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Journal of Magnetic Resonance 167 (2004) 31–35
JMR Journal of Magnetic Resonance
www.elsevier.com/locate/jmr

Detecting intermolecular NOEs by means of a novel DPFGSE pulse sequence. Application to the solvation of carbohydrates in binary mixtures

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Received 1 August 2003; revised 22 October 2003

Abstract

We present a pulse sequence based on solute-to-solvent NOE enhancement and aimed at the detection of intermolecular NOE's. Thus, a W3 pulse cluster is used to selectively filter the solvent signals in a DPFGSE sequence. The sequence has been tested on a sample of glucose dissolved in two binary aqueous mixtures (water–acetonitrile and water–DMSO). We show how the resulting enhancements may derive from intermolecular cross-relaxation or, in the water–DMSO sample, also from chemical exchange. In each case, a quantitative interpretation of the data is also supplied, both in terms of local enrichment in one specific solvent (preferential solvation), and by means of a kinetic model for a two-site chemical exchange.

Keywords: Intermolecular NOE; DPFGSE; W3 pulse trains; Preferential solvation; Carbohydrates

1. Introduction

Several thermodynamic aspects may concur to the non-ideal behavior of real solutions. For instance, it is well known that a molecular species dissolved into binary mixtures may undergo a *preferential solvation* by altering the composition of its inner solvation shell with respect to that of the bulk solution. This phenomenon, which can affect many physico-chemical properties of the solute, is generally explained on the basis of several concomitant effects, ranging from a different interaction strength between solute and solvents to possible micro-heterogeneities within the mixture (clustering) or even to a size exclusion of the solvents themselves [1,2].

In this respect, carbohydrates are extremely interesting systems, due to their widespread involvement in many biochemical processes which take place in complex mixed media. Thus, the strong interactions between carbohydrates and various solvents [3] or lanthanide ions [4] suggest that this class of molecules may exhibit a marked preferential solvation when dissolved into binary aqueous systems, as also confirmed by MD simulations [5].

Many recent studies have employed two-dimensional NOESY to probe preferential solvation [1–3], but the use of such a technique is often limited by the low sensitivity which stems from the intrinsic weakness of the observed intermolecular dipolar interactions. However, one-dimensional NOESY experiments based on double pulsed field gradient spin-echo (DPFGSE) sequences [6] can partially overcome the aforementioned limitations by delivering high sensitivity at low time costs. In typical applications of these 1D techniques [7], the solvent resonance is selected and a solvent-to-solute NOE enhancement is observed. Yet, this straightforward approach may suffer from subtle drawbacks when applied to the case of binary mixtures of non-deuterated solvents. In order to detect small *inter*molecular NOEs, it is desirable that both solvents provide high proton concentrations, so as to increase the rate of *inter*molecular dipole–dipole relaxation between solute and solvents (the opposite requirement holds for a typical *intra*molecular NOE experiment). Nonetheless, only a small fraction of the solvent magnetization is transferred to

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doi:10.1016/j.jmr.2003.11.009

the solute (usually present in small amount) while its remaining magnetization also appears in the final spectrum and therefore has to be suppressed. Hence, in order to attain a higher efficiency, we have sought to implement an NOE experiment relying on *solute-to-solvent* magnetization transfer. This approach, in principle, allows for simultaneous observation of NOE buildup on multiple solvent signals, provided that the whole solute spectrum is effectively filtered by a uniform, tunable, and band-selective excitation scheme. Following this idea, we have set up a pulse sequence tailored to investigate the preferential solvation of carbohydrates in binary aqueous mixtures.

2. Results and discussion

As a first approach, we have chosen to examine water–acetonitrile and water–DMSO binary mixtures with glucose as solute, since both systems have the advantage of showing only two solvent signals which do not overlap with the solute spectrum (Fig. 1).

In order to achieve selective filtering of the solute spectrum, a 3–9–19 pulse cluster (W3) [8] was employed as refocusing element in a DPFGSE sequence. Within a W3 binomial pulse, the total π rotation is split into a $[3\alpha–\tau_p–9\alpha–\tau_p–19\alpha–\tau_p–19\bar{\alpha}–\tau_p–9\bar{\alpha}–\tau_p–3\bar{\alpha}–\tau_p]$ cluster, where the overbar indicates phase inversion of the single pulse and $\alpha = \pi/62$. When used as a refocusing element in a DPFGSE, such a composite pulse provides periodical nulling points at offsets of $\pm n/\tau_p$ Hz ($n = 1, 2, \ldots$), yet retaining a uniform excitation profile between the nulls. Other kinds of clusters, for example W5, lead to similar excitation profiles, as shown in Fig. 2. By finely adjusting τ_p it was possible to let the null points fall above the solvents resonances, so as to obtain the solute spectrum with minimum phase and intensity distortions (see Fig. 3). Finally, a gradient-selected NOE pulse scheme was appended to the properly phase-cycled W3-DPFGSE filter, to give the full sequence shown in Fig. 4.

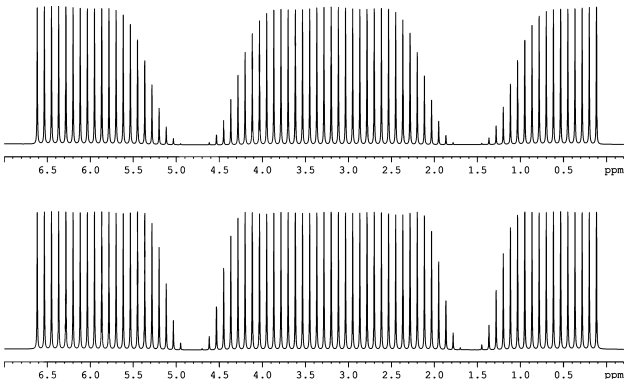

Fig. 2. Simulated excitation profiles emerging from a DPFGSE pulse scheme with W3 (top) and W5 (bottom) clusters as refocusing elements. Although W5 exhibits narrower dips, in practice W3 was found to perform better. Simulated profiles were obtained at 600 MHz ^{1}H frequency and $\tau_p = 519\,\mu s$, according to the actual experimental parameters.

^{1}H NOE spectra were obtained with the pulse scheme of Fig. 4 by setting the transmitter offset on resonance with the water signal and $\tau_p = |\nu(\text{water}) - \nu(\text{cosolvent})|^{-1}$. Ten mixing time ($\tau_m$) increments were sampled in the range 0.1–1.5 s and all FIDs (16 scans each) were arranged into a single, pseudo-2D experiment. The overall acquisition time was 20–30 min. The resulting array of spectra was post-processed along F2 with a 1-Hz line broadening and baseline correction. Water and cosolvent signals were then integrated and plotted against τ_m to give the graphs of Figs. 5 and 6.

In order to check that the observed NOE buildups actually stem from solute–solvent interactions (rather than from solvent–solvent interactions or other spurious

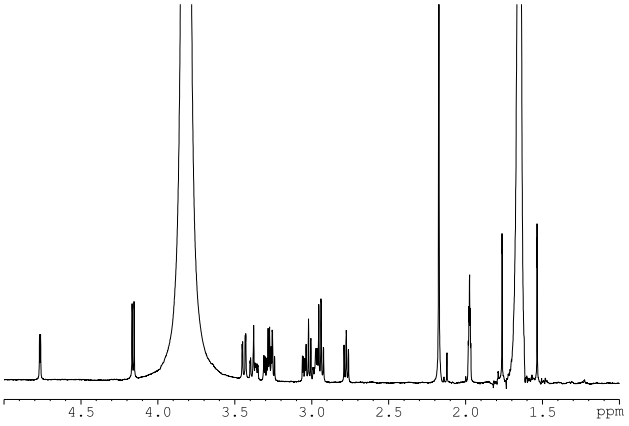

Fig. 1. ^{1}H 600 MHz spectrum of glucose (ca. 167 mM) in 2:3 v/v water–acetonitrile.

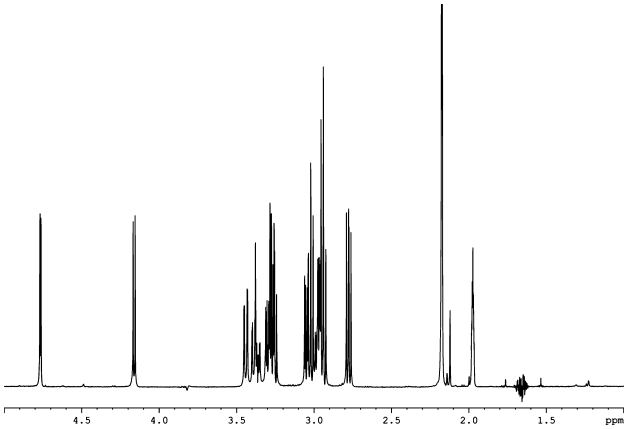

Fig. 3. W3-filtered DPFGSE spectrum of glucose in water–acetonitrile, showing effective suppression of solvent resonances. The sharp singlet at 2.2 ppm originates from residual water in the CD_3CN employed for external locking.

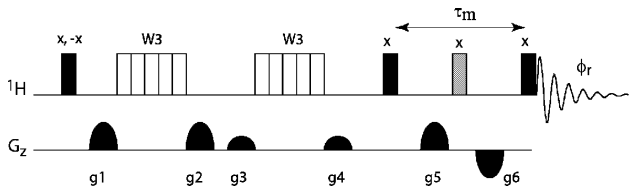

Fig. 4. W3-filtered DPFGSE-NOE pulse scheme. Filled rectangles: $\pi/2$ pulses, grey rectangle: π pulse, open rectangles: variable α pulses of the W3 cluster (see Section 4). Gradients duration: 0.6 ms (g1–g4) and 1 ms (g5 and g6). Gradient intensity ratios (100 = 64 G/cm): 53:53:32:32:40:−40. Each gradient pulse is sine-shaped and is followed by a 100-μs recovery delay. Phase cycles: $\alpha = (x)_2, (y)_2, (-x)_2, (-y)_2$ for the first W3 cluster and $\alpha = (x)_8, (y)_8, (-x)_8, (-y)_8$ for the second W3 cluster; $\phi_r = (x, -x, -x, x)_2, (-x, x, x, -x)_2$.

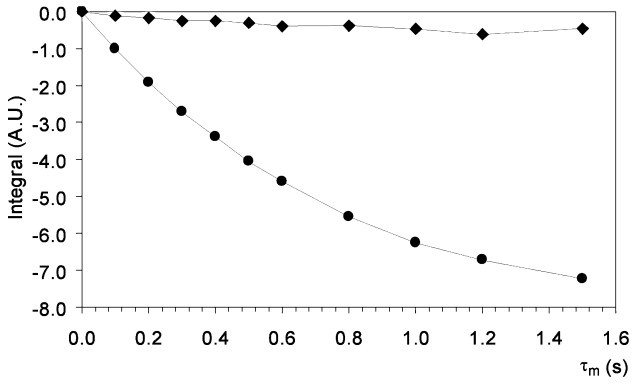

Fig. 5. Intermolecular NOE buildup curves for water (●) and acetonitrile (◆).

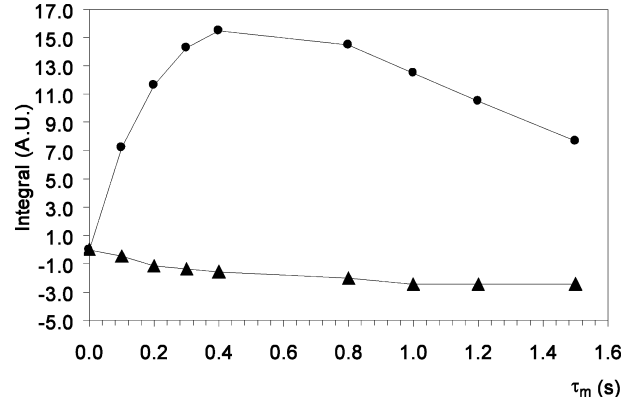

Fig. 6. Intermolecular NOE and exchange buildup curves for water (●) and DMSO (▲).

phenomena) we ran a control experiment where the solute (glucose) was kept physically separated from the solvent mixture (water–acetonitrile) by dissolving it in the outer compartment of the coaxial tube containing the lock (D_2O), while the inner tube contained only the solvent mixture (see Section 4). Application of the proposed sequence led to no observable buildup, even when $\tau_m = 1$ s.

2.1. Water–DMSO system

Fig. 5 shows that the intermolecular NOE buildup is negative for both water and acetonitrile, as expected in the extreme narrowing regime. Conversely, in water–DMSO mixtures, the intermolecular NOE buildup is negative for DMSO but positive and much stronger for water (Fig. 6). This behavior can be explained by noting that, in this sample, signals of the glucose hydroxyl protons are clearly detectable in a simple 1D spectrum at 298 K, due to their slow exchange with water protons. Part of the OH magnetization can therefore bypass the W3 filter and generate positive signal enhancements by chemical exchange on the τ_m timescale. This contribution counteracts the (much smaller) negative signal enhancement coming from the intermolecular NOEs in the extreme narrowing regime (cf. the DMSO curve of Fig. 6). Even though the number of non-exchangeable and exchangeable sugar protons is the same, the resulting buildup curve for water is by far dominated by contributions from OH protons, thus showing large, positive enhancements all over the explored τ_m range. A conventional 2D NOESY spectrum of this sample also reveals strong and positive cross-peaks between OH and water signals, while intermolecular NOE peaks are nearly invisible. Therefore, under the assumption that intermolecular NOE buildup is negligible and that glucose OH protons exchange independently of each other, we have fitted the experimental data with the equation describing an EXSY signal enhancement for a two-site slow symmetrical exchange [9], Eq. (1):

$$a(\tau_m) = \sinh(k\tau_m)\exp[-(k + 1/T_1)\tau_m], \quad (1)$$

where k is the exchange rate constant and T_1 is the spin–lattice relaxation time, assumed to be identical for the two sites. A non-linear least-squares fitting of the experimental data yielded $T_1 = (0.85 \pm 0.06)$ s and $k = (0.91 \pm 0.14)\,s^{-1}$. A graphical representation of the fitting curve is given in Fig. 7.

Similar estimates of T_1 for water (0.88 s) and OH protons (0.89 s) are also obtained by inversion-recovery even if, in principle, such T_1 values will be partially averaged over the two sites by the process of chemical exchange. However, due to the low concentration of glucose, the T_1 value for water can be assumed to represent a fair estimate of the actual longitudinal relaxation rate, since all contributions from OH protons are statistically negligible. Moreover, from the good fit of experimental data, we conclude that the proposed model adequately describes the observed trend. Indeed, the average exchange rate for hydroxyl sucrose protons in a

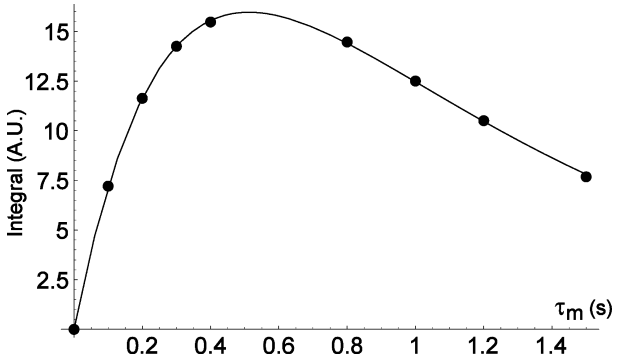

Fig. 7. Water–DMSO sample: integral of water signal as a function of the mixing time. The curve was obtained by fitting the two-site slow symmetrical exchange model (Eq. (1)) to the experimental data.

3:1 acetone-d_6/water mixture has been reported to be $4.8\,s^{-1}$ at 273 K [10].

2.2. Water–acetonitrile system

Let us assume the case of two protons (i.e., $I = S = 1/2$) diffusing apart on different molecules. In this system, the intermolecular dipole–dipole relaxation rate ρ_{IS} is given by Eq. (2) [11]:

$$\rho_{IS} = \left(\frac{\mu_0}{4\pi}\right)^2 \frac{2\pi}{15}\gamma_H^4\hbar^2\frac{1}{D_{IS}b_{IS}}, \tag{2}$$

where $D_{IS} = (D_I + D_S)/2$ is the mutual diffusion coefficient, and b_{IS} is the distance of closest approach for the spin pair [1]. While Eq. (2) gives the theoretical efficiency of dipole–dipole relaxation for an isolated spin pair, the actual cross-relaxation rate σ_{IS} also depends on the local spin concentrations, i.e., on the density of I spins surrounding S and vice versa. Hence, if σ_{gw}^{inter} and σ_{ga}^{inter} are the total intermolecular cross-relaxation rates between glucose–water and glucose–acetonitrile spins, respectively, the following ratio applies to the binary mixture:

$$\left(\frac{\sigma_{gw}^{inter}}{\sigma_{ga}^{inter}}\right)_{calc} = \frac{N_w}{N_a}\frac{D_{ga}b_{ga}}{D_{gw}b_{gw}}, \tag{3}$$

where N_i is the spin density of solvent i, defined as number of spins per unit volume, and the pedices g, a, and w stand for glucose, acetonitrile, and water, respectively.

The spin densities appearing in Eq. (3) are determined simply by the mixing ratio of each solvent. Nonetheless, if preferential solvation causes the local spin densities (N_w^{loc}, N_a^{loc}) to differ from those of the bulk solution, the experimental ratio $(\sigma_{gw}^{inter}/\sigma_{ga}^{inter})_{exp}$ will be different from $(\sigma_{gw}^{inter}/\sigma_{ga}^{inter})_{calc}$, and its value allows one to recalculate N_w^{loc}/N_a^{loc} by inverting Eq. (3). The experimental value obtained in the initial rate approximation is $(\sigma_{gw}^{inter}/\sigma_{ga}^{inter})_{exp} = 10.8$, so that $N_w^{loc}/N_a^{loc} = 8.9$. The same ratio for the bulk solution is 1.3, thus indicating that the solvation shell of glucose is strongly enriched in water molecules. The extent of preferential solvation is perhaps more easily appreciated by the imbalance between bulk and local mole fractions of the two solvents (Table 1), which shows that the mole fraction of acetonitrile (34% in the bulk) is depleted to 7% in the solvation shell of glucose.

Table 1
Diffusion coefficients, bulk and local mole fractions of the glucose–water–acetonitrile system

	D (10^{-9} m^2/s)	x_{bulk}	x_{local}
Water	2.26	0.66	0.93
Acetonitrile	2.29	0.34	0.07
Glucose	0.66		

2.3. Evaluation of the proposed pulse sequence

A potential drawback of this sequence lies in its capability to probe only the overall solvation shell of the solute, regardless of its possible site-specific features. While site-specific information is indeed lost, there is a number of issues where our proposed approach is expected to perform better, as follows. (a) Improved sensitivity: all solute protons add up their contributions to produce NOE buildups on solvent single resonances, rather than on solute multiple signals. (b) Shorter and simpler sequence: no need to suppress the outcoming solvent signal before acquisition. (c) Faster and more consistent data collection: a single experiment needs to be run, rather than one for each solvent. (d) Wider scope: the ability to use fully non-deuterated solvents enables one to study unusual solvent systems too. (e) Suppression of intermolecular magnetization transfers between the protonated solvents, which may also be active during the mixing time.

3. Conclusions

We have implemented a W3-filtered DPFGSE NOE pulse scheme suitable for measuring intermolecular NOEs in binary mixtures. Since this technique relies on solute-to-solvent magnetization transfer via intermolecular dipolar interactions, only a single experiment is needed to record a full buildup curve for both solvents. In particular, this last feature makes our pulse scheme a suitable tool for measuring and comparing NOEs as well as chemical exchange rates.

4. Experimental

The sample of glucose in water–acetonitrile was prepared by dissolving α-D-glucose (0.015 g) in twice-distilled water (200 μl) and HPLC-grade acetonitrile

(300 μl). The sample in water–DMSO was prepared by dissolving α-D-glucose (0.011 g) in twice-distilled water (100 μl) and HPLC-grade DMSO (450 μl). A 60-μl coaxial insert was filled with either solution and fit into a standard 5-mm NMR tube. CD_3CN and DMSO-d_6 were used as external lock, respectively. NMR measurements were carried out at 298 K on a Bruker AVANCE DMX 600 spectrometer equipped with a 5-mm TXI (^1H, ^{13}C, and ^{15}N) *xyz*-gradient inverse probe. Diffusion coefficients were determined on the same samples through a BPPLED sequence [12], by fitting the echo signal as a function of the applied gradient strength.

References

[1] A. Bagno, M. Campulla, M. Pirana, G. Scorrano, S. Stiz, Preferential solvation of organic species in binary solvent mixtures probed by intermolecular ^1H NOESY NMR spectroscopy, Chem. Eur. J. 5 (1999) 1291–1300, and references cited therein.

[2] A. Bagno, G. Scorrano, S. Stiz, Preferential solvation of neutral species in binary solvent mixtures characterized by ^1H NOESY NMR spectroscopy, J. Am. Chem. Soc. 119 (1997) 2299–2300.

[3] S. Berger, M.D. Diaz, C. Hawat, The solvation of carbohydrates in dimethylsulfoxide and water, Pol. J. Chem. 73 (1999) 193–197.

[4] M.D. Diaz, S. Berger, Studies of the complexation of sugars by diffusion-ordered NMR spectroscopy, Carbohydr. Res. 329 (2000) 1–5.

[5] A. Vishnyakov, G. Widmalm, A. Laaksonen, Carbohydrates exhibit a distinct preferential solvation pattern in aqueous solvent mixtures, Angew. Chem. Int. Ed. Engl. 39 (1999) 140.

[6] K. Stott, J. Stonehouse, J. Keeler, T.-L. Hwang, A.J. Shaka, Excitation sculpting in high-resolution nuclear magnetic resonance spectroscopy: application to selective NOE experiments, J. Am. Chem. Soc. 117 (1995) 4199–4200;
K. Stott, J. Keeler, Q.N. Van, A.J. Shaka, One-dimensional NOE experiments using pulsed field gradients, J. Magn. Reson. 125 (1997) 302–324.

[7] M. Fioroni, M.D. Diaz, K. Burger, S. Berger, Solvation phenomena of a tetrapeptide in water/trifluoroethanol and water/ethanol mixtures: a diffusion NMR, intermolecular NOE, and molecular dynamics study, J. Am. Chem. Soc. 124 (2002) 7737–7744;
M. Angulo, C. Hawat, H.-J. Hofmann, S. Berger, Site-specific solvation determined by intermolecular nuclear Overhauser effect—measurements and molecular dynamics, Org. Biomol. Chem. 1 (2003) 1049–1052.

[8] M. Liu, X. Mao, C. Ye, J.K. Nicholson, J.C. Lindon, Improved WATERGATE pulse sequences for solvent suppression in NMR spectroscopy, J. Magn. Reson. 132 (1998) 125–129.

[9] M.H. Levitt, Spin Dynamics: Basics of Nuclear Magnetic Resonance, Wiley, New York, 2002.

[10] B. Adams, L. Lerner, Observation of hydroxyl protons of sucrose in aqueous solution: no evidence for persistent intramolecular hydrogen bonds, J. Am. Chem. Soc. 114 (1992) 4827–4829.

[11] S. Macura, R.R. Ernst, Elucidation of cross relaxation in liquids by two-dimensional NMR spectroscopy, Mol. Phys. 41 (1980) 95–117;
R.R. Ernst, G. Bodenhausen, A. Wokaun, Principles of Nuclear Magnetic Resonance in One and Two Dimensions, Clarendon Press, Oxford, 1987.

[12] W.S. Price, Pulsed-field gradient nuclear magnetic resonance as a tool for studying translational diffusion: part II. Experimental aspects, Concepts Magn. Reson. 10 (1998) 137–197;
M.D. Pelta, H. Barjat, G.A. Morris, A.L. Davis, S.J. Hammond, Pulse sequences for high-resolution diffusion-ordered spectroscopy (HR-DOSY), Magn. Res. Chem. 36 (1998) 706–714.