a function of the distance between the SCN and NO species of nitrosyl thiocyanate. The triplet states are in the EWMO model represented by the LUMO and HOMO energy difference[16] $\Delta\epsilon = |E_{HOMO} - E_{LUMO}|$, and the single state should be located 1–2 eV above the triplet state. The results are shown in Figure 7 where $\Delta\epsilon$ is plotted as a function of the stretching of the S–N bond. The total atomic net charge on the SCN and NO parts are given as well.

The dotted curve in Figure 7 which represents the triplet excitation energy in nitrosyl thiocyanate reaches a minimum at a stretching length of 1.2 Å. This is evidence of an avoided crossing of the molecular orbital energies. The full line which represents the total atomic net charge on the SCN and NO parts, respectively, tends to zero at $r = 1.2$ Å. These simple considerations indicate the presence of a biradical state close in energy, and it can be concluded that nitrosyl thiocyanate might dissociate to NO and SCN radicals. Seel and Wesemann[17] have shown that nitrosyl thiocyanate decomposes to nitrogen oxide and thiocyanogen (**5**); the decomposition probably takes place via SCN radicals.

$$2ON\text{–}SCN \rightarrow 2NO + \underset{\mathbf{5}}{NCS\text{–}SCN}$$

Smokers secrete ca. 200 mg of thiocyanate per day more than nonsmokers.[2b] The thiocyanate secreted in saliva must be reabsorbed in the alimentary tract and secreted again in the saliva. Nitrite can be present in the stomach from bacterially reduced nitrate or from nitrite added to food. The acidic conditions in the stomach might cause nitrite to form the nitrosyl cation which can react with the thiocyanate anion under formation of nitrosyl thiocyanate. The higher content of thiocyanate in the saliva of smokers may then accelerate the rate of formation of *N*-nitrosamines as stated earlier or it might cause formation of reactive NO and SCN radicals which can be involved in the carcinogenesis in the stomach or in the alimentary tract. On the other hand, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine affords after incubation in rat liver homogenate an ESR spectrum with a *g* value of 2.035,[18] due to a cysteine–ferrous ion–nitrogen oxide complex which is known to inhibit the binding of carcinogens to proteins. Since nitrogen oxide has a pronounced tendency to couple with free radicals, it might be released in the liver and quench the carcinogenic free radicals and, thereby, inhibit their action.

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Registry No. 1, 3985-25-9; **2**, 90991-48-3; NO^+, 14452-93-8; SCN^-, 302-04-5; nitrogen, 7727-37-9; sulfur, 7704-34-9.

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Skewed Exchange Spectroscopy. Two-Dimensional Method for the Measurement of Cross Relaxation in ^{1}H NMR Spectroscopy

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Abstract: A theoretical treatment of the two-dimensional measurement of nuclear magnetic cross relaxation leads to a method in which all rates are obtained through the back-transformation of a matrix of NOESY mixing coefficients. The theory of a two-dimensional pulse sequence, skewed exchange spectroscopy, is also developed. This experiment is applicable to the study of nuclear Overhauser effects in ^{1}H NMR spectroscopy. *J*-coupling effects are absent, and spectral crowding along the diagonal is avoided by the projection of diagonal-peak information onto the cross peaks. The back-transformation method is used to identify a relay NOE in a three-spin system.

I. Introduction

The nuclear Overhauser effect (NOE) is an important exchange phenomenon which can be studied by both one-dimensional (1-D) and two-dimensional (2-D) NMR spectroscopy.[1,2] Its importance stems from the fact that NOE's are used to determine the structure and dynamics of molecules in solution. In proton NMR spectroscopy, NOE's are usually absent when nuclei are more than 500 pm apart, and thus the mere occurrence of an NOE provides valuable information on connectivity[3] and structure.[4,5] Quantitative information, however, is obtained only by the measurement of cross relaxation rates between interacting nuclei. The rates are normally measured by 1-D techniques in which the buildup of the NOE is plotted as a function of saturation time.[6,7] In these experiments, measurements must be restricted to the regime of short saturation times to avoid the effects of multispin exchange, which arise in the steady state.

An efficient way to study cross relaxation is found in the NOESY experiment, which was first described by Jeener et al.[8] Unlike 1-D methods, NOESY allows the simultaneous observation of all exchange phenomena in one experiment and is free of the problems associated with selective irradiation. Semiquantitative analysis of the relaxation pathways obtained from NOESY has

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led to the determination of polypeptide conformations[4,5] via Crippen's distance algorithm.[9]

Further advances can be expected if, in addition, techniques are developed for the 2-D measurements of cross relaxation rates. Such measurements would also facilitate the identification of relay NOE's, which arise not from the close proximity of nuclei but from a relay effect in which magnetization is passed from one nucleus to another by spin diffusion.

In a study of bovine pancreatic trypsin inhibitor, Kumar et al.[10] examined the variation in NOESY cross peaks as a function of mixing time. They demonstrated a qualitative correlation between the time rate of increase of cross peak intensity and the interatomic distances found from X-ray analysis. In addition, they reported relay NOE's, which exhibited a time dependence similar to the 1-D case.

More recently, Bodenhausen and Ernst[11,12] have proposed a new kind of exchange experiment, accordion spectroscopy, which is capable of measuring cross relaxation rates. By stepping through a range of mixing times, the accordion experiment leads to a NOESY-type spectrum in which the rate constants are reflected in the line shapes of the cross and diagonal peaks. The experiment has been successfully applied to the measurement of chemical exchange using ^{13}C NMR spectroscopy[12] but is unsuitable so far for ^1H NMR spectroscopy because of zero-quantum effects, which arise in J-coupled systems.[12,13]

In this paper a new experiment is described that is also capable of measuring exchange and cross relaxation rates. As will be shown, the experiment results in a skewed NOESY spectrum and has thus been named SKEWSY for skewed exchange spectroscopy. SKEWSY requires no line-shape analysis, and all information is theoretically obtainable from the cross peaks, thus avoiding the (usually) crowded diagonal region of the 2-D spectrum. Like NOESY, SKEWSY uses a randomized mixing period to eliminate zero-quantum coherence and is therefore well suited to ^1H NMR spectroscopy and the study of NOE's. The description and theory of the experiment will be given in section II.

In both 1-D and 2-D NMR spectroscopy, the approach to the measurement of cross relaxation rates has so far relied on a time series of experiments.[6,7] It will be shown in section III that all rates can be measured in a single 2-D experiment by solving the eigenvalue problem for a matrix of NOESY coefficients. The relaxation rates are then obtained through a linear transformation of the NOESY matrix. In this general context the proposed SKEWSY experiment is only one of several possible approaches to the problem.

Finally, section IV is devoted to a case study of a three-spin system, in which the transformation method is used to identify a relay NOE.

II. Background Theory

The SKEWSY experiment may be thought of as a NOESY experiment that is perturbed by a 180° pulse in the preparation period (Figure 1) and is broadly similar to the sequence used in 2-D exchange difference spectroscopy.[13] Magnetization exchange is allowed to occur during time τ_s, after which spins are frequency labeled in the t_1 period by the second 90° pulse. The third 90° pulse again promotes magnetization exchange for a period τ_m, after which the spins are observed in the time period t_2 following the fourth pulse. A series of free-induction decays, $S(t_1,t_2)$, are recorded in the usual manner and Fourier transformed to obtain the 2-D spectrum $S(\omega_1,\omega_2)$.

In this treatment, we consider a system of n spin-$^1/_2$ nuclei, which are uncoupled and in thermal equilibrium. The initial state is described by the vector

$$\mathbf{M}_0 = \{M_{01},M_{02},M_{03}...M_{0n}\}$$

where M_{0i} is the equilibrium magnetization of the ith spin. Just after the nonselective π pulse, all of the spins are oriented in the $-z$ direction and undergo dipolar relaxation according to

$$d\mathbf{M}_z/dt = -\mathbf{R}\{\mathbf{M}_z - \mathbf{M}_0\} \quad (1)$$

$\mathbf{R}$ is a matrix of rate constants describing the dipolar relaxation process, in which off-diagonal terms mediate the NOE effect (i.e., cross relaxation) and the spin–lattice relaxation is described by the diagonal rate constants. The process of dipolar relaxation for a system of three spins is shown schematically in Figure 2. The principles of reversibility and energy conservation ensure that the matrix $\mathbf{R}$ is both symmetric and positive definite; thus a system of n spins can be described by at most $(n/2)(n+1)$ independent rate constants. Solving eq 1, we obtain

$$\mathbf{M}_z(\tau_s) = [\hat{\mathbf{I}} - 2\exp(-\mathbf{R}\tau_s)]\mathbf{M}_0 \quad (2)$$

$\hat{\mathbf{I}}$ = identity matrix

Following the $(\pi/2)_{-x}$ pulse, we describe the initial transverse

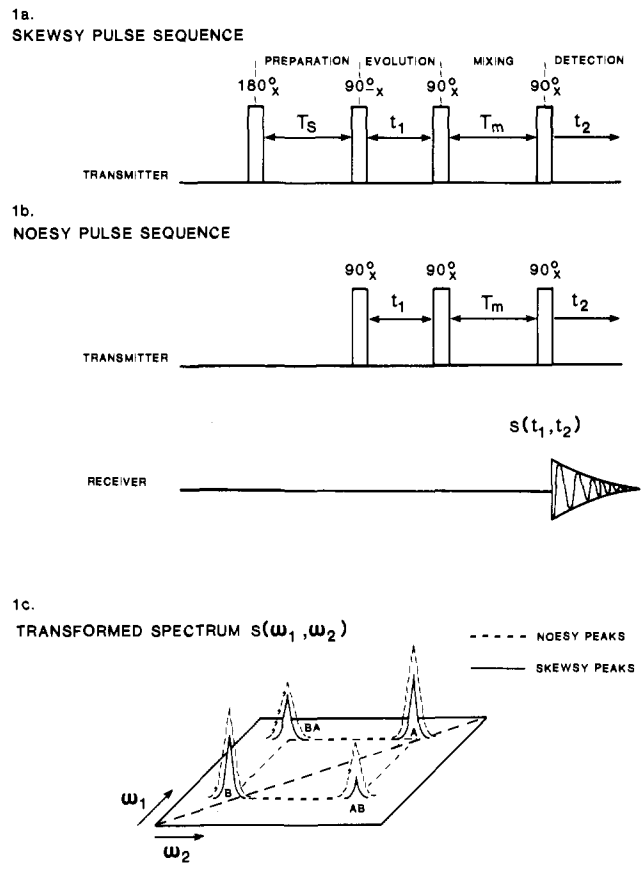

Figure 1. Pulse sequences used in SKEWSY (a) and NOESY (b) experiments. The transformed spectrum of a hypothetical two spin system is shown in (c), in which the cross peaks of the SKEWSY spectrum are asymmetric.

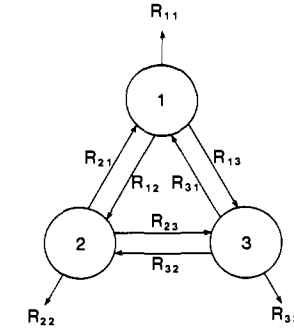

Figure 2. Cross relaxation between a system of three spins. Each term of the relaxation matrix R_{ij} corresponds to an exchange of magnetization as shown. The diagonal terms R_{11}, R_{22}, and R_{33} are the rates of spin–lattice relaxation and are thus responsible for energy losses to the surroundings.

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magnetization in complex form as

$$\mathbf{M}^+(\tau_s) = i[\hat{\mathbf{I}} - 2\exp(-\hat{\mathbf{R}}\tau_s)]\mathbf{M}_0 \quad (3)$$

where imaginary components are used to denote y magnetization. The system then evolves under the influence of the complex spectral matrix Ω, which describes both the precession and relaxation of the transverse magnetization. In addition, $\hat{\Omega}$ may include the effects of chemical exchange which we assumed to be negligible. Thus:

$$\mathbf{M}^+(\tau_s,t_1) = i\exp(i\hat{\Omega}t_1)[\hat{\mathbf{I}} - 2\exp(-\hat{\mathbf{R}}\tau_s)]\mathbf{M}_0 \quad (4)$$

After the third pulse, the z magnetization is given by

$$\mathbf{M}_z(\tau_s,t_1,\tau_m = 0) = \mathrm{Im}\{\mathbf{M}^+(\tau_s,t_1)\} = \mathrm{Re}\{\exp(i\hat{\Omega}t_1)[\hat{\mathbf{I}} - 2\exp(-\hat{\mathbf{R}}\tau_s)]\mathbf{M}_0\} \quad (5)$$

Again the system undergoes magnetization exchange according to eq 1 leading to

$$\mathbf{M}_z(\tau_s,t_1,\tau_m) = \mathbf{M}_0 + \exp(-\hat{\mathbf{R}}\tau_m)[\mathrm{Re}\{\exp(i\hat{\Omega}t_1)[\hat{\mathbf{I}} - 2\exp(-\hat{\mathbf{R}}\tau_s)]\mathbf{M}_0\} - \mathbf{M}_0] \quad (6)$$

Finally the observation pulse results in transverse magnetization whose precession is governed by the spectral matrix $\hat{\Omega}$ and described as

$$\mathbf{M}^+(\tau_s,t_1,\tau_m,t_2) = i\exp(i\hat{\Omega}t_2)\exp(-\hat{\mathbf{R}}\tau_m)\mathbf{M}_0 - i\exp(i\hat{\Omega}t_2)\mathbf{M}_0 - i\exp(i\hat{\Omega}t_2)\exp(-\hat{\mathbf{R}}\tau_m)\,\mathrm{Re}\{\exp(i\hat{\Omega}t_1)[\hat{\mathbf{I}} - 2\exp(-\hat{\mathbf{R}}\tau_s)]\mathbf{M}_0\} \quad (7)$$

The first two terms have no dependence on t_1 and result in unwanted axial peaks in the 2-D spectrum. The axial peaks can be eliminated by appropriate phase cycling and thus will be ignored in the present treatment. The last term corresponds to the 2-D SKEWSY spectrum prior to transformation, where the observed signal $S(t_1,t_2)$ is formed by summing each element of the vector $\mathbf{M}^+(\tau_s,t_1,\tau_m,t_2)$.

In circumstances of slow chemical exchange, the spectral matrix $\exp(i\hat{\Omega}t)$ is diagonal (see eq 15, ref 8), and eq 7 can be rearranged to give

$$\mathbf{M}^+(\tau_s,t_1,\tau_m,t_2) = i[\exp(i\hat{\Omega}t_2)\hat{\mathbf{S}}\,\mathrm{Re}\{\exp(i\hat{\Omega}t_1)\}]\mathbf{M}_0 \quad (8)$$

The new exchange matrix $\hat{\mathbf{S}}$ is a composite of the exchange processes taking place in the mixing and preparation periods. Using a_{ij} to denote the elements of $\exp(-\hat{\mathbf{R}}\tau_m)$ and assuming $\tau_s = \tau_m$, we express the mixing coefficients S_{ij} as

$$S_{ij} = -a_{ij} + 2a_{ij}\sum_{k=1}^{n} a_{jk} \quad (9)$$

The mixing coefficients S_{ij} modulate the SKEWSY spectrum in the same way that the coefficients a_{ij} modulate a NOESY spectrum; that is, the intensities of cross peaks are proportional to S_{ij}, M_0, and the line-shape functions. A typical cross peak takes the form given by Macura and Ernst[2]

$$S_{ij}^{\mathrm{cross}}(\omega_1,\omega_2) = \frac{1}{2}\frac{1/T_{2i}}{(\omega_2 - \omega_i)^2 + 1/T_{2i}^2}S_{ij}\frac{1}{2}\frac{1/T_{2j}}{(\omega_1 - \omega_j)^2 + 1/T_{2j}^2}M_{0j} \quad (10)$$

where ω_i represents the Larmor frequency of the ith spin and T_{2i}, the transverse relaxation time. Unlike NOESY coefficients, SKEWSY coefficients S_{ij} are not symmetric, and thus the two-dimensional spectrum has a skewed appearance.

In a two-spin system, the skewed appearance has a simple interpretation. Using eq 9 and the expression for a_{ij} derived by Jeener et al.,[8] we obtain

$$S_{AB} - S_{BA} = a_{AB}(a_{BB} - a_{AA}) = (2\delta R_{AB}/D^2)e^{-2\sigma\tau_m}\sinh^2 D\tau_m \quad (11)$$

$$\delta = (1/2)(R_{AA} - R_{BB})$$

$$D = \sqrt{\delta^2 + R_{AB}^2}$$

$$\sigma = (1/2)(R_{AA} + R_{BB})$$

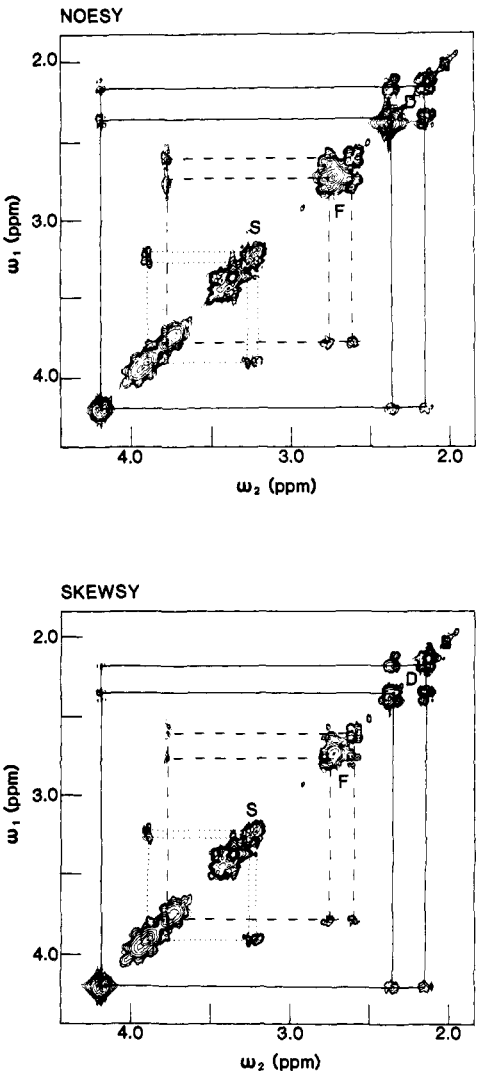

Figure 3. Two-dimensional NOESY (top) and SKEWSY (bottom) contour plots of the nonapeptide Phe-Lys-Leu-Gly-Gly-Arg-Asp-Ser-Arg (FKLGGRDSR) taken at 400 MHz. Concentration was 35 mM in dimethyl-d_6 sulfoxide at 298 K. $\tau_s = \tau_m = 0.2$ s with t_1 ranging from 0.2 to 102.4 ms in steps of 0.2 ms and a recycle delay of 2.1 s. Free-induction decays, $S(t_1,t_2)$, were interleaved with a total of 8192 scans for NOESY and 16 384 scans for SKEWSY. The spectra are absolute value mode, with sine bell apodization along t_1 and t_2. Sample preparation and assignments are reported in ref 14.

This equation shows that the SKEWSY cross peaks have equal intensity if $R_{AA} = R_{BB}$, and thus the difference in height reflects a difference in spin–lattice relaxation times of nuclei A and B. Figures 3–5 depict SKEWSY and NOESY spectra of a nonapeptide that has previously been studied by other NMR methods.[14] The asymmetries of the SKEWSY cross peaks are clearly evident. The α-CH–β-CH$_2$ region from 2.0 to 4.5 ppm is shown in contour plot in Figure 3, where intraresidue NOE's are indicated for the serine, phenylalanine, and aspartic acid redisues. The NOESY cross peaks show similar intensities on both sides of the diagonal (Figures 4 and 5). The SKEWSY cross peaks, however, do not. This "skewness" can be observed in detail in the cross sections shown in Figure 5, which are in marked contrast to the NOESY cross sections.

III. Cross Relaxation between Several Spins

In a system of n spins, the NOESY mixing coefficients are related to the rate constant matrix by

$$\hat{\mathbf{a}} = \exp(-\hat{\mathbf{R}}\tau_m) \quad (12)$$

(14) Mendz, G. L.; Moore, W. J.; King, G.; Wright, P. E. *Int. J. Pept. Protein Res.*, in press.

The equation is evaluated by solving the eigenvalue problem,

$$\hat{\mathbf{R}}\mathbf{u} = \lambda\mathbf{u} \tag{13}$$

Since $\hat{\mathbf{R}}$ is real, symmetric, and positive definite, there are n eigenvalues and n distinct eigenvectors, from which a modal matrix $\hat{\mathbf{U}}$ can be formed. The matrix has the following two properties:

$$\hat{\mathbf{U}}^{\mathrm{T}}\hat{\mathbf{U}} = \hat{\mathbf{I}}$$

$$\hat{\mathbf{U}}^{\mathrm{T}}\hat{\mathbf{R}}\hat{\mathbf{U}} = \hat{\lambda} \tag{14}$$

where $\hat{\lambda}$ denotes a diagonal matrix whose elements are the n eigenvalues. By formation of a second diagonal matrix with elements $e^{\lambda_i\tau_m}$, it can be shown that

$$\hat{\mathbf{a}} = \hat{\mathbf{U}}[e^{-\lambda\tau_m}]\hat{\mathbf{U}}^{\mathrm{T}} \tag{15}$$

It is important to note at this stage that the terms $e^{-\lambda_i\tau_m}$ are actually the eigenvalues of the matrix $\hat{\mathbf{a}}$, as can be proven by post multiplying (15) by $\hat{\mathbf{U}}$ to give

$$\hat{\mathbf{a}}\hat{\mathbf{U}} = \hat{\mathbf{U}}[e^{-\lambda\tau_m}] \tag{16}$$

Here the matrix equation represents the n solutions of the eigenvalue problem,

$$\hat{\mathbf{a}}\mathbf{u} = \gamma\mathbf{u}$$

$$\gamma = e^{-\lambda_i\tau_m} \tag{17}$$

Thus, the multispin relaxation problem can be restated in the form "find the matrix of NOESY mixing coefficients". Given that $\hat{\mathbf{a}}$ is known, the relaxation matrix $\hat{\mathbf{R}}$ is then obtained, in reverse order, by solving (17) to obtain

$$\hat{\mathbf{R}} = -(1/\tau_m)\hat{\mathbf{U}}[\ln\gamma]\hat{\mathbf{U}}^{\mathrm{T}} \tag{18}$$

In practical circumstances, linear equations for the mixing coefficients $\hat{\mathbf{a}}$ can be formed by using data from a SKEWSY and a NOESY spectrum.

To illustrate, we consider a three-spin system, in which all spins cross relax to produce six cross peaks as shown in Figure 6. Where $\tau_s = \tau_m$, the SKEWSY mixing coefficients are then

$$\begin{aligned}
S_{12} &= a_{12}(2a_{21} + 2a_{22} + 2a_{23} - 1)\\
S_{13} &= a_{13}(2a_{31} + 2a_{32} + 2a_{33} - 1)\\
S_{23} &= a_{23}(2a_{31} + 2a_{32} + 2a_{33} - 1)\\
S_{21} &= a_{21}(2a_{11} + 2a_{12} + 2a_{13} - 1)\\
S_{31} &= a_{31}(2a_{11} + 2a_{12} + 2a_{13} - 1)\\
S_{32} &= a_{32}(2a_{21} + 2a_{22} + 2a_{23} - 1)
\end{aligned} \tag{19}$$

Denoting the NOESY peak heights by N_{ij} and the SKEWSY peak heights by M_{ij}, ratios can be formed to yield the equations

$$\begin{bmatrix}
2 & 0 & 0 & 2 & 2 & 0\\
0 & 2 & 0 & 2 & 0 & 2\\
0 & 0 & 2 & 0 & 2 & 2\\
0 & 0 & 0 & 1 & 0 & -\frac{M_{12}}{M_{32}}\\
0 & 0 & 0 & 0 & 1 & -\frac{M_{13}}{M_{23}}\\
1 & 0 & 0 & -\frac{M_{11}}{M_{21}} & 0 & 0
\end{bmatrix}
\begin{bmatrix} a_{11}\\ a_{22}\\ a_{33}\\ a_{12}\\ a_{13}\\ a_{23} \end{bmatrix}
=
\begin{bmatrix}
1+\frac{M_{21}}{M_{21}}\\
1+\frac{M_{32}}{M_{32}}\\
1+\frac{M_{13}}{M_{13}}\\
0\\
0\\
0
\end{bmatrix} \tag{20}$$

It should be noted that the last three equations depend on the similarity of the line shape functions $f_i(\omega) = T_{2i}/(1+(\omega_i-\omega_0)^2T_2^2)$.

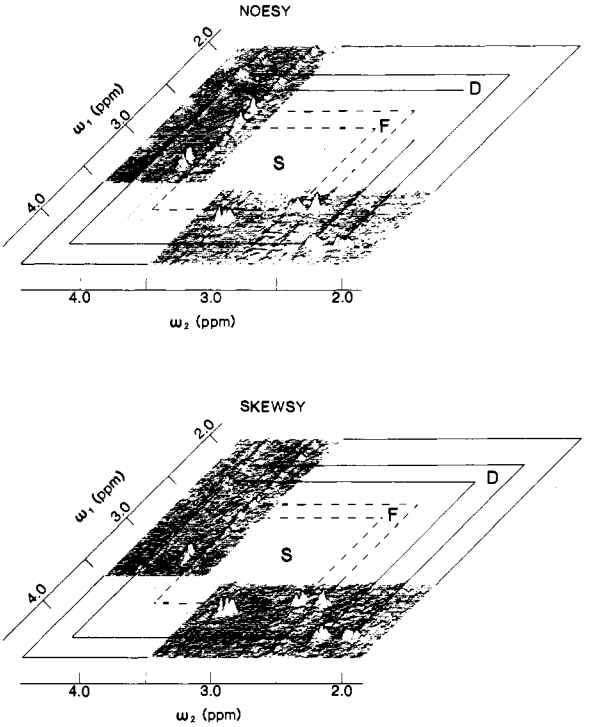

Figure 4. Stacked-plot presentation of the SKEWSY and NOESY spectra shown in Figure 3, with diagonal peaks removed to allow the observation of cross peaks. The spin systems of serine (S), phenylalanine (F), and aspartic acid (D) show symmetric cross peak intensities for NOESY and asymmetric intensities for SKEWSY.

If the line shapes are dissimilar, M_{ij} and N_{ij} should be normalized by integrating each peak through the ω_1 and ω_2 cross sections.

In general, for a system of n spins with m cross peaks, the SKEWSY/NOESY ratios between the cross peaks will yield $n + m - 1$ equations. n Equations originate from ratios of the form M_{ij}/N_{ij}, $j = 1, n$ $(i \neq j)$ and $m - 1$ equations from ratios of the form $M_{ij}/M_{kj} = N_{ij}/N_{kj}$ $(i, k \neq j)$.

The last equation must be obtained by using a cross peak to diagonal peak ratio. Where spectral crowding is a problem this restriction can be relaxed by noting that $\mathrm{Tr}[\mathbf{a}_{ij}]$ is invariant under transformation and therefore

$$\sum_i^n a_{ii} = \sum_i^n e^{-\lambda_i\tau_m} \tag{21}$$

In using eq 21 as the $(n + m)$th equation the right-hand side can be estimated and then refined iteratively by successive solution of the eigenvalue problem (eq 17).

IV. A Three-Spin Case Study

Assuming that magnetization is relayed between spins 1 and 3, we choose a rate constant matrix,

$$\hat{\mathbf{R}} = \begin{bmatrix} 2.50 & -1.70 & 0\\ -1.70 & 2.50 & -1.80\\ 0 & -1.80 & 3.0 \end{bmatrix} \tag{22}$$

as typical of a molecule with a long correlation time.[1,2] In such a case, the NOESY cross peaks are all large and positive.[2] Figure 7 shows the simulated NOESY and SKEWSY spectra obtained with $\tau_s = \tau_m = 0.8$ s. The time dependence of the mixing coefficients is shown in Figure 8.

Several points are illustrated in this simulation. First, the relay NOE is evident at positions 13 and 31 of the NOESY spectrum. Although $R_{13} = 0$, it can be seen that magnetization is efficiently exchanged along the path $1 \rightleftharpoons 2 \rightleftharpoons 3$. From the theory developed in section III, the relay NOE should be identifiable from the matrix of NOESY coefficients. When the SKEWSY/NOESY ratios are calculated to one decimal place accuracy (i.e., two

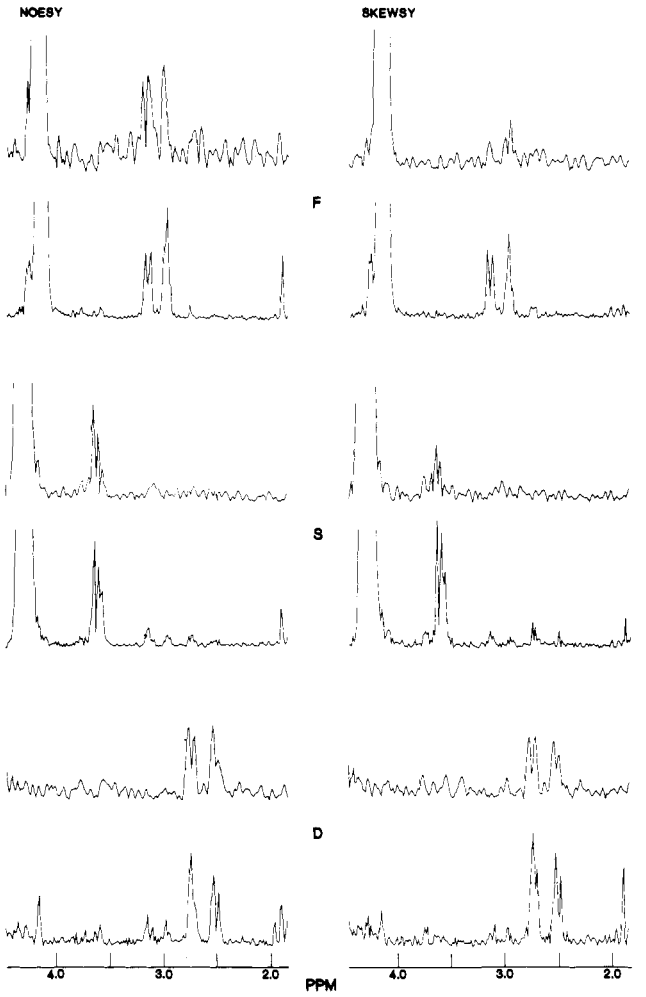

Figure 5. Cross sections taken from the SKEWSY and NOESY spectra of Figure 3. ω_1 and ω_2 cross sections are depicted for phenylalanine (top), serine (middle), and aspartic acid (bottom). In each case the ω_2 cross section is directly above the ω_1 cross section. Comparable cross sections for NOESY and SKEWSY can be compared from left to right.

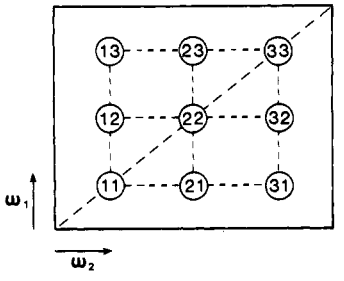

Figure 6. Schematic representation of the contour map for a three spin system. Locations are marked with the corresponding subscript of the NOESY mixing coefficient a_{ij}.

significant figures) and back-transformed according to eq 20, 17, and 18, the following rate matrix is obtained. The low value of

$$\hat{R} = \begin{bmatrix} 2.43 & -1.65 & -0.08 \\ -1.65 & 2.41 & -1.66 \\ -0.08 & -1.66 & 2.82 \end{bmatrix}$$

R_{13} is sufficient in itself to identify the (13) cross peak as a relay effect.

A second outcome of the SKEWSY experiment is that it can produce an absolute enhancement of certain cross peaks relative to the NOESY spectrum of comparable mixing time. In particular, the cross peak S_{32} and diagonal peak S_{22} (Figures 7 and 8) are larger than their NOESY counterparts. It is tempting to conclude that SKEWSY might be useful in cases where cross peak intensities are small. Unfortunately, however, SKEWSY tends to attenuate weak cross peaks, as evidenced by the coefficients

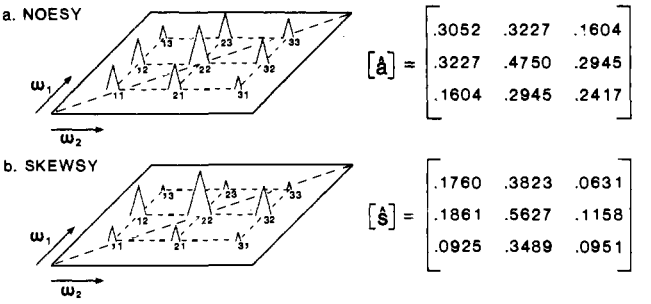

Figure 7. Simulated NOESY spectrum (a), $\tau_m = 0.8$ s, and SKEWSY spectrum (b), $\tau_s = \tau_m = 0.8$ s, with the rate constants of eq 22. The NOESY and SKEWSY mixing coefficients $\hat{\mathbf{a}}$ and $\hat{\mathbf{S}}$ are listed in standard matrix form; i.e., the diagonal runs from top left to bottom right of the matrix.

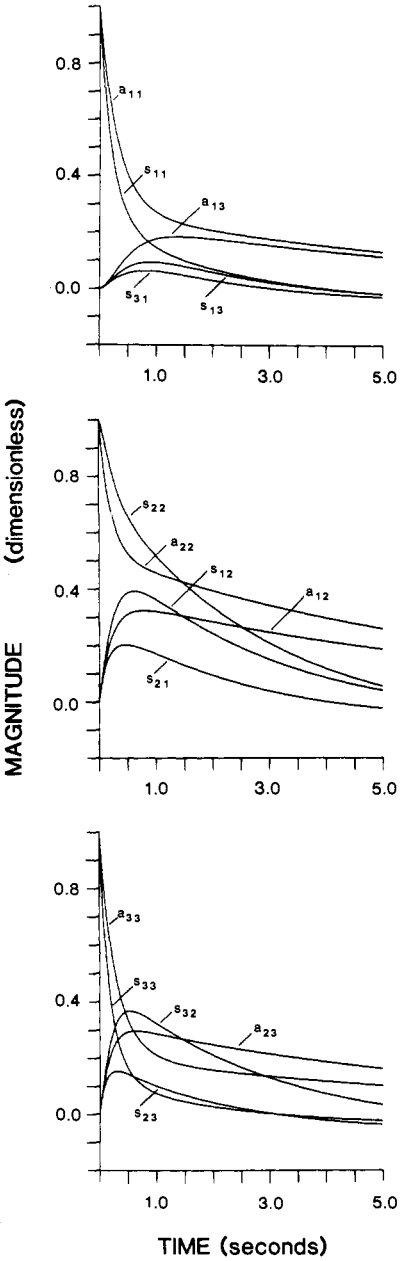

Figure 8. Variation in the SKEWSY and NOESY mixing coefficients as a function of the mixing time τ_m for the system described by eq 22. The SKEWSY coefficients S_{ij} are calculated for the case $\tau_s = \tau_m$.

S_{13} and S_{31}. The effect may be likened to a kind of Darwinian competition, in which the strong get stronger and the weak eventually succumb.

Previous simulations of the two spin NOE[2,8] have always shown the cross peak intensity to be less than or equal to the diagonal peak intensity. In the present case, it is evident that in three-spin and probably in higher order systems, it is possible for the cross peak to be larger than the diagonal peak.

V. Discussion and Conclusions

The analysis presented here offers a simple and verifiable approach to rate-measurement problems, by back transforming the NOESY mixing coefficients. In particular, the SKEWSY experiment is well suited to studies of the nuclear Overhauser effect in ^{1}H NMR spectroscopy, owing to the information obtainable from cross peaks and the absence of zero-quantum coherences.

In view of the general nature of the $\hat{\mathbf{a}} \rightarrow \hat{\mathbf{R}}$ transformation, the question arises as to why a combined SKEWSY/NOESY experiment is preferred over either a single NOESY or a single SKEWSY experiment. In the former case a NOESY spectrum might be expected to give a direct map of the $\hat{\mathbf{a}}$ matrix, in which n diagonal peaks correspond to n diagonal coefficients and m cross peaks to m off-diagonal terms. Two problems arise in this instance. The first stems from the need to calibrate the spectrum by measuring the equilibrium magnetization M_0. Failure to do so leads to a false $\hat{\mathbf{R}}$ matrix, in which each diagonal element must corrected by the additive constant $(-1/\tau_m)$ ln C, C being the calibration constant. If ratios are used to eliminate the dependence on M_0, as in eq 20, then the NOESY spectrum can yield only $n + m - 1$ equations.

This result obviates the second problem associated with NOESY experiments. The $n + m - 1$ equations can only be formed if all of the diagonal peaks are resolved, a situation rarely encountered in practice. In the worst case, where no diagonal peaks can be distinguished, only $m - 1$ equations are obtained.

By contrast, the SKEWSY experiment projects diagonal peak information onto the cross peaks, and $m + n - 2$ equations can be obtained from cross peak/cross peak ratios. Thus, if two diagonal peaks are resolved, a single SKEWSY spectrum will contain sufficient information for a tractable solution. In this context the combination of a SKEWSY and NOESY experiment diminishes the required number of resolvable diagonal peaks, from two to one, or, in the case of the iterative solution (eq 21), to none.

Like all matrix solutions, a two-dimensional approach to the measurement of n-site exchange will be prone to error propagation. For this reason, it may prove useful to conduct several SKEWSY experiments and subject the solution to a least-squares analysis. In such circumstances the SKEWSY mixing time τ_s could be changed while the NOESY mixing time is kept constant. All the consequent SKEWSY peaks could then be used with the same reference NOESY spectrum. A natural extension of this approach would be to apply an accordion-type increment[11,12] to τ_s and obtain the time dependence of the cross peaks by deconvolution of selected cross sections in the ω_1 domain.

The two-dimensional measurement of cross relaxation promises considerable time savings compared to one-dimensional methods. In the experimental scheme presented here two principal obstacles, J-cross peaks and the overlap of diagonal peaks, have been removed. If, in addition, the back-transform method can provide accurate relaxation data, a more efficient approach to the study of molecular structure and dynamics will be possible.

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Functional Capsule Membranes. 8.[1] Signal-Receptive Permeability Control of NaCl from a Large Nylon Capsule Coated with Phospholipid Bilayers

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Abstract: Nylon ultrathin capsules coated with bilayers of synthetic phospholipids (1,3-dipalmitoylglycero-2-phosphocholine and/or 1,3-dipalmitoylglycero-2-phosphoethanolamine) were prepared. Coating phospholipids were proved to exist as multilamellar bilayers from studies of X-ray diffraction and electron microscopy. The permeation of NaCl trapped in the inner aqueous phase was reduced by factors of 10–500 relative to that of the uncoated capsule and drastically decreased above the phase transition temperature (T_c) of coating phospholipid bilayers. When the capsule was coated with phospholipid bilayers containing the phosphatidylethanolamine moiety, the permeation of NaCl was *reversibly* controlled by the interaction and then the removal of divalent cations; the NaCl permeation was increased by treating with aqueous Ca^{2+} from outside and reverted by washing capsules with aqueous EDTA. The permeation mechanism of NaCl was also discussed from the activation energy data of Arrhenius plots.

Recently we prepared newly functional nylon capsules whose porous membranes were coated with synthetic amphiphile bilayers.[1-7] The capsule has some advantages of both nylon capsules and bilayer vesicles: the large inner aqueous phase, the physically strong wall, and the characteristics of bilayer properties.

We describe in this paper the nylon capsule membrane coated with biologically interesting phospholipid bilayers of 1,3-di-

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