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Two-Dimensional Inverse Laplace Transform NMR: Altered Relaxation Times Allow Detection of Exchange Correlation

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Abstract: A pulse sequence for a two-dimensional inverse Laplace transform NMR experiment is proposed and demonstrated. The experiment is analogous to the two-dimensional Fourier transform protocol called EXSY, but detects exchange by monitoring alterations in the transverse relaxation time rather than the NMR frequency. The sequence may be useful for measurement of exchange and diffusion of water *in vivo* and for detecting slow exchange phenomena in glassy polymers.

The application of the Fourier transform (FT) to nuclear magnetic resonance spectroscopy[1] and its extension to multiple dimensions[2] has radically altered modern chemistry and medical science; NMR imaging[3] and the determination of the structure of proteins in solution[4] are two important advances that would be either impossible or orders-of-magnitude more difficult without the FT. The crucial feature of the transformation is the increase in available spectroscopic resolution. If two signals have different precession frequencies ν_I and ν_S as a result of some coherent interaction, the time evolution of the total transverse nuclear magnetization $\mathbf{M}(t)$ will be the sum of the time evolution of these two species ($\mathbf{M}_I(t) + \mathbf{M}_S(t)$), and at every point in time it will be dependent on ν_I and ν_S. However, the FT will still show resolved signals in frequency space, thus discriminating the contributions of the two species.

The enormous success of modern NMR is evidence enough that the constraint requiring species to have different precession frequencies is not usually a severe hindrance. Nonetheless, there exist several important systems where detailed investigation by NMR has been inhibited by the fact that the heterogeneity of the sample is not reflected in anisochronicity in the NMR frequency spectrum. The NMR signals from water, from quadrupolar ions[5] in living systems, and from ^{13}C in polymers,[6] are but three examples. While chemical shift heterogeneity can be manufactured (often very successfully) by externally perturbing the system, for example with shift reagents,[5,7] one would ideally like to get resolution without any extraneous manipulations.

In many cases, the heterogeneity of a sample, unresolved in a conventional FT spectrum, is nonetheless reflected in the spin relaxation times T_1 and T_2. There is a compelling but deceptive similarity between the rate equations for the evolution of an NMR signal as a result of a coherent evolution and that due to incoherent relaxation. If for convenience we describe the vectorial transverse magnetization as a complex scalar I, with $\mathrm{Re}(I) = M_x$, $\mathrm{Im}(I) = M_y$, then we can write the rate of change of the signal as the result of coherent precession,

$$\delta I/\delta t = i\omega_I I \tag{1}$$

and the evolution of the signal as a result of transverse relaxation.

$$\delta I/\delta t = -R_2 I, \qquad R_2 = 1/T_2 \tag{2}$$

However, the absence of the factor i in the expression for spin relaxation means that the time evolution $f(t)$ of a system with a "spectrum" of relaxation rate constants $g(R)$ is not an inverse Fourier transform of g, as it would be of coherent evolution, but a Laplace transform.

$$f(t) = \int_0^\infty g(R_2) \exp(-R_2 t)\, dR_2 = \mathbf{L}(g(R_2)) \tag{3}$$

A possible source of confusion here is that time in the present example is the variable in the image space of the transform, rather than the object space, as is more conventional in the literature on Laplace transforms.[8]

The FT and its inverse (the IFT) differ only in the sign of an exponent. The inverse of a Laplace transform (ILT), however, is very different from the LT itself. The major practical consequence lies in the stability of the operation. While the FT and IFT are of similar, moderate stability with respect to noise, the LT is highly stable (addition of a small amount of random noise to $g(R)$ does not significantly change $f(t)$), while the ILT is conversely highly unstable. Therefore, while all four operators (FT, IFT, LT, ILT) are formally single valued, the ILT is in practice highly indeterminate if a small amount of uncertainty is introduced into the argument. This problem has been successfully addressed by constraining the solutions $L^{-1}(f(t))$ using some criterion of "physical reasonableness", that is, instead of attempting to compute the exact value of $L^{-1}(f(t))$, seeking a physically reasonable $g(R)$ for which $L(g(R))$ is identical to $f(t)$ within the statistical uncertainty of the experiment. This approach has been implemented using several different constraints, among them maximum entropy[9] and non-negative least squares (NNLS).[10] In this work we have used CONTIN,[11] a regularized NNLS algorithm, modified to impose smoothness in both

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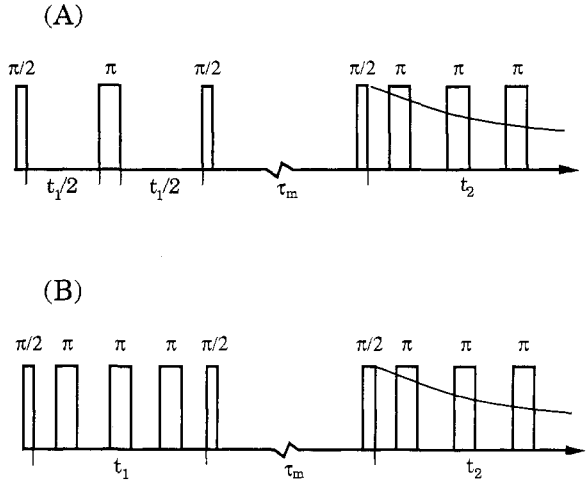

Figure 1. Pulse sequences used in the present work. (A) Simple ARTDECO sequence, which uses a single refocusing pulse during the evolution (t_1) period. The latter is split symmetrically about the π pulse and incremented from slice to slice in the 2D data matrix. (B) Symmetrical ARTDECO sequence, which uses similar Carr–Purcell echo trains in the t_1 and t_2 periods to avoid shifting of the diagonal peaks due to asymmetrical scaling of the exchange contribution to T_2 by the echo train during t_2. From one slice to the next the evolution period is incremented by a $t_1/2-\pi-t_1/2$ interval.

dimensions. This algorithm has been successfully employed in a variety of other situations[12] to implement the ILT.

Given spectral resolution, regardless of whether it is in ω or R space, one can begin to construct NMR experiments to exploit it.[9b,10] In this paper we report a two-dimensional exchange experiment analogous to the EXSY experiment of Jeener *et al.*[13] The EXSY experiment probes slow exchange between species with different resonance frequencies; our new experiment, which we have dubbed ARTDECO (altered relaxation times detect exchange correlation), detects exchange between species with different transverse relaxation rate constants.

The fundamental ARTDECO pulse sequence is shown in Figure 1A. The transverse magnetization is excited with a simple $\pi/2$ pulse; there follows a delay of length t_1, in the middle of which is inserted a π pulse, which refocuses the chemical shift, resonance offset, and any other linear coherent contributions to the evolution. Consequently, a spin echo is produced at the end of the t_1 period. The intensity of the echo as a function of t_1 is a decaying exponential or distribution of exponentials. At this point, a second $\pi/2$ pulse, $\pi/2$ radians out of phase with the first, puts the residual magnetization, labeled now by the transverse relaxation, along the z direction. There follows an interval τ_m in which slow exchange processes have a significant probability. After τ_m, the magnetization is detected, using a Carr–Purcell echo train to suppress coherent contributions and allow measurement of the "real" T_2 (however, *vide infra*).

Signals which have evolved with a transverse relaxation rate constant $R_{2I} = 1/T_{2I}$ during the t_1 period and have not undergone exchange will give rise to a detected signal,

$$I(t_1,t_2) = I_0 \exp(-R_{2I}t_1) \exp(-R_{2I}t_2) \qquad (4)$$

which upon inverse Laplace transformation gives a peak at (R_{2I}, R_{2I}), on the diagonal of the 2D relaxation spectrum. Species which have undergone exchange such that the relaxation rate constant during t_2 is $R_{2S} \neq R_{2I}$ will give an off-diagonal peak at (R_{2I}, R_{2S}). The overall spectrum, just as in EXSY, should therefore consist of diagonal peaks from the part of the magnetization that has not undergone exchange, and cross peaks from the part that has.

However, in reality the situation is somewhat more complicated. If the exchange process itself contributes significantly to T_2, then the asymmetry between the evolution period (with a single π pulse) and the detection period (with a Carr–Purcell train, which suppresses the exchange and scalar contribution to the broadening) will cause the position of the diagonal to be scaled by a factor that depends on τ_{ex}/τ_{CP}, where τ_{ex} is the time constant for exchange plus scalar relaxation and τ_{CP} the interval between pulses in the echo train. This scaling effect is actually not a drawback, since the diagonal ordinarily contains 2-fold redundant information, while here the scaling factor allows an estimate of the spectral density of the exchange and scalar relaxation correlation function at $\omega = 1/\tau_{CP}$.

A rigorous solution of the Bloch equations for two-site exchange[14] confirms the essentials of this descriptive treatment, with the *caveat* that neither the signal intensities nor the exchange rate constants detected in the ARTDECO experiment are those of the individual species *per se*, but rather are linear combinations of these intensities and rate constants, weighted by coefficients which depend on the relative magnitudes of the exchange and intrinsic relaxation rate constants.

To establish the feasibility of this experiment, we have tested it on concentrated solutions of urea in water. In aqueous solution, urea protons exchange with those of water at a rate that depends on urea concentration, temperature, and pH. The urea proton signal is 1.1 ppm higher in frequency than the water signal; this allowed rigorous characterization of the exchange reaction by conventional NMR methods.[15] This sample was chosen also because the proton exchange rate can be changed by adjusting the pH; the high solubility of urea means that concentrated solutions can be prepared, reducing the dynamic range problem ordinarily encountered with proton NMR in water; and the intrinsic T_2 of urea protons is rather short, because of scalar coupling to the quadrupolar ^{14}N spin, which has a T_1 on the order of 10^{-3} s in aqueous solution at room temperature.[15] In addition, EXSY and ARTDECO spectra can be run on the same material, allowing the earlier experiment to be used as a control.[16]

Figure 2A shows as a stacked plot the conventional 2D-EXSY spectrum of a 16.6 M solution of urea in water, adjusted to pH 9.1, obtained with a τ_m of 100 ms. Under these circumstances, the expected time constant for proton exchange is around 50 ms, consistent with the ratio of cross- to diagonal-peak intensities. Figure 2C shows the 2D-ARTDECO relaxogram (as we have termed the distribution in T space[12]) of the same sample at the same mixing time, while Figure 2D shows a relaxogram obtained at a much shorter τ_m of 1 ms. It is clear that the cross peaks in the τ_m = 100 ms relaxogram are absent in that obtained at the shorter mixing times; relaxograms at intermediate times show that the ARTDECO cross peaks grow with a time constant experimentally indistinguishable from those of the EXSY spectra; both of these facts confirm that these are true exchange cross

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(16) Experimental details: 10 g of urea (Sigma), without further purification, was dissolved in 10 mL of distilled water to give a concentration of approximately 16.6 M. It was adjusted to pH 9.1 using a sodium borate/HCl buffer. All NMR measurements were performed using a Bruker MSL-400 spectrometer, operating at a proton frequency of 400 MHz and a temperature of 25 ± 1 °C. The magnetic field was not locked during data acquisition. 2D-EXSY experiments employed the standard NOESY/EXSY protocol, using time-proportional phase incrementation to achieve quadrature in the first dimension. A 256 × 1024 point data matrix was collected, with a spectral width of 3.333 kHz in both dimensions. ARTDECO experiments using either the symmetric of asymmetric pulse sequences were cycled through 32 phase combinations; the collected 20 × 128 time-domain data matrix was processed on a VAX Station 3100; the 2D relaxograms were plotted on a 24 × 24 grid.

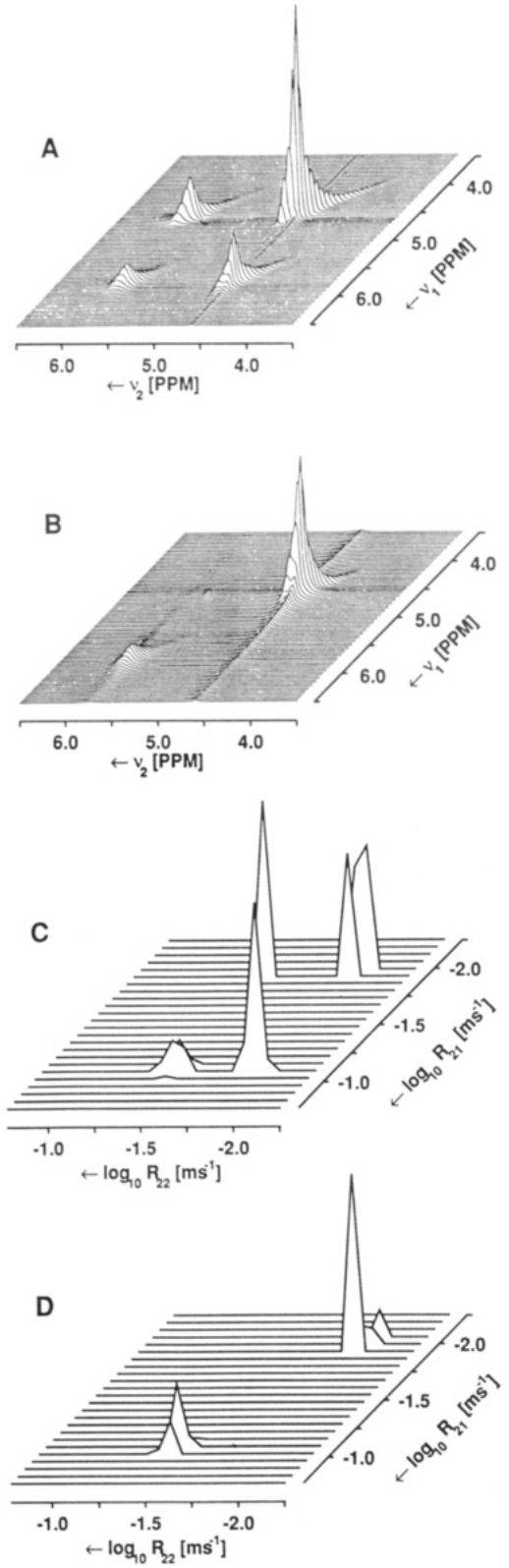

Figure 2. Stacked plots of EXSY spectra and ARTDECO relaxograms of 16.6 M urea in water, pH 9.1, collected under similar conditions to allow comparison of the results of the two experiments: (A) EXSY with a mixing time τ_m of 100 ms; (B) EXSY with τ_m of 1 ms; (C) ARTDECO with τ_m of 100 ms; (D) ARTDECO with τ_m of 1 ms.

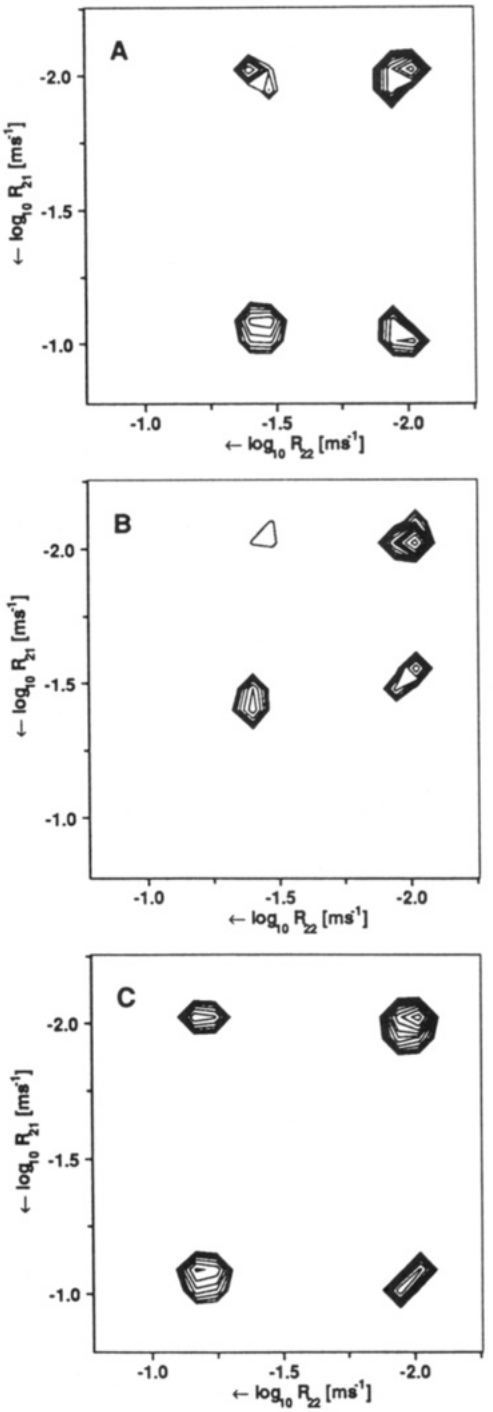

Figure 3. ARTDECO relaxograms of 16.6 M urea in water, pH 9.1, plotted as contour plots to show the scaling of the diagonal-peak positions. (A) Relaxogram obtained with the asymmetric pulse sequence (Figure 1A); the urea "diagonal" peak (lower left) has a significantly longer T_2 in the R_{22} dimension (in which it is scaled by a Carr–Purcell train with a repetition time (τ_{CP}) of 2 ms) than in the R_{21} dimension where there is only a single π pulse. (B) Relaxogram obtained with the symmetric pulse sequence (Figure 1B), with identical Carr–Purcell sequences (τ_{CP} = 1 ms) in both dimensions; scaling of the "diagonal" peak relaxation time is therefore equal in both dimensions, so the peak really lies on the diagonal. (C) Relaxogram obtained with the asymmetric pulse sequence but with τ_{CP} = 4 ms; with the lower duty cycle, scaling of the T_2 in the R_{22} dimension is much less severe, so the urea peak lies close to the diagonal again. The water peak (upper right) is close to the diagonal in all cases; because the water contains a larger number of protons, exchange broadening is less severe, so scaling of the T_2 is much less significant. The mixing time for all spectra was 100 ms.

peaks. However, there are two major discrepancies which should be noted. First, it is clear that the relative intensities of the diagonal urea and water peaks are different in the two experiments. As mentioned above, the eigenvectors of the relaxation matrix in the presence of exchange are linear combinations of the urea and water magnetizations; it is the intensities of these linear combinations that occur in the ARTDECO spectrum or relaxogram. Second, the diagonal-peak positions are scaled as discussed above;

while the "mostly water" diagonal peak has very similar R (=1/T_2) values in the two dimensions, the "mostly urea" peak has R = (12 ms)$^{-1}$ in the R_{21} dimension and R = (30 ms)$^{-1}$ in the R_{22} dimension. This shift of the "diagonal peak" can be avoided by applying an echo train during t_1 as well as t_2. This is shown in Figure 3, in which we compare the ARTDECO relaxogram obtained with the asymmetrical pulse sequence in Figure 1A (plotted in Figure 3A) with the relaxogram obtained using a symmetrized sequence (Figure 1B), plotted in Figure 3B. The diagonal peaks are now symmetrical with respect to the relaxation time coordinates.

While in the present work we deliberately chose a simple chemical system, in which the two exchanging species are distinguishable by other means, in order to demonstrate the feasibility and examine the limitations of the method, we believe its real usefulness will lie in investigations of complex systems such as glasses and living organisms, in which the simplicity of the conventional FT NMR spectrum belies the complexity of the material. In particular, we believe that ARTDECO may be particularly useful in visualizing the exchange of water molecules across cell membranes separating compartments where the relaxation times are significantly different, as is the case for many cell suspensions. Such investigations are in progress.

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