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Intrinsic Asymmetry in Multidimensional Solid-State NMR Correlation Spectra

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Solid-state MAS correlation spectra involving cross polarization will generally provide spectra which are asymmetric about the main diagonal. A quantitative treatment of this effect is provided and is demonstrated experimentally with examples of two-dimensional spin-diffusion spectra. The effect, which is due to the nonuniform nonequilibrium initial coherent state induced by cross polarization, is usually pronounced, and cannot be eliminated simply. It must be taken into account when quantifying solid-state CPMAS correlation spectra. © 1998 Academic Press

Nuclear magnetic resonance correlation spectroscopy applied to powders has recently experienced a rapid expansion. A promising route toward structure determination in solids by NMR (*1*) appears to be the combination of magic-angle sample spinning (MAS) (*2, 3*), which yields high-resolution spectra during the detection periods of a two-dimensional experiment, with different methods of reintroducing dipolar interactions during the mixing time. These experiments all conform to the basic spin-exchange scheme shown in Fig. 1 (*4, 5*) and encompass a wide variety of mixing schemes varying from simply waiting long enough for exchange to occur spontaneously (in the original spin-diffusion experiment) (*6, 7*), to sophisticated recoupling schemes using rotor-synchronized RF pulses. Usually, the experiments are performed on fully or partially isotopically enriched carbon-13 or nitrogen-15 samples, in which sensitivity is enhanced by cross polarization (once again using some sort of more or less sophisticated sequence of RF pulses) (*8, 9*). The spectra that can be recorded are currently of sufficient quality that attention can be given to detailed quantification and interpretation of the observed correlations in terms of molecular geometry.

In this article we point out that contrary to their liquid-state cousins such as COSY and NOESY, which under conditions of complete relaxation yield symmetric correlation spectra (*5*), solid-state MAS correlation spectra involving cross polarization will always provide spectra which are asymmetric about the main diagonal. This effect, which is due to the nonuniform nonequilibrium initial conditions induced by cross polarization, is usually very pronounced, cannot be eliminated simply, and must be taken into account in quantitative analysis.

The possibility of liquid-state correlation spectra being asymmetric is well recognized (*5*). In general it is sufficient that the preparation of the magnetization is nonuniform, and it has been remarked that this occurs whenever selective pulses are used, or if the delay between successive experiments is too short to allow full recovery to thermal equilibrium in the presence of different relaxation rates. This second mechanism, originally exploited by Bremer *et al.* (*10*), has recently been discussed in some detail by Griesinger and co-workers (*11, 12*). In most liquid-state correlation experiments, however, asymmetry can be avoided by simply using a sufficiently long relaxation delay. This is not the case in solid-state experiments in which cross polarization is used to prepare the initial coherent state, since the cross-polarization step always results in a state which is normally not uniformly proportional to the equilibrium condition. This is a well-known effect which is at the origin of the fact that it is difficult to obtain ‘‘quantitative’’ CPMAS spectra in which the integrals are simply proportional to the number of carbons. The consequences of this situation on two-dimensional correlation spectra can be easily appreciated if we consider the predicted cross-peak intensities in two possible versions of the MAS exchange experiment (*4, 13, 14*) shown in Figs. 1b and 1c. Using standard procedures one finds that in the case of Fig. 1b where excitation is achieved by a single nonselective carbon pulse, the time-domain signal is (neglecting relaxation during t_1 and t_2) (*5*)

$$s(t_1, \tau_m, t_2) = -\sum_{k,l} \exp(i\omega_k t_2)[\exp(\mathbf{L}\tau_m)]_{kl}\exp(i\omega_l t_1)M_{l0}, \quad [1]$$

where ω_i is the precession frequency of spin i, τ_m is the

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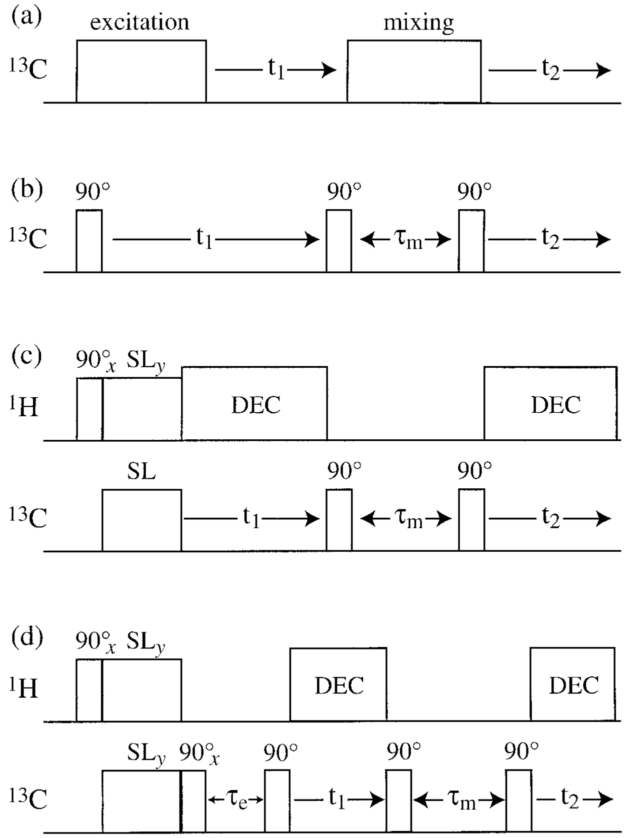

FIG. 1. Pulse sequences used in this work. A schematic representation of MAS correlation spectroscopy is shown in (a). In (b) we show the sequence for a two-dimensional exchange/spin-diffusion experiment without cross polarization in which the magnetization is excited by a single carbon 90° pulse before t_1. In (c) we show the same experiment, but with a cross-polarization sequence for carbon excitation, and in (d) we show an experiment which includes a ''preequilibration'' period after cross polarization and before t_1. See the text for an explanation of the relevance of the different sequences.

duration of the mixing time, **L** is the kinetic rate matrix, and M_{l0} is the equilibrium magnetization of the spin l. After two-dimensional Fourier transformation, the integrated amplitude I_{kl} of a cross peak between two magnetically different spins k and l is given by

$$I_{kl}(\tau_m) = a_{kl}(\tau_m)M_{l0}, \qquad [2]$$

where

$$a_{kl}(\tau_m) = [\exp(\mathbf{L}\tau_m)]_{kl}. \qquad [3]$$

Under such conditions we find that $I_{kl} = I_{lk}$ and the spectrum is always symmetric. This situation is slightly modified if we consider the sequence of Fig. 1c, where excitation is achieved through cross polarization. In this case we can describe the nonuniform excitation with $\mathbf{M}_+^{\mathrm{CP}} = \mathbf{T}^{\mathrm{CP}}\mathbf{M}_0$, where $\mathbf{T}^{\mathrm{CP}}$ is a diagonal matrix having (nontrivial) elements T_{ll}^{CP} which provide a phenomenological reflection of the fact that cross polarization scales the value of M_{l0} (normally one would hope that $T_{ll}^{\mathrm{CP}} > 1$, corresponding to polarization enhancement). As a consequence as previously shown by Luz *et al.* (*14*)

$$I_{kl}(\tau_m) = a_{kl}(\tau_m)T_{ll}^{\mathrm{CP}}M_{l0} \qquad [4]$$

and under these conditions we recover spectral symmetry only if

$$T_{ll}^{\mathrm{CP}} = T_{kk}^{\mathrm{CP}}, \qquad [5]$$

i.e., if polarization transfer is uniform for all correlated spins. Normally this is by no means the case. The asymmetry of the spectrum will now be given in a straightforward manner by the degree of the nonuniformity of polarization transfer,

$$\eta_{kl} = \frac{I_{kl}(\tau_m)}{I_{lk}(\tau_m)} = \frac{T_{ll}^{\mathrm{CP}}}{T_{kk}^{\mathrm{CP}}}, \qquad [6]$$

where η_{kl} is defined as an asymmetry parameter for the cross-peak pair.

Curiously, it appears that the consequences of Eq. [4] on the spectrum have not previously been explicitly considered. Luz *et al.* (*14*) have provided a quantitative analysis of two-dimensional exchange spectra with, to our knowledge, the only previous statement of Eq. [4], but no further mention of the consequences of the T_{ll}^{CP} terms was made. They assume throughout that cross polarization was uniform (the equivalent of Eq. [5]), which is probably valid since they were particularly interested in exchange between sidebands for a single site. In this article we remark simply that the T_{ll}^{CP} terms can have a large effect on the spectrum in multisite exchanging systems, and we provide experimental examples.

Magnetization dynamics during cross polarization are such that the nonequilibrium polarization-enhanced state of the system is only likely to be uniformly polarized in systems containing very similar types of carbons (or nitrogens). In fact, cross-polarization dynamics are so different for CH_3, CH_2, CH, and quaternary carbons that the dynamics can be used as a means of spectral editing (*15–17*). Even for a given type of carbon, significant differences in cross-polarization dynamics result from differences in local motion and environment. Indeed, since they involve a many-body problem, the details of cross-polarization dynamics are notoriously difficult to calculate (especially in the presence of MAS), and the quasi-equilibrium states that result can vary greatly from one carbon to another (*9, 18, 19*). Thus, for multisite systems, we expect the asymmetry effects predicted above to be large and widespread.

A typical example of spectral asymmetry is given in Fig. 2 which shows a contour plot of a two-dimensional proton-

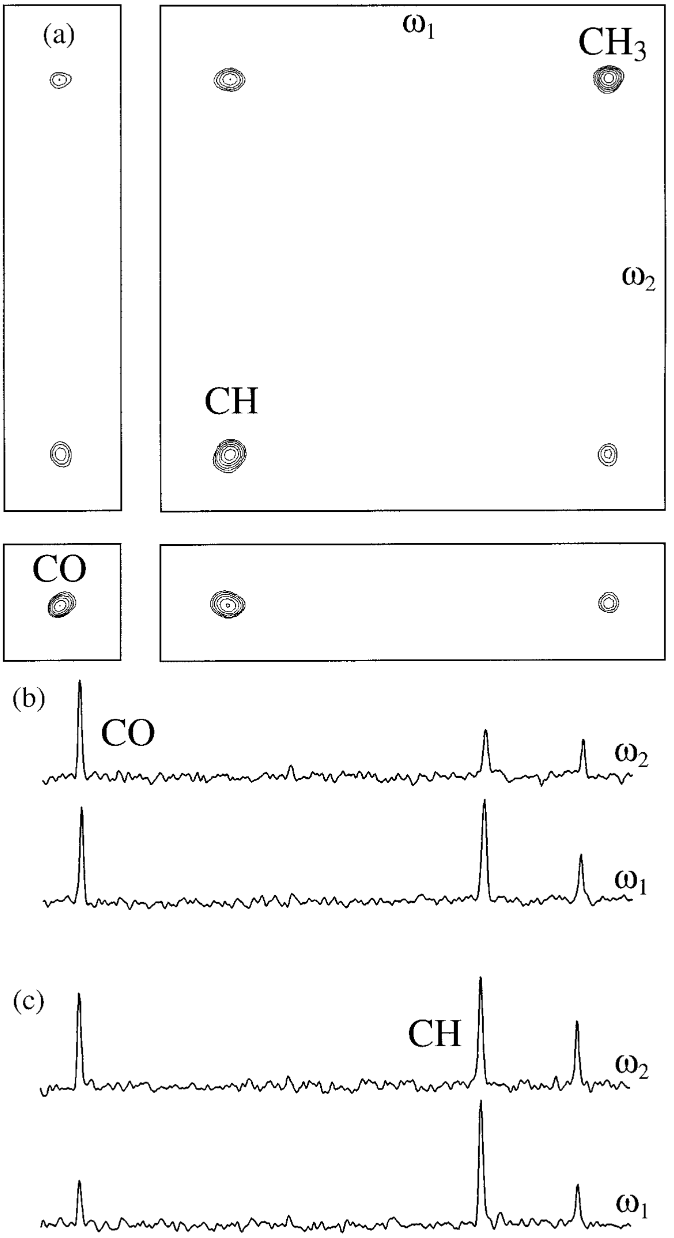

FIG. 2. (a) Contour plot of a two-dimensional magic-angle-spinning spin-diffusion spectrum of a powdered sample containing 10% uniformly enriched $^{13}C_3$-L-alanine cocrystallized with 90% natural abundance L-alanine. The spectrum was obtained with the sequence of Fig. 1c and highlights the intrinsic asymmetry in this kind of spectrum. The cross-polarization time was 0.5 ms, and the mixing time was 30 ms. The spectrum was acquired at a proton frequency of 500 MHz on a Varian Unity + spectrometer with 128 complex points in t_1 and 1600 points in t_2, zero filled to 2K × 4K before apodization with identical Gaussian weighting functions in t_1 and t_2 and Fourier transformation. The asymmetry is all the more visible in (b) and (c) where we show equivalent rows and columns extracted from the spectrum. In (b) we show the row (ω_2) and column (ω_1) that pass through the CO diagonal peak. If the spectrum were symmetric the two traces would be identical, but in fact large deviations from symmetry can be seen in the two cross peaks on the right-hand side of each trace. Similar effects are observed in (c), this time for the traces passing through the CH diagonal peak.

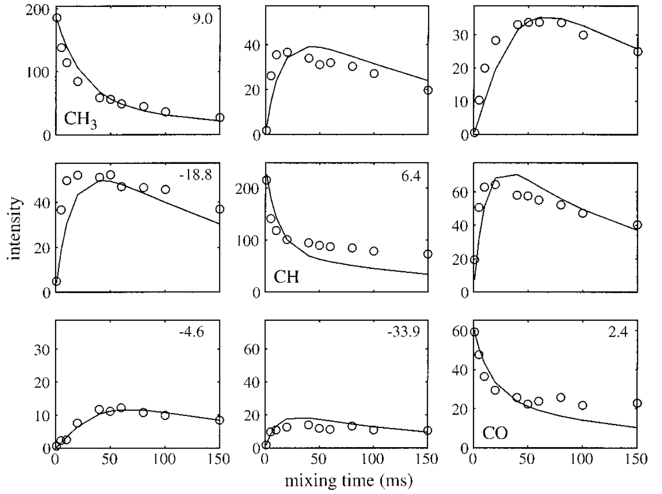

FIG. 3. Peak volumes as a function of the mixing time (τ_m) for spectra of the type shown in Fig. 2. The spectra were recorded at a proton frequency of 400 MHz using a Bruker DSX spectrometer. Each panel of the figure shows the cross-peak volumes for a particular peak, with the diagonal peaks shown diagonally and being correlated by the appropriate cross peaks. The figure demonstrates that the asymmetry of the spectrum is independent of the mixing process, as the ratio between the cross peaks can be seen to remain constant for all mixing times. The solid line represents a fit of the data to Eq. [4] using the crude model of Eq. [3], and the numbers in the upper right corner of the panels give the corresponding rates derived from the fit in s^{-1}.

driven spin-diffusion spectrum obtained with the sequence of Fig. 1c for a sample of uniformly ^{13}C-enriched alanine. The spectrum was acquired with a cross-polarization time of 0.5 ms, a mixing time of 30 ms, and a spinning speed of 8.5 kHz. In this case we observe asymmetry parameters of $\eta_{CH_3,CH} = 0.63$, $\eta_{CO,CH} = 0.48$, and $\eta_{CO,CH_3} = 0.73$. One should note that the asymmetry does not depend on the method of coherence transfer that gives rise to the cross peak (proton-driven spin diffusion in this case) nor on the mixing time, as is shown in Fig. 3 where we have plotted the integrals of the peaks in the spectrum as a function of mixing time. For example, the asymmetry is not affected by unequal relaxation times during the mixing period (or by any other effect which is purely due to the correlation step) (*5*). Using Eq. [3] as a model for spin dynamics we can determine spin-diffusion ''rates'' by fitting the curves of Fig. 3 to Eq. [4], and the fit is also shown in the figure (mainly to highlight the predicted asymmetry, rather than to determine any molecular information, since Eq. [3] only provides a crude phenomenological model for spin diffusion).

Since the asymmetry does not depend on the mixing function (as long as the mixing function is symmetric), we expect that *all CPMAS dipolar correlation spectra should be asymmetric*, irrespective of whether the mixing is achieved through spin diffusion or through dipolar recoupling by RF pulses. In addition, there does not appear to be a simple way to avoid this asymmetry, in contrast with the liquid-state

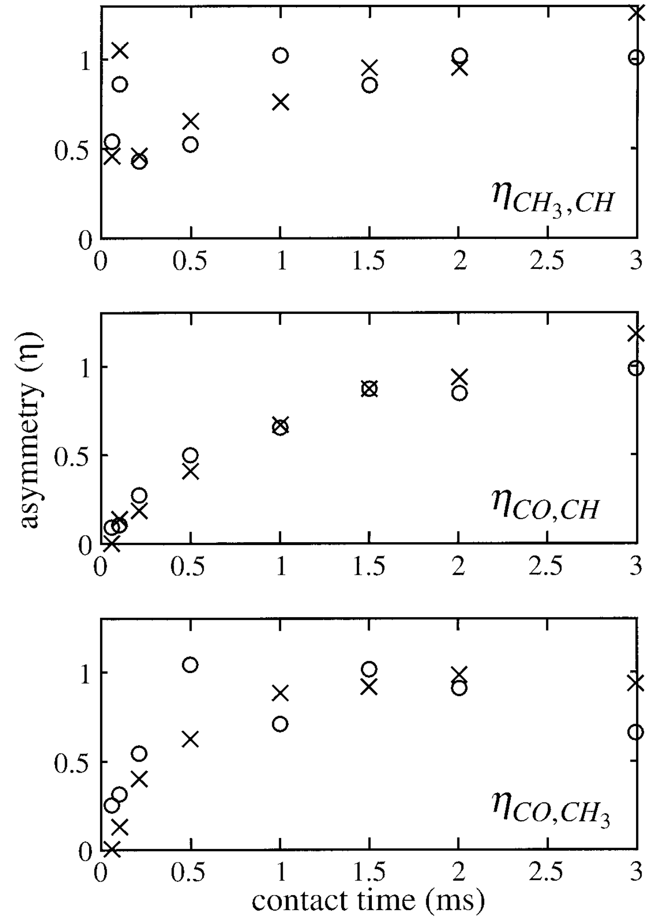

FIG. 4. A comparison as a function of cross-polarization contact time of the observed asymmetry parameters in two-dimensional spin-diffusion spectra, η_{kl}, shown as open circles and predicted values (crosses) obtained from the intensities observed in corresponding one-dimensional cross-polarization experiments. The experimental conditions were the same as those for Fig. 2.

situation. In Fig. 4 we show the observed asymmetry parameters associated with cross-polarization dynamics for the sample of alanine. We see that at short CP times the carbonyl carbon is only weakly polarized, leading to very pronounced asymmetry. As the CP contact time increases the asymmetry becomes slightly less pronounced, but at long CP times differential $T_{1\rho}$ relaxation leads to a modulation of the asymmetry. We note that there is no single value where the spectrum is fully symmetric ($\eta_{k,l} = 1$ for all k, l), and therefore any CPMAS spin-diffusion spectrum of alanine using the sequence of Fig. 1c will be asymmetric. We note also that since cross-polarization dynamics are a sensitive function of spinning speed (becoming increasingly coherent as spinning speed increases), the details of spectral asymmetry are also a function of spinning speed.

It is important to notice that the asymmetry parameters observed in the two-dimensional experiment will simply follow the peak-to-peak ratios observed in a normal one-dimensional CPMAS experiment, which are shown for comparison in Fig. 4. The agreement between these predicted ratios and the observed asymmetry parameters is seen to be good. Thus, a quantitative analysis of MAS correlation spectra either can include the T_{ll}^{CP} values as variables in the fitting procedure of Eq. [4] or can include them as known parameters measured from the corresponding one-dimensional CPMAS spectrum. Note that Eq. [4] represents a universal description of CPMAS correlation experiments in which the model function for the $a_{kl}(\tau_m)$ varies according to the particular mixing sequence used.

It may seem at first sight that, if necessary, the asymmetry can be corrected for by including a ''preequilibration'' period after CP and before t_1. However, it appears to us that methods based on spin diffusion are unlikely to work since equal intensities can be achieved in an accidental rather than an asymptotic fashion, except for the case (of little interest to the spectroscopist) of true equilibration. Even in the absence of oscillatory behavior (which may be observed at very high spinning rates), any earlier steady state will correspond to a quasi-equilibrium (*9, 20*), and there is no guarantee (similarly to the case with CP itself) that the condition of Eq. [5] will be met for this state. As a demonstration we have used the sequence of Fig. 1d to show that while the asymmetry parameters depend on the preparation sequence, the spin-diffusion preparation sequence does not yield symmetric spectra. Figure 5 shows how the amplitudes do not equilibrate (except at very long times as they tend to zero!) and that indeed the asymmetry parameters behave in a somewhat unpredictable way, with values evolving from $\eta < 1$ to $\eta > 1$ as a function of τ_e.

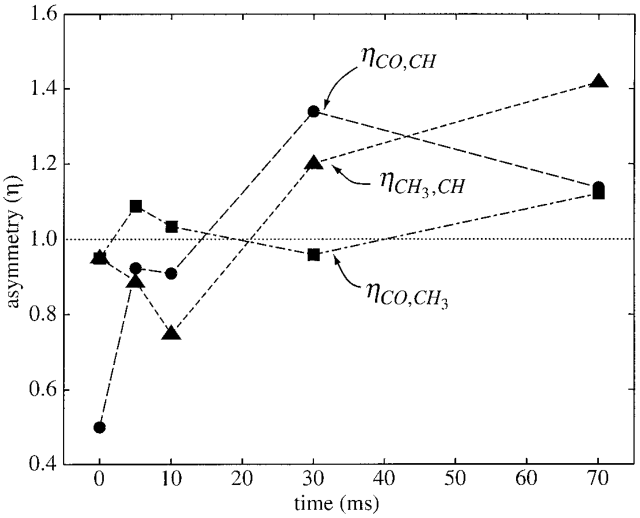

FIG. 5. Observed asymmetry parameters η_{kl} as a function of the τ_e period obtained using the sequence of Fig. 1d. Note that the dynamics during this period are not monotonic and that at no point in time do the asymmetry parameters of all the peaks converge toward 1, the value corresponding to a symmetric spectrum. The experimental conditions were the same as those for Fig. 2.

In order to provide symmetric correlation spectra, a preparation sequence is required which equalizes the populations of each carbon resonance on a sufficiently short time scale to avoid significant losses due to relaxation. This requirement is the same as that necessary to obtain quantitative CPMAS spectra, i.e., in which the integrals are proportional to the number of carbon spins. This latter problem is relatively old, and no simple universal solution comes to mind.

In conclusion, we have demonstrated that cross-polarization magic-angle-spinning correlation spectra in solids are intrinsically asymmetric about the main diagonal. The effect is independent of the mixing function, and is therefore relevant to all forms of dipolar recoupling experiments and to multisite dynamic exchange spectroscopy. The asymmetry results from the nonequilibrium many-body dynamics inherent to cross-polarization sequences. While it does not pose a real problem, asymmetry must be taken into account in a quantitative analysis of the data, since a simplified model assuming symmetry can lead to large errors. An accurate measure of the data, including asymmetry as described by Eq. [4], is all the more important since dipolar correlations are relatively difficult to interpret in terms of molecular models as they often not only depend on internuclear distance but also on the details of secondary dipolar couplings, the anisotropy parameters of the chemical shift, and experimental factors such as spinning speed and pulse imperfections. Similarly, symmetrization of the spectrum by data processing will also lead to errors in quantification. The effect may appear obvious in hindsight, but the degree of asymmetry we observe can be surprisingly large, and the effect on experimental spectra, which is observable in many published spectra (*4, 21, 22*), has not to the best of our knowledge received any previous attention in the literature.

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