APPLICATIONS OF TWO-DIMENSIONAL NMR SPECTROSCOPY TO ORIENTED MOLECULES

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ABSTRACT

Two-dimensional (2D) NMR spectroscopy has proved exceedingly useful in the study of oriented molecules. In this article the applications of this technique to the study of molecules oriented in liquid crystalline solvent are reviewed. The main applications have been, (a) in simplification of NMR spectra by symmetry filtering, multiple quantum filtering and multiple quantum 2D spectroscopy, (b) in enhancing information content of spectra by spin-echo and correlated 2D spectroscopies, and (c) in the study of multiple quantum relaxation rates by 2D spectroscopy.

INTRODUCTION

Two-dimensional nuclear magnetic resonance (NMR) spectroscopy, in which NMR spectrum is obtained as a function of two-frequency variables, has had a profound effect on the study of biomolecules and other systems which yield complex NMR spectra. One such class is the NMR of molecules partially oriented in liquid crystal matrices. Such molecules have finite intramolecular dipolar interactions, with the intermolecular dipolar interactions averaged to zero. This yields a rich but tractable NMR spectrum which in turn contains detailed information on the conformation of the molecules. The spectra are often analysed using iterative computer programs such as LAOCOON and its various modifications.

Two-dimensional (2D) NMR methods have been employed, with significant advantage, for the study of spectra of molecules oriented in liquid crystal matrices. This article aims to review the use of the 2D methods in the study of oriented molecules. The main applications are: (a) the use of 2D resolved spectroscopy and 2D correlated spectroscopy, both of which enhance the information content of the spectra, (b) the simplification of the spectra of oriented molecules by symmetry filtering, multiple quantum filtering and multiple quantum spectroscopy, and (c) study of multiple quantum relaxation rates of oriented molecules.

The methodology of 2D NMR spectroscopy has been very well described in two recent books and several articles. Application of 2D NMR techniques to the study of biomolecules has also been extremely well described in another recent book. The NMR of oriented molecules has been periodically reviewed in several articles and in specialist periodical reports. This is a first dedicated review on the application of 2D techniques to oriented molecules and need-based periodic reviews may be planned in future.

Brief outlines of:

2D NMR techniques

In 2D NMR one obtains an NMR spectrum as a function of two frequency variables \( F_1 \) and \( F_2 \) rather than one. The main advantage of the technique is that information transfer takes place between various lines in the spectrum simultaneously in a non-selective manner. This is achieved by allowing the signals to precess in the evolution period \( t_1 \), affecting the information transfer (by either a pulse, a series of pulses or a change of Hamiltonian) and detecting the signal during the detection period \( t_2 \) (figure 1). A complete set of time domain data matrix \( s(t_1,t_2) \) is collected which on Fourier transformation with respect to \( t_1 \) and \( t_2 \) yields the two-dimensional spectrum \( S(F_1,F_2) \).
Figure 1. Pulse schemes for various 2D NMR experiments, (a) Correlated 2D spectroscopy (COSY), (b) Multiple quantum filtered COSY (MQFC), (c) Multiple quantum transitions spectroscopy (MQT), (d) Nuclear Overhauser effect spectroscopy (2D NOE or NOESY), (e) spin-echo 2D spectroscopy, (f) Spin-echo multiple quantum transitions spectroscopy (SEMQT). The various orders are separated by time proportional phase incrementation (TPPI).

Schemes (b), (e) and (f) require phase cycling for selection of the desired multiple quantum order, Scheme (d) requires phase cycling for cancellation of transverse and retention of longitudinal magnetization during $\tau_m$. All schemes expect (e) require phase cycling for cancellation of axial magnetization. Details of phase cycling can be obtained from ref. 2 and references therein.

The various types of two-dimensional experiments are distinguished by the nature of information transfer taking place in the experiment. If a non-selective coherence transfer is effected by the application of a pulse at the end of $t_1$, then this leads to the correlated 2D spectroscopy (COSY), figure 1(a). If the coherence transfer is restricted through an intermediate state of well-defined multiple quantum order (achieved by the application of two pulses with well defined phases) then one obtains what is known as multiple-quantum-filtered COSY (MQFC): of which the most popular is the double-quantum-filtered COSY (DQFC), figure 1(b). On the other hand, if the multiple-quantum orders are allowed to proceed during $t_1$ (generated prior to $t_1$ by two pulses with a fixed time interval between them) and a coherence transfer to detectable single-quantum transitions is effected by a pulse at the end of $t_1$, then one obtains multiple-quantum-transitions (MQT) 2D spectroscopy, figure 1(c). If the information transfer is a relaxation transfer (achieved by the application of two pulses at the end of $t_1$ with a fixed interval $\tau_m$ between them — the mixing period, and retaining only longitudinal magnetization during $\tau_m$), one obtains nuclear Overhauser effect 2D spectroscopy (2D NOE or NOESY), figure 1(d). If on the other hand no pulse is applied at the end of $t_1$ and only the effective Hamiltonian of the spins is changed from $t_1$ to $t_2$, one obtains what is known as resolved 2D spectroscopy, figure 1(e).

Spectra of molecules oriented in liquid crystal matrices

Solute molecules partially oriented in liquid crystalline media exhibit a high order of anisotropic reorientations and significant translational diffusion, as a result all intermolecular interactions are averaged, in the first order, to zero, like in liquids, while the intramolecular interactions are anisotropically averaged to residual values dependent on the value of order parameter. The effective Hamiltonian
for this case can be represented as
\[ \mathcal{H} = \mathcal{H}_z + \mathcal{H}_J + \mathcal{H}_D + \mathcal{H}_Q, \]
where the first term is the Zeeman term containing the chemical shift \( (1 - \sigma_i - \sigma_{ia}) \) of the spins, the second is the indirect spin-spin coupling \( (J_{ij}) \) term, the third describes the intramolecular dipolar interactions \( (D_{ij}) \) and the last is the quadrupolar interaction term. \( \sigma_i \) and \( J_{ij} \) represent one third of the traces of corresponding tensors and are identical to the chemical shift and indirect spin-spin couplings observed in high resolution NMR of liquids (isotropic cases). \( \sigma_{ia} \) is the anisotropic part of the chemical shift and for axially symmetric tensor has two components \( \sigma_1 \) and \( \sigma_2 \). For spin 1/2 nuclei the quadrupolar term is absent. The intermolecular dipolar interactions are averaged to zero due to large translational diffusion of the molecules. The intramolecular dipolar interactions are reduced to small but finite values governed by the order parameter which defines the degree of orientation of molecules. The high resolution proton spectra of oriented molecules thus exhibit sharp lines with many more lines than the corresponding isotropic spectrum. However, unlike the isotropic case, the spectrum is often not a first order spectrum mainly because the magnitude of the intramolecular dipolar interactions is usually comparable to or larger than the chemical shift differences. This necessitates explicit diagonalization of the Hamiltonian matrix to calculate the spectra and iterative computer programs to find a fit to the experimental spectra. In the case of spins with \( I > 1/2 \), the quadrupolar interaction dominates the spectra and the dipolar interactions give additional splittings (1).

2D SPIN-ECHO RESOLVED SPECTROSCOPY

Single-quantum transitions of spin 1/2 systems

The first application of two-dimensional techniques for the study of oriented molecules was a calculation and observation of the 2D spin-echo spectrum of a coupled two-spin system (each spin having \( I = 1/2 \)) (figure 2), showing that due to strong coupling features inherent in the spectra of oriented molecules, one gets additional lines in the 2D spin-echo spectrum some of which appear with negative intensity and that the spin-echo 2D spectrum gives additional information useful in the analysis of the spin-systems\(^{11,12} \). In the above example it was shown that one obtains 8 lines in the 2D spectrum, 4 of which have equal intensities, the difference in the frequencies of which unambiguously determines the coupling parameter \( (J + 2D) \), an information which could not be obtained from 1D single resonance spectrum.

A related experimental study, not utilizing two-dimensional algorithm but recording spin-echo spectra of AA'BB' spin systems of 4-cyanopyridine, and 4-acetylpyridine-d₃ (CD₃)

![Figure 2. Spin-echo 2D spectrum of two strongly coupled protons of 1-thio-3-selenole-2-thione oriented in MBBA [obtained using scheme (e) of figure 1] recorded at 270 MHz. The phase sensitive spectrum gives 8 lines, 2 of which are of negative intensity, in conformity with the calculations (11) [From ref. 12].](image-url)
dissolved in phase V, 4-[\text{H}_2] pyridine dissolved in E5* and fluorine echo spectra of 2,3,5,6-tetra fluorothioanisole-d₃ dissolved in E5 have been recorded and analysed using modified LAOCOON computer programs¹³. Significant r.f. field inhomogeneity gave rise to artifacts in the spectra which were simulated as error in the 180° refocusing pulse and the spectra were fit to variable angle \( \alpha \) of the refocusing pulse in the spin-echo sequence 90-t-\( \alpha \)-t. The angle \( \alpha \) varied between 150° and 162° for a good fit. The feasibility of such an analysis was demonstrated as an alternative to spin decoupling for measurements of homonuclear dipolar couplings¹³.

**Single and multiple quantum transitions of spin 1 system of deuterium**

The quadrupolar splittings and the chemical shift of deuterium have been separated out by two methods using 2D spectroscopy¹⁴. The first method utilizes the sequence 90°-(\( \pi/2 \))-180°-(\( \pi/2 \))-90°-t₁-90°-t₂(aq). In this method the double quantum coherence is excited in the preparation period by the sequence 90°-(\( \pi/2 \))-180°-(\( \pi/2 \))-90°, which is frequency-labelled during t₁ period, at the end of which a coherence transfer is effected by the third 90° pulse into detectable single quantum coherences of deuterium, which are detected during period t₂. The double quantum frequency during t₁ depends only on twice the chemical shift [2(\( \Delta \omega \))] and is free of quadrupolar coupling while the single quantum frequencies in t₂ depend on both quadrupolar coupling and chemical shift (contains two transitions at frequencies \( \Delta \omega \pm \omega_q \)). Two-dimensional spectroscopy allows separation of these two interactions in two frequency dimensions. The experiment has been applied to identify the quadrupole split, chemically shifted resonances of seven non-exchangeable deuterons of per-deuterated mannose partially oriented in a liquid crystal matrix. Because of low chemical shift dispersion and larger linewidths of the double quantum coherences, the separation of the chemical shift in these experiments was limited¹⁴. In the second method utilizing the sequence 90°(\( \phi_1 \))-t₁/2-90°(\( \phi_2 \))-t₁/2-t₂(aq), quadrupolar echoes are formed at the end of the t₁ period. The quadrupolar interaction is focussed at the echo point and the t₁ domain contains only the chemical shifts, while the t₂ domain contains both chemical shifts and quadrupolar interactions. This experiment also yielded only a limited resolution in the F₁ axis¹⁴.

In another study, separation of dipolar and quadrupolar splitting in partially deuterated and oriented systems (CD₂HI oriented in Merck Phase IV* liquid crystal) has been carried out by using 2D NMR techniques for simplification of the spectrum¹⁵. The pulse scheme used is shown in figure 3a. Heteronuclear decoupling during the double quantum preparation period \( \tau \) facilitates selection of \( \tau \) for optimal creation of double quantum coherence of interest and during t₂ eliminates \( ^1\text{H}-^2\text{H} \) dipolar interactions. The evolution of double quantum (DQ) coherences is studied during the period t₁. Simultaneous application of proton and deuterium \( \pi \) pulses in the middle of the t₁ period refocusses off-set effects, without affecting the evolution of the double quantum coherences. The last 90° pulse converts the DQ coherences into observable deuterium signals. By the use of phase cycling, only DQ coherences are retained during the t₁ period. The F₁ axis contains only dipolar splittings while the F₂ axis contains the quadrupolar splitting and chemical shift. A clear-cut separation of these interactions was obtained in the 2D spectrum (figure 3b), allowing the homonuclear and heteronuclear dipolar couplings and the quadrupolar coupling to be measured directly from the spectrum. The use of spin-echo and DQ precession allows high resolution to be obtained in F₁ dimension resolving the various dipolar couplings¹⁵.

**Study of deuterium relaxation**

In order to study the molecular motions the deuterium relaxation has been a subject of several studies¹⁶-¹⁹. The \( \text{d}^\text{2} \)uterium relaxation is studied using spin-echo sequences, for the single and multiple quantum coherences of deuterium. In the first of these studies the

* Eutectic mixtures of Merck liquid crystals.
(a) Pulse sequence for obtaining double quantum spectrum of deuterium via two-dimensional MQT spectroscopy which yields a separation of dipolar and quadrupolar splittings along $\omega_1$ and $\omega_2$ axes respectively, of partially deuterated molecules oriented in liquid crystal phase. The phase of pulses is cycled such that all unwanted signals (other than double quantum) are suppressed from $\omega_1$ dimension (2). The resulting spectrum of CD$_2$HI, oriented in Merck Phase IV, recorded at 11.7 tesla, is shown in (b) [from ref. 15].

The relaxation rate of the DQ coherence of deuterium of CDC$_3$ oriented in liquid crystal matrix was studied by using multiple-quantum spin-echo spectroscopy. DQ coherence was excited by the sequence 90-τ-90. The excited DQ coherence was allowed to evolve during the detection period $t_1$, at the end of which another 90° pulse affects a coherence transfer to single quantum coherences detected in period $t_2$. Phase cycling was employed to cancel the axial and single quantum peaks of period $t_1$. The spin-echo of deuterium DQ was created by an $\alpha$ pulse in the middle of $t_1$ period and it was shown that for $\alpha = 180°$, complete refocusing
of the DQ coherence takes place. The decay rate of this spin-echo DQ coherence, free of magnetic field inhomogeneity was shown to be due to quadrupolar relaxation mechanism. This was extended to the study of relaxation of two coupled deuterons of CDCl₂ partially oriented in a liquid crystal matrix. The relaxation of the coupled deuterons is governed by three auto-correlation and three cross-correlation spectral densities. Two of the auto correlation spectral densities were measured by the use of semiselective and nonselective inversion recovery experiments while the remaining spectral densities were determined by 2D techniques using both single and double quantum spin-echoes. The single quantum echoes were obtained by the sequence π/2-t₁/2-π-t₁/2-t₂(aq) with 4 step EXORCYCLE to cancel the artifacts arising due to imperfections of pulses. The widths of the lines along the t₁ dimension yield the relaxation rates from which the two additional spectral densities could be extracted. The DQ coherence was selectively excited by a weak DQ pulse applied near the resonance. The spin-echo of the excited DQ coherence was obtained by applying a π pulse in the middle of the t₁ period. The 90° pulse at the end of t₁ as usual affects coherence transfer to single quantum coherences detected during t₂. The line-width of the DQ spin-echo spectrum in the F₁ dimension was used to obtain the relaxation rates, which were used to extract the remaining spectral densities. Limited information on the motional processes which contribute to the relaxation of CD₂Cl₂ in phase V could be extracted from this study.

In a continuing study, the deuterium relaxation of partially oriented methyl group in acetonitrile-d₃ oriented in phase V was calculated and measured. Multiple-quantum spin-echo spectra were recorded by the usual sequences. The C₃v symmetry of the spins was utilized for obtaining symmetrized basis functions and the total spectrum was divided into A₁, A₂ and E irreducible representations. DQ coherences were correlated to single quantum coherences of the same irreducible representation and this aspect was utilized for assignment of the DQ spectrum. All the zero and multiple quantum frequencies were classified into 3 further classes: class 1 in which the frequencies depend only on ν₁₁, the chemical shift; class 2 in which the frequencies depend also on J and D; and class 3 in which the frequencies depend in addition on ωQ. The lines belonging to class 3 usually gave broader lines and all the single quantum transitions belong to this class. The class 1 coherences are focussed in the spin-echo experiment and appear at zero frequency in the F₁ dimension of the 2D spectrum. The most striking feature of the observed DQ spectrum is its enhanced resolution. This was due to refocussing of the static field inhomogeneity in the F₁ dimension, bunching of class 1 peaks at F₁ = 0 and non-excitation of class 3 peaks due to the particular value of interval τ chosen in the excitation sequence.

For studying the relaxation features, these coherences were further classified into degenerate and nondegenerate types. The relaxation behaviour of nondegenerate coherences is straightforward, as they decay as single exponentials whose rates can be calculated from the assumed relaxation model. An ideal 180° pulse reverses the class 1 coherences into their complex conjugates while each class 2 coherence is converted into the complex conjugate of its image under spin inversion. The image decays exponentially at the same rate as the original coherence and the decay rate remains single exponential even in the spin-echo experiment and the corresponding line is a simple Lorentzian whose width in F₁ dimension directly yields a relaxation matrix element. Degenerate coherences show multieponential relaxation and additional complications due to the refocussing 180° pulse. Even order multiple-quantum spin-echo relaxation rates of classes 1 and 2 transitions have been measured, and their relation to various spectral densities pointed out.

2D SINGLE QUANTUM CORRELATION SPECTROSCOPY (COSY)

Two-dimensional single quantum correlation spectroscopy (COSY) has been applied for
studying the relationship of various transitions in the dipolar coupled spectra of molecules oriented in liquid crystal matrices. Conventionally double resonance experiments were used for this purpose in high resolution NMR of liquids, but these have had little success in dipolar coupled cases mainly due to the second order nature of the spectra of the oriented molecules. The first attempt in this direction was the study of an AA’BB’ spin system formed by the protons of benzoselenodiazole oriented in liquid crystal EBBA. The 2D COSY spectrum obtained at 270 MHz using the pulse sequence 90-\(t_1\)-90-\(t_2\) (aq), yielded cross-peaks only within the transitions belonging to the symmetric and antisymmetric types, leading to a complete separation of all transitions into the symmetric and antisymmetric manifolds. These manifolds were then separately analysed. It was found that identification of the transitions of the antisymmetric manifold allowed analytical analysis of the transitions of this manifold. From this several parameters governing the spectrum could be extracted. The symmetric manifold peaks were classified into three groups, depending on the intensity of diagonal peaks compared to their intensity in one-dimensional spectrum. In strongly coupled spins the diagonal peaks of 2D spectrum have intensities different from the square of their 1D intensities due to contribution of 3-spin-1-quantum type of matrix elements in the 90° pulse rotation operator. Such an element is not possible if a pure state is involved in the transition. The class 1 peaks were thus identified to be those involving a pure state. This identification leads to information useful in the analysis of the spectrum.

It was further shown that additional information from the COSY spectrum could be obtained by changing the flip angle of the coherence transfer pulse. By decreasing the flip angle below 90°, the relative intensity of the connected to unconnected cross-peaks in a COSY spectrum is increased. This was explained by using a pathway description. The intensity of a cross-peak between connected transitions is dependent on matrix elements in the pulse operator which take a total path of lower order than the unconnected transitions. The higher order pathways contribute to the cross-peaks between unconnected transitions, and can be enhanced by increasing the flip angle above 90°. The study demonstrated the utility of COSY in such studies.

The absence of cross-peaks between symmetric and antisymmetric transitions was further utilized in systems with higher symmetry, leading to a dramatic symmetry, filtering off the spectra of oriented molecules by COSY. In the 2D COSY experiment coherence transfer is affected by the second 90° pulse. The operator representing the 90° (or \(\alpha\)) pulse is given by

\[
P_\alpha = \exp (i\alpha I_{12}) = 1 + i\alpha I_{12} + [(i\alpha)^2/2!] I_{12}^2 + \ldots. \tag{2}
\]

Each term \(I_{ij}\) has matrix elements only between states of the same irreducible representation. The coherence transfer operator, therefore, does not effect transfer of coherence between transitions belonging to different irreducible representations of the symmetry group. Spectra of two symmetric molecules, having spin systems denoted by \(A_1A_2\) and \(AA'AA''A'''A''''\) arising respectively from protons of acetone and benzene oriented in a nematic phase have been studied. In the case of acetone (\(A_1A_2\)), there are two rotating methyl groups each having \(C_3\) symmetry and the spin system is characterized by an intermethyl and an intramethyl H-H dipolar coupling and an internmethyl spin-spin coupling. The eigenstates of the Hamiltonian can be grouped into four irreducible representations \(A_1, A_2, G_1\), and \(G_2\), and analytical expressions are available for the frequencies of all the transitions. The 2D COSY spectrum along with the 1D spectrum is shown in figure 4. The cross-peak linkages in the 2D spectrum separate the spectrum into four sets which are uniquely identified, in a straightforward manner, to the respective irreducible representations.

* EBBA is \(p\)-ethoxybenzylidene \(p\)-n-butylaniline and MBBA is \(p\)-methoxybenzylidene \(p\)-n-butylaniline.
representation has only two transitions. These are immediately identified in the COSY spectrum and yield the value of one of the dipolar couplings. Similarly other representations are also uniquely identified yielding the spectral parameters without the need of complicated calculations.\(^2\)

Benzene has \(D_{6h}\) symmetry and its proton NMR spectrum in oriented phase is characterized by six parameters namely \((D_o, J_o)\), \((D_m, J_m)\), \((D_p, J_p)\) respectively the ortho, meta and para dipolar and spin-spin couplings. However, for regular hexagon the dipolar couplings are interrelated reducing the independent para-
meters to only 4. The spin states are divided into six irreducible representations \((A_1, A_2, B_1, B_2, E_1, E_2)\). The \(A_2\) representation has only one spin state and no transition. Transitions belonging to the remaining 5 irreducible representations have been identified with the help of the COSY spectrum. Separate iterations on each irreducible representation using a modified LAOCOONOR programme yielded all the parameters in an efficient manner\(^{21}\).

2D MULTIPLE QUANTUM TRANSITIONS SPECTROSCOPY

In the previous section, utility of the experiments which correlate single quantum transitions with each other (COSY) was described. In this section the utility of correlation of multiple quantum transitions to single quantum transitions and the use of multiple quantum filtering in the study of oriented systems are described.

\(MQT\) spectroscopy

2D multiple quantum spectra are obtained by the use of sequence shown in figure 1(c). The multiple quantum transitions are excited at the end of second 90° pulse; they are frequency-labelled during \(t_1\) and converted into observable single quantum magnetization by the last 90° pulse. This observable magnetization is detected as a function of \(t_2\), yielding a 2D spectrum in which the \(F_1\) axis contains the various multiple quantum transitions and the \(F_2\) axis contains the single quantum transitions (SQT). The 2D spectrum indicates the correlation of the MQT with the SQT. The magnetization in \(t_2\) can also be detected at a single fixed point \(r\), resulting in a one-dimensional spectrum as a function of \(F_1\). This one-dimensional spectrum is equivalent to a projection of the 2D absolute mode spectrum on the \(F_1\) axis. While most of the applications have been demonstrated using such one dimensional spectra\(^{22-30}\), 2D spectra have also been recorded\(^{31-34}\). Figure 5 shows the 2D multiple quantum NMR spectrum of oriented benzene\(^{34}\). The spectrum becomes progressively simple as one goes to higher multiple quantum orders. For example the 6th quantum has only one transition which correlates to many single quantum transitions. There are two 5 quantum transitions and seven 4 quantum transitions.

The various multiple quantum orders are separated out by offsetting the carrier from exact resonance\(^{22}\). If \(\Delta \omega\) is the offset of carrier from the centre of a single quantum spectrum, then the centre of the \(n\)th order appears at \(n(\Delta \omega)\). The linewidths due to field inhomogeneity along \(F_1\) axis are increased proportionately for various orders. To refocus the field inhomogeneity, a 180° pulse has been introduced in the middle of \(T_1\) period [figure 1(f)]. This focuses the field inhomogeneity as well as offset dependence of various orders. The orders in such cases are separated out by time proportional phase incrementation.

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(TPPI)\(^{22,34}\). In this method the phase of the first two 90° pulses is incremented in sequential \(t_1\) experiments as \(\phi = (\Delta \omega) t_1\). This results in a separation of various multiple quantum orders by an offset of \(n(\Delta \omega)\). In case the chemical shifts are present along with dipolar couplings, the 180° pulse in scheme (f) of figure 1 causes coherence transfer within each order, giving rise to new transitions and additional frequencies in a manner similar to the strong coupling isotropic cases discussed earlier\(^{35}\).

It is possible, by additional phase cycling, to retain only one of the orders along \(F_1\) axis resulting in a much simpler spectrum; say a DQ spectrum\(^{23,31}\). Figure 6 shows a part of the two quantum proton spectrum of 81% randomly deuterated \(n\)-hexane\(^{31}\). The deuterium spins have been decoupled during the experiment. Deuteration by large amount reduces the number of coupled protons. Molecules having only one proton give no DQ spectrum and the spectrum essentially consists of molecules having two protons. The number of molecules having more than two protons is negligibly small. The two protons can be in any of the two methyl (M) or four methylene (\(E_1\) and \(E_2\)) positions yielding a possible eight AB and eight \(A_2\) type dipolar coupling patterns. The \(A_2\) type are formed by the molecules having the two protons in the configuration \(MM, MM', E_1E_1, E_1E_1'\) (cis). \(E_1E_2\) (trans), \(E_2E_1, E_2E_2'\) (cis), \(E_2E_2'\) (trans) and the \(AB\) type by \(ME_1, ME_1', ME_2, ME_2, E_1E_2\) (cis), \(E_1E_2\) (trans), \(E_2E_2\) (cis), \(E_1E_2\) (trans). In figure 6, vertical lines parallel to the single quantum axis identify six of the DQ frequencies\(^{31}\).

The 6 quantum and 7 quantum spectra of \(n\)-hexane fully deuterated at both the methyl positions have also been reported recently and the various spectral parameters have been derived\(^{32}\). There are 8 coupled protons in this case and it is shown that the \((n-1)\) and \((n-2)\) order spectra yield the various dipolar couplings and their signs. From these results an estimate of dynamic molecular structure in the form of conformational probabilities was obtained\(^{32}\).

In another study of 34% randomly deuterated benzene oriented in a nematic liquid crystal, 5-quantum versus 1-quantum proton 2D spectrum was recorded. The 5-quantum spectrum can be obtained either from fully protonated benzene or from singly deuterated species\(^{33}\). The singly deuterated species has only one 5 quantum transition while the fully protonated species has two 5 quantum transitions. From their correlation to the single quantum transitions, various spectral parameters could be derived\(^{33}\).

**Multiple-quantum filtered COSY**

Instead of obtaining multiple quantum 2D spectra which need large computer memory and yield rectangular data matrices, one can obtain a COSY type spectrum, in which the single quantum transitions are correlated to...
CONCLUSIONS

Two-dimensional NMR spectroscopy has been successfully utilized in the study of spectra of molecules oriented in liquid crystal matrices. The spin-echo experiment has been used for enhancing the information content of the spectra and for studying the relaxation behaviour of the single and the multiple quantum coherences. The COSY experiment has been utilized for finding interrelation between various transitions and for symmetry filtering of the spectra. The MOT and MOFC have proved highly effective for filtering the spin systems and for identification of coupling networks. These have been applied to partially deuterated species leading to information on the spectral parameters and the conformations.

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