occurs as a result of imbalance in the quadrature detector commonly encountered only in older spectrometers and is easily fixed by acquiring an even number of scans. The effect of the number of scans on sensitivity (or signal-to-noise) is shown in Figure 9.

3.2.1 | Solution

Clearly, performing more scans leads to an increase in the sensitivity, however, in most cases, NMR time is limited so that there is a practical need to optimize the experiments to achieve maximum sensitivity in a given time. The crucial factors to consider in optimizing NMR experiments are the total time available specifically the time for each scan (i.e., the "repetition time," RT: the total time for each that includes the recycle delay time, pulse sequence time and the signal acquisition time), the longitudinal relaxation time T_1 of the slowest relaxing component of interest and how long the signal persists (related to its transverse relaxation time T_2).

It is recommended that the acquisition time be shortened so that the resulting FID just contains the decaying signal (and excludes the flat baseline that contain mostly noise). As noted above this increases sensitivity. Many NMR users also employ a smaller pulse angle usually 30° and 45° and higher repetition rates arguing that this smaller angle allows shorter recovery times. This argument has since been contested[9] and there is thus no real benefit in using smaller pulse angle and thus a relatively large pulse angle should be employed. Nevertheless, for increased sensitivity in simple pulse and acquire method, there is a recommended pulse angle (β) for a given RT and longitudinal time T_1 which is referred to as the "Ernst angle" given by,[9–11]

$$\cos(\beta) = e^{-RT/T_1}. \quad (1)$$

Note that it may be necessary to tune and match the probe and perform accurate pulse calibration as the 90° pulse duration can vary considerably for different samples especially with broadband probes that have been used previously on other nuclei. If accurate integration of peaks is required full relaxation between scans is required and this is discussed in detail in Section 4.

3.3 | Receiver gain set too low

The receiver gain can be set manually by the NMR user but can be set automatically by the instrument based on the largest signal detected. This parameter is optimized so that the biggest signal efficiently utilize the ADC range. This will result in the largest signal (and other signals) being "digitized" maximally to better discriminate it from the noise. Thus, this ensures an overall increase in S/N for all signals. Very low receiver gain results in poor S/N (see Figure 10). This is because of the

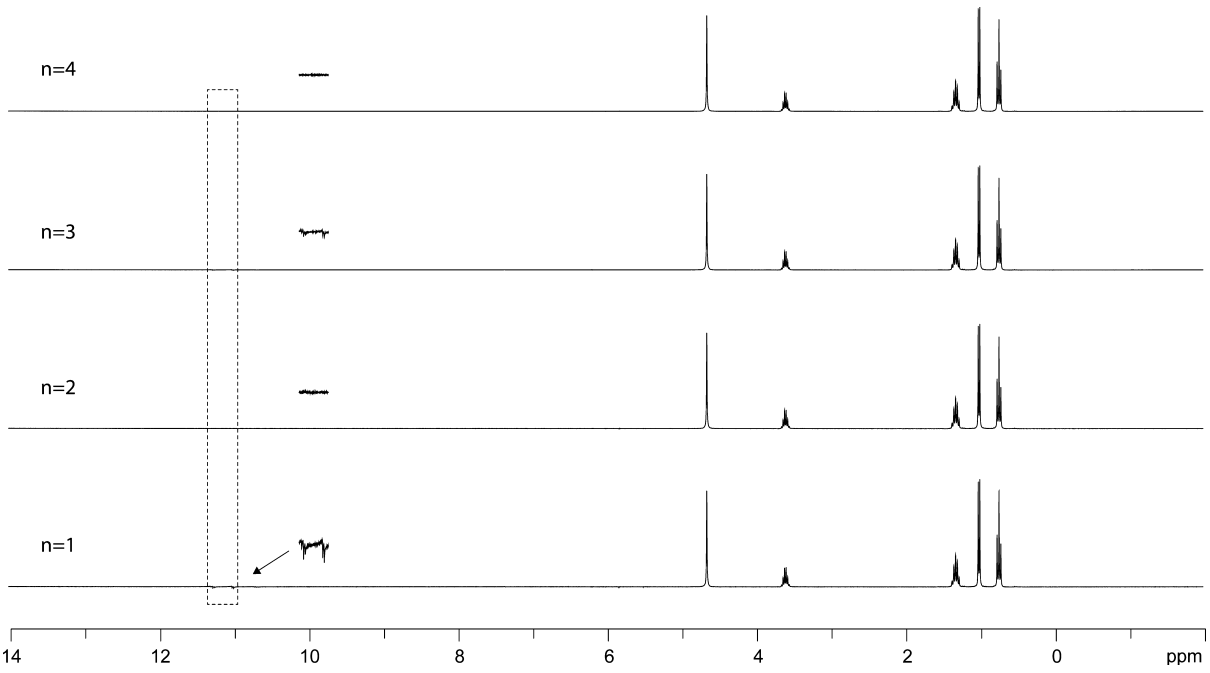

FIGURE 8 Effect of number of scans (n) on "folded peaks" in the ^{1}H 300 MHz 1D of spectrum of 10% (v/v) 2-butanol in D_2O. Regions surrounded by the dotted rectangle are magnified (inset after arrow) to illustrate folded peaks. n=1 with "folded peaks" (bottom), n=2, n=3 with small "folded peaks" and n=4 no folded peak (top). Experiments were performed on an old Varian Mercury 300 spectrometer

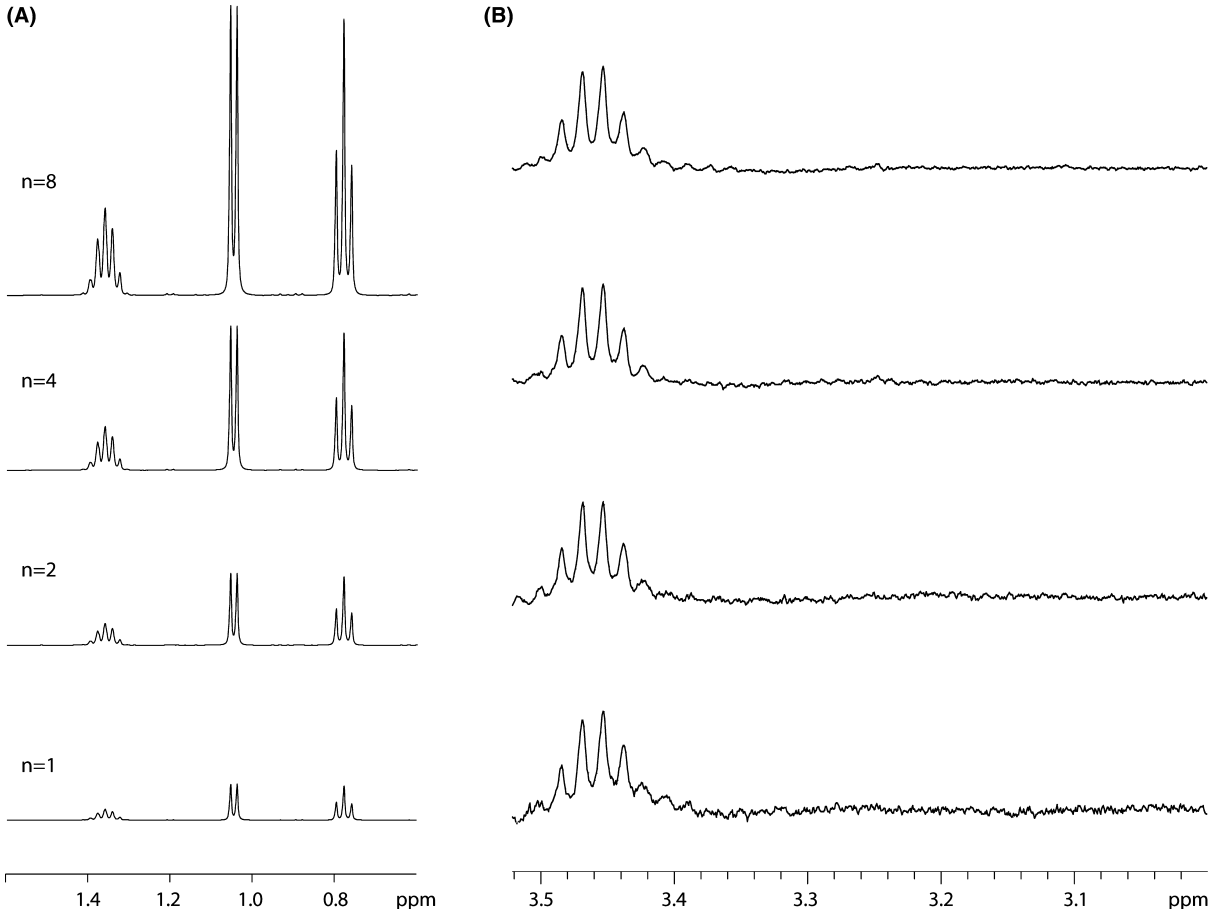

FIGURE 9 Effect of number of scans (n) on sensitivity of 1D spectrum of 10% (v/v) 2-butanol in D_2O at 400 MHz. (A) Spectra are scaled in absolute intensity to illustrate the increase in signal with scans. (B) Spectra are rescaled with equal maximum intensity to illustrate the increase in sensitivity or S/N. The peak shown here is the multiplet of one the ^{13}C satellites

ADC range (or levels) being not fully utilized by the signals leading to the signals and noise being digitized to a similar extent. Increasing the receiver gain would lead to higher S/N as the signals which are larger (than the noise) will start occupying more ADC levels than the noise. Again, as discussed previously, care has to be taken in increasing the receiver gain so as not to clip the FID.

3.3.1 | Solution

Increase the receiver gain until clipping of the signal is obtained. The final receiver gain setting should then be set two dB lower than this clipping receiver gain value.

3.4 | Strong solvent signals

Very intense solvent signals not only obscure nearby signals but also deleteriously affect the S/N of the desired solute component. While the intense solvent signal is digitized maximally, the much smaller solute signals are not digitized to the same extent, thus resulting in sensitivity loss.

3.4.1 | Solution

Decreasing or suppressing the solvent signals relative to other signals would therefore allow the receiver gain to be increased and thus increase the S/N of the solute. There are a variety of ways that solvent signals can be suppressed or reduced,[12] and the most common of these methods are solvent presaturation and WATERGATE (WATER-suppression by GrAdient-Tailored Excitation).[13,14] Various spectra obtained with and without solvent suppression are shown in Figure 11. As these methods also suppress and reduce solute signals near the solvent care has to be taken in using them.

In presaturation, a weak but long continuous RF field is applied selectively at the solvent resonance frequency before the excitation period. This solvent saturation process is performed usually during the recovery delay time which typically ranges from one to a few seconds. The simplicity and the ease of implementation are the drawcards for this technique, however, compared with other techniques such as WATERGATE, it is not very efficient at suppressing solvent signals and spin diffusion can occur especially in

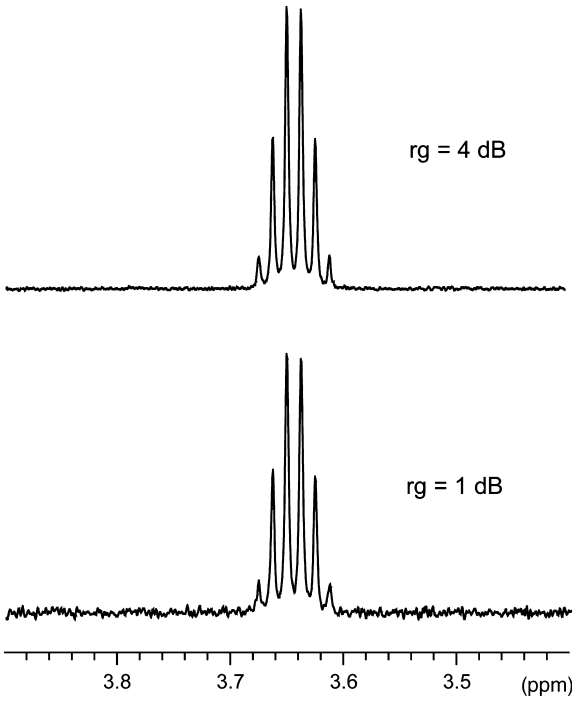

FIGURE 10 Effect of receiver gain (rg) on sensitivity in the ^{1}H 400 MHz 1D spectrum of 10% (v/v) 2-butanol in D_2O. Sensitivity or signal-to-noise at rg=4 dB (top) is much higher than that at rg=1 dB (bottom)

macromolecules where resonances near the solvent peak that are also suppressed affect the intensity of others peaks by cross relaxation. It is also recommended that the power of the RF field be kept to low values as possible to minimize sample heating. Note the sample heating is internal to the sample and will take the sample above the temperature set for the experiment.

The WATERGATE solvent suppression technique involves the use of a frequency selective pulse train and magnetic field gradient pulses in a spin-echo pulse sequence. This highly efficient solvent suppression technique works by selectively dephasing the solvent signals. Although the technique is very popular in that it can substantially reduce the solvent, it has some serious limitations in selectivity as it also obliterates resonances near the solvent and those at some fixed distances from the solvent resonance near the edge of the spectrum. Thus, WATERGATE has been a subject of numerous studies for further improvement. Among these include the use of five pairs of binomial selective pulses referred to as W5[15] and incorporation of excitation sculpting scheme[16] that uses two WATERGATE sequences. Most recently, a new extremely selective solvent suppression method referred to as WaterControl[17] has been devised. This technique incorporates two W5 based WATERGATE sequences and utilizes the difference in diffusion coefficient between the solute and solvent for selectively attenuating the solvent signal.

4 | INACCURATE INTEGRALS

Accurate peak integrals especially in 1D ^{1}H pulse and acquire experiments are a necessary part of structure determination as they reflect the relative number of protons in the molecule. This is generally not important for ^{13}C NMR as signals are usually obtained with ^{1}H decoupling resulting in NOE enhancements which add to the peak integrals. One important factor to consider in obtaining accurate integral is the baseline which is usually overlooked and not corrected during spectral processing. Baseline artifacts can occur if there is a big difference in the intensities of the solute and solvent peaks so that the strong solvent signal peak is carried over to the solute resulting in a baseline that is not flat. There are many baseline correction procedures available within the processing software that can remedy this problem.

Besides the baseline, there are two important parameters that need to be looked at in obtaining accurate integrals: dummy scans and the repetition time of each scan. Dummy scans or steady state scans are implemented to shorten the experimental time as waiting for the spins to relax back to equilibrium value could be unacceptably long.

These scans implement the pulse sequence without data acquisition and are tailored to ensure that the spin systems are in a "steady state" before acquisition of the signal so that subsequent scans are obtained in similar condition as the first scan. This requires that each nucleus relaxes back to the same initial magnetization at the start of each subsequent scan. The repetition time for each scan on the other hand is mainly controlled by the relaxation delay employed at the start of the NMR pulse sequence. This delay is usually set to 1 to 5 times the T_1 value of the slowest relaxing spin. Note that to obtain very accurate integrals without dummy scans, the repetition time has to be set to at least 5 times T_1. For many experiments, employing dummy scans with relaxation times of 2-3 s for pulse and acquire is sufficient to obtain acceptable integrals. However, this is not always the case and error in the integral could lead to confusion, or worse, incorrect analysis. The problem that results when the repetition time and number of dummy scans are set to non-optimal values is illustrated in Figure 12.

4.1 | Solution

Properly set the repetition time and the number of dummy scans. For accurate integrals, the repetition time should be set to $5T_1$ to ensure the spins have relaxed back to 99.3% of their equilibrium value while avoiding the use of dummy scans. It has been shown through more thorough analysis that pulse repetition time of $4.5T_1$ is sufficient in most cases.[9]

Note that a common mistake in setting-up NMR experiment is improperly setting of the number of dummy scans. One dummy scan is almost certainly insufficient to reach a

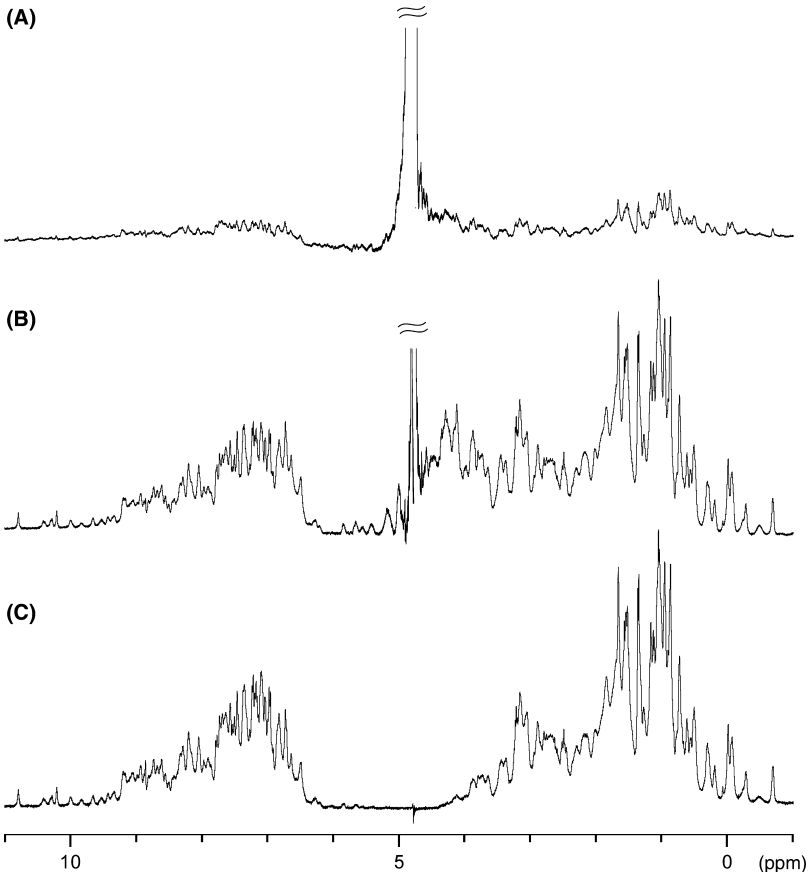

FIGURE 11 Effect of strong solvent signal in ^{1}H 400 MHz 1D spectra of 2 mM lysozyme in 90% H_2O and 10% D_2O. (A) Pulse and acquire experiment with no solvent suppression, (B) with solvent presaturation and (C) with WATERGATE solvent suppression

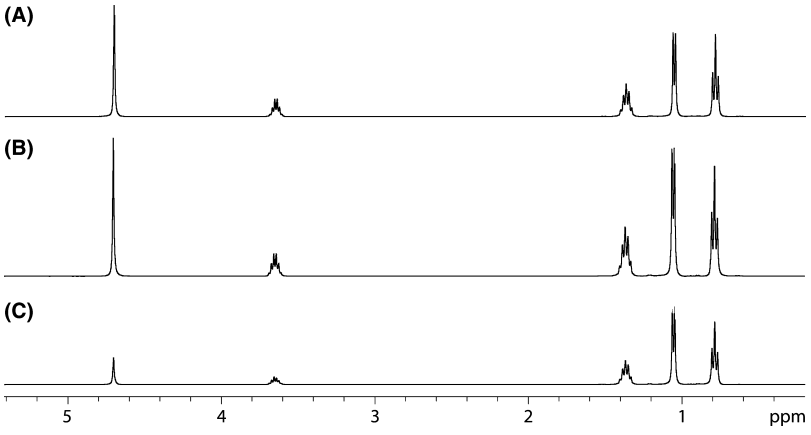

FIGURE 12 Effect of repetition time and dummy scans on the ^{1}H 400 MHz spectrum of 1D spectrum of 10% (v/v) 2-butanol in D_2O. The HOD signal has the slowest T_1 of ~14 s. (A) Fully-relaxed spectrum obtained with 1 scan and no dummy or steady state scan, (B) with repetition time set to ~0.04 × T_1 of HOD (4.7 ppm) for 4 scans and no dummy scan (C) with repetition time set to ~0.04 × T_1 of HOD for 4 scans and 4 dummy scans

steady state. At least four dummy scans should be set to ensure that the spin system has reached a steady state before acquisition. However, if the repetition time is set to allow full relaxation it is pointless to include dummy scans.

5 | FOLDED OR ALIAS PEAKS DUE TO INSUFFICIENT SPECTRAL WIDTH

5.1 | 1D NMR

This is caused by using a spectral width (SW) insufficient to encompass all of the peaks in the spectrum. In older spectrometers with no oversampling and digital filtering, insufficient spectral width could result in the peaks outside spectral range being "folded in" or "aliased" in the 1D spectrum. This is illustrated for 2-butanol in Figure 13.

These artifactual peaks can be usually distinguished from other peaks as they will be harder to phase or move in a non-linear and seemingly illogical manner when the transmitter offset is changed. Becaue of the introduction of oversampling and digital filtering in newer spectrometers, such outlying peaks do not usually appear when working with common nuclei (e.g., ^{1}H).

The signal sampling rate determines the SW of the resulting NMR spectrum. The Nyquist theorem states that

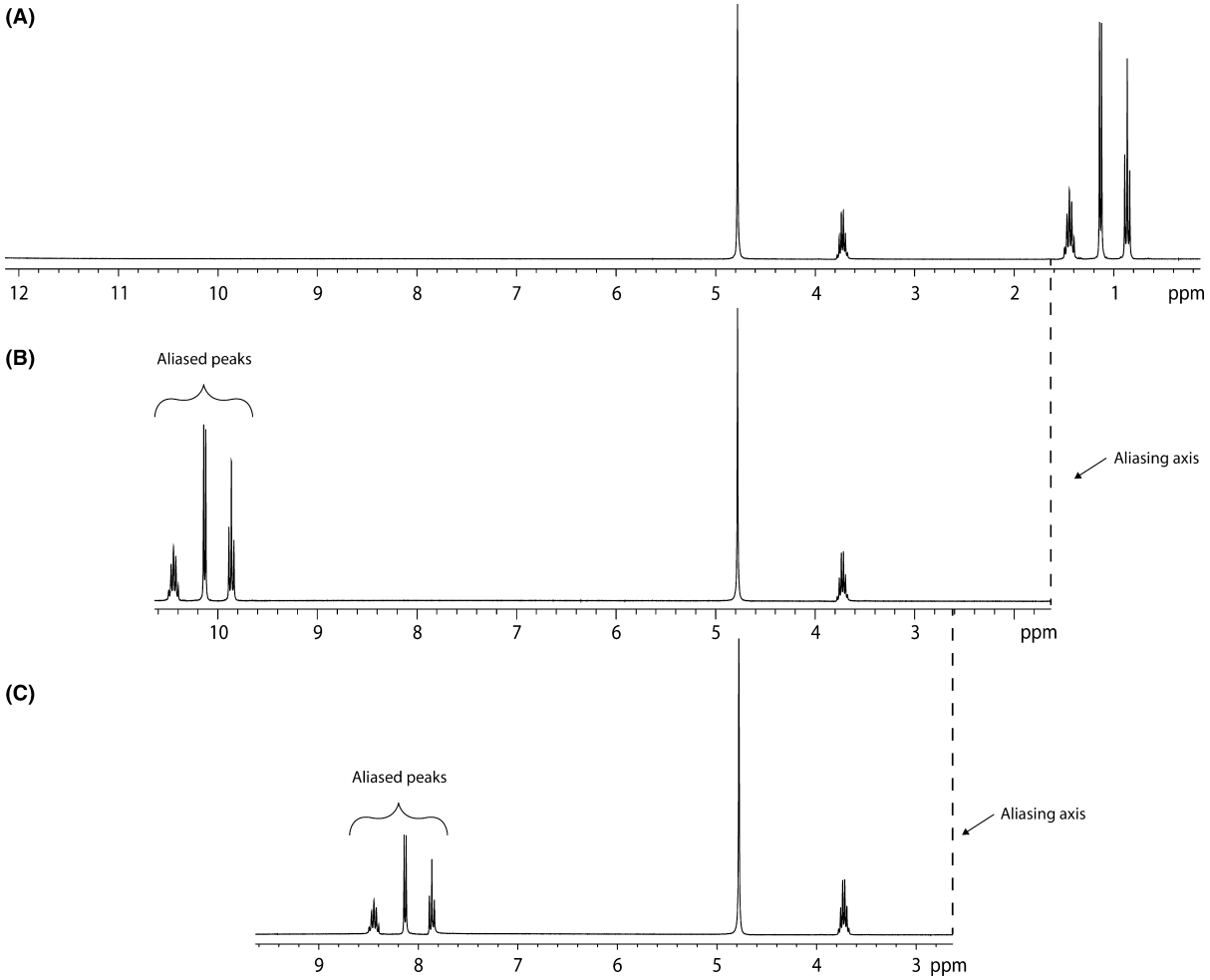

FIGURE 13 Aliasing of methyl and methylene peaks of 2-butanol because of insufficient SW in 1D ^{1}H NMR experiments. The sample consists of 10% (v/v) 2-butanol in D_2O acquired at 300 MHz using an old Varian Mercury 300. (A) Reference spectrum with wide SW showing peaks at correct positions. (B) Spectrum with insufficient SW causing peaks outside the SW to be aliased to the left end of the spectrum. The aliased peaks are located at exactly one SW to the left of their true position. (Note that in old Bruker spectrometer, the aliased peak would appear as folded peak located to the right end of the spectrum because of different modes of quadrature detection.) The aliasing axis shown by the dotted vertical line aids in visualizing the position of alias peaks in comparison with the reference spectrum in (A). (C) Spectrum with much narrower SW than (A) and (B). In comparison with those in (B), alias peaks are more centrally situated

the sampling rate which is inversely proportional to the dwell time (DW) or time between two sampling points in the (digitized) FID has to set twice the width or simply 1/DW=2 × SW. Besides sampling rate, there are other factors to consider in setting-up the spectral width in an NMR experiment. These include the digital resolution needed, the relaxation or signal decay rate and the possible RF pulse excitation range.

The frequency excitation range of an RF pulse is limited and is determined by the duration of the pulse which is usually in tens of microseconds. The RF excitation of a pulse of duration t_p has an effective spectral width SW given by SW=2/t_p.[6] Thus, for a 10 μs RF pulse this translates to spectral width of 200 000 Hz. This is not a big problem for 1D NMR proton and carbon but for other nuclei such as fluorine, platinum, and cobalt chemical shifts can vary widely, so that setting-up the right spectral width can present a big challenge. In fact for some nuclei with extremely large chemical shift ranges (e.g., ^{59}Co) it is generally not possible to set the SW to span the entire possible chemical shift range in one go.

5.1.1 | Solution

To check for the presence of folded or aliased peaks, increase SW to the maximum value and move the transmitter frequency slightly; examine the positions of the peaks relative to each other. Do the positions in the spectrum remain in the same relative position when you change the transmitter frequency? – if not it is almost certainly a fold

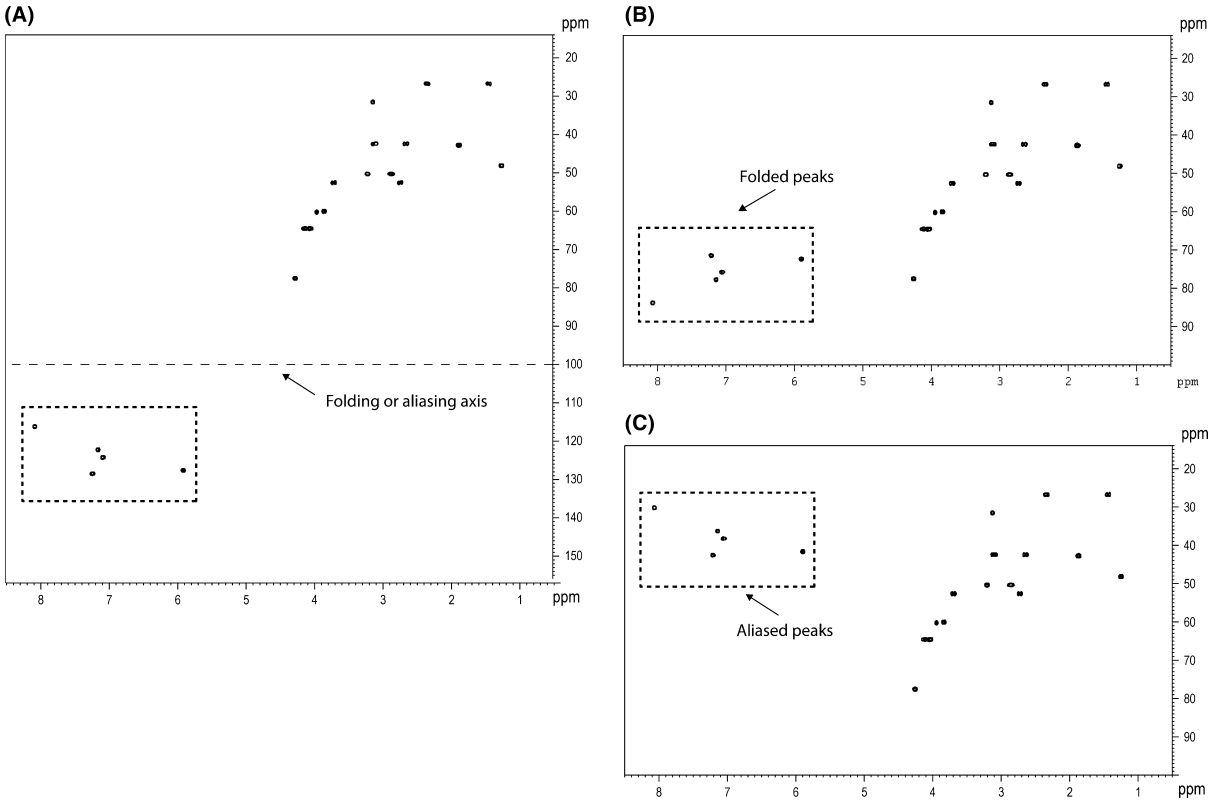

FIGURE 14 Folding or aliasing of peaks in 400 MHz 2D HMQC spectrum of strychnine in $CDCl_3$ (50 mg/0.5 mL) because of the insufficient SW in the F_1 dimension. (A) reference spectrum with sufficient SW. Indicated by dashed line is the folding or aliasing axis which indicates the edge of SW for experiments with insufficient SW (B, C). The peaks enclosed by the dashed rectangle are those which will lie outside the SW. (B) Spectrum with insufficient SW acquired in TPPI mode causing peaks outside of SW to be folded and (C) Spectrum with insufficient SW acquired in States mode peaks causing peaks outside of SW to be aliased

in or (an alias). Attach an analog Butterworth filter (an electronic device that removes unwanted frequencies) to the preamplifier to remove or minimize the occurrence of these peaks, however folded peaks could still appear especially if they are very intense.

For other nuclei besides proton and carbon, it might be necessary to perform a series of NMR experiments at various transmitter frequencies to cover the possible SW. It is also important to consider the digital resolution needed and the sampling rate limitation in setting-up the spectral width.

5.2 | 2D NMR

The occurrence of out of range artifact peaks is more common in 2D NMR than in 1D NMR as no oversampling or digital filtering can be performed in the indirect dimension of a 2D experiment. There are two major of phase sensitive acquisition modes that are popularly used in detecting signals indirectly in multidimensional NMR experiments, the Time Proportional Phase Incrementation (TPPI[18]) and the States method introduced by States, Haberkorn and Ruben.[19] Although these two methods yield equivalent spectra, the location of the out of range artifacts peaks differ. In the TPPI method, these artifacts appear as folded peaks while in the States they appear as aliased peaks. 2D spectra obtained with sufficient SW and with insufficient SW acquired using TPPI and States are presented in Figure 14.

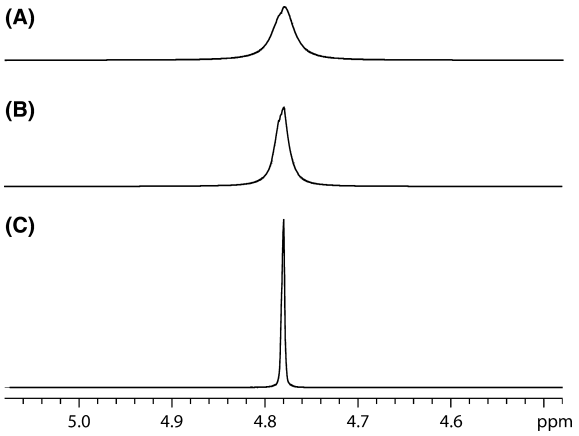

FIGURE 15 Effect of radiation damping on the 400 MHz ^1H 1D spectrum of sample with various H_2O and D_2O concentrations. (A) 90% H_2O/10% D_2O, (B) 50% H_2O/50% D_2O and (C) 1% H_2O/99% D_2O

5.2.1 | Solution

Similar approaches to those given for the 1D can be adopted.

6 | RADIATION DAMPING

Radiation damping effects[20–22] are caused by an intense signal usually associated with the water signal – however in theory any strong resonance. It is observed in aqueous samples where the water signal is so intense that it generates its own effective RF field that acts on water nuclei itself, ultimately leading to effectively enhanced water relaxation. This effect is analogous to the feedback audio noise generated in a public-address system when a microphone is held too close in front of a speaker. Radiation damping manifests in the NMR spectrum as unusually broadened water peaks that can obscure peaks that are close to the water peaks (see Figure 15). This also distorts the intensity, phase and symmetry of the strong signal making it tricky to perform RF pulse calibrations with such a peak. For example, the application of the "true" 90° pulse will not actually yield the maximum signal intensity while the supposedly 180° pulse will still shows substantial signal intensity.

6.1 | Solution

The effect of radiation damping can minimized by using solvent suppression as described above or by simply using a smaller sample volume. Another effective way is detuning the probe making it less sensitive or reactive to RF induction from intense solvent signal. Additionally, employing lower receiver gain settings can also be useful. Note that these remedies (besides the solvent suppression technique) also decrease the overall sensitivity. Q-switching is the best choice but few spectrometers are capable of this.[21]

As mentioned earlier, calibrating the 90° and 180° pulse durations in the presence of radiation damping can be difficult as the additional RF field complicates the spectrum. The determination of a 360° pulse duration in this case is more appropriate.

7 | CONCLUDING REMARKS

NMR experiments incorporate many parameters that need to be set properly to obtain useful or meaningful results. It is important for NMR users to be able to identify many of these common problems and artifacts that may occur and be able to eliminate them.

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REFERENCES

1. Richards SA, Hollerton JC. *Essential Practical NMR for Organic Chemistry*. New York: Wiley; 2011.
2. Jacobsen NE. *NMR Spectroscopy Explained Simplified Theory, Applications and Examples for Organic Chemistry and Structural Biology*. Hoboken, NJ: Wiley-Interscience; 2007.
3. Berger S, Braun S. *200 and More NMR Experiments*. New York: Wiley; 2004.
4. Fukushima E, Roeder SBW. *Experimental Pulse NMR: a Nuts and Bolts Approach*. Reading, MA: Addison-Wesley; 1993.
5. Martin ML, Martin GJ, Delpuech JJ. *Practical NMR Spectroscopy*. London: Heyden & Son Ltd; 1980.
6. Claridge TDW. Chapter 3 - Practical aspects of high-resolution NMR. In: Timothy DWC, ed. *Tetrahedron Organic Chemistry Series*, Volume 27. New York: Elsevier; 2009:35-98.
7. Price WS, Stilbs P, Jönsson B, Söderman O. Macroscopic background gradient and radiation damping effects on high-field PGSE NMR diffusion measurements. *J Magn Reson*. 2001;150: 49-56.
8. Chmurny GN, Hoult DI. The ancient and honourable art of shimming. *Concepts Magn Reson*. 1990;2:131-149.
9. Traficante DD. Optimum tip angle and relaxation delay for quantitative analysis. *Concepts Magn Reson*. 1992;4:153-160.
10. Ernst RR, Anderson WA. Application of Fourier transform spectroscopy to magnetic resonance. *Rev Sci Instrum*. 1966;37:93-102.
11. Ernst RR. Sensitivity enhancement in magnetic resonance. *Adv Magn Reson*. 1966;2:1-135.
12. Zheng G, Price WS. Solvent signal suppression in NMR. *Prog Nucl Magn Reson Spectrosc*. 2010;56:267-288.
13. Piotto M, Saudek V, Sklenar V. Gradient-tailored excitation for single-quantum NMR spectroscopy of aqueous solutions. *J Biomol NMR*. 1992;2:661-665.
14. Zheng G, Stait-Gardner T, Anil Kumar PG, Torres AM, Price WS. PGSTE-WATERGATE: an STE-based PGSE NMR sequence with excellent solvent suppression. *J Magn Reson*. 2008;191: 159-163.
15. Liu M, Mao X-a, Ye C, Huang H, Nicholson JK, Lindon JC. Improved WATERGATE pulse sequences for solvent suppression in NMR spectroscopy. *J Magn Reson*. 1998;132:125-129.
16. Hwang TL, Shaka AJ. Water suppression that works. Excitation sculpting using arbitrary wave-forms and pulsed-field gradients. *J Magn Reson Ser A*. 1995;112:275-279.
17. Zheng G, Torres AM, Price WS. WaterControl: self-diffusion based solvent signal suppression enhanced by selective inversion. *Magn Reson Chem*. 2016; doi:10.1002/mrc.4420.
18. Marion D, Wüthrich K. Application of phase sensitive two-dimensional correlated spectroscopy (COSY) for measurements of 1H-1H spin-spin coupling constants in proteins. *Biochem Biophys Res Commun*. 1983;113:967-974.
19. States DJ, Haberkorn RA, Ruben DJ. A two-dimensional nuclear overhauser experiment with pure absorption phase in four quadrants. *J Magn Reson*. 1982;48:286-292.

20. Szántay C, Demeter Á. Radiation damping diagnostics. *Concepts Magn Reson*. 1999;11:121-145.
21. Price WS, Wälchli M. NMR diffusion measurements of strong signals: the PGSE-Q-switch experiment. *Magn Reson Chem*. 2002;40:S128-S132.
22. Bloembergen N, Pound RV. Radiation damping in magnetic resonance experiments. *Phys Rev*. 1954;95:8-12.

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