Improving pulse sequences for 3D DOSY: COSY-IDOSY

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An improved pulse sequence for measuring diffusion-ordered COSY spectra is achieved by incorporating the diffusion encoding directly into the evolution and detection periods of a gradient-enhanced COSY sequence, giving improved sensitivity and a 32-fold reduction in minimum experiment time.

Diffusion ordered spectroscopy (DOSY) is a very efficient tool for mixture analysis, separating the signals in a conventional spectrum according to their apparent diffusion coefficient. This is achieved by adding diffusion encoding to a parent pulse sequence and fitting the resultant decays of signals to the appropriate expression. The fundamentals of DOSY have been thoroughly reviewed elsewhere.[1–3] In the basic experiment (2D DOSY), ^{1}H spectra with increasing diffusional attenuation are recorded and diffusion coefficients for the resonances calculated from the signal decays.[4] High resolution in the diffusion dimension (less than 1% difference in diffusion coefficient) can be obtained with a simple two-parameter fit if resonances are well separated in the spectral dimension.[5] Unfortunately where signals overlap, as is commonly the case, it is much more difficult to extract accurate diffusion information. Many different data fitting procedures are available which seek to extract the underlying spectra and their diffusion coefficients, but all suffer from much poorer diffusion resolution.[6–13] Most applications require good diffusion resolution, so there is a pressing need for DOSY techniques which minimise signal overlap. The most general solution here is to add a diffusion dimension to a 2D (or higher dimensionality) spectrum. Numerous pulse sequences for 3D DOSY have been published.[14–23] In principle, there are three strategies for creating a DOSY pulse sequence: prepending the diffusion encoding (DOSY-X), appending it (X-DOSY), and incorporating it internally (X-IDOSY). Almost all published experiments to date have been X-DOSY or DOSY-X, but where the parent pulse sequence can accommodate an extra diffusion delay Δ of a few tens of ms the IDOSY approach can be simpler, quicker and more sensitive. Thus the mixing period in TOCSY[16] or NOESY can double as the diffusion delay. Long-range HMQC allows the incorporation of two separate diffusion weighting segments, to form an HMQC-IDOSY sequence.[21] While 2DJ spectroscopy is largely obsolete, 2DJ-DOSY[19] is a useful tool for mixture analysis; very recently 2DJ-IDOSY has been shown to give a two-fold increase in signal-to-noise and four-fold reduction in minimum experimental time.[20]

In the present investigation a simplified and improved pulse sequence for diffusion-ordered COSY is presented. Previous DOSY-COSY experiments have used a phase cycled COSY sequence preceded by a BPPLED (bipolar pulse pair longitudinal eddy-current delay) sequence[14] (Fig. 1a). The COSY-IDOSY experiment of Fig. 1b is derived from the basic gradient-enhanced COSY experiment[24] by adding two short extra delays $\Delta/2$ and using the same field gradient pulses for coherence transfer pathway selection and for diffusion encoding (Table 1). This reduces the minimum phase cycle from 32 transients to 1, and removes the two-fold sensitivity penalty incurred by the stimulated echo in Fig. 1a. The extra diffusion delay is short compared to typical T_2 values in small molecules, and the conventional absolute value processing masks the phase modulation introduced. While the pulse sequence of Fig. 1a uses *n*-type coherence transfer, COSY-IDOSY may be used in either *p*-type (Fig. 1b) or *n*-type (Fig. 1c) mode. The *p*-type version will have lower t_1-noise,[25] but at the expense of slightly lower F_2 signal-to-noise ratio in inhomogeneous fields. The (optional) first gradient pulse in the *n*-type sequence helps to minimise field and field-frequency lock disturbance.

The efficacy of the COSY-IDOSY experiment is illustrated in Fig. 2 for an approximately equimolar mixture of propanol, isobutanol and isopentanol (10% v/v in D_2O). This mixture of medium chain alcohols is a model system for alcohols found in port wine.[26] Despite being a simple mixture, the crowded cross-peaks provide quite a rigorous test of the technique. The *p*-type (Fig. 1b) experiment was carried out non-spinning on a Varian UNITY 500 MHz spectrometer fitted with a ^{1}H/^{13}C/^{15}N triple probe equipped with a 50 G cm^{-1} gradient coil, using 512 t_1 increments each of 1 transient and 512 complex points and with 6

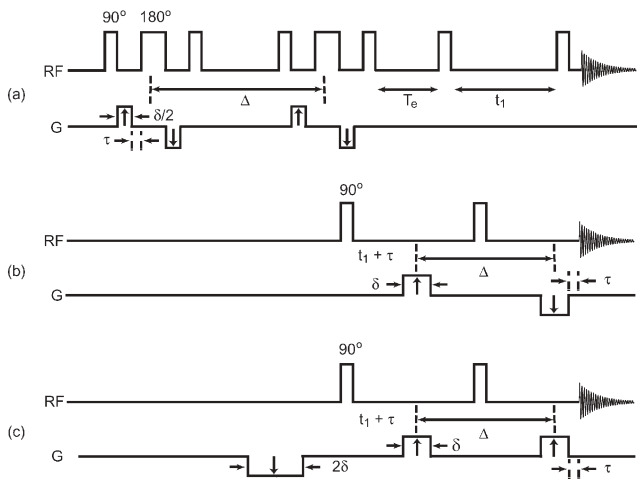

Fig. 1 Pulse sequences for 3D DOSY derived from the COSY experiment. (a) BPPLED-COSY sequence of Wu *et al.*;[14] (b) proposed COSY-IDOSY sequence (*p*-type); (c) proposed COSY-IDOSY sequence (*n*-type), showing radio-frequency (RF) and gradient (G) pulses. Gradient pulses with vertical arrows indicate gradient levels which are changed to vary the diffusion weighting of signals; a delay τ is allowed for field stabilization after each gradient pulse.

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Table 1 Phase cycling for COSY-IDOSY

ϕ_1[a]	0123	
ϕ_2	$0_4 1_4 2_4 3_4$	
ϕ_R	0123	*p*-type (Fig. 1b)
ϕ_R	0321 2103	*n*-type (Fig. 1c)

[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.

gradient amplitudes spaced between 5 and 25 G cm^{-1} in equal steps of gradient squared. The diffusion time Δ was 50 ms and diffusion encoding pulse width (δ) 4 ms, and the total experiment time was 5 h. 2D Fourier transformation was carried out following sinebell time-domain weighting, and 3D DOSY spectra were calculated as described previously[15] by monoexponential fitting of cross-peak volumes. The latter were corrected for baseplane noise by subtraction of the appropriate proportion of the integrated volume of a large sample of baseplane from an empty region of the spectrum.[19] In the 1D ^{1}H spectrum, the cluster of peaks at 0.9 ppm contains unresolved signals from all the alcohols. In the COSY spectrum all the relevant cross peaks are resolved, enabling COSY-IDOSY to produce a separate COSY subspectrum for each species. The only signals not successfully separated are the unresolved diagonal peak at (0.9, 0.9) ppm, which shows an apparent diffusion coefficient which is a weighted average and hence falls outside the three diffusion ranges used, and two very weak isopentanol cross-peaks which are barely visible in the parent COSY spectrum.

To measure the signal-to-noise improvement, *p*- and *n*-type COSY-IDOSY spectra were acquired using 256 complex points and one gradient amplitude (0.5 G cm^{-1}). The improvement in total signal amplitude, integrated over the spectral area shown in Fig. 2, after adjustment for the 32-fold disparity in number of transients, was a factor of 1.5 for the *p*-type (Fig. 1b) and 2.0 for the *n*-type (Fig. 1c) experiment.

High resolution DOSY is at its most effective when applied to complex mixtures of relatively simple molecules, as for example in some biofluids. The IDOSY family of 3D DOSY experiments, of which COSY-IDOSY is a member, is particularly efficient in such systems because the relatively long T_2's encountered minimise relaxation losses during the delay Δ. In general, the principal disadvantage of the IDOSY approach is the potential for evolution of scalar couplings during Δ, but this is turned into an advantage

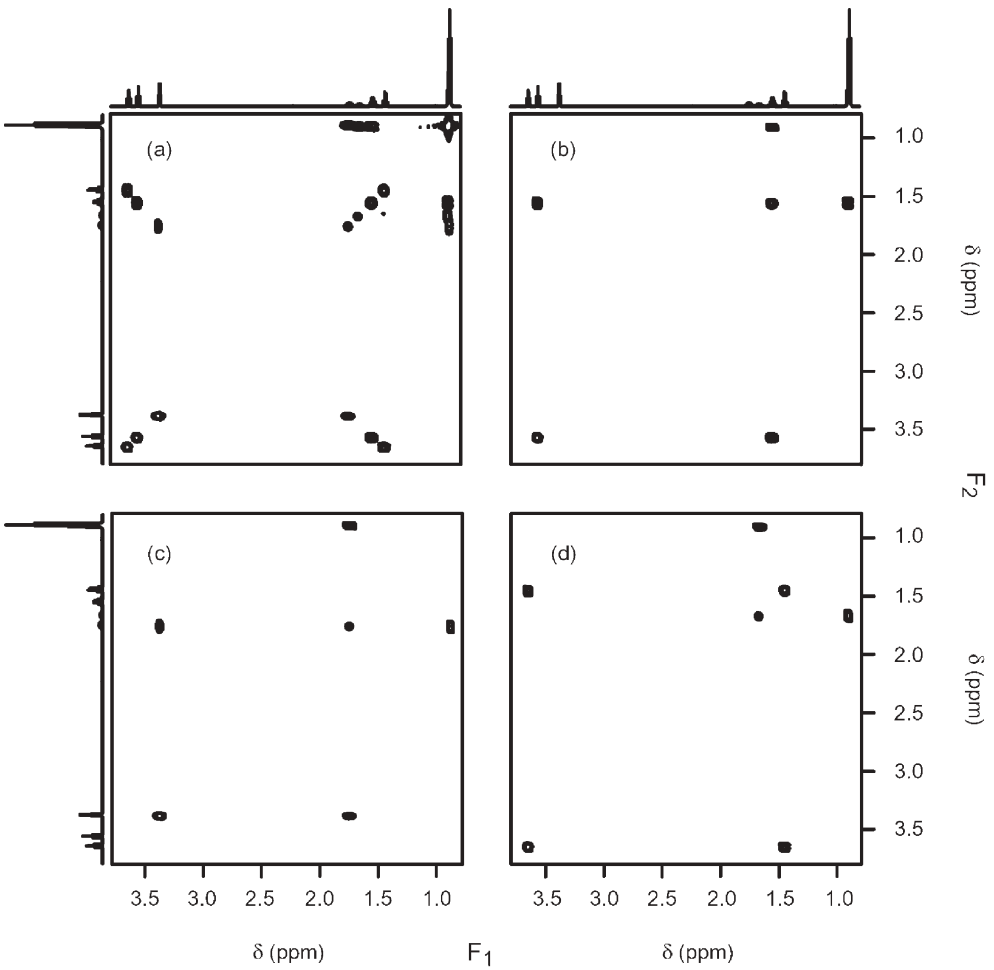

Fig. 2 COSY-IDOSY data for a mixture of medium chain alcohols. (a) COSY spectrum; (b) signals with diffusion coefficients between 8.3 and 8.6 × 10^{-10} m^2 s^{-1}, (propanol); (c) with diffusion coefficients between 7.3 and 7.8 × 10^{-10} m^2 s^{-1}, (isobutanol); (d) with diffusion coefficients between 6.8 and 7.2 × 10^{-10} m^2 s^{-1}, (isopentanol). The 1D ^{1}H spectrum is shown top and left.

in COSY-IDOSY as the extra evolution reduces the number of t_1 increments needed.[27]

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