Suppressing Exchange Effects in Diffusion-Ordered NMR Spectroscopy

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Abstract

In diffusion-ordered spectroscopy (DOSY) the aim is to separate signals from different molecular species according to their different diffusion coefficients. Each species has its individual diffusion coefficient (that may accidentally coincide with that of another species, e.g. if they are of very similar size). In exchanging systems, however, there is a serious complication in that the apparent diffusion coefficient of an exchanging signal will be a compromise that depends, among other factors, on the diffusion coefficients of the exchange partners and the rate of exchange between them. The DOSY spectrum will be much harder to interpret and can often give the appearance of extra (spurious) components in the mixture. Here a new and surprisingly simple experiment is described that suppresses the effects of exchange on apparent diffusion coefficients, restoring the simplicity of interpretation enjoyed by non-exchanging systems.

Keywords

DOSY; diffusion; chemical exchange; spin echo

Introduction

Diffusion-ordered spectroscopy (DOSY) is a family of NMR experiments used in mixture analysis to allow signals belonging to a given species to be correlated through their rate of diffusion. The technique is widely used[1-10] but is well-known to give misleading results when applied to systems undergoing chemical exchange[11]. While such effects can be put to good use[12, 13], when using DOSY to identify mixture components they are a serious nuisance[14]. Thus, for example, where hydroxyl signals are seen in DOSY spectra they routinely appear at higher diffusion coefficients than non-exchanging signals from the same species, because of exchange with residual water[15] and other labile protons.

Almost all DOSY pulse sequences in common use, such as the Oneshot45[16, 17] sequence used to acquire the spectrum of Figure 1a, use the stimulated echo (STE). The primary reason is to minimise J-modulation: the STE stores spatially-encoded magnetization along the z-axis for most of the diffusion time, minimising the time for which J acts. Any exchange of magnetization during this storage period, whether by chemical exchange[18-20] or NOE[21], will affect the attenuation as a function of gradient pulse area for the signals involved. This changes the apparent diffusion coefficients and complicates analysis. The practical effect is that DOSY spectra show peaks with apparent diffusion coefficients intermediate between those of the exchanging sites, with the exact positions determined by the interplay between experimental parameters and the rate(s) of exchange, making it appear that more different species are present than is actually the case.

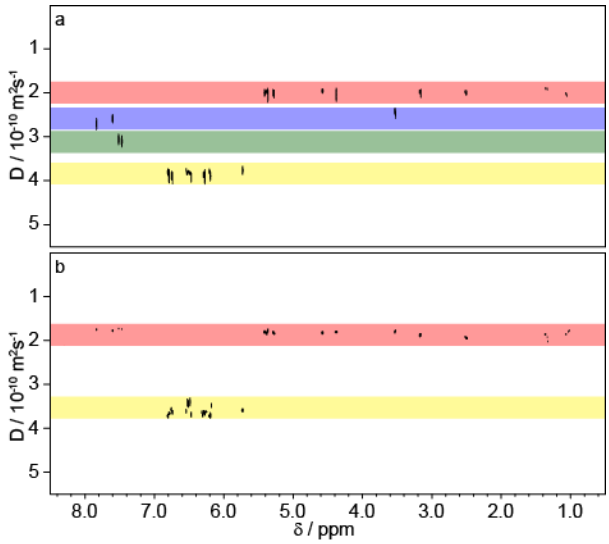

Figure 1. 500 MHz ^{1}H DOSY spectra of a mixture of flavone and catechin in [D6]dimethylsulfoxide (DMSO), measured using (a) the convection compensated BPPSTE pulse sequence[22] and (b) the PROJECTED pulse sequence of Figure 2 (with all gradient pulses present and no 45° radiofrequency pulse). Spectrum (a) suggests the possible presence of multiple species, but from spectrum (b) it is clear that there are only two.

The effects of exchange are particularly frustrating in analysis problems such as mixtures of flavonoids[23], and in general in samples containing glycans[15], where OH signals are much better resolved than CH[24], but exchange with residual water causes them to show increased apparent diffusion coefficients. It can be possible to suppress such exchange by addition of acid[24], but this is chemically invasive and risks sample degradation. Where magnetization exchange is mediated by the nuclear Overhauser effect (NOE), on the other hand, no general suppression method has been reported[21].

It is possible to suppress the effects of exchange (whether chemical or by cross-relaxation) on DOSY experiments in the special case where exchange with only a single species X (e.g. water) is concerned. If the initial excitation has a notch at the X frequency, then X magnetization is not encoded and therefore exchange with it does not lead to refocused signal at the end of a DOSY experiment. This approach has been used for determining protein NH exchange rates[25], but is not general. In the specific case that one of the exchanging spin pools is immobile, it is also possible to use a T_2 filter to suppress the effects of exchange.[26]

In principle, a general solution to the problem of exchange is to use not the stimulated echo but the spin echo (SE). Here the magnetization remains transverse throughout the experiment. Because the phases of spins with different Larmor frequencies evolve at different rates, magnetization exchange (whether by chemical exchange or cross-relaxation) does not result in net magnetization transfer: exchange is incoherent, with spins exchanging at different times having different phases, and leads simply to signal loss. Thus a simple pulsed field gradient spin echo experiment would be expected to yield correct diffusion coefficients for species with different frequencies, even in the presence of exchange; the effects of the latter will only survive for chemical shift differences between exchange partners of the order of the inverse of the echo time or less. Unfortunately, for realistic diffusion times (of the order of

tenths of a second), such experiments show severe J-modulation. Not only does this complicate the interpretation of spectra, it greatly increases signal overlap (because of the dispersion mode tails of signals) and thus degrades the accuracy of the diffusion data obtained[16].

The classic way to suppress J-modulation of spin echoes is to use the Carr-Purcell-Meiboom-Gill (CPMG) experiment[27-30], in which a train of spin echoes is performed, with a short echo time 2τ of the order of the inverse of the chemical shift difference between the coupled spins. Unfortunately this requires a high radiofrequency pulse duty cycle, causing sample heating and risking convection (anathema to diffusion experiments), and in any case the rapid pulsing would restore the unwanted effects of chemical exchange and cross-relaxation (here the ROE, as opposed to the NOE in STE experiments).

As has recently been pointed out[31], however, there is a simple and general way to suppress J modulation in a CPMG pulse sequence without resorting to rapid pulsing (i.e. to very short echo times 2τ). The PROJECT (Periodic Refocusing Of J Evolution by Coherence Transfer) approach uses a CPMG sequence with quadrature 90° pulses inserted in the middle of each double spin echo, and is based on the so-called perfect echo[32, 33]. The extra 90° pulses refocus J-evolution, for arbitrary τ in AX spin systems and for all spin systems if $\tau J << 1$. If diffusion weighting is added, for example by including field gradient pulses in each echo as in the PROJECTED (PROJECT Extended to DOSY) sequences of Figure 2, then spin echo DOSY spectra may be obtained free of both exchange effects and J modulation if τk, $\tau J << 1$, where k is the exchange rate constant.

Results and Discussion

The DOSY spectrum of Figure 1a was acquired for a mixture of flavone and catechin. At first sight there appear to be two impurities present. In fact these signals are simply the flavone hydroxyl resonances, but their diffusion coefficients are increased by exchange with the small amount of (protio-) water present in the sample. As noted above, such signals are typically better dispersed than backbone proton signals, but serve only to confuse in the spectrum of Figure 1a. If exchange effects are suppressed using the PROJECTED sequence (Figure 2, all gradient pulses present, no 45° pulse), however, the assignment of the signals becomes obvious and hydroxyl and backbone signals alike align correctly in the diffusion domain as shown in Figure 1b. Of course the effect is not limited to exchanging OH signals (which can, if appropriate, be suppressed by addition of D_2O), but is general (and extends to magnetization exchange through the Overhauser effect).

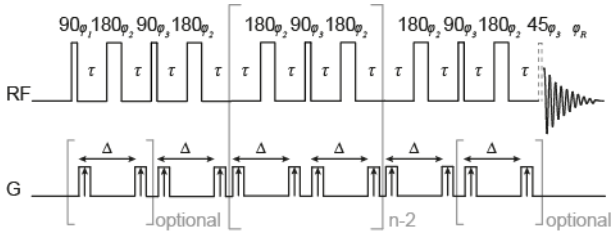

Figure 2. The PROJECTED pulse sequence. The diffusional attenuation has the form $\exp[-2nD\gamma^2G^2\delta^2(\Delta - \delta/3)]$, where D is the diffusion coefficient, G the gradient amplitude, γ the magnetogyric ratio, Δ the unit diffusion time, δ the gradient pulse width, and n the number of diffusion-encoding echoes. The field gradient pulses in the last echo can be dropped to minimize eddy currents affecting the FID; convection compensation can be implemented simply by dropping the field gradient pulses from

the first and last echoes; and a 45° pulse can be used to purge any residual dispersive components produced when the PROJECT condition $\tau J << 1$ is partially violated. The sequence was implemented with the phase cycle $\varphi_1 = (0\ 2)_2$, $\varphi_2 = 1_4$, $\varphi_3 = 1_2\ 3_2$, and $\varphi_R = (0\ 2)_2$. PROJECTED pulse sequence code for Bruker and Agilent NMR spectrometers is included in the supplementary material.

The use of PROJECT-based DOSY experiments is not limited to cases where exchange is a problem; if, as in small and medium-sized molecules, T_2 is not too short, they can be significantly more sensitive than their STE counterparts, because the SE retains the full signal while the STE discards half. However, as there is signal loss due to T_2 during the SE delay, a compromise between degree of diffusion weighting and signal-to-noise ratio may be required for signals with a short T_2. Other important examples of applications for PROJECTED include T_2-filtered DOSY and convection compensation. T_2-filtration is commonly used where broad signals, for example from polymers or proteins, obscure signals of interest, and is typically implemented in STE-based DOSY pulse sequences by adding CPMG sequences either before[34], or after[35] the STE element. With PROJECTED, diffusion encoding and T_2-filtering can be performed simultaneously, minimising signal losses, sample heating and J-modulation.

Convection compensation is a particularly attractive application. In STE-based DOSY experiments, convection compensation is normally achieved using a double stimulated echo (DSTE)[22], with a fourfold loss in signal compared to a SE experiment. In contrast, convection compensation can be implemented in a PROJECTED experiment without this sensitivity penalty, simply by dropping the field gradient pulses from the first and last echoes, as indicated by the outer square brackets in Figure 2. To illustrate the potential, Figure 3 compares Oneshot45 and convection-compensated double stimulated echo with PROJECTED DOSY spectra of the trisaccharide maltotriose in warm [D6]DMSO. In Figure 3a convection causes all signals to show artificially high apparent diffusion coefficients. The conventional solution, the DSTE experiment (Figure 3b), restores the CH signals to the correct positions in the diffusion domain, but leaves the exchanging signals with faster diffusion and shows poorer signal-to-noise ratio. The PROJECTED method (Figure 3c) restores all signals to their correct positions, with good signal-to-noise ratio.

Conclusion

The new PROJECTED experiment can simultaneously suppress the effects of chemical exchange, J-modulation and convection. It offers two to three times better sensitivity than the double stimulated echo pulse sequence (Supporting material, Fig. SI-1), and allows exchanging signals such as those of hydroxyl groups to be used in the identification of species in mixtures. The new method has the potential to find routine application in DOSY experiments on small molecules.

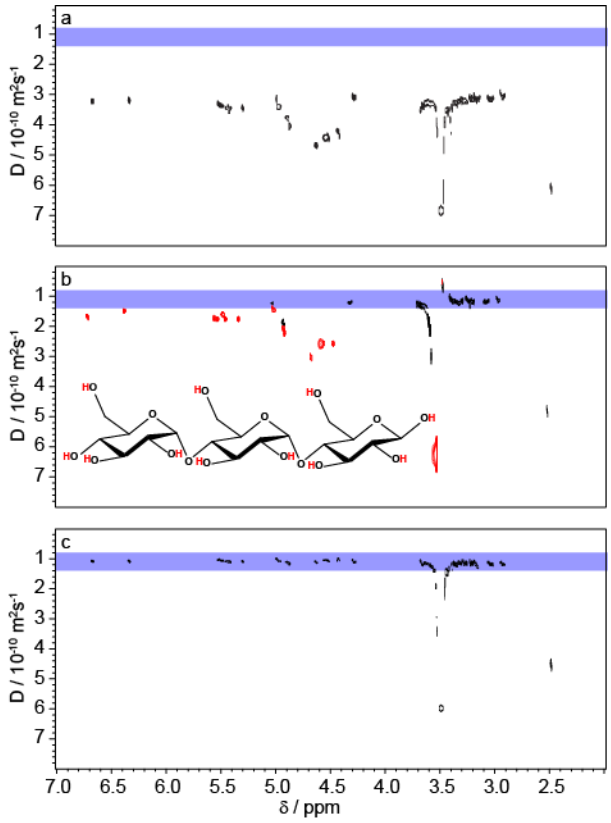

Figure 3. DOSY spectra of 77 mM maltotriose in [D6]DMSO at 27 °C, (a) measured using the Oneshot45 pulse sequence, and showing both gross errors in apparent diffusion coefficient arising from convection, and a range of apparent hydroxyl diffusion coefficients caused by exchange; (b) measured using the convection compensated bipolar double stimulated echo sequence, with the gross errors corrected but still showing the effects of hydroxyl exchange with water, marked in red; and (c) measured using the PROJECTED pulse sequence, showing that all the maltotriose signals correctly aligned. (References to colour in this figure legend refer to the online version).

Experimental

A 1 mL sample of a mixture of catechin hydrate (53 mM) and flavone (47 mM) in [D6]DMSO was measured at 30 °C on a 500 MHz Varian VNMRS spectrometer equipped with a 5 mm triple resonance probe with a z-gradient coil giving a maximum gradient of 66 G cm^{-1}. DBPPSTE convection compensated and PROJECTED pulse sequences were used. In both cases 4 transients of 10739 complex points were averaged for each of 10 gradient increments ranging from 10.5 to 56.4 G cm^{-1} nominal amplitude. Equal increments in gradient squared were used, with rectangular gradient pulses of 1 ms duration, and the total experiment time was 4 min. A 0.7 mL sample of maltotriose (77 mM) in [D6]DMSO containing 10% H_2O was measured at 27 °C using the Oneshot45, BPPSTE and PROJECTED pulse sequences. 4 transients of 16k complex points were averaged for each of 10 gradient increments, ranging from 10.4 to 56.4 G cm^{-1} nominal amplitude, in equal steps of gradient squared; 1.0 ms rectangular gradient pulses were used. For experiments using the PROJECTED sequence 15 cycles, n, were performed with cycle times, 4τ, of 30 ms and 20 ms for the catechin-flavone and the maltotriose samples respectively. The gradient duty cycle was kept at 10% or below to minimise systematic errors in gradient pulse area due to amplifier or coil heating.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/<copy ed please supply>

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