

NOTES

Magnetization Transfer in the Rotating Frame: A New Simple Kinetic Tool for the Determination of Rate Constants in the Slow Chemical Exchange Range

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A number of different NMR techniques have been developed in the past for the determination of slow chemical exchange rates (1-12). These include measurements of linewidths (1), transverse relaxation times, T_2 (2, 3), longitudinal relaxation times, T_1 (4), longitudinal relaxation times in the rotating frame, $T_{1\rho}$ (5, 6), and magnetization transfer experiments (7-12). The latter, first proposed by Forsén and Hoffman (7, 8) using cw-NMR, are very useful in connection with pulsed FT-NMR. Dahlquist *et al.* (9) studied slow chemical exchange by selectively inverting one line of a pair of resonances and then following the changes in magnetization of both resonances. Campbell *et al.* (10) have treated the theory behind such experiments. They took into account magnetization transfer by both chemical exchange and nuclear Overhauser effects. This method is, however, difficult to apply if the frequency difference between the exchanging lines is smaller than approximately 100 Hz. In order to fulfill the requirement of line selective pulses one has to apply long and weak pulses during which relaxation may become important. As a result the lines can no longer be inverted. An alternative, the DANTE pulse sequence of Morris and Freeman (13), poses similar problems because of its long duration.

We propose here a solution to this problem by carrying out the experiments in the rotating frame in the presence of a nonselective spin-locking field B_1 . Our method may be regarded as a combination of the magnetization transfer and the $T_{1\rho}$ method, which has been used until now mainly for the determination of rate constants in the fast exchange range (5, 6, 14-17). Spin locking of a magnetization in the y direction of the rotating frame characterized by the coordinates x, y, z , where z is the axis of the static field B_0 , can be achieved either according to Solomon (18) by adiabatic half rapid passage or according to Freeman and Hill (19) by applying a 90°_x pulse followed by a ϕ_y pulse of the length $t = \phi_y / \gamma B_1$, where γ is the gyro-magnetic ratio. Since 90°_x and ϕ_y have a 90° phase shift the magnetization is collinear to $B_1 = B_{1y}$ and, thus, spin-locked. For spin locking it is necessary that the angle α between the effective field $B_{\text{eff}} = B_1 + B_z$,

$$\tan \alpha = \frac{B_z}{B_1} = \frac{2\pi\Delta\nu}{\gamma B_1} = 2\Delta\nu t_{180^\circ}, \quad [1]$$

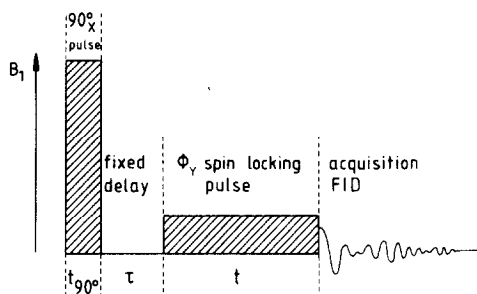


FIG. 1. Pulse scheme for the magnetization transfer experiment in the rotating frame. Experiment (ii): $\tau = 0$, experiment (iii): $\tau = (2\Delta\nu)^{-1}$.

does not exceed values of about 5 to 10° (20). The quantity $\Delta\nu$ is the frequency offset between the magnetization and ϕ_y , which must, thus, be applied in or near resonance. For the case of fast exchange and a coalesced line i the spin-locking condition is easy to fulfill even for $B_1 \rightarrow 0$ by setting $\Delta\nu = 0$. For this case it is generally accepted (14–17) that

$$\frac{1}{T_{1\rho}(i)} = \frac{1}{T_2(i)} \approx \frac{1}{T_1(i)} + W_{\text{exch}}(i), \quad B_1 \rightarrow 0, \quad [2]$$

where $W_{\text{exch}}(i)$ is the exchange broadening of the line i . The problem of spin locking in the case of slowly exchanging lines has not yet been adequately discussed in the literature. We distinguish two cases: (i) only one magnetization is selectively spin-locked by the on-resonance B_1 field, which implies an offset $\Delta\nu$ to the other lines equal to the chemical shift difference; (ii) the strength of B_1 is sufficient for locking the magnetization of all exchanging lines. Simple exponential solutions of the Bloch equations with time constants given by Eq. [2] are obtained for case (i) only if the spin-locked site loses but does not gain magnetization through the exchange process. This is true if the non-spin-locked sites have a very short $T_{2\text{eff}}$ or if they are present only in very small concentrations as in Meiboom's case (5), where the following equation, similar to Eq. [2], is valid:

$$\frac{1}{T_{1\rho}(i)} = \frac{1}{T_1(i)} + \sum_{j \neq i} k_{ij}. \quad [3]$$

The k_{ij} are the pseudo-first-order rate constants. In general the time dependence of the magnetizations will be complicated and the determination of the k_{ij} will be very difficult for case (i). If case (ii) is realized all magnetizations precess with the same frequency in the laboratory frame given by the frequency of ϕ_y , and, contrary to our initial proposition (6), $T_{1\rho}(i)$ is much less affected by the k_{ij} than predicted by Eq. [3], as will be shown below. We, therefore, modify experiment (ii) for the two-site problem in the following way (iii): a delay $\tau = (2\Delta\nu)^{-1}$ is introduced between the two pulses as shown in Fig. 1. The frequency of ϕ_y is set to the frequency of line 1; $\Delta\nu$ is the frequency difference between the two lines. During the time τ the magnetization I of line 1 remains in the y direction, whereas the magnetization S of line 2 precesses about the z axis and points at the end of this period in the

— y direction. During ϕ_y the two magnetizations are locked to B_{1y} . The situation resembles a selective inversion–recovery experiment in the laboratory frame. Since the time dependence of I and S is now considerably affected by the exchange, the rate constants k_{IS} and k_{SI} can be obtained without assumptions by performing experiments (ii) and (iii). These experiments can be described by the modified Bloch equations (21, 22)

$$\frac{dI_y}{dt} = -\rho_I I_y - \sigma S_y - k_{IS} I_y + k_{SI} S_y, \quad [4]$$

$$\frac{dS_y}{dt} = -\rho_S S_y - \sigma I_y - k_{SI} S_y + k_{IS} I_y, \quad [5]$$

where ρ and σ are the usual (10) relaxation constants of a dipolar-coupled system, here, however, in the rotating frame. The solution of Eqs. [4] and [5] is similar to equations given by Campbell *et al.* (10) for the case of magnetization transfer in the laboratory frame with the only difference that in the rotating frame $I_\infty \approx S_\infty \approx 0$:

$$I_y = A \exp(\lambda_+ t) + B \exp(\lambda_- t), \quad [6]$$

$$S_y = \frac{A(\rho_I + k_{IS} + \lambda_+) \exp(\lambda_+ t)}{(k_{IS} - \sigma)} + \frac{B(\rho_I + k_{IS} + \lambda_-) \exp(\lambda_- t)}{(k_{IS} - \sigma)}, \quad [7]$$

where

$$\lambda_{\pm} = \frac{1}{2} (-(\rho_I + k_{IS} + \rho_S + k_{SI}) \pm \{[(\rho_I + k_{IS}) - (\rho_S + k_{SI})]^2 + 4(k_{SI} - \sigma)(k_{IS} - \sigma)\}^{1/2}). \quad [8]$$

The constants A and B are given by the values of I_y and S_y at $t = 0$. For experiment (ii)

$$I_y(0) = I_{z0} = cC_I,$$

$$S_y(0) = S_{z0} = cC_S,$$

where c is a constant and C_i the concentration of i . For experiment (iii) we obtain

$$I_y(0) = I_{z0} \exp(-\tau/T_{2\text{eff}}^I) = c'_I C_I,$$

$$S_y(0) = -S_{z0} \exp(-\tau/T_{2\text{eff}}^S) = -c'_S C_S.$$

If $T_{2\text{eff}}^I$ and $T_{2\text{eff}}^S$ are not too different—which is, generally, the case especially if $T_{2\text{eff}}$ is determined by the homogeneity of the magnetic field—then

$$c'_I = c'_S = c'.$$

If τ is not too long

$$c' = c, \quad I_{y(0)} = I_{z0}, \quad S_{y0} = S_{z0}.$$

In order to see the rationale behind these experiments we discuss the special case where

$$|k_{IS} - k_{SI}| \gg |\rho_I - \rho_S|; \quad k_{IS}, k_{SI} \gg \sigma. \quad [9]$$

Defining

$$\frac{1}{T_{1\rho}^0} = \frac{\rho_I + \rho_S}{2}$$

one obtains from Eqs. [6] to [8] for experiment (ii)

$$I = \frac{k_{SI}}{k_{IS}} S = cC_I \exp(-t/T_{1\rho}^0) \quad [10]$$

and for experiment (iii)

$$I = c'C_I[(x_I - x_S) \exp(-t/T_{1\rho}^0) + 2x_S \exp(-t/T_{1\rho}^0 - (k_{IS} + k_{SI})t)], \quad [11]$$

$$S = c'C_S[(x_I - x_S) \exp(-t/T_{1\rho}^0) + 2x_I \exp(-t/T_{1\rho}^0 - (k_{IS} + k_{SI})t)], \quad [12]$$

where x_i are the mole fractions.

A particular simple case arises from Eqs. [6] to [8] if

$$k_{IS} = k_{SI} = k, \quad I_{x0} = S_{x0}, \quad k \gg \sigma, \quad k \gg \frac{\rho_I - \rho_S}{2}. \quad [13]$$

Then, for experiment (ii)

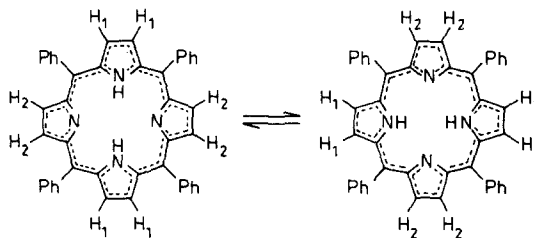
$$I = S = cC_I \exp(-t/T_{1\rho}^0) \quad [14]$$

and for experiment (iii)

$$I = -S = c'C_I \exp(-t/T_{1\rho}^0 - 2kt). \quad [15]$$

Only if Eqs. [9] and [13] do not hold are I and S a function of k_{IS} and k_{SI} in experiment (ii) in contrast to experiment (iii). The strategy is now to determine $T_{1\rho}^0$ in experiment (ii) and k_{IS} , k_{SI} in experiment (iii).

As an example of the method we present the results of experiments (ii) and (iii) carried out at -70°C on the separate H-1/H-2 signals of meso-tetraphenylporphine (TPP) dissolved in tetrahydrofuran- d_8 . These signals broaden at higher temperatures and coalesce because of the intramolecular hydrogen migration (6, 21):



The experiments were performed at 90 MHz using a Bruker CXP 100 FT NMR spectrometer, which has the possibility of reducing pulses in strength during a pulse sequence. The solvent was purchased from ICN-Chemikalien, Munich, and dried over anthracene/sodium-potassium alloy. The sealed sample was prepared using a vacuum line as described previously (6). TPP was synthesized according to the literature (22). The temperature was determined according to van Geet (23) using a methanol sample. The results are shown in Fig. 2. As discussed before (6, 21), the lower-field signal H-1 contains an unresolved coupling with the inner NH atom. A total of 100 scans was accumulated with a repetition time of 10 sec. The strength

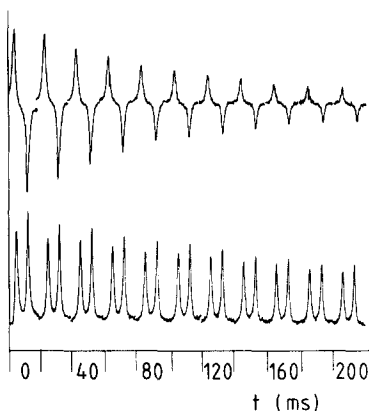


FIG. 2. Magnetization transfer experiment in the rotating frame carried out at -70°C and 90 MHz on the H-1/H-2 signals of TPP dissolved in tetrahydrofuran- d_8 . Lower trace: experiment (ii); upper trace: experiment (iii) with $\Delta\nu = 25$ Hz. For further explanation see text.

of the spin-locking field created by ϕ_y was determined as usual from the length $t_{180^{\circ}}$ of the 180° pulse. In the experiments shown in Fig. 2, $t_{180^{\circ}}$ was set to a value of 0.4 msec. Since the frequency of ϕ_y was set to the frequency of H-1 using quadrature detection the angle α between B_1 and the magnetization of H-2 did not exceed a value of 1.2° according to Eq. [1] with $\Delta\nu = 25$ Hz. Thus, the spin-locking condition was well fulfilled for both signals (6). If α increases the phase correction changes with the time t and it is no longer possible to obtain absorption spectra in one set of experiments using the same phase correction. At higher values of α a complicated time dependence of the magnetizations was observed. The lower trace in Fig. 2 corresponds to experiment (ii), the upper trace to experiment (iii). As shown in Fig. 3 the decay of all signals was found to be exponential within the error limits. The identical slopes of curves 1 and 2 prove the validity of Eqs. [13] and [14], those of curves 3 and 4 the validity of Eq. [15] in these experiments. As a result we obtain $1/T_{1\rho}^0 = 2.6 \pm 0.4 \text{ sec}^{-1}$ and $1/T_{1\rho}^0 + 2k = 8.4 \pm 0.4 \text{ sec}^{-1}$ from the slope of the curves which leads to a value of $k = 2.9 \pm 0.4 \text{ sec}^{-1}$. The value

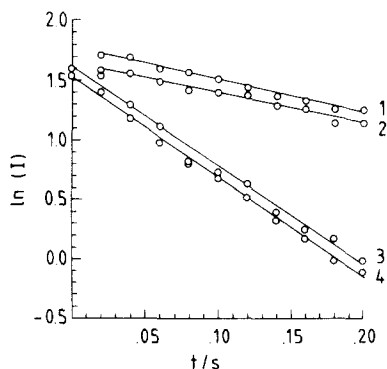


FIG. 3. Evaluation of the data of Fig. 2. I = peak height. 1, 3: H-2 signal, 2, 4: H-1 signal. The difference in the slope of curves 1 and 3 (2 and 4) is equal to $2k$ according to Eqs. [14] and [15].

of k was found to be independent of the strength of B_1 as long as the spin-locking condition was fulfilled. The experiments shown in Figs. 2 and 3 were performed on a nonspinning sample. Spinning did not affect the k value; however, the time fluctuation of the B_0 homogeneity was found to be greater, which lead to fluctuations in the signal heights. In this case the evaluation of the line integrals had to be used for the calculation of $T_{1\rho}^0$ and k instead of the peak heights. Before we undertook our $T_{1\rho}^0$ experiments we tried to determine k using the selective inversion-recovery method described in Ref. (10). However, we did not succeed in inverting the H-1 or H-2 signals in a selective way because of the small frequency difference between the signals and the rather short T_1 values (6). The value of k reported here using our improved $T_{1\rho}$ method or "magnetization transfer in the rotating frame" method is smaller than the value of about 5 sec^{-1} reported previously by us (6). There we had used Eq. [2] instead of Eq. [14] to explain experiment (ii). Therefore, the difference in the k values reported by us and by Stilbs *et al.* (24) is not due to imperfections in our previous $T_{1\rho}$ measurements, as suggested by these authors. A part of this difference probably arises also from solute-solvent interactions. The anomalous kinetic HH/HD/DD primary kinetic isotope effects which we have succeeded in determining for the first time for a defined double-proton transfer reaction (25) can only be explained by the vibrational model of tunneling proposed by us (6, 25, 26). A full account on the relaxation behavior of TPP with respect to the HH migration will be reported elsewhere and will be discussed (27) in relation to the kinetics of other double-proton and deuteron exchange reactions studied in our laboratory (28-32).

In summary we may state that our magnetization transfer in the rotating frame method permits the determination of rate constants in a simple way especially when it is difficult to perform the usual magnetization transfer experiments, as, for example, in high resolution solid-state NMR. No selective pulses are required nor a specific strength of the spin-locking pulse. A further advantage is that the equilibrium magnetizations in the rotating frame are very close to zero, which simplifies the magnetization decay curves considerably as compared to the laboratory frame experiments. We are convinced that our method, for which one can easily imagine variations, will take its place among the methods for the study of slow chemical exchange rates.

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