Low-Field NMR is Back

Real-Time Chemical-Shift Scaling in High-Resolution NMR Spectroscopy

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Superconducting magnets have brought many blessings to NMR spectroscopy, but these are not entirely unmixed. Problems arise particularly with systems undergoing chemical exchange, since linewidth is proportional to the square of the field strength in the fast exchange limit; here, lower-field spectra can show both higher sensitivity and higher resolution. Unfortunately, relatively few laboratories now have

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access to low-field NMR instrumentation. A new technique, real-time chemical-shift scaling (δ scaling) is proposed which causes the apparent chemical-shift to be scaled down during signal acquisition, allowing the effects of chemical exchange and scalar coupling on spectra to be controlled.

The selective scaling of NMR parameters such as chemical shifts and scalar and dipolar couplings has been exploited extensively in solid-state NMR spectroscopy.[1] Applications to high-resolution NMR spectroscopy have been more limited, although scaling has been used with success in indirect (parameter time) dimensions in multidimensional NMR spectroscopy.[2] Heteronuclear J scaling in high-resolution NMR spectroscopy has been carried out in parameter time[3,4] and in real time,[5] but since its first proposal[6] and one subsequent refinement,[7] only a single application of real-time δ scaling in high-resolution NMR spectroscopy appears to have been reported.[8] Paradoxically, this last report made no use of the scaling effect, rather exploiting the sensitivity to imperfections of the pulse sequence to achieve spatial localization. A special case of real-time δ scaling, refocussed acquisition, has however been used in the past to improve sensitivity in parameter time experiments for J scaling[3] and for the spectroscopy of systems with inhomogeneously broadened signals,[9] and recently in multiple-quantum NMR spectroscopy.[10]

A new approach to real-time δ scaling is proposed here, based on repeated refocussing[3] rather than phase-alternated pulses of variable flip angle.[6,7] Figure 1 shows one practical

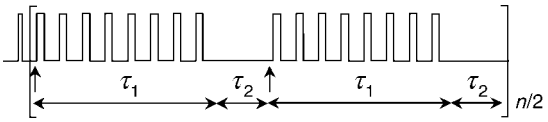

Figure 1. Prototype pulse sequence for the measurement of a spectrum with δ scaling. Narrow boxes indicate 90°, broad boxes 180° pulses; vertical arrows indicate acquisition of one complex data point. The phases of successive 180° pulses follow the *XY*-16 supercycle $x,y,x,y,y,x,y,x,-x,-y,-x,-y,-y,-x,-y,-x$, with a total of n complex points being acquired.

realization. The signal elicited by a simple 90° pulse is repeatedly refocussed by alternate sequences of 180° pulses forming the two *XY*-8 subcycles of an *XY*-16 supercycle,[11] with one complex data point measured at the end of the precession delay τ_2 following each subcycle. *XY*-16 refocussing has previously been used successfully for chemical-shift suppression ($\lambda = 0$) in the INEPT[12] sections of two-dimensional ^{15}N-correlated NOESY experiments,[13] and there is an interesting parallel in the use of windowed acquisition during DIPSI-2 heteronuclear Hartmann–Hahn transfer by Luy and Glaser.[14] Many other configurations of refocussing blocks, phase sequences, precession intervals and data sampling are possible. The average effect of the chemical shift is scaled down by a factor $\lambda = \tau_2/(\tau_1 + \tau_2)$ for shifts within the spectral window. The use of a phase sequence such as *XY*-16 is essential if the cumulative effect of pulse imperfections is not to result in the rapid attenuation of any transverse magnetization components orthogonal to the radiofrequency pulse axis. The phase sequence is remarkably effective; even with conventional hardware, signals survive several thousand refocussing steps, allowing high resolution to be achieved. All experiments were carried out using a Varian INOVA 400 spectrometer with no relevant hardware modifications. A 5 mm broadband probe with pulsed field gradient inverse geometry was used, with a 90° pulse width of 5 μs.

Figure 2 shows the scaling of a typical high-resolution proton spectrum using the sequence of Figure 1. A clean straightforward scaling of the chemical shifts is achieved; as

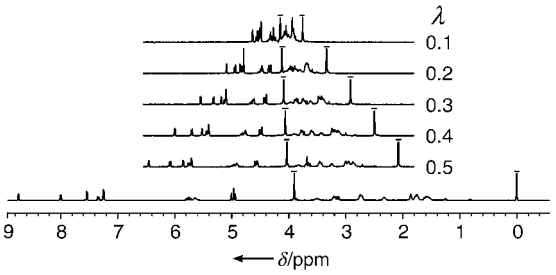

Figure 2. 400 MHz proton NMR spectra of quinine in $CDCl_3$, acquired using (bottom) a single 90° pulse, and (remainder) the sequence of Figure 1 with different scaling factors λ.

expected, the relative signal intensities change as the spin systems become more strongly coupled. Figure 3 illustrates the potential of δ scaling for manipulating the spectra of dynamic systems. The result of applying the pulse sequence of Figure 1 with different scaling factors λ to a simple two-site proton-exchange system (CD_3OH and HDO) which is in the

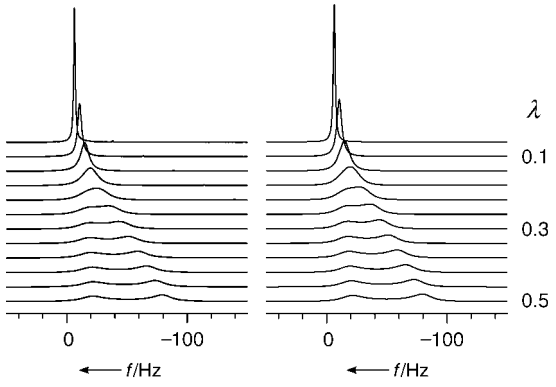

Figure 3. Left: 400 MHz proton NMR spectra of a solution of H_2O in CD_3OD at 40 °C, measured with the sequence of Figure 1. Right: spectra calculated for this spin system with chemical shifts scaled down by the factor λ.

slow-exchange regime under normal conditions ($\lambda = 1$) is compared with the behavior expected from theory.[15] As λ decreases, the system first shows coalescence, then moves into the fast exchange limit, with the peak intensity increasing as the inverse square of the scaling factor. δ scaling is also of potential use in the analysis of spin systems, where it is unclear whether a particular splitting is caused by chemical shift or

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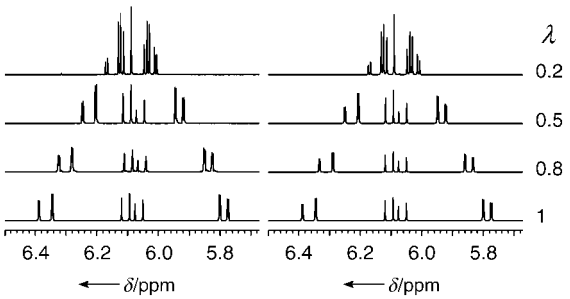

Figure 4. Left: 400 MHz proton NMR spectra of a solution of methyl acrylate in $CDCl_3$, measured with the sequence of Figure 1 (top three spectra) and with a single 90° pulse (bottom spectrum). Right: spectra calculated for this spin system with chemical shifts scaled down by the factor λ and unchanged coupling constants.

homonuclear coupling, or where the relative signs of coupling constants are required. Figure 4 shows scaled spectra for a simple three-spin system, methyl acrylate, comparing theory and experiment.

Real-time δ scaling gives the experimenter considerable freedom to control the apparent chemical-shift scale of a spectrum while retaining close to the full sensitivity of a high-field instrument; the principal drawback is the high radio-frequency power required. The method presented here appears both efficient and much more robust than preceding methods, and is relatively easy to implement on modern spectrometers. Potential applications include the scaling of spectra to improve the sensitivity of signals lost through exchange broadening, the investigation of chemical exchange processes, distinguishing between homogeneous and inhomogeneous broadening, and the analysis of spin systems. Complete scaling ($\lambda = 0$) may be used to reduce by one the dimensionality of multidimensional experiments where proton chemical-shift resolution is either impossible (e.g. because of inhomogeneous broadening in heterogeneous samples) or unnecessary (as for example in some applications of multiple-quantum NMR spectroscopy), while retaining high sensitivity; the use of *XY*-16 or similar phase sequences greatly improves the efficacy of such techniques.

Particularly promising is the application to biomolecules such as proteins. Chemical-shift heterogeneity in biological systems can arise from conformational exchange (e.g. exchange between free and complexed forms), proteins containing flexible or conformationally dynamic regions, and exchange between folded and unfolded forms. Often the exchange is on the microsecond to millisecond timescale, causing a modulation of the isotropic chemical shifts and leading to deterioration of the NMR spectrum. In many cases, resonances from important protein residues are not detectable, ironically rendering the NMR method unsuitable for obtaining the very type of structural and dynamic information that NMR spectroscopy can best provide. Hence, ways need to be found to recover the missing resonances. δ scaling, possibly combined with parameter-time scaling, should be able to restore sensitivity even in cases of severe broadening.

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