Pure shift proton DOSY: diffusion-ordered ^{1}H spectra without multiplet structure

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The problem of NMR signal overlap in DOSY is significantly diminished by suppressing multiplet structure in the spectral dimension.

In recent years, diffusion-based methods for mixture analysis have provided some of the most exciting developments in NMR. The use of pulsed field gradient spin and stimulated echo experiments allows signals originating from molecular species of different sizes in a mixture to be distinguished. In high resolution diffusion-ordered spectroscopy (DOSY[1–3]), even modest differences in diffusion coefficient between species are sufficient to allow the spectrum of an intact mixture to be decomposed into the sub-spectra of individual components. High resolution in the diffusion dimension is dependent on signals being well-resolved in the spectral dimension. When signals overlap, it is much more difficult to extract the diffusion coefficients of individual components; this requires some approximation to the inverse Laplace transform, a classic ill-posed problem.[4] Almost all DOSY experiments suffer to a greater or lesser extent from problems caused by such overlap; in very complex mixtures it can largely defeat useful analysis. Spectral resolution can be greatly improved by using 3D DOSY, where diffusion weighting is added to a 2D experiment (*e.g.* COSY-DOSY,[5,6] 2DJ-DOSY[7,8] or HMQC-DOSY[9–11]) at the cost of increased experiment duration and more complex data processing and display.

One alternative to extending spectral dimension(s) is to simplify existing ones, *e.g.* by suppressing the multiplet structure in a proton spectrum to give a ^{1}H-homodecoupled, or "pure shift", experiment. Such an experiment would give an improvement in resolution of about an order of magnitude – comparable to that obtainable from a (hypothetical) 5 GHz spectrometer. Early attempts to obtain broadband proton homonuclear decoupling used the 45° projection of a 2DJ-experiment,[12] but unfortunately the phase sensitive projection along this direction is zero. This necessitates the use of absolute value projection, in turn requiring the use of time-symmetrising weighting functions such as the pseudo echo.[13] The net result is a projection in which peak intensities are distorted, and in which lines are significantly broadened. This experiment has nevertheless been extended with diffusion weighting[14] to give what is in essence a pure shift 2D DOSY experiment (although no DOSY spectrum was synthesised) but with a greatly reduced resolution advantage. Non-linear data processing methods can alleviate the problems with 45° 2DJ projection,[15–18] but have not found wide application. Constant-time experiments can also produce pure shift spectra, but with relative intensities dependent on the scalar coupling J.[19] Only recently have general solutions to obtaining such a pure shift proton spectrum been proposed, by Zangger and Sterk[20] and most recently by Keeler and coworkers.[21] The former experiment uses spatially selective pulses to measure different spectral regions using different parts of the sample, while Keeler's method uses a (phase sensitive) 45° projection of diagonal peaks in a 2D anti z-COSY spectrum.[22] Both can readily be extended to give pure shift DOSY experiments; some initial results have been obtained by the z-COSY method.[22]

It is instructive to compare the Keeler/z-COSY and Zangger–Sterk approaches to pure shift ^{1}H NMR. While using rather different pulse sequences, both rely on restricting signal observation to a small subset of spins in order to refocus J coupling effects, and both involve the intermediacy of an indirect dimension t_1. In both cases sensitivity and experimental simplicity are traded for a large improvement in spectral resolution. Strong coupling affects the two types of spectrum differently: in the Zangger–Sterk method strongly coupled multiplets emerge substantially unaffected, while in the z-COSY method partial decoupling is accompanied by artefact peaks at intermediate apparent chemical shifts. It seems likely that these complementary methods will both prove useful for pure shift DOSY experiments.

We show here that pure shift DOSY spectra can be obtained by the Zangger–Sterk method,[20] in which the homonuclear decoupling is achieved by using spatially selective pulses first to restrict the signal measured for each spectral region to a given slice of the sample, and then to apply a 180° rotation to the remaining spins only. (This technique parallels, and predates, the ultrafast nD experiments introduced by Frydman and others[23]). The result is that for weakly coupled spins the evolution of the measured magnetization has the effects of J coupling refocused, leaving just the effects of the chemical shift. In the prototype sequence of Fig. 1a, the initial selective 270° pulse in the presence of a weak z gradient excites a different horizontal slice of the sample for each chemical shift range. The combination of the selective and nonselective 180° pulses ensures that all but the spins that were initially excited experience a 180° rotation, refocusing the J coupling. The bandwidth of the selective pulses determines the minimum chemical shift difference for which decoupling is achieved. Measuring successive data points by incrementing the time t_1 allows an interferogram to be constructed which will transform to a pure shift spectrum. Decoupling is purchased at the expense of a reduction in signal-to-noise ratio, since only a fraction of the sample contributes to any given chemical shift range in the spectrum.

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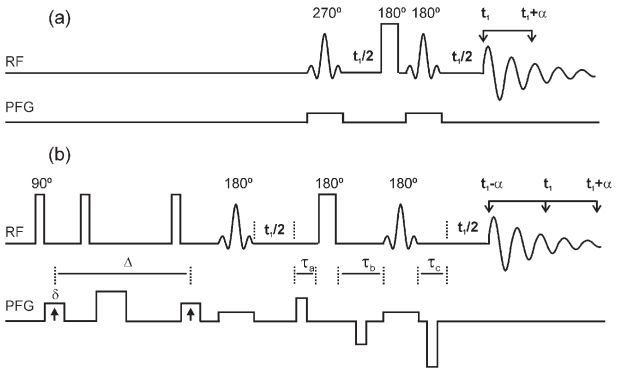

Fig. 1 Pulse sequences for pure shift spectroscopy (a) and pure shift DOSY (b), showing radio-frequency (RF) and pulsed z field gradient (PFG) pulses. Gradient pulses with vertical arrows indicate gradient levels which are changed to vary the diffusion weighting of signals. The gradient pulses for coherence pathway selection (during delays τ_a, τ_b and τ_c) are in the ratio $1 : -1 : -2$. The chemical shift should be refocused at the beginning of acquisition for $t_1 = 0$, requiring that $\tau_a + \tau_c = \tau_b$. The free induction decay is recorded from $t_1 - \alpha$ to time $t_1 + \alpha$ for each t_1 value except the first ($t_1 = 0$), where only the first α s is recorded.

As formulated so far, this experiment has a double sensitivity penalty: only part of the sample contributes to a given shift range, and that signal is measured a single t_1 point at a time. Zangger and Sterk proposed an elegant palliative, constructing the interferogram from chunks of data acquired in real time from time t_1 to $t_1 + \alpha$, reducing the experiment duration by a factor $SW \times \alpha$ where SW is the spectral width. Provided that $J\,\alpha \ll 1$, this introduces negligible error; if the requirement is violated, weak sidebands appear every $1/\alpha$ Hz in the spectrum. Zangger and Sterk's trick can be extended to give a double time saving by acquiring data from time $t_1 - \alpha$ to time $t_1 + \alpha$, reducing the sensitivity penalty, as indicated in the pure shift DOSY sequence in Fig. 1b, except for $t_1 = 0$, where only the first α s of the free induction decay is recorded. The pure shift ^{1}H 2D DOSY sequence is constructed by concatenating the basic experiment with a stimulated echo, here replacing the original soft 270° pulse with a corresponding 180° pulse (Fig. 1b). For cleaner coherence transfer pathway selection, gradient pulses are placed in the τ_a, τ_b and τ_c delays with areas in the ratios $1 : -1 : -2$. These delays are needed to accommodate the selective radiofrequency pulses and gradient pulses; they should be kept short to minimise signal losses due to J modulation, with $\tau_a + \tau_c = \tau_b$ to ensure that the chemical shift refocuses at the start of the acquisition for $t_1 = 0$.

Fig. 2 shows the results of conventional and pure shift ^{1}H DOSY experiments on a mixture of 2-methyl-1-propanol (150 mM) and 2,3-dimethyl-2-butanol (130 mM) in D_2O containing TSP as a chemical shift reference. All measurements were carried out non-spinning on a 400 MHz Varian Inova instrument, using a 5 mm diameter indirect detection probe equipped with a z-gradient coil allowing gradient pulses up to 30 G cm^{-1}. No sample temperature control was used and the experiments were carried out in a room air-conditioned at about 20 °C. A diffusion delay Δ of 0.1 s was used, with a diffusion-encoding pulse width δ of 2 ms, and 8 gradient strengths ranging from 3.0 to 27.3 G cm^{-1} chosen to give equal steps in gradient squared. The standard 2D DOSY spectrum of Fig. 2a was acquired using the Oneshot

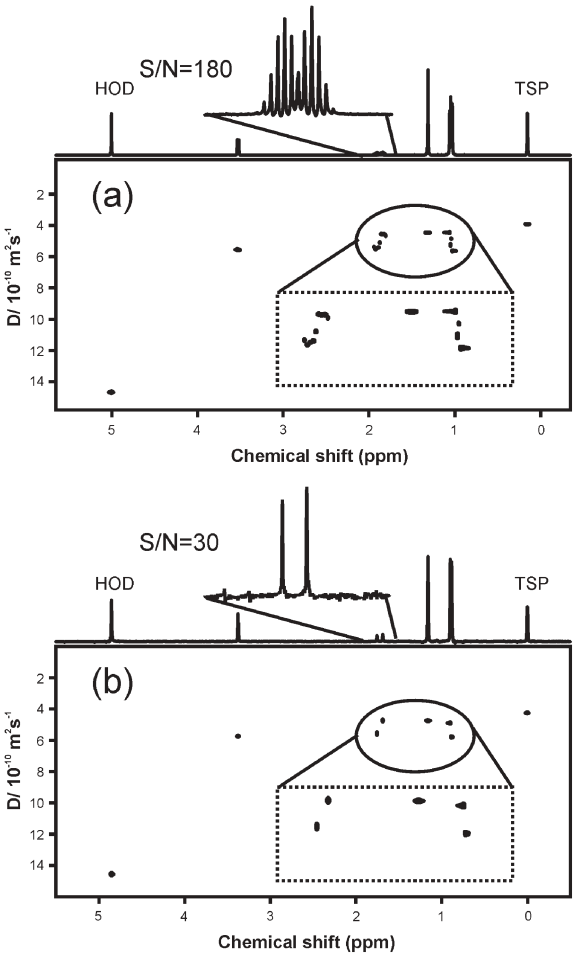

Fig. 2 DOSY (a) and pure shift DOSY (b) spectra of a solution of 2-methyl-1-propanol, 2,3-dimethyl-2-butanol, and TSP in D_2O, acquired in 11 min and 2 h 10 min respectively. The ^{1}H spectrum with the lowest attenuation is shown on top of each DOSY spectrum, with an expansion of the area around 1.7 ppm; the indicated signal-to-noise ratios (S/N) are corrected for time averaging and represent the value for 4 transients. HOD and TSP signals are indicated in the spectra at 4.8 and 0 ppm respectively. Peaks from 2-methyl-1-propanol are at 3.38, 1.75 and 0.88 ppm and from 2,3-dimethyl-2-butanol at 1.69, 1.16 and 0.90 ppm.

sequence[24] at a spectral width of 4000 Hz with 16 transients of 8192 complex data points for each gradient strength.

The pure shift DOSY spectrum of Fig. 2b was acquired using the sequence of Fig. 1(b) with the phase cycling of Table 1, at a

Table 1 Phase cycling for the pure shift DOSY sequence of Fig. 1(b). Phases are given for the 6 radiofrequency pulses, in order from left to right, and for the receiver phase ϕ_R

ϕ_1[a]	0202 1313
ϕ_2	0
ϕ_3	0
ϕ_4	0
ϕ_5	$0_{16}1_{16}2_{16}3_{16}$
ϕ_6	$0011_2\ 2233_2$
ϕ_R	$\phi_1 + 2\phi_5 + 2\phi_6$

[a] Phases are notated as multiples of 90° (0 = 0°, 1 = 90°, 2 = 180°, 3 = 270°), with subscripts denoting repetition; thus the cycle $0_4 1_4 2_4 3_4$ corresponds to the sequence of phases 0°, 0°, 0°, 0°, 90°, 90°, 90°, 90°, 180°, 180°, 180°, 180°, 270°, 270°, 270°, 270° on successive transients.

spectral width of 3000 Hz with 8 transients at 50 t_1 values using the initial 16 ms (8 ms for the first increment) of each free induction decay to construct an interferogram of 2376 complex data points at each gradient strength. The interferogram was constructed using a macro written in-house which took less than 1 minute to run on a Sun Blade 100. The Gaussian selective pulses had a bandwidth of 200 Hz and duration 9.4 ms; the gradient strength during these pulses was 0.5 G cm^{-1}. Exponential weighting corresponding to a 1 Hz line broadening was applied to all FIDs before Fourier transformation and base line correction. Processing of the standard DOSY spectrum was corrected for the effects of pulsed field gradient non-uniformity by using a modified Stejskal–Tanner equation derived from experimental maps of field gradient and signal strength as a function of z, determined using Oneshot experiments on a sample of known diffusion coefficient, in this case 1% H_2O in D_2O, with a weak read gradient applied during signal acquisition.[3,25,26] The accuracy of such calibration is limited largely by the quality of the temperature calibration; in our hands, calibration with HDO results in measured diffusion coefficients for a range of common solvents that agree with literature values to better than 1%.

In the pure shift DOSY experiment, the effect of non-uniformity of the gradient is different: the diffusional attenuation at each chemical shift depends on the gradient strength at the corresponding slice of the sample, but for each slice the attenuation follows the normal Stejskal–Tanner equation[27] very closely. The spatial variation of the diffusional attenuation is known from the map of field gradient as a function of z, so a simple chemical-shift dependent correction of D is inserted into the standard macro for DOSY processing between fitting of the peak attenuations and the construction of the DOSY spectrum, resulting in excellent fit statistics and very narrow peaks in the diffusion dimension. The extra programming, including the macro for constructing the pure shift interferogram and the correction for non-uniform field gradient, required fewer than 120 lines of code.

In the standard DOSY spectrum of Fig. 2a the signal overlap around 2.7 and 0.9 ppm causes parts of the corresponding multiplets to show apparent diffusion coefficients that lie between the actual values for the two alcohols. The simplification of the proton dimension of the pure shift DOSY removes this overlap and enables complete resolution of all peaks, resulting in a DOSY spectrum where all signals can be assigned unambiguously to the correct species. The new method achieves a practical resolution for ^{1}H DOSY comparable to the best obtainable using 3D DOSY methods such as DOSY-HMQC, but without the need to involve low abundance heteronuclei. The advantages of pure shift methods should increase with static field strength, as problems with strong coupling are reduced, while the sensitivity penalty becomes less onerous because of the increased signal strength. It should be straightforward to extend this proof of principle to more complex systems; the technique is compatible with a number of water suppression methods and produces good results even with (as here) modest signal-to-noise ratio in the pure shift spectra. It remains to be seen whether, for example, the cumulative effect of the small artefacts that result from scalar coupling evolution during the periods α (just visible in the expanded trace in Fig. 2b) and/or strong coupling will prove troublesome.

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