High-Resolution DOSY NMR with Spins in Different Chemical Surroundings: Influence of Particle Exchange

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The effect of chemical exchange in the diffusion-ordered (DOSY) spectra of a two-site system in the slow-exchange limit with respect to the chemical shift is studied. The problem is addressed both theoretically and experimentally. The relationship between diffusion time (t) and mean lifetimes (τ) is studied by the simulation of the magnetization attenuations as a function of the gradient strength, under PFG conditions. The influence of the difference in populations and diffusion coefficients of the two sites is also considered. In analogy to the limiting cases of fast- and slow-exchange with respect to the chemical shift, limiting cases with respect to the diffusion dimension are defined. The slow-exchange limit in diffusion corresponds to the relation of t and τ that allows us to observe the two spins in exchange associated with the individual diffusion coefficients of the two sites when no exchange is present. The fast-exchange limit in diffusion is reached when the relation of t and τ is such that the two spins present the same apparent diffusion coefficient. By using a model system consisting of water/t-butanol it is shown that by recording several DOSY experiments with increasing diffusion times it is possible to estimate the value of the exchange rate. © 2002 Elsevier Science (USA)

Key Words: exchange; DOSY; diffusion.

INTRODUCTION

The use of pulsed field gradient (PFG) spin-echo NMR experiments for the determination of translational diffusion in studies related to the size and shape of molecules has become a field of growing interest in the last years (*1*). The number of applications of this technique in different areas reflects the establishment of the diffusion experiment as a valuable tool for studies of aggregation and binding (*1–5*). These applications have also incorporated the latest modifications and improvements of the traditional PFG NMR spin echo technique, and more modern and reliable pulse programs are now commonly used (*6–10*). With the introduction of diffusion-ordered 2D (DOSY) NMR spectroscopy the traditional diffusion experiment was subject to

a remarkable advance, since the resolution and assignment of even complex mixtures is greatly facilitated (*1*).

DOSY spectra are usually constructed from the analysis of the signal amplitudes of pulsed field gradient (PFG) datasets where the areas of the gradient pulses were incremented. The 2D datasets generated in this manner are basically stack plots of attenuated 1D spectra which, by using an appropriate analysis program, can be converted into 2D spectra with chemical shifts on one axis and the distribution of diffusion coefficients on the other. Therefore, each line in the NMR spectra is expected to generate one or more lines in the diffusion dimension at the values of the corresponding diffusion coefficients of the contributing species. However, in the presence of chemical exchange, alterations to this distribution are expected.

In contrast to the effects of chemical exchange on the lineshape of NMR signals, that are well known and have been the subject of much research (*11*), the study of the effects of chemical exchange in the distribution of diffusion coefficients from PFG NMR datasets is rarely addressed in literature (*12–14*). The effects of chemical exchange in DOSY NMR have been investigated specifically for the case of two-site exchange for a system in the fast-exchange limit (*15*). In this study a comprehensive analysis of the characteristics of the diffusion spectra has been made and simulated diffusion spectra for different exchange rates and site populations are presented. There is however a lack of experimental data to confirm the calculations reported or the extension of the study to the case of slow-exchange in chemical shift.

We report here our studies of the effect of chemical exchange in the DOSY spectra of a two-site system in the slow-exchange limit with respect to the chemical shift. The problem has been addressed both theoretically, by the simulation of exchange data in different situations (varying populations, diffusion coefficients, and rate constants), and experimentally through the careful choice of a system in conditions comparable to the simulations. In the first part of this paper we present some calculated curves showing the effects of different chemical exchange scenarios and diffusion times on the observed attenuation of

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magnetization under typical pulsed field gradient conditions, as used to acquire DOSY spectra. These curves model the behavior of a hypothetical two-site exchange, in the slow-exchange limit with respect to chemical shift (i.e., the lines corresponding to the two sites can be observed separately in the chemical shift dimension), assuming pseudo-first-order kinetics. In the second part of the study we show some experimental data for a model system, the exchange of the hydroxyl proton of t-butanol with water in a water : DMSO mixture. We show that for this model system, the concepts that are usually associated with chemical exchange in chemical shift can be extended to the diffusion dimension and that by the proper choice of the diffusion time, different chemical exchange regimes can be observed in the diffusion dimension.

RESULTS AND DISCUSSION

We initiate our study by determining the expressions that describe the diffusion attenuation of the magnetization for a two-site exchange system (Scheme 1) under pulsed field gradient NMR experiments. For this the following assumptions have been made:

—The spin under observation may be located in different chemical surroundings i with the probability p_i and the mean life time τ_i;

—The principle of detailed equilibrium requires $p_i/\tau_i = const$;

—The distances between the different lines ($\Delta\omega_{ij}$) and the mean life times in these states (τ_i) are large enough so that all lines may be observed separately from each other ($\Delta\omega_{ij}\tau_i \gg 1$);

—The transport of each component is described by Fick's 2nd law with a diffusivity D_i (if different spins belong to the same molecule, their D_i are clearly identical).

This is exactly the situation we have to expect with the experimental system under study, as represented in Scheme 1. In it the symbols A-H and B-H stand for t-butanol and water, respectively, and one is concerned with the mobility of the exchanging protons.

The evolution of the nuclear magnetization during the diffusion-sensitive time interval has been addressed before based on the Bloch equations and the simple exchange theory. The set of equations and the solutions that describe the magnetization can be found in literature and have been discussed in detail for special cases, as, for example, the case where the two sites have the same chemical shift or the exchange rate is much larger than the difference in shifts and only one line is observed in the NMR spectra (15–17).
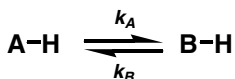
If the influence of relaxation is negligibly small (or identical in all states), in PFG NMR experiments the diffusion-induced evolution of the relative magnitude M_i of magnetization in state i obeys the relation

$$\frac{dM_i}{dt} = -m^2 D_i M_i - M_i/\tau_i + M_j/\tau_j \qquad [1]$$

with $m = \gamma\delta g$ ("area" of the gradient pulse, δ and g stand for the gradient length and strength, respectively); $\tau_i =$ mean time of (direct) transition from i to j;

$$k_i = 1/\tau_i \qquad [2]$$

and the initial condition $M_i(t=0) = p_i$ with $t =$ diffusion time $=$ distance between the two gradient pulses (16, 17).

For a two-site exchange in the slow-exchange limit with respect to the chemical shift the two different magnetizations M_i—corresponding to the two different lines—may be measured separately. In this case the set of equations becomes

$$\frac{dM_A}{dt} = -m^2 D_A M_A - \frac{M_A}{\tau_A} + \frac{M_B}{\tau_B} \qquad [3]$$

$$\frac{dM_B}{dt} = -m^2 D_B M_B + \frac{M_A}{\tau_A} - \frac{M_B}{\tau_B}. \qquad [4]$$

These magnetization intensities may be determined analytically and the resulting solutions are

$$M_A = \left[\frac{M_A(0)}{2} - \left(\frac{\delta M_A(0) - k_B M_B(0)}{2\lambda}\right)\right]\exp[(-\sigma+\Lambda)t]$$
$$+ \left[\frac{M_A(0)}{2} + \left(\frac{\delta M_A(0) - k_B M_B(0)}{2\lambda}\right)\right]\exp[(-\sigma-\Lambda)t] \qquad [5]$$

$$M_B = \left[\frac{M_B(0)}{2} + \left(\frac{\delta M_B(0) + k_A M_A(0)}{2\lambda}\right)\right]\exp[(-\sigma+\Lambda)t]$$
$$+ \left[\frac{M_B(0)}{2} - \left(\frac{\delta M_B(0) + k_A M_A(0)}{2\lambda}\right)\right]\exp[(-\sigma-\Lambda)t]. \qquad [6]$$

Here the functions λ, σ, and Λ are defined as (12)

$$\sigma = \frac{1}{2}[k_A + k_B + D_A m^2 + D_B m^2] \qquad [7]$$

$$\lambda = \frac{1}{2}[k_A - k_B + D_A m^2 - D_B m^2] \qquad [8]$$

$$\Lambda = \sqrt{\lambda^2 + k_A k_B}. \qquad [9]$$

It turns out that M_A and M_B are superpositions of the same two exponentials, however, with different weights.

$$\text{A–H} \underset{k_B}{\overset{k_A}{\rightleftharpoons}} \text{B–H}$$

SCHEME 1

Effect of the Relation between the Diffusion Time (t) and Exchange Time (τ_A) on the Observed Magnetization Attenuation

In a typical PFG NMR experiment the diffusion time t is kept constant and m is varied. In a dimensionless representation the following quantities need to be specified:

D_B/D_A	diffusivity ratio
p_i	relative state populations
$m^2 D_A \tau_A = x$	gradient intensity normalized with respect to a value which leads to an attenuation of $1/e$ if $D = D_A$ and $t = \tau_A$
$t/\tau_A = y$	observation time related to exchange time.

For visualizing different typical attenuation patterns we have considered the ratios $D_B/D_A = 2$, 5, and 10 (i.e., B is always the faster component) and for all of these ratios the cases where $p_A = 0.05$, 0.1, and 0.5. We are interested in simulating systems where the exchange occurs between a large, slower diffusing molecule, and a small, faster diffusing species that can be present as the solvent (like the exchange of NH protons of a peptide with water in aqueous solution (*14*), or the exchange of hydroxyl protons with water for some organic molecules). For each of these cases the relative observation time, as a parameter of these representations, was set equal to $t/\tau_A = y = 0.1$ (close to no exchange) 0.3, 1, 3, 10 (close to fast exchange). As we will see the parameter y reflects the exchange limit in the diffusion dimension and should be considered independent of the exchange limit in the chemical shift dimension. Starting from $x = 0$, as a reasonable maximum value we consider $x = x_{max} = 3/y$. This means that $3 = m_{max}^2 D_A t$, i.e., an echo attenuation to e^{-3} for $D = D_A$ and the given diffusion time (see supplementary material).

The analysis of the plots of $\ln M_i$ vs m^2 for the different cases and for each of the y values allows us to advance with some general observations concerning the behavior of the magnetization decays of such systems.

The population and the diffusion coefficient ratio of the two sites determine the range of values of y where M_A and M_B can be approached to monoexponential decays and the exchange limits can be observed. We can define as a limit for slow exchange in diffusion the relation of t and τ_A that allows us to observe the two spins in exchange associated with the individual diffusion coefficients of the two sites when no exchange is present (see the example for $D_B/D_A = 2$ with $t/\tau_A = 0.1$ in Fig. 1). This means that during the considered diffusion time the exchange process is too slow to attenuate the detected magnetization of the exchanging spins. On the other hand, we will consider that the fast-exchange limit has been reached when the relation of t and τ_A is such that the two spins present the same apparent diffusion coefficient (see the example for $D_B/D_A = 2$ with $t/\tau_A = 10$ in Fig. 1). For this limiting case, the exchange process is so frequent during the diffusion time that the diffusion is completely dominated by the exchange process and only a population-weighted

average diffusion coefficient is measured for the two spins. With these definitions, we see that the values of y for which the limits of slow- and fast-exchange are reached will depend on the differences of population and diffusion coefficients.

As can be seen in Fig. 1, in spite of the fact that the general expressions for M_A and M_B, that describe the attenuation of the magnetization are biexponential, when approaching the limiting cases of slow (low t/τ_A ratio) and fast-exchange (high t/τ_A ratio), there is a tendency for only one exponential for M_A and M_B to remain, being almost identical for fast exchange and with deviations from the respective diffusivities of the two sites for slow-exchange. Therefore, and independently of the series of data considered, the increase of the diffusion time (t) has always the same effect over the attenuation profiles, i.e., to approach the values of the exponential decays of the two magnetizations to the same exponential decay (Fig. 1). Due to this fact, in the limit of fast-exchange the same diffusion coefficient will be computed for the two spins. However, the position of this "apparent" diffusion coefficient in the diffusion dimension will depend on the population difference of the two sites in exchange (Fig. 2).

The simulations show that for small D_B/D_A ratios (<5) the behavior of the magnetization attenuation can be approached by a monoexponential decay over the total range of population differences and for all the t/τ_A ratios considered (i.e., considering attenuation over 2 orders of magnitude in no case the attenuation curves M_A and M_B were found to deviate by more than a factor of 1.3 from single exponentials). For these cases the conditions of slow- and fast-exchange in the diffusion dimension are fulfilled within the ratios of t/τ_A previously mentioned (slow exchange, $t/\tau_A \leq 0.1$, and fast-exchange, $t/\tau_A \geq 10$, Fig. 1).

By increasing the difference among the diffusion coefficients of the two sites in exchange ($D_B/D_A > 5$), the biexponential nature of the magnetization attenuation becomes evident within the values of y considered. As a consequence, the value of y for observing the limit of slow exchange decreases and that for fast exchange increases. This means that longer diffusion times have to be used in order to observe the two spins with the same diffusion coefficient (fast-exchange limit), and that shorter diffusion times have to be used to observe the two spins with the characteristic diffusion coefficient of the sites in the absence of exchange (slow-exchange limit). This effect can be clearly observed in the simulations for $D_B/D_A = 5$ and $D_B/D_A = 10$ in Fig. 1. The slow-exchange limit becomes particularly difficult to achieve, mainly for the fast component (D_B) for D_B/D_A ratios ≥ 10. However, in all ranges of D_B/D_A considered, a value of y of 4 to 5 times that of D_B/D_A seems to define the fast limit exchange.

These observations have direct implications in the way that DOSY data of systems in chemical exchange should be treated and interpreted, especially when dealing with processing techniques that display the data as 2D plots of diffusion versus chemical shift. In spite of the fact that, for a correct analysis of the diffusion attenuated data in the presence of chemical exchange, a biexponential fitting should be performed, a great number of cases can be approached to monoexponential behavior without
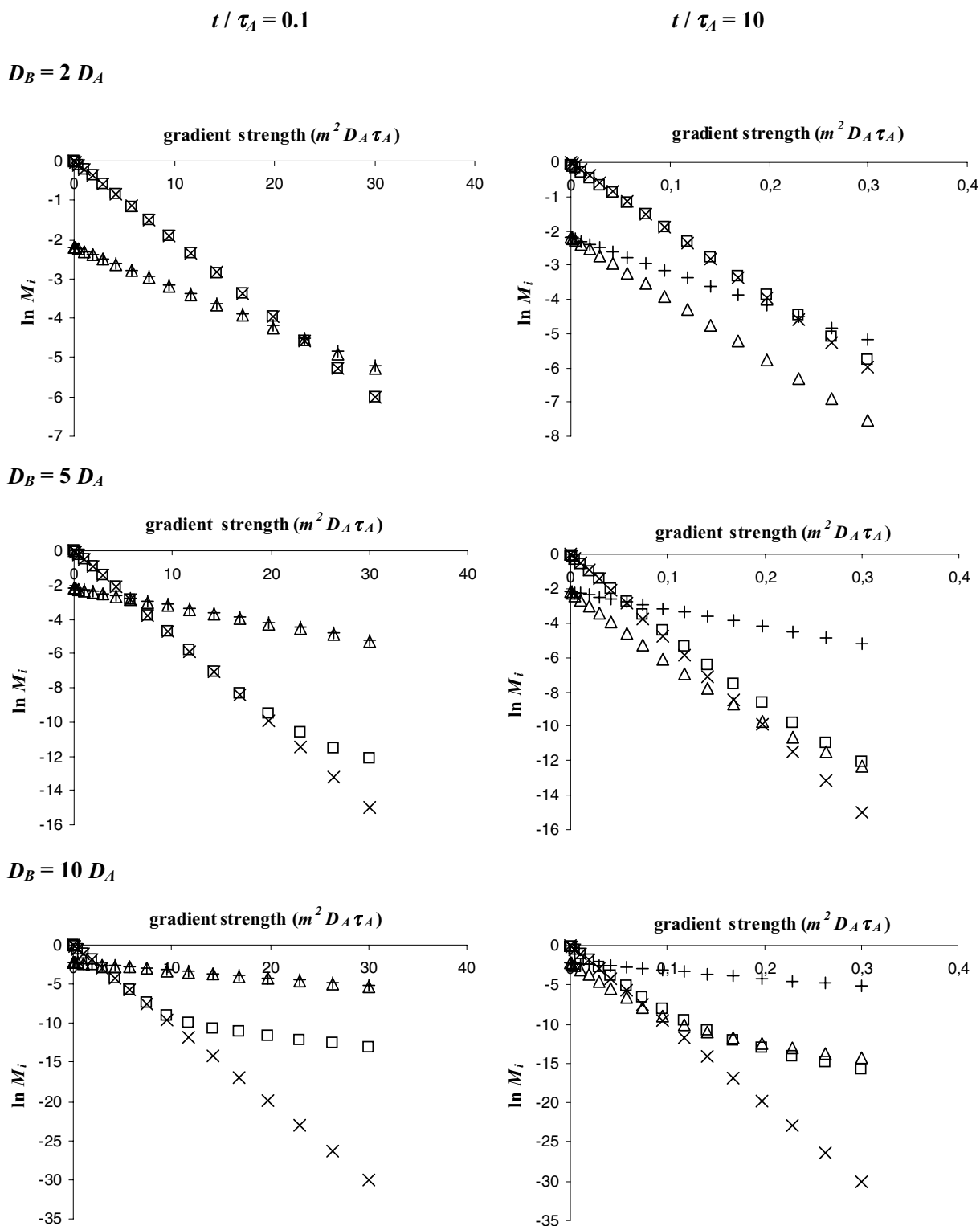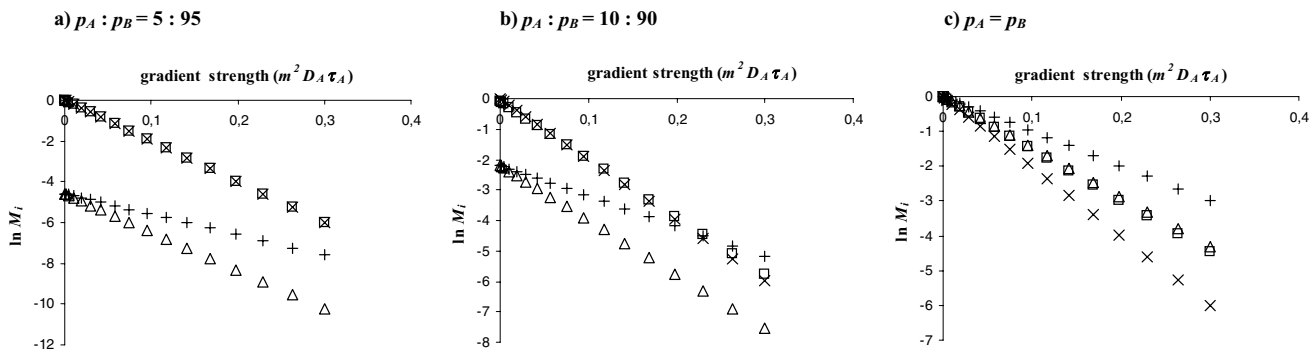
FIG. 1. Effect of the variation of the diffusion time (t) and diffusion coefficient ratio (D_B/D_A) on the magnetization decays ($\triangle$, M_A; and $\square$, M_B) of a two-site system in chemical exchange. The simulations were done considering a relative A : B population of 1 : 9. The attenuation profiles for the sites A and B, simulated considering the same population difference but without the effect of exchange, are also shown in order to allow a better visualization of the influence of the exchange phenomena ($+$, M_A site no exchange; and $\times$, M_B site no exchange).

$$D_B = 2\,D_A, \quad t/\tau_A = 10$$

a) $p_A : p_B = 5 : 95$

b) $p_A : p_B = 10 : 90$

c) $p_A = p_B$

FIG. 2. Effect of the variation of the population ratio (p_A/p_B) on the magnetization decays of the exchanging spins ($\triangle$, M_A; and $\square$, M_B) close to the limit of fast-exchange in diffusion (high t/τ_A). All data were simulated using $D_B = 2D_A$ and $t/\tau_A = 10$. The attenuation profiles for the sites A and B, simulated considering the same population differences but without the effect of exchange, are also shown in order to allow a better visualization of the influence of the exchange phenomena (+, M_A site no exchange; and ×, M_B site no exchange).

compromising the quality of the information obtained, and thus simplifying the data treatment and analysis. This simplification arises from the fact that these cases can be analyzed by taking into account only one diffusion coefficient for each of the exchanging spins. Therefore, information about the exchange rate can be obtained just by considering the observed diffusion coefficient value of the exchanging spin, the values of the diffusion coefficients of the two sites and their populations, and the diffusion times used in the experiments. On the other hand, if biexponential analysis is required, then each one of the two exchanging spins will be associated with two diffusion coefficients. The analysis becomes more complex since not only the position but also the width of the two distributions associated with the two diffusion coefficients calculated for each spin will vary with y.

The following example shows how the diffusion experiment can be manipulated in order to obtain information about a system in exchange. As a model system we have taken a solution of t-butanol in a mixture of water : DMSO (2 : 8). The composition of the mixture was chosen so that the conditions were that of the slow-exchange limit in chemical shift. DMSO was used to adjust the exchange limit so that the signals of water and the hydroxyl group would be well separated. Several DOSY spectra were acquired with the BPPLED pulse sequence with different diffusion times (6, 7). In order to facilitate the comparison with the previous simulations the experimental DOSY data were plotted in the same way, as the natural logarithm of the signal intensity versus $m^2 D_A$ (Fig. 3). In this case the t-butanol diffusion is represented by D_A, and the water, being the faster component, is associated with D_B. The exact populations of t-butanol and water were determined by integration of the normal ^{1}H-NMR spectrum (8 : 1). The diffusion coefficient of the alcohol (D_A) was determined in each DOSY experiment from the attenuation of the methyl group signal and the average value was used (2.87×10^{-10} m^2 s^{-1}). An alcohol-free water : DMSO

solution was used to determine the H$_2$O (D_B) diffusion coefficient (5.57×10^{-10} m^2 s^{-1}). These are the data which are used for an estimate of the respective diffusivities in the mixture. A D_B/D_A ratio of approximately 2 is obtained, so the experimental data are directly comparable to the simulations. The data were also processed as normal 2D DOSY spectra in order to obtain the corresponding plots of chemical shift versus diffusion.

The agreement between the simulated and the experimental data is obvious through the comparison of the experimental plots with the sets of simulated data for the $D_B = 2D_A$ case with a 90 : 10 population ratio and for t/τ_A values between 1 and 10. As can be seen in Fig. 3, by increasing the diffusion time, the magnetization attenuation profile of the alcohol's OH and the water became more similar. For the initial value of 25 ms diffusion time the system can still be considered to be in moderately slow-exchange in diffusion and for values superior to 100 ms the fast-limit exchange is reached (as it becomes clear from the 500 ms data). Since the diffusion time is known for every experiment, a value of approximately 4.0×10^{-2} s^{-1} (corresponding to a τ_A value of 25 ms) for the rate constant of the exchange process can be estimated only from the observation of the plots.

In the 2D DOSY spectra the chemical shift is displayed in one dimension and the diffusion in the other; this type of data display greatly facilitates the processing and the interpretation of the diffusion exchange data. In our example the data were processed as monoexponential decays as we have seen that this approximation is valid for this case. In the 2D DOSY spectra the effect of the increase of t/τ_A over the exchange regime in the diffusion dimension is very clear (Fig. 4). In the first spectrum the water and the OH signals are associated with different diffusion coefficients, with the water being faster than the alcohol exchangeable proton. As the diffusion time is increased, the difference in the diffusion coefficients is averaged down by the exchange process and tends to the same value. Since water is present in great excess, the effect seen in the diffusion dimension
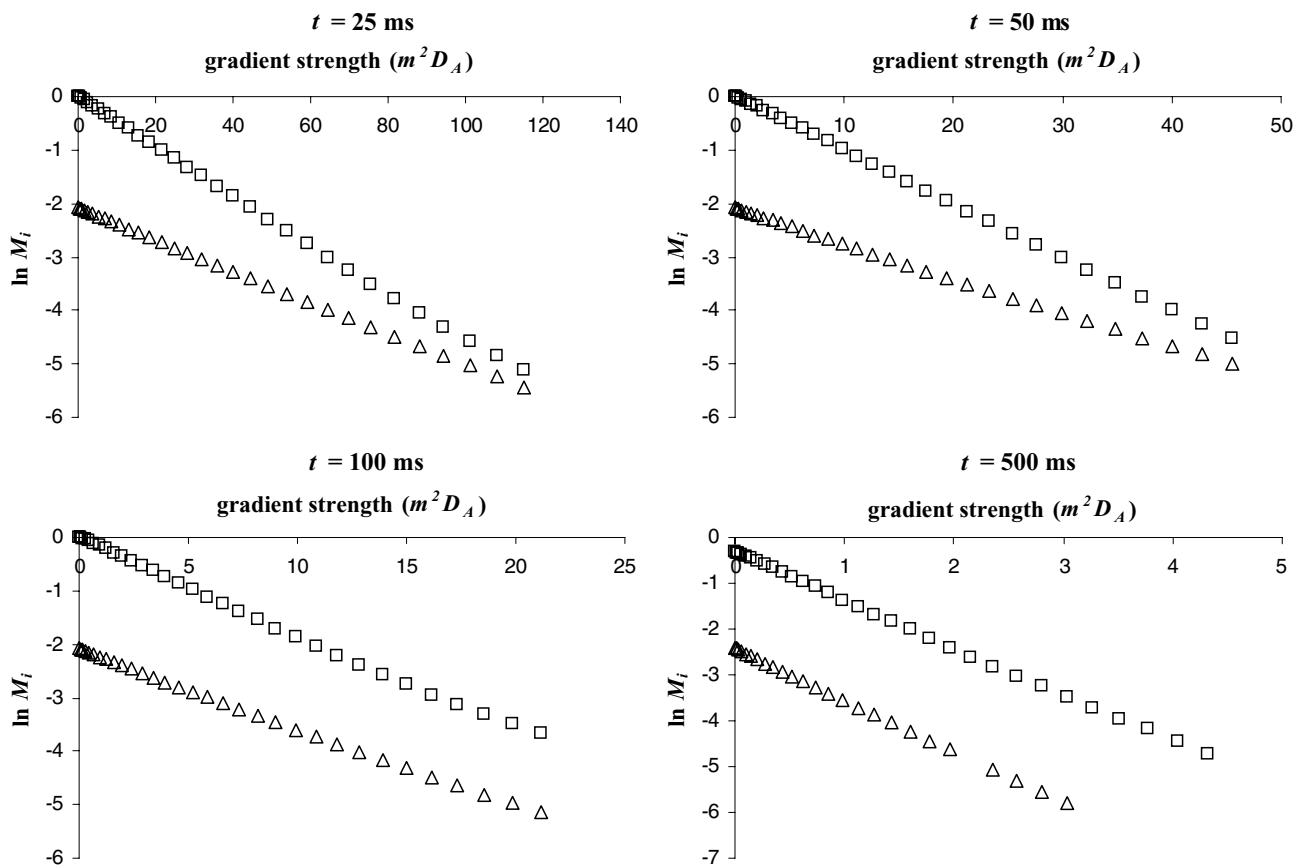
$t = 25$ ms
gradient strength ($m^2 D_A$)

$t = 50$ ms
gradient strength ($m^2 D_A$)

$t = 100$ ms
gradient strength ($m^2 D_A$)

$t = 500$ ms
gradient strength ($m^2 D_A$)

FIG. 3. Effect of the variation of the diffusion time (t) on the magnetization attenuation of the OH group of t-butanol ($\triangle$, –OH) and water ($\square$, H_2O) for a solution of t-butanol in DMSO : H_2O (80 : 20 v/v). The data were acquired with the BPPLED pulse sequence for diffusion times of 25, 50, 100, and 500 ms.
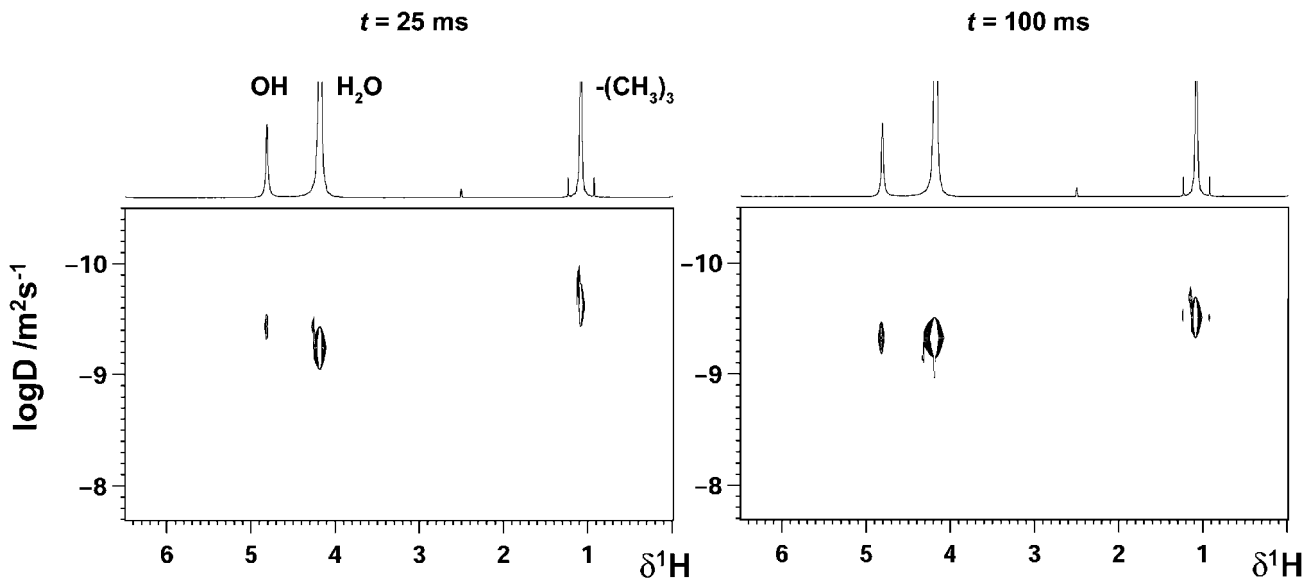
$t = 25$ ms

$t = 100$ ms

OH H_2O -$(CH_3)_3$

FIG. 4. The 2D-DOSY spectra of a solution of t-butanol in DMSO : H_2O (80 : 20 v/v) obtained for the diffusion times of 25 and 100 ms. The same data as those shown in Fig. 3 were used.

of the DOSY spectra is the approaching of the hydroxyl signal to the water signal. When the condition for fast-exchange is reached the two signals are located at the same diffusion coefficient value.

CONCLUSIONS

The use of the translational diffusion of exchangeable protons to characterize exchange phenomena is only possible if in the appropriate timescale these protons do not belong really to a single site or molecule but instead, depending on the system, to two or more different sites or molecules with different diffusion coefficients. The diffusion of these protons will then be affected by the exchange process. The effects of this phenomenon in the DOSY spectra, i.e., the chemical exchange attenuation of the diffusion data, will depend on the exchange rate, the diffusion-sensitive experimental time (i.e., the diffusion time), and on the difference of the diffusion coefficients and populations of the sites.

For a two-site exchange system in the slow-exchange limit in chemical shift we have shown that it is possible to obtain information about the rate of the exchange process by performing several DOSY experiments with increasing diffusion time. In spite of the fact that the attenuation of the magnetization in these experiments is correctly described by a biexponential decay, simulations of the diffusion attenuation of the magnetization show that the attenuation can be approximated to monoexponential behavior if the ratio of the diffusion coefficients is smaller than 5, over the total range of population differences. Exchange limits can be defined in the diffusion dimension depending on the relation of the diffusion time (t) and the exchange rate ($1/\tau_A$). In the slow-exchange limit ($t/\tau_A < 0.1$) the two spins in exchange will be associated with the individual diffusion coefficients of the sites when no exchange is present. In the fast-exchange limit ($t/\tau_A > 10$) the two spins will have the same diffusion coefficient, with the value being dependent on the population difference ($D_{obs} = p_A D_A + p_B D_B$).

The observation of DOSY spectra acquired with increasing diffusion times will allow an estimation of the value of the exchange rate if special relations among diffusion time and exchange rate can be fulfilled in the experiments, since by the appropriate choice of the diffusion time it should be possible to observe the system in fast (one diffusion coefficient) or slow exchange (two diffusion coefficients) in the diffusion dimension.

EXPERIMENTAL

NMR experiments were recorded with a Bruker DRX 400 spectrometer (9.4 T) equipped with a pulsed gradient unit capable of producing magnetic field pulse gradients in the z-direction of 56 G·cm^{-1}. All spectra were acquired in a 5-mm inverse probehead at 300 K in 5-mm tubes. The gradient strength was calibrated using water and benzene. In order to minimize convection effects (6), DOSY experiments were performed using the

bipolar pulse longitudinal eddy current delay (BPPLED) pulse sequence.

The duration of the magnetic field pulse gradients (δ) was optimized for each diffusion time (t) in order to obtain 1–5% residual signal with maximum gradient strength. Typically in each PFG-NMR experiment a series of 32 BPPLED spectra on 16 k data points were collected, the values of δ were 0.8 to 5.0 ms duration (depending on the preset diffusion time), and the eddy current delay (T_e) was set to 5 ms in all experiments. The pulse gradients (g) were incremented from 2% to 95% of the maximum gradient strength in a linear ramp. The temperature was set and controlled to 300 K with an air flow of 545 l/h in order to avoid any temperature fluctuations due to sample heating during the magnetic field pulsed gradients. For the 2D DOSY plots, after Fourier transformation and baseline correction the diffusion dimension was processed with the Bruker xwinnmr software package (version 3.0). The apparent linewidth in the diffusion dimension in the DOSY spectra is correlated to the fitting error, namely to the standard deviation. The value of 2.87×10^{-10} m^2 s^{-1} for the diffusion coefficient of t-butanol is the average from all experiments performed with different diffusion time (25, 50, 100, 200, and 500 ms). Deviations of less than $\pm 5\%$ from this value were found among experiments and are considered to be within the experimental error.

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