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Investigation of exchange processes by two-dimensional NMR spectroscopy

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A new general technique for the investigation of exchange processes in molecular systems is proposed
and demonstrated. Applications comprise the study of chemical exchange, of magnetization transfer by
inter- and intramolecular relaxation in liquids, and of spin diffusion and cross-relaxation processes in
solids.

I. INTRODUCTION

Molecular dynamics is dominated by a number of rate processes, the most dominant ones being molecular relaxation, molecular diffusion, and chemical exchange processes. These processes are governed by very similar principles. They are all described by a master equation in the form of a set of coupled differential equations. The experimental techniques for investigating molecular rate processes are also rather similar. A considerable number of such techniques has been worked out.

Some of the most versatile techniques are based on nuclear magnetic resonance (NMR). In particular, NMR enables one to investigate systems in dynamic equilibrium, whereas with most other techniques, like stopped flow, temperature or pressure jump methods for the investigation of chemical exchange, the system has to be put into an initial nonequilibrium state to allow the observation of its return to equilibrium.

The hithertofore known NMR techniques can be grouped into three general classes:

(a) *Line shape analysis*[1-5]: Magnetic or chemical exchange processes are manifested by characteristic changes of the NMR line shape involving exchange broadening, coalescence, and exchange narrowing. Two-site exchange processes can be analyzed virtually by inspection. However, for more complex reaction networks, a computer simulation and least squares fitting are required. Line shape analysis is of major importance for the analysis of chemical exchange processes in dynamic equilibrium. In some cases, it can also be used for the determination of relaxation rates.

(b) *Double resonance*[6-8]: By selective double resonance irradiation, it is possible to obtain a more detailed insight into dynamics utilizing Overhauser effects which are strongly dependent on the relaxation mechanisms,[6,7] or by saturation transfer which allows one to trace out the pathways of chemical exchange.[8]

(c) *Pulse experiments*[9-11]: Radio frequency pulses are applied to create the initial magnetic nonequilibrium state. The subsequent return to equilibrium under the influence of exchange and relaxation processes is being

observed. A special class of pulse experiments is formed by spin echo experiments, which are quite sensitive to exchange processes and to relaxation processes.[11]

In this paper, we propose and demonstrate another general technique for the investigation of magnetic or chemical rate processes in liquids and in solids. It is based on the concept of two-dimensional NMR spectroscopy.[12,13] The technique can lead to a particularly vivid representation of the exchange process in the form of a two-dimensional exchange diagram which puts the exchange network into clear evidence.

In the proposed method (Fig. 1), the various components of the initially created transverse spin magnetization are at first frequency labeled by letting them precess at their characteristic resonance frequencies during the "evolution period" of length t_1. The exchange process to be investigated takes place predominantly during the following "mixing period" of fixed length τ_m. This period is of central importance in acquiring knowl-
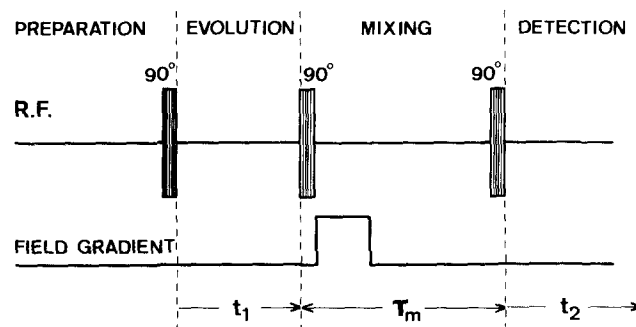

FIG. 1. Basic scheme of 2D exchange spectroscopy involving four successive time periods: preparation period, evolution period, mixing or exchange period, and detection period. Initially, transverse magnetization is prepared by a 90° pulse. In the course of the evolution period, the magnetization vectors acquire precession frequency information and become "frequency labeled." The exchange process predominantly takes place during the mixing period while the magnetization is longitudinal. Remaining transverse components are destroyed by a magnetic field gradient pulse. A third 90° pulse rotates the longitudinal magnetization after exchange into the xy plane for detection.
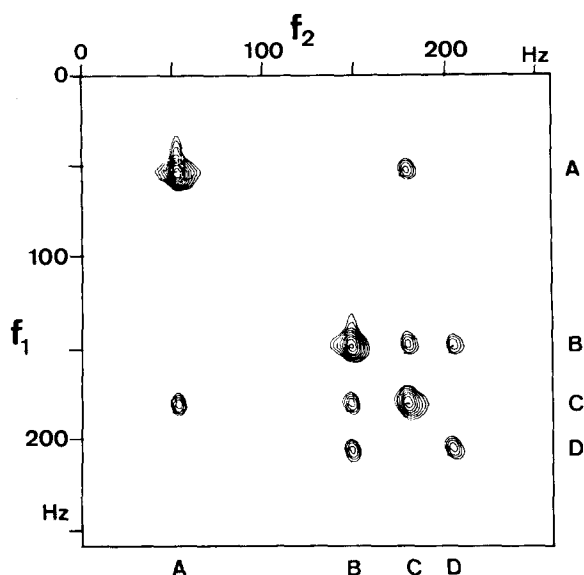
FIG. 2.   2D exchange spectrum of heptamethylbenzenonium ion. A contour plot of the 60 MHz 2D proton resonance spectrum, recorded at 40 °C in 9.4 M H_2SO_4, is shown.

edge about the exchange process in question. Finally, the exchanged magnetization components are measured by letting them precess at their new resonance frequencies and by recording the induced signal as a function of the time variable t_2.

As it is usual in 2D spectroscopy techniques, the experiment has to be repeated for a number of equally spaced values of the evolution time t_1. The result is a data matrix $s(t_1, t_2)$, the double Fourier transform of which is the desired 2D spectrum $S(\omega_1, \omega_2)$. The appearance of an off-diagonal peak at frequencies ω_1, ω_2 in this 2D spectrum indicates that an exchange process during the mixing period has transferred magnetization components of precession frequency ω_1 to a transition of frequency ω_2. When the experiment is performed for a sufficiently short mixing time, the resulting 2D spectrum can be considered as a qualitative map of the kinetic matrix describing the exchange process which is of particular value for the elucidation of complex exchange networks. This experiment allows one, in addition, to utilize all the tricks of 2D spectroscopy, e.g., creating different spin Hamiltonians during evolution, mixing, and detection periods and using more sophisticated pulse sequences. [13,14]

II. A SIMPLE EXAMPLE

Let us briefly consider as an example the chemical exchange in the heptamethylbenzenonium ion, which has recently been analyzed by 2D spectroscopy. [15] This molecule undergoes a 1–2 methyl shift of the following kind [16]:
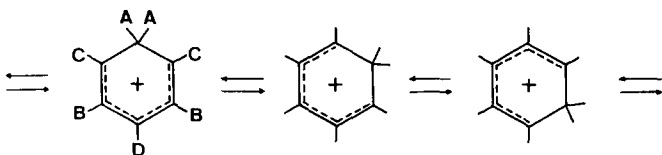
Each methyl group can assume the four possible states A, B, C, and D (labeled in the order of the corresponding proton chemical shifts). The kinetic matrix of this system can easily be found [16]:

$$K = \begin{bmatrix} -R/2 & 0 & R/2 & 0 \\ 0 & -R & R/2 & R \\ R/2 & R/2 & -R & 0 \\ 0 & R/2 & 0 & -R \end{bmatrix} .$$

An experimental proton magnetic resonance 2D exchange spectrum obtained for this system is shown in Fig. 2. Its 10 peaks correspond in a one-to-one relationship to the 10 nonzero elements of the kinetic matrix. A 2D exchange spectrum can therefore be regarded as a qualitative map of the kinetic matrix, and it permits one to deduce the exchange network by inspection. The intensities, however, are usually not proportioned to the elements of the kinetic matrix. Nevertheless, they give a qualitative indication of the exchange rates.

III. THE THREE-PULSE TECHNIQUE FOR 2D-EXCHANGE SPECTROSCOPY

A number of schemes are conceivable to measure a 2D exchange spectrum. The techniques differ mainly by the structure of the mixing period. This is, as mentioned before, the relevant period during which the exchange process predominantly occurs. We will limit the discussion in this paper to one specific nuclear magnetic resonance technique which is most suitable for the investigation of chemical exchange processes and for the study of cross relaxation in liquids.

The particular experimental technique is indicated in Fig. 1. Transverse magnetization is created during the preparation period by a nonselective 90° pulse. After evolution for a time t_1, the x component of the precessing magnetization is selected and rotated along the z axis by a second 90° pulse which is applied along the y axis. The remaining transverse y components are destroyed by a magnetic field gradient pulse. The z components, which form the initial state for the mixing or exchange process, appear now modulated by the precession angles acquired during the evolution period. This provides the desired frequency labeling of the magnetization components. After exchange, the resulting distribution of longitudinal magnetization components is again converted into observable transverse magnetization by a third 90° pulse. It enables the detection of final state of the system. The information necessary to determine the original state before exchange is obtained by a systematic variation of t_1 from experiment to experiment. The use of a magnetic field gradient pulse to destroy transverse magnetization is often undesirable because of the disturbance of the field frequency lock system. An alternative is provided by sequences involving phase-shifted rf pulses for compensation of transverse magnetization.

We limit the analysis of this experiment to a particularly simple situation where each of the N nuclear sites l is characterized by a single characteristic frequency

ω_l (corresponding to its chemical shift). Spin–spin couplings are assumed to be absent.

For convenience, we combine the two transverse magnetization components $M_{lx}(t)$ and $M_{ly}(t)$ and define a complex transverse magnetization $M_l^*(t)$,

$$M_l^*(t) = M_{lx}(t) + iM_{ly}(t) \quad . \tag{1}$$

The N complex transverse magnetizations $M_l^*(t)$ are collected into an N-dimensional transverse magnetization vector $\mathbf{M}^*(t)$. The initial nonselective $\pi/2$ pulse is assumed to effect a positive rotation about the y axis. Then,

$$\mathbf{M}^*(0) = \mathbf{M}_0 \tag{2}$$

where $\mathbf{M}_0$ is the vector of equilibrium z magnetizations.

The evolution during the evolution period is governed by the complex spectral matrix $\boldsymbol{\Omega}$ which describes precession by the resonance frequencies ω_l and possibly decay and exchange of the magnetization components during t_1:

$$\mathbf{M}^*(t_1) = \exp(i\boldsymbol{\Omega} t_1)\mathbf{M}^*(0) \quad . \tag{3}$$

The mixing process after the second $(\pi/2)_y$ pulse starts with the initial magnetization

$$\mathbf{M}_z(t_1, \tau_m = 0) = -\operatorname{Re}\{\mathbf{M}^*(t_1)\} \quad . \tag{4}$$

The dynamics of mixing is governed by the differential equation

$$(d/dt)\mathbf{M}_z = \mathbf{L}\{\mathbf{M}_z - \mathbf{M}_0\} \quad . \tag{5}$$

$\mathbf{L}$ is thereby the exchange matrix which describes relaxation and chemical exchange. Its characterization is the principal purpose of the experiment. The solution of this differential equation is

$$\begin{aligned}\mathbf{M}_z(t_1, \tau_m) &= \mathbf{M}_0 + \exp(\mathbf{L}\tau)[\mathbf{M}_z(t_1, \tau_m = 0) - \mathbf{M}_0]\\ &= 1 - \exp(\mathbf{L}\tau)[\operatorname{Re}\{\exp(i\boldsymbol{\Omega} t_1)\} + 1]\mathbf{M}_0 \quad . \end{aligned} \tag{6}$$

At the end of the mixing period, $\mathbf{M}_z(t_1, \tau)$ is transferred into the xy plane by a third $(\pi/2)_y$ pulse. The evolution, finally, during the detection period (as a function of t_2) is again dominated by the spectral matrix $\boldsymbol{\Omega}$:

$$\mathbf{M}^*(t_1, \tau_m, t_2)$$

$$= \exp(i\boldsymbol{\Omega} t_2)\{1 - \exp(\mathbf{L}\tau_m)[\operatorname{Re}\{\exp(i\boldsymbol{\Omega} t_1)\} + 1]\}\mathbf{M}_0 \quad . \tag{7}$$

The acquired complex magnetization $\mathbf{M}^*(t_1, \tau, t_2)$ or its real or imaginary part is recorded as a function of t_2 for an equidistant set of t_1 values. The resulting 2D data matrix is then treated by the standard procedures of 2D spectroscopy.[13]

Equation (7) consists of terms oscillatory in both time variables t_1 and t_2 and of terms oscillatory exclusively in t_2. The latter terms originate from longitudinal magnetization which recovered during the evolution period. They do not convey information directly related to the exchange mechanism and give rise to the so-called "axial peaks."[13] They will be disregarded in the following. The relevant part of $\mathbf{M}^*(t_1, \tau_m, t_2)$ is

$$\mathbf{M}^{*n}(t_1, \tau_m, t_2) = -\exp(i\boldsymbol{\Omega} t_2)\exp(\mathbf{L}\tau_m)\operatorname{Re}\{\exp(i\boldsymbol{\Omega} t_1)\}\mathbf{M}_0 \quad . \tag{8}$$

Evaluated in the eigenbase of the spectral matrix $\boldsymbol{\Omega}$, it is represented by the following explicit form:

$$\begin{aligned}\mathbf{M}^{*n}(t_1, \tau_m, t_2) = &-\sum_k \sum_l \exp(i\Omega_k t_2)[\exp(\mathbf{L}\tau_m)]_{kl}\\ &\times \operatorname{Re}\{\exp(j\Omega_l t_1)\}\mathbf{M}_{0l} \quad , \end{aligned} \tag{9}$$

with the eigenvalues Ω_k and Ω_l of $\boldsymbol{\Omega}$.

The complex spectrum $S^*(\omega_1, \tau_m, \omega_2)$ is finally obtained by a 2D Fourier transformation

$$\begin{aligned}S^*(\omega_1, \tau_m, \omega_2) = &-\sum_k \sum_l \frac{1}{i(\Omega_k - \omega_2)}[\exp(\mathbf{L}\tau_m)]_{kl}\\ &\times \operatorname{Re}\left\{\frac{1}{j(\Omega_l - \omega_1)}\right\}\mathbf{M}_{0l} \quad . \end{aligned} \tag{10}$$

Each term in this double sum represents a peak in the 2D spectrum at the frequency coordinates $\operatorname{Re}\{\Omega_l\}$ and $\operatorname{Re}\{\Omega_k\}$ with the corresponding line widths $\operatorname{Im}\{\Omega_l\}$ and $\operatorname{Im}\{\Omega_k\}$, respectively. Intensities and phases of the diagonal peaks $(k = l)$ and cross peaks $(k \neq l)$ are determined by the exchange process $\mathbf{L}$. The 2D spectrum is, in essence, just a pictorial representation of the mixing operator $\exp\{\mathbf{L}\tau_m\}$ whereby cross peaks indicate exchange of magnetization between two nuclear sites either due to chemical exchange or due to cross relaxation.

At this point, it should be noticed that the spectral matrix $\boldsymbol{\Omega}$ can be quite complicated, as it usually involves effects of the exchange process as well. A simple illustrative example will be treated next.

IV. RATE PROCESSES IN A SYSTEM WITH TWO SITES

We consider in this section a system with two sites or two molecules A and B. A first order chemical exchange process is assumed,

$$A \underset{k_{BA}}{\overset{k_{AB}}{\rightleftharpoons}} B, \tag{11}$$

described by the kinetic matrix $\mathbf{K}$,

$$\mathbf{K} = \begin{bmatrix} -k_{AB} & k_{BA} \\ k_{AB} & -k_{BA} \end{bmatrix} \quad . \tag{12}$$

The rate constants k_{AB} and k_{BA} are related through the mole fractions x_A and x_B in chemical equilibrium,

$$k_{AB} = x_B k, \qquad k_{BA} = x_A k \quad . \tag{13}$$

It is assumed that each of the two sites (two molecules) possesses a single Larmor frequency ω_A and ω_B, respectively, each associated with one nuclear spin.

We also introduce relaxation processes into our model system. Longitudinal relaxation shall involve two different processes: (a) The two nuclei may relax independently with relaxation rates R_{1A} and R_{1B} due to external relaxation. (b) In addition, there may be a direct dipolar interaction between the two nuclei A and B leading to dipolar relaxation with the relaxation rate R_D. We make here the extreme narrowing approximations for nonviscous solutions. This gives then the longitudinal relaxation matrix.

$$W = \begin{bmatrix} -R_{1A} - 2x_B R_D & -x_A R_D \\ & \\ -x_B R_D & -R_{1B} - 2x_A R_D \end{bmatrix} . \tag{14}$$

When the resonance lines of A and B do not appreciably overlap, transverse relaxation will be exponential and can be described by the two transverse relaxation rates $R_{2A} = 1/T_{2A}$ and $R_{2B} = 1/T_{2B}$. This model leads to the following spectral matrix Ω:

$$i\Omega = \begin{bmatrix} i\omega_A - (R_{2A} + k_{AB}) & k_{BA} \\ & \\ k_{AB} & i\omega_B - (R_{2B} + k_{BA}) \end{bmatrix} . \tag{15}$$

To describe the effects of the spectral matrix Ω during evolution and detection periods, it is convenient to determine its eigenvalues Ω_1 and Ω_2, obtained by the transformation

$$T_\Omega^{-1}(i\Omega)T_\Omega = \begin{bmatrix} i\Omega_1 & 0 \\ & \\ 0 & i\Omega_2 \end{bmatrix} , \tag{16}$$

$$i\Omega_{1,2} = -\tfrac{1}{2}\{R_{2A} + R_{2B} + k_{AB} + k_{BA} - i(\omega_A + \omega_B) \pm w\} ,$$

$$w = \{\Delta^2 + 4k_{AB}k_{BA}\}^{1/2} ,$$

$$\Delta = R_{2A} - R_{2B} + k_{AB} - k_{BA} - i(\omega_A - \omega_B) .$$

The eigenvectors are given by the column vectors of T_Ω,

$$T_\Omega = T_\Omega^{-1} = \{(w+\Delta)^2 + 4k_{AB}k_{BA}\}^{-1/2} \begin{bmatrix} -(w+\Delta) & 2k_{BA} \\ & \\ 2k_{AB} & (w+\Delta) \end{bmatrix} . \tag{17}$$

Because 2D spectroscopy is particularly suited to the investigation of slow processes, we make the simplifying assumption that chemical exchange and relaxation are sufficiently slow that we may use first order perturbation theory to determine their effects during evolution and detection periods. The two eigenvalues of $i\Omega$ are then

$$i\Omega_1 = i\omega_A - (R_{2A} + k_{AB}) ,$$

$$i\Omega_2 = i\omega_B - (R_{2B} + k_{BA}) . \tag{18}$$

The exchange matrix L which governs the mixing process is given for this system by

$$L = K + W = \begin{bmatrix} -R_{AA} & -x_A R_C \\ & \\ -x_B R_C & -R_{BB} \end{bmatrix} , \tag{19}$$

with

$$R_{AA} = R_{1A} + 2x_B R_D + x_B k ,$$

$$R_{BB} = R_{1B} + 2x_A R_D + x_A k ,$$

$$R_C = R_D - k . \tag{20}$$

Integration of the equations of motion during the three periods leads, finally, to the signal observed during the detection period. We assume detection of the x component of the precessing magnetization and obtain

$$M_x(t_1, \tau_m, t_2) = -\sum_{j,l=A,B} a_{jl}(\tau_m) \cos\omega_j t_1 \cos\omega_l t_2$$

$$\times \exp\{-t_1(R_{2j} + (1-x_j)k)\}\exp\{-t_2(R_{2l} + (1-x_l)k)\} . \tag{21}$$

For the mixing coefficients $a_{jl}(\tau_m)$, which describe the exchange process, the following expressions are found:

$$a_{AA}(\tau_m) = x_A e^{-\sigma\tau_m}[\cosh(D\tau_m) - \frac{\delta}{D}\sinh(D\tau_m)] ,$$

$$a_{BB}(\tau_m) = x_B e^{-\sigma\tau_m}[\cosh(D\tau_m) + \frac{\delta}{D}\sinh(D\tau_m)] ,$$

$$a_{AB}(\tau_m) = a_{BA}(\tau_m) = -x_A x_B \frac{R_C}{D} e^{-\sigma\tau_m}\sinh(D\tau_m) , \tag{22}$$

with

$$\sigma = \tfrac{1}{2}(R_{AA} + R_{BB}) , \tag{23}$$

$$\delta = \tfrac{1}{2}(R_{AA} - R_{BB}) , \tag{24}$$

$$D = (\delta^2 + x_A x_B R_C^2)^{1/2} . \tag{25}$$

The mixing coefficients $a_{jl}(\tau_m)$ determine the integrated intensities of the two diagonal and the two cross peaks of the resulting 2D spectrum. The two cross peaks have identical intensity.

In practical applications, the mixing time τ_m should be selected properly to obtain significant intensities for the off-diagonal peaks. The optimum mixing time to maximize the cross peaks is computed to be

$$\tau_{opt} = \frac{1}{2D} \ln\frac{\sigma + D}{\sigma - D} , \tag{26}$$

with the following intensity ratio of cross- and diagonal peaks:

$$r(\tau_{opt}) = \frac{a_{AB}(\tau_{opt})}{[a_{AA}(\tau_{opt})a_{BB}(\tau_{opt})]^{1/2}}$$

$$= -\frac{R_C}{D} \frac{\sinh(D\tau_{opt})x_A x_B}{[\cosh^2(D\tau_{opt}) - (\delta/D)^2 \sinh^2(D\tau_{opt})]^{1/2}} . \tag{27}$$

The general formulas will now be applied to a few specialized cases to illustrate their principal features.

A. Spin system with chemical exchange

We consider here a one-spin system exchanging between two sites with equal mole fractions $x_A = x_B = \tfrac{1}{2}$. In addition, we take into account an external relaxation mechanism acting equally on both sites. It should be noticed that chemical exchange will cause exchange of transverse magnetization also during evolution and detection periods. However, for slow chemical exchange, i.e., $k_{AB}, k_{BA} \ll |\omega_A - \omega_B|$, the exchange effects during evolution and detection will be small and the Ω matrix may be approximated by its diagonal part, Eqs. (15) and (18). The exchange during evolution and detection will then affect only the line width and cause the following effective transverse relaxation rates:

$$R_{2A}^{eff} = R_{2A} + \tfrac{1}{2}k, \quad \text{and} \quad R_{2B}^{eff} = R_{2B} + \tfrac{1}{2}k . \tag{28}$$

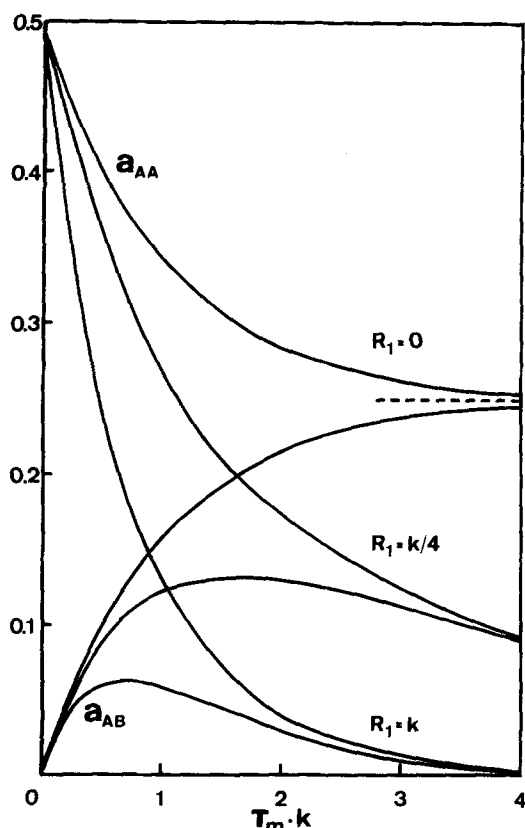The behavior during the mixing period is determined by the parameter values

FIG. 3. Amplitudes of the diagonal peaks, $a_{AA} = a_{BB}$, and of the cross peaks, $a_{AB} = a_{BA}$, in the presence of chemical exchange in a two-site system as functions of the product of rate constant k and exchange time τ_m for three random field relaxation rates $R_1 = 0$, $k/4$, and k.

$$R_{AA} = R_{BB} = \sigma = R + \tfrac{1}{2}k \ ,$$

$$R_C = -k \ ,$$

$$\delta = 0, \qquad D = \tfrac{1}{2}k, \tag{29}$$

and leads to the peak amplitudes

$$a_{AA}(\tau_m) = a_{BB}(\tau_m) = \tfrac{1}{4}e^{-R_1\tau_m}\left[1 + e^{-k\tau_m}\right] \ ,$$

$$a_{AB}(\tau_m) = a_{BA}(\tau_m) = \tfrac{1}{4}e^{-R_1\tau_m}\left[1 - e^{-k\tau_m}\right] . \tag{30}$$

It is important to note that diagonal and cross peaks have the same phase, irrespective of τ_m and of the rate of external relaxation R_1. This is in contrast to magnetization transfer caused by relaxation in the extreme narrowing regime. The quantitative behav-

ior of the peak amplitudes is visualized in Fig. 3. For $R_1 = 0$, all intensities tend to the same constant value when $\tau_m \rightarrow \infty$. However, for the more realistic case $R_1 > 0$, the cross-peak amplitude goes through a maximum for

$$\tau_{opt} = \frac{1}{k}\ln\frac{R_1 + k}{R_1} \tag{31}$$

with the intensity ratio, Eq. (27),

$$r(\tau_{opt}) = \frac{1}{2R_1 + k} . \tag{32}$$

To obtain cross peaks of appreciable intensity, it is necessary to select the exchange rate (i.e., temperature) such that $k \gtrsim R_1$.

We verified the theoretical predictions for exchange in a two-site system by experiments on N, N-dimethyl-acetamid. The hindered rotation about the C–N bond provides an exchange mechanism for the two N-methyl groups. A complete 2D exchange spectrum recorded at 30 °C is shown in Fig. 4. The three diagonal peaks correspond to the two N-methyl groups and to the acetic methyl group, respectively. Cross peaks appear exclusively between the two N-methyl groups and indicate the well-known exchange process between these two groups.

For faster exchange, the characteristic broadening, coalescence, and line narrowing features, known from conventional exchange spectra, appear in a 2D exchange spectrum as well. Figure 5 demonstrates these effects for four different temperatures. The most informative range for recording 2D exchange spectra is under slow exchange conditions. Here, the intensities of the cross peaks provide direct information on the rate of exchange and on the exchange pathways. For faster exchange, all peaks become of equal amplitude and no quantitative information is available anymore.

We would like to mention that similar cross peak intensities are also obtained for magnetization exchange by dipolar relaxation with sufficiently long correlation times. In the following two sections, we will treat the other extreme, dipolar relaxation with very short correlation times.

B. Longitudinal magnetization exchange by dipolar relaxation in a two-spin system

We assume a system consisting of two-spin molecules without chemical exchange in a spin-free solvent. The relevant relaxation processes shall be intramolecular
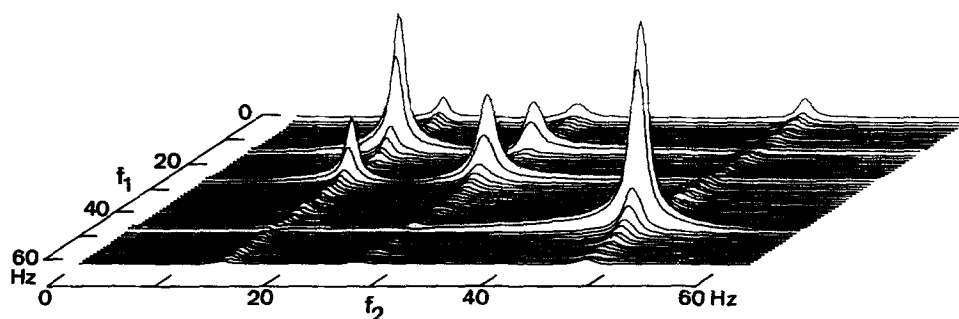

FIG. 4. 2D exchange spectrum of N, N-dimethylacetamide. The 60 MHz proton resonance spectrum has been recorded at 30 °C using the experimental scheme of Fig. 1 with 128 equidistant t_1 values from 0 to 1016 ms. Each free induction decay has been represented by 256 samples. A mixing time of 0.5 s was employed.
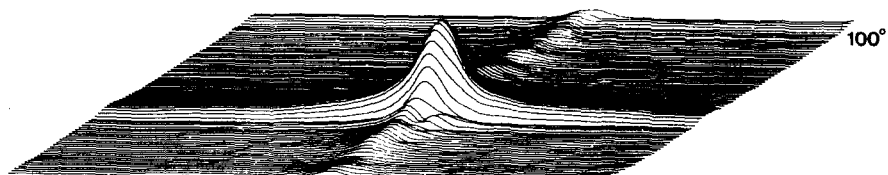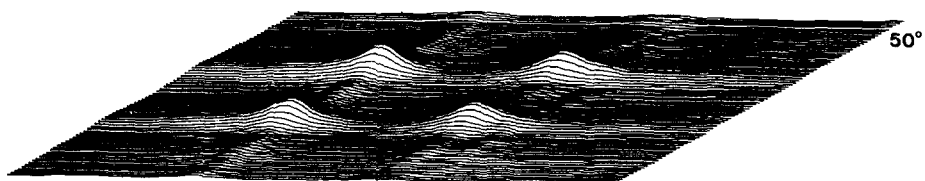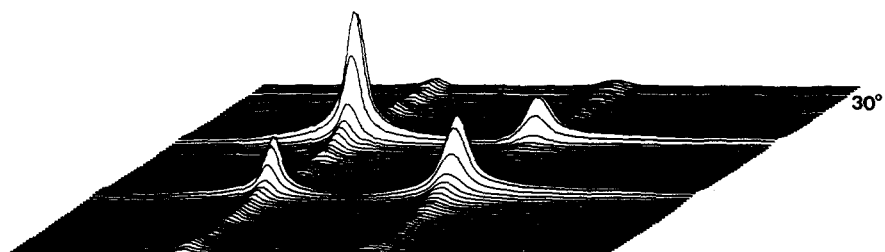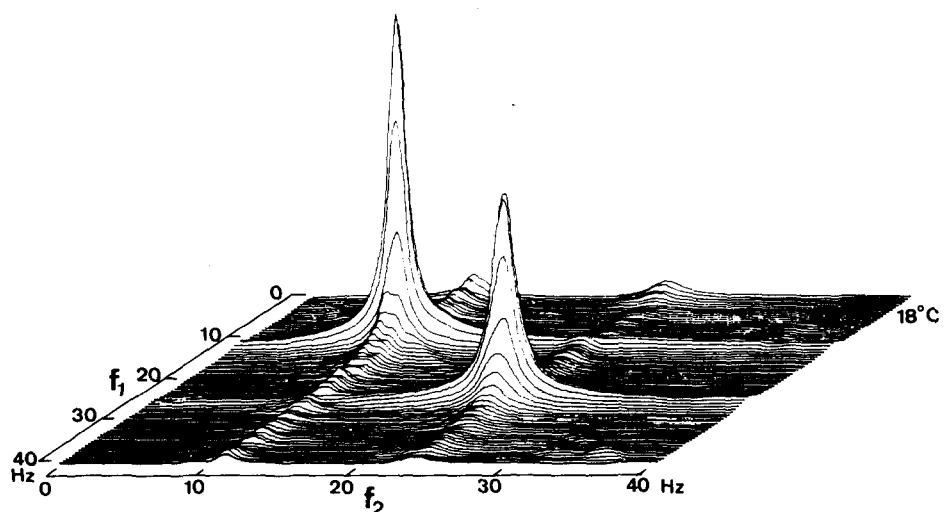
FIG. 5. Partial 2D exchange spectra of N, N-dimethylacetamide (same conditions as for Fig. 4) for five different temperatures to demonstrate the exchange effects at higher exchange rates.

dipolar relaxation assuming extreme narrowing (rate constant R_D) and external relaxation identical for both spins (rate constant R_1). A possible magnetization exchange is caused in this case by intramolecular dipolar relaxation. We obtain the following expressions for the relevant parameters in Eqs. (22):

$$R_{AA} = R_{BB} = R_1 + R_D \quad , \qquad R_C = R_D/2 \quad ,$$

$$\sigma = R_1 + R_D, \qquad \delta = 0, \qquad D = \tfrac{1}{2}R_D \quad . \tag{33}$$

This leads then to the peak intensities

$$a_{AA}(\tau_m) = a_{BB}(\tau_m) = \tfrac{1}{4} \exp[-(R_1 + R_D/2)\tau_m][1 + e^{-R_D\tau_m}] \quad ,$$

$$a_{AB}(\tau_m) = a_{BA}(\tau_m) = -\tfrac{1}{4} \exp[-(R_1 + R_D/2)\tau_m][1 - e^{-R_D\tau_m}] \quad . \tag{34}$$

The intensity coefficients $a_{AA}(\tau_m)$ and $a_{AB}(\tau_m)$ are plotted in Fig. 6 as functions of $\tau_m R_D$ for $R_1 = 0$ and $R_1 = R_D$. It is obvious that the cross peaks are in general rather weak compared to the maximum possible intensity of a_{AA} for $\tau_m = 0$. Even in the ideal case of pure dipolar relaxation within the nuclear pair A and B, the maximum cross-peak intensity reaches only 19% of $a_{AA}(0)$. External relaxation provides some leakage. If, for example, external relaxation is of equal strength, $R_1 = R_D$, the cross-peak intensity is reduced to 9% of $a_{AA}(0)$.

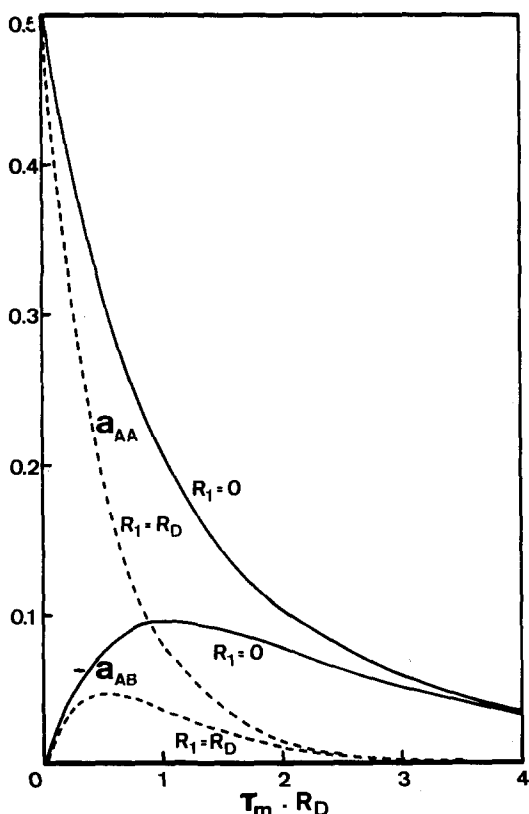The optimum τ_m for maximum cross-peak amplitude is

FIG. 6. Peak amplitudes in a 2D magnetization exchange spectrum of a two-spin system. The exchange is caused by dipolar relaxation between the two spins with relaxation rate R_D. The amplitudes of the diagonal peaks, $a_{AA} = a_{BB}$, and of the cross peaks, $a_{AB} = a_{BA}$, are plotted as functions of the product $\tau_m R_D$ for vanishing random field relaxation, $R_1 = 0$, and for $R_1 = R_D$.
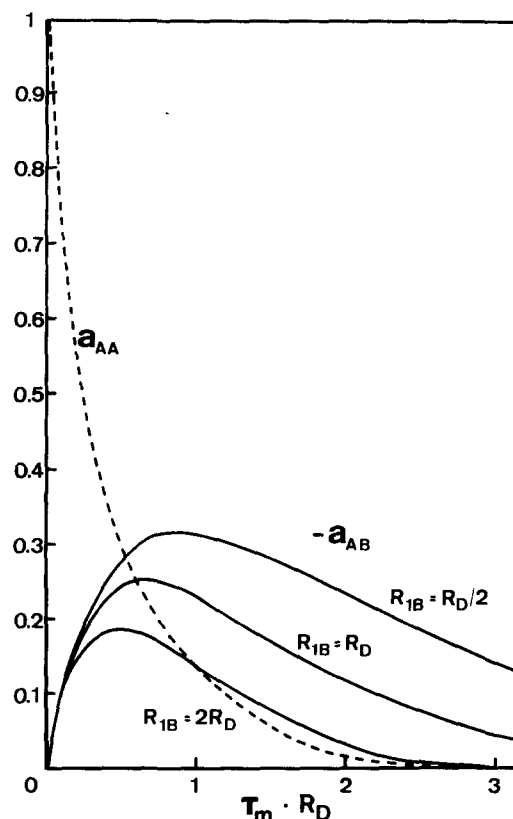

FIG. 7. Peak amplitudes in a 2D magnetization exchange spectrum of a dilute one-spin system in a one-spin solvent. The amplitudes of the diagonal peaks, $a_{AA} = a_{BB}$, and the cross peaks, $a_{AB} = a_{BA}$, are plotted as functions of the product of exchange time τ_m and dipolar relaxation rate R_D for three random field relaxation rates of the solvent, $R_{1B} = R_D/2$, R_D, and $2R_D$.

$$\tau_{opt} = \frac{1}{R_D} \ln \frac{R_1 + \tfrac{3}{2}R_D}{R_1 + \tfrac{1}{2}R_D} \tag{35}$$

and the corresponding ratio of intensities $r(\tau_{opt})$ becomes

$$r(\tau_{opt}) = -\frac{1}{2} \frac{R_D}{R_1 + R_D} \quad . \tag{36}$$

For sufficiently long mixing time τ_m, the four peaks in the 2D spectrum tend to assume equal but vanishingly small intensity. It is of importance to note that in this case diagonal and cross peaks have opposite phase in contrast to the case treated in the previous section.

C. Longitudinal magnetization exchange by dipole relaxation between one-spin solute and one-spin solvent

As a final example, we assume a dilute solution of A spin molecules in a B spin solvent with $x_A \ll 1$ and $x_B \simeq 1$. The B spins relax independently with rate R_{1B} while the solute spins A relax exclusively through interaction with the solvent spins. Therefore

$$R_{AA} = 2R_D \quad , \qquad R_{BB} = R_{1B} \quad , \qquad R_C = R_D \quad ,$$

$$\sigma = R_D + \tfrac{1}{2}R_{1B} \quad , \quad \delta = R_D - \tfrac{1}{2}R_{1B} \quad , \quad D = R_D - \tfrac{1}{2}R_{1B} \quad , \tag{37}$$

and

$$a_{AA}(\tau_m) = x_A\, e^{-2R_D\tau_m} \quad , \qquad a_{BB}(\tau_m) = e^{-R_{1B}\tau_m} \quad ,$$

$$a_{AB}(\tau_m) = x_A\, \frac{R_D}{2R_D - R_{1B}} [e^{-R_{1B}\tau_m} - e^{-2R_D\tau_m}] \quad . \tag{38}$$

We have plotted $a_{AA}(\tau_m)/x_A$ and $a_{AB}(\tau_m)/x_A$ in Fig. 7

as functions of $\tau_m R_D$. It is of interest that the cross peaks can become of larger amplitude than the diagonal A peak, in particular for the case $R_{1B} < R_D$. Assuming a homonuclear system, however, usually the opposite ratio is observed, $R_{1B} > R_D$. The cross peaks will be attenuated by the leakage relaxation R_{1B}, in a manner similar to that of the case described in Sec. IV.B. The maximum cross-peak amplitude is obtained for

$$\tau_{opt} = \frac{1}{2R_D - R_{1B}} \ln \frac{2R_D}{R_{1B}} \quad,$$

with

$$r(\tau_{opt}) = -\frac{a_{AB}(\tau_{opt})}{a_{AA}(\tau_{opt})} = \frac{R_D}{R_{1B}} \quad. \tag{39}$$

It should be noted that the two nuclei A and B can also be different nuclear species. Then, the three 90° pulses would have to be applied simultaneously to both nuclei. If, for example, the solvent B spin is of lower gyromagnetic ratio than the solute A spin, it becomes quite possible that $R_{1B} < R_D$, and the cross peaks can become of considerable relative amplitude.

Again, diagonal and cross peaks have opposite phase, which is typical for magnetization transfer by relaxation under extreme narrowing conditions.

V. EXPERIMENTAL

The experiments have been performed on a Fourier spectrometer based on a Varian DA 60 NMR spectrometer equipped with a Varian 620/L-100 computer and a Diablo 43 disk unit. Five-millimeter sample tubes have been used. The samples contained 25 vol% hexafluorobenzene for the fluorine field-frequency lock.

A general 2D NMR program has been used. This versatile program, suitable for almost all conceivable 2D experiments including data accumulation, data transformation, and plotting, uses an overlay technique and requires only $4k$ of memory space. The experiment-specific part is contained in a separate user subroutine. For short mixing periods without sufficient delay between field gradient pulse and data acquisition, the perturbation of the field frequency lock by the applied field gradient causes unstable conditions during observation. To avoid these disturbances, the lock was disabled during and immediately after the field gradient pulse. At present, phase alternation schemes are under development to completely replace field gradient pulses used for the elimination of transverse interference.

A contour plot program has been developed for operation on a minicomputer, which is capable of plotting very large data matrices. The data matrix to be plotted defines a rectangular grid of cells with the data points forming the corners of the cells. A contour line at a given level crosses all those cells with corners simultaneously above and below the selected level. To determine the entry and exit points, linear interpolation along the edges of the cell is used. The continuation of a contour line through the neighbor cells is unique as long as the cells have two entry or exit points, only. For cells with four entry or exit points, there is an am-

biguity about the continuation which, however, does not influence the general appearance of a contour plot.

VI. CONCLUSIONS

It has been demonstrated that it is possible to detect chemical exchange processes and magnetization exchange due to relaxation by means of 2D spectroscopy. The method is of particular value for the detection of exchange networks in systems with many sites. 2D exchange spectra lead to an intuitive understanding of the involved exchange network.

Applications of 2D magnetization exchange studies can be envisioned in molecular biology to measure magnetization transfer by inter- and intramolecular Overhauser effects. The same scheme can also be utilized to investigate cross relaxation in solids. It is possible to investigate homonuclear and heteronuclear cross relaxation, for example during heteronuclear cross-polarization processes used to enhance sensitivity of rare nuclei.

2D spectroscopy has hitherto mainly been used to investigate coherent effects in coupled spin systems. The scheme proposed in this paper now makes it feasible to elucidate incoherent processes by 2D spectroscopy techniques, as well.

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