Two-dimensional Fourier transform ESR in the slow-motional and rigid limits: 2D-ELDOR ☆

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The two-dimensional Fourier transform ESR techniques of stimulated SECSY and 2D-ELDOR are shown to be powerful methods for the study of slow motions for nitroxides. Stimulated SECSY provides magnetization transfer rates, whereas 2D-ELDOR displays how the rotational motions spread the spins out from their initial spectral positions to new spectral positions, as a function of mixing time. The role of nuclear modulation in studies of structure and dynamics is also considered.

1. Introduction

Patyal et al. [1] in the preceding Letter (hereafter referred to as I) demonstrated the feasibility of performing two-dimensional Fourier transform ESR (2D-FT-ESR) in the slow-motional and rigid limits by means of a two-pulse SECSY-ESR technique. This approach was shown to be useful for the study of structure, utilizing the 2D-FT-ESR nuclear modulation patterns, as well as for studying motional dynamics by means of the line broadening it induces in the various parts of the 2D spectrum. In this Letter we focus on 2D-FT-ESR techniques based upon the three-pulse sequence: $\pi/2$–t_1–$\pi/2$–T–$\pi/2$–t_1–t_2, which in conventional spin echo ESR is just the stimulated echo sequence. We primarily address its use for the study of motions, but nuclear modulation is necessarily also considered. In particular, we consider two distinct but related forms. In the first, t_1 is held constant and the echo decay is collected as a function of t_2 for different values of mixing time T followed by FFT (or equivalent) in both T and t_2. We refer to this technique as stimulated SECSY-ESR because of analogies with two-pulse SECSY-ESR, discussed in I. In the second form the mixing time T is held constant, and the echo decay is collected along t_2 for different values of t_1 followed by FFT (or equivalent) in both t_1 and t_2. In this form it is the slow-motional analogue of the technique, 2D-FT-ELDOR (electron–electron double resonance), previously introduced [2–6] for the study of cross-relaxation in fast-motional ESR spectra.

Stimulated SECSY-ESR is the FT analogue of the earlier field-swept 2D-ESE technique introduced by Schwartz et al. [7] for studying the rate of magnetization transfer (MT) out of each spectral region, which they showed to be quite sensitive to molecular motion. The principal advantages of stimulated SECSY-ESR in the slow-motional regime over the related field-swept 2D-ESE are the same as those of SECSY-ESR, discussed in I, viz. greatly reduced data acquisition times and the use of very short non-selective pulses that minimize dead-time and are short compared to T_2. There is also the potential for the use of stimulated SECSY-ESR to study nuclear modulation by analogy to the use of stimulated echoes in ordinary ESEEM studies [8–10].

As shown in the fast-motional studies, the epitome of techniques for the study of MT is 2D-ELDOR, because one observes the cross-peaks corresponding to the cross-relaxation from each spectral position to each of the other positions. The cross-peaks may be observed to grow in time T, and their intensities are quantitative measures of the accumulation of such cross-transitions. For short times T, this is directly related to the transition

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probabilities between these spectral positions. The principal advantage of ELDOR over the MT-ESE methods (both the field-swept and stimulated SECSY varieties) is that the latter merely provide the rate of loss of magnetization from a given spectral region, whereas ELDOR shows to which region(s) of the spectrum they are delivered. (This feature is also true for cw-ELDOR and other forms of pulsed ELDOR [8,11,12].)

A spin echo ELDOR technique has previously been developed and applied to the study of slow motions [13,14]. One first inverts the z-magnetization of some spin packets, and at a later time, one observes whether this spin inversion has been transmitted to another part of the spectrum. This approach is based upon selective "pumping" and "observing" pulses, and each such experiment merely examines a single "cross-peak region". Initially such experiments were performed with a single ESR frequency source but the magnetic field was rapidly stepped after the first inverting pulse. More recently [15,16], in order to avoid long delay times (≈ 1 μs) after the magnetic field step, two frequency sources were used in conjunction with a low-Q resonator. The important advantages of our new 2D-FT-ELDOR method applied to slow motions are twofold. First of all, one *simultaneously* observes *all* the cross-peaks representing all the rotationally induced transitions between spectral positions. It would be exceedingly time consuming to map out the full 2D plot step-by-step by conventional spin echo ELDOR especially in the slow-motional regime. Secondly, since there is only a single coherent frequency source, it is possible to observe MT between spectral positions as close together as the natural limits of the spectral resolution allow. (In conventional spin echo ELDOR [13–16], one must observe at a sufficient spectral displacement from the "pump" region so as to avoid direct effects of the pump pulse on the "observing" region.) This feature is important in order to address such questions as small-step (i.e. Brownian) versus large-step rotational reorientation.

A ^{2}H NMR technique analogous to the 2D-FT-ELDOR experiment we describe below has been developed by Spiess and co-workers [17,18]. Whereas the 2D-NMR method applies to ultra-slow motions (i.e. $\tau_R \geq 10^{-2}$ s), the 2D-ELDOR method would be applicable for considerably faster motions ($10^{-3} < \tau_R < 10^{-7}$ s).

2. Experimental

The 2D-FT-ESR spectrometer has been described elsewhere [3,19]. The modifications needed to perform 2D-FT-ESR in the slow-motional regime are described in I, and they apply in the present work. Both for stimulated SECSY and for 2D-FT-ELDOR the stimulated echo decay was collected for 200 ns with 256 sampling points and apodized with a function that approximates unity for the first 80 ns dropping rapidly but smoothly to zero thereafter. Either T (stimulated SECSY) or t_1 (2D-FT-ELDOR) was stepped out in 128 steps. The step size in T was either 5 or 70 ns as described below, and the initial value of T was 110–130 ns. The step size in t_1 was 5 ns, and the initial value of t_1 was 100 ns. Each echo decay was averaged 256 times (including the phase cycling). In all cases we used a special eight-step phase cycle (cf. table 4, case (a) in ref. [20]) that cancels all transverse and axial terms together with DC offsets and provides the full effect of CYCLOPS. An additional eight-step phase cycle supplies the dual quadrature output needed to form the S_{c-} signal defined by eq. (1) of I. A full collection takes about 1 h.

All experiments were with ^{15}N-protonated tempone or ^{14}N-perdeuterated tempone (PDT) dissolved in 85% glycerol–15% H_2O and degassed to better than 10^{-6} Torr. The stimulated SECSY experiments were on 0.5 mM solutions; those for 2D-FT-ELDOR were on 1 and 2 mM solutions. At the low temperatures studied we found that the results were independent of concentration over this range.

3. Results

3.1. Stimulated SECSY

We show in figs. 1a and 1b the stimulated SECSY-ESR spectra of ^{15}N-tempone and for ^{14}N-PDT respectively at $-83°$C. One observes both the main ESR spectrum along the $f_1=0$ axis as well as nuclear modulation peaks due to the protons (which occur at ± 14 and ± 28 MHz) and the deuterons (at ± 2 and ± 4 MHz). They are weaker than the comparable peaks seen in the two-pulse SECSY-ESR spectra in I. The basic equation for nuclear modulation in three-pulse FT-ESR experiments for the case of a single proton ($I=1/2$) is [20]:

$$
\begin{aligned}
S_{c-}(t_1, T, t_2) &= \exp[(-2t_1, +t_2)/T_2] \\
&\times (\exp(-T/T_1)\{k_+ \cos(\omega_- t_2) + k_- \cos(\omega_+ t_2) \\
&+ \tfrac{1}{8}k[\cos(\omega_\alpha t_1 + \omega_- t_2) + \cos(\omega_\beta t_1 - \omega_- t_2) + \cos(\omega_\alpha t_1 + \omega_+ t_2) + \cos(\omega_\beta t_1 + \omega_+ t_2)]\} \\
&+ \tfrac{1}{8}k \exp(-T/T_2)\{\cos[\omega_\alpha(t_1+T) + \omega_- t_2] + \cos[\omega_\beta(t_1+T) - \omega_- t_2] \\
&+ \cos[\omega_\alpha(t_1+T) + \omega_+ t_2] + \cos[\omega_\beta(t_1+T) + \omega_+ t_2] - \cos[\omega_\alpha T + \omega_-(2t_1+t_2)] \\
&- \cos[\omega_\beta T - \omega_-(2t_1+t_2)] - \cos[\omega_\alpha T + \omega_+(2t_1+t_2)] - \cos[\omega_\beta T + \omega_+(2t_1+t_2)]\}) . \qquad (1)
\end{aligned}
$$

In eq. (1), S_{c-} is the complex stimulated echo signal as defined in eq. (1) of I, and all other parameters are defined in I (cf. eqs.(3)–(5) of I).

Since $T_2 \ll T_1$ in these slow-motional (and rigid-limit spectra), the terms within the second curly brackets will be suppressed (compared to those in the first curly brackets) by their greater breadth after FT with respect to T in the stimulated SECSY-ESR experiment. The nuclear modulation terms that involve T are just of this type. We have found that further reduction in the nuclear modulation can be achieved by a careful selection of t_1. Fig. 1a (where $t_1=170$ ns) corresponds to such a minimum. The terms in the first curly brackets of eq. (1) show that there is also nuclear modulation along the t_2 (or f_2) direction of the main ESR spectrum, which is in fact given by the k_+ term. Fig. 1a has the additive effects of all the methyl and solvent protons, which is difficult to write down in analytic form, but theoretical simulations [20] are shown in figs. 2a and 2b for

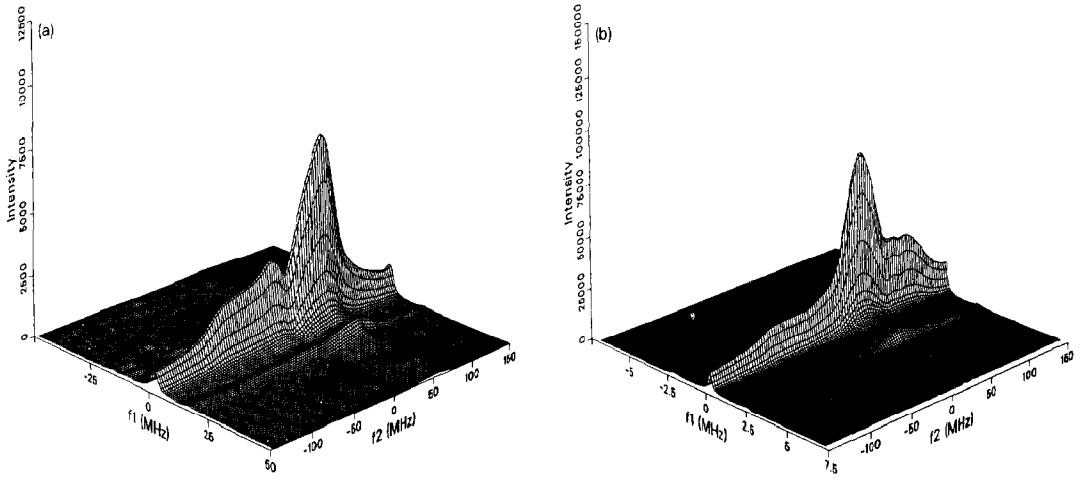

Fig. 1. Stimulated SECSY spectra of (a) ^{15}N-tempone; (b) PDT at $-83°$C. Experimental conditions as described in text. These spectra have been processed by LPSVD.

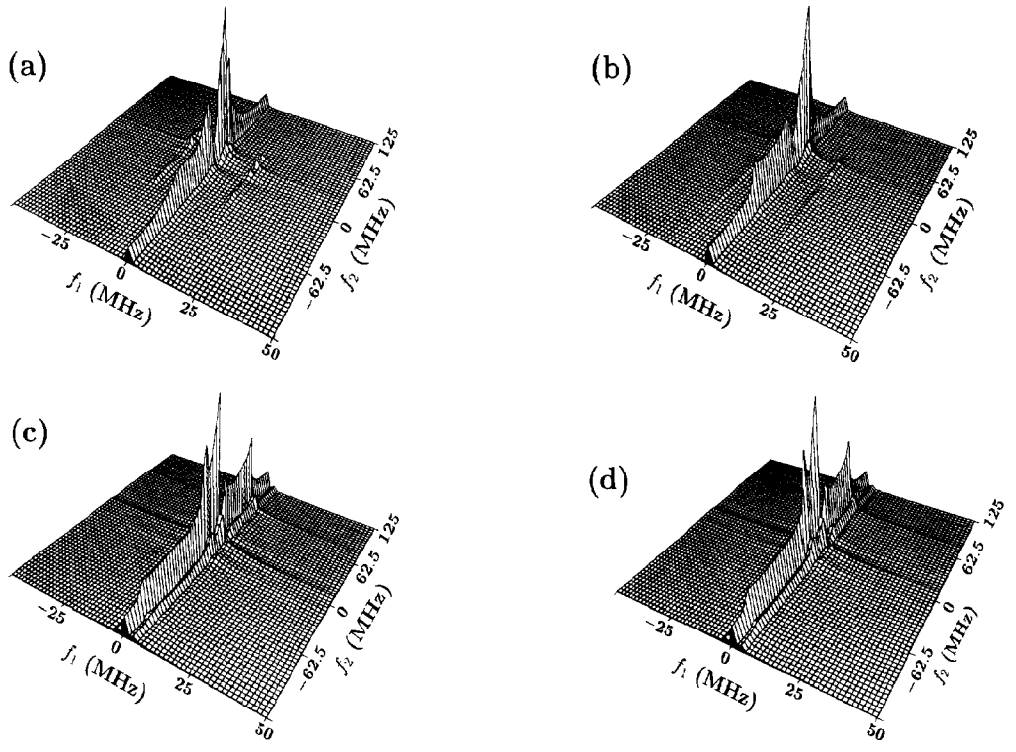

Fig. 2. Simulations of 2D-ESR spectra for stimulated SECSY and 2D-FT-ELDOR in the rigid limit. Cases (a) and (b) are for stimulated SECSY for ^{15}N-tempone with (a) 12 equivalent methyl protons and (b) 4 groups of 3 equivalent methyl protons (cf. I, fig. 3b). Cases (c) and (d) are for ELDOR of ^{14}N-PDT with 4 groups of 3 equivalent methyl deuterons and with mixing time (c) $T=3$ μs and (d) 20 μs.

the case of $T_1=T_2$. (For many nuclei, the more sophisticated computer algorithm required for the case of $T_1\neq T_2$ leads to exceedingly long computational times at present.) For this case there is prominent nuclear modulation. Figs. 2a and 2b differ in that the former assumes 12 equivalent methyl protons, whereas the latter has 4 groups of 3 equivalent methyl protons oriented as in the known crystal structure (cf. I). Just as for the two-pulse SECSY experiment (cf. I), the main ESR spectrum along $f_1=0$, as well as the nuclear modulation patterns, are significantly affected by these choices.

The relative lack of nuclear modulation appears to make stimulated SECSY-ESR a little more desirable for motional studies than two-pulse SECSY-ESR. However, we find that given the long T_1 values, the spectrum shown in fig. 1a is not optimized for accurate measurements of T_1 and of T_A, the MT rate. This is because the choice of steps of size $\Delta T=5$ ns, which produces a bandwidth of ± 100 MHz (with dual quadrature), will have a $T_{max}=T_1+128\,\Delta t=770$ ns, but this is less than T_1, which is typically several microseconds. Instead, since the nuclear modulation is not of principal interest, it is preferable to take larger steps. In particular, when we take $\Delta T=70$ ns, corresponding to $T_{max}=9.1$ μs, we are able to accurately discriminate from the LPSVD analysis of these results two dominant components which appear at $f_1=0$ MHz (within experimental error) and with the same phase. This is the expected behavior for this type of MT experiment.

The theory for this experiment leads to the following approximate form [7,19,21]:

$$S(T)=B\exp(-2W_e T)+C\exp(-T/T_A)\,, \tag{2}$$

where $2W_e=T_1^{-1}$ and $T_A^{-1}\approx T_1^{-1}+5\tau_R^{-1}$. The results of $T_1=4.83$ μs and $T_A=1.38$ μs then lead to an estimate

of $\tau_R \approx 10$ μs. For the case of PD-tempone (fig. 1b) we obtain from a similar analysis with $T_1 = 7.84$ μs and $T_A = 1.67$ μs that $\tau_R \approx 9$ μs. (Note that in this case C/B (cf. eq. (2)) is small, ≈ 0.08.) This is to be compared with the estimate from stepped-field spin echo ELDOR by Hornak and Freed [13] (HF) of $\tau_R \approx 6$ μs for PD-tempone in 85% perdeuterated glycerol–15% D_2O at the same temperature (but the more recent theoretical analysis [7,21] of this experiment showed that the simple theory of HF requires correction by about a factor of 2 to yield a $\tau_R \approx 12$ μs). Thus there seems to be reasonable agreement between these methods considering experimental uncertainties and the approximate application of theory. However, we do note that the measured T_1's are quite sensitive to isotopic labelling. Thus we find that the T_1's vary in the following fashion: ^{14}N-PDT in fully deuterated solvent [13] > ^{14}N-PDT in fully protonated solvent > ^{15}N-(protonated) tempone in fully protonated solvent. It could be that internal rotation of methyl protons as well as low-frequency motions of solvent protons have a significant influence on the T_1's observed at low temperatures in the viscous solvent.

But we must now point out that Schwartz et al. [7] have criticized the earlier interpretation by HF of their spin echo ELDOR data. Schwartz et al. argue that the ELDOR data can equally well be interpretated as due to an orientation-independent nuclear spin flip process, W_n. Their model avoids HF's conclusion of strong jumps and of very low activation energy for τ_R at low temperatures in this viscous medium. It would also account for the fact that the 2D-ESE T_2 contours of Millhauser and Freed [22] (MF) clearly favor a Brownian motion model over a jump model, and MF's τ_R values are consistent with the Arrhenius extrapolation from higher temperatures with an $E_a = 15.2$ kcal/mol (but see ref. [16]). This matter is reconsidered below in the context of the new 2D-FT-ELDOR experiment.

3.2. 2D-ELDOR

We show, in fig. 3, a series of 2D-ELDOR spectra for PD-tempone at −73°C taken over a range of values of mixing time, T from 3 to 40 μs. (For $T > 40$ μs the effects of $T_1 \approx 6$ μs and $T_A \approx 1$ μs lead to serious loss of S/N.) No appreciable spectral changes occur during the first 10 μs, but as can be clearly seen, for $T > 10$ μs noticeable changes do occur. They may be described as a broadening out of the structure principally along the f_1 direction, as though the widths of the main peaks and the modulation peaks were increasing with T.

This is actually the slow-motional equivalent of the development of cross-peaks, as T increases, for the case of a continuum of cross-peaks, i.e. they develop between all orientations (or all the spectral positions). Since it is the echo decay that is collected, one has the auto-peaks appearing along $f_1 = 0$ instead of along a diagonal corresponding to $f_1 = f_2$ (which is the case when the FID is collected [2–5]) [#1]. The interpretation of the present display is that cross-peaks are read as frequency differences along f_1 (e.g. if one follows along f_1 from the main ESR peak at $f_2 = 50$ MHz and $f_1 = 0$ MHz, the signal at $f_1 = 25$ MHz corresponds to a cross-peak between $f_2 = 50$ MHz and the auto-peak at 75 MHz). Thus, the interpretation of the observed broadening is that as T increases the rotational motion is gradually spreading spins out from their initial orientation and spectral position to new orientations and spectral positions. These changes are not uniform across the spectrum (as may be seen in the spectrum along $f_2 \approx 80$ MHz, where spectral detail is broadened out, as well as around $f_2 \approx -80$ MHz) showing that there are different rates of growth of cross-peaks in different spectral regions.

We also note that the 2D-ELDOR lineshape for small T is very similar to that for two-pulse SECSY (cf. I) including the significant modulation peaks. This is as expected from theory, which also predicts no significant change as a function of T in the rigid limit [20] (cf. figs. 2c and 2d). (Note that the one-proton case is again predicted by eq. (1) in the rigid limit.) One also finds that the experimental modulation peaks change with mixing time T, showing that they also develop "rotational cross-peaks".

These features can be seen from another vantage point by considering constant f_2 slices taken at several values

[#1] The latter could be achieved by addition of a fourth pulse, which refocuses the FID from the third pulse into an echo. Instead, if desired, one may achieve the equivalent by rotating the present spectra by the following axis transformation: the new f_1 axis is equal to the old $-f_1 + f_2$ and the f_2 axis is unchanged.

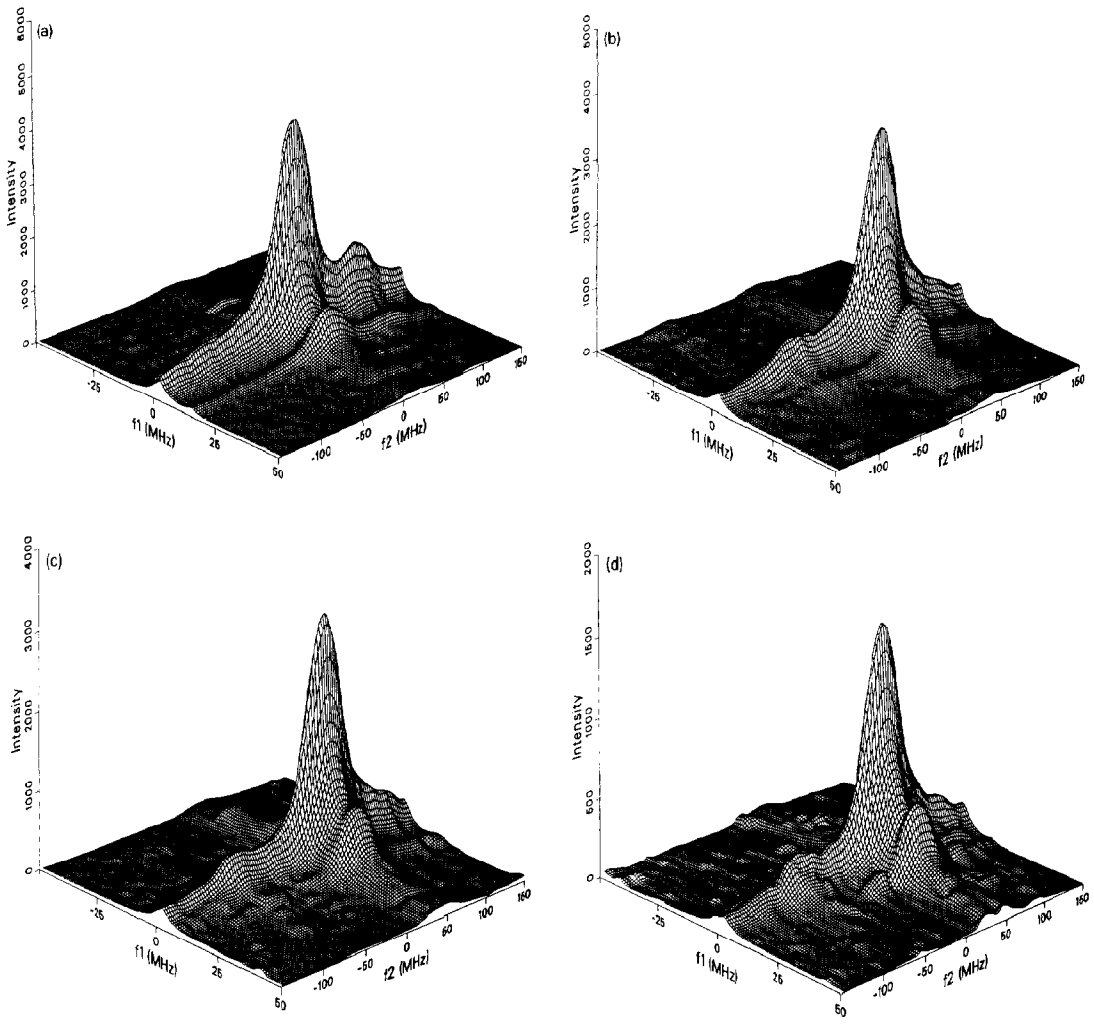

Fig. 3. 2D-FT-ELDOR spectra of PDT at −73°C as a function of mixing time (a) $T=3$ μs, (b) 20 μs, (c) 30 μs, (d) 40 μs. These spectra have been processed by LPSVD. Other experimental conditions as described in the text.

of f_2 and the same T (figs. 4a and 4b) and for different values of T but the same f_2 (figs. 4c and 4d). One observes that the spectra broaden out and even change shape as T increases, and that these effects do depend upon spectral position f_2. The LPSVD results indicate that the main structure along each f_2 slice primarily broadens (by a factor of about 2) as T increases from 3 to 20 μs, but from 20 to 40 μs there is definite shifting of location of the structural features. There is also an indication from the LPSVD analysis of some growing in of cross-peaks at large frequency differences (± 60 to 80 MHz) from the central spectrum region in particular for slices around $f_2=0$.

It is clear then that there are many rich spectral features that relate to the motional dynamics as a function of mixing time T. It is premature to attempt to analyze all of them in this Letter, so we confine ourselves to a few remarks of a preliminary nature.

First of all, the gradual spread of cross-peaks with T appears to be consistent with small-step reorientations characteristic of Brownian rotation. This is further confirmed by variation in behavior in different spectral

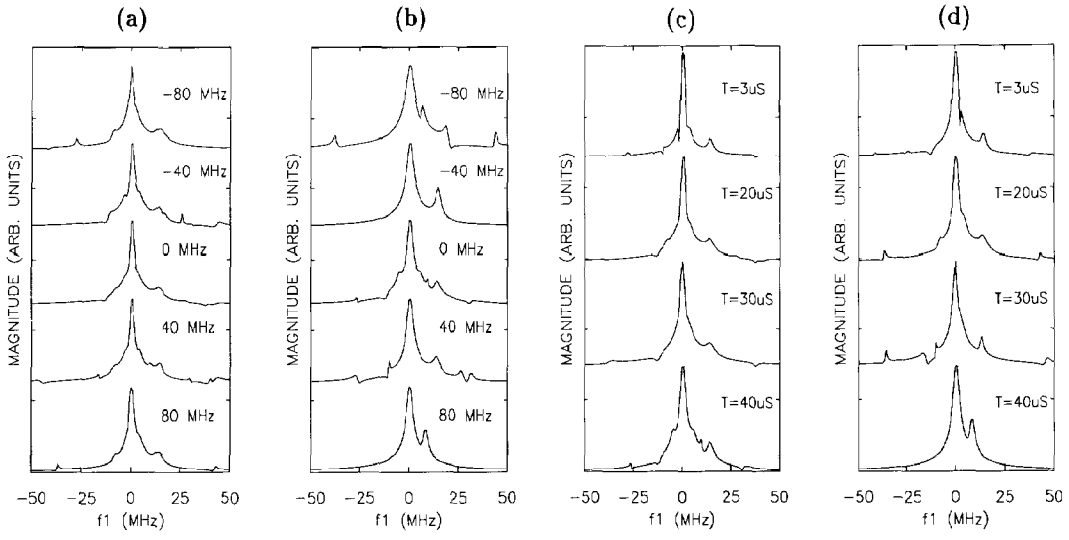

Fig. 4. Constant f_2 slices versus f_1 for 2D-FT-ELDOR spectra of PDT at -73°C for mixing time, (a) $T=20$ μs, (b) 40 μs. Also variation of (c) $f_2=0$ MHz and (d) $f_2=80$ MHz slices versus f_1 for different values of T. These are results after processing by LPSVD. (Note that in this figure (unlike fig. 3) the ELDOR spectra for the different values of T have been adjusted to a common reference value of $f_2=0$ corresponding to the maximum in the ESR spectrum.)

regimes as expected from Brownian motion models [7,19,21,22]. Also, it is the virtue of 2D-FT-ELDOR that it permits the observation of the evolution of cross-peaks characterized by small frequency differences. On the other hand, spin echo ELDOR is more restricted to cross-peaks characterized by large frequency differences (e.g. 62 MHz in the work of HF). The fact that major changes in the 2D-ELDOR spectral shape are not induced over mixing times of the order of 20 μs implies that $\tau_R > 20$ μs. This is consistent with the Arrhenius value (and the MF estimates) of $\tau_R \approx 50$–100 μs as opposed to a value of ≈ 10 μs (emerging from T_A in stimulated SECSY). This tends to support our belief that the analysis of HF's spin echo ELDOR experiment and the present stimulated SECSY experiment are affected by a faster (≈ 10 μs) orientation-independent W_n as outlined above. The appearance of high-frequency cross-peaks could well be the effects of nuclear spin flips. Rotational modulation of the ^{14}N (or ^{15}N) hf interaction is a source of such spin flips which must be present whether or not there is an orientation-independent W_n process [11,21].

In order to comment on how the nuclear modulation cross-peaks evolve with T, we first note that in eq. (1) only the terms in the first curly brackets (i.e. those multiplied by $\exp(-T/T_1)$) are dominant, because we have preset $T > T_2 = 594$ ns. When we adapt this one-proton equation to a nitroxide we have to note that the actual spectral location with respect to f_2 is largely controlled by the ^{14}N hf tensor and the orientation of the molecule. Thus we find that these terms in eq. (1) predict simple modulation peaks such that the "rotational cross-peaks" are expected to spread out along f_1 just as the main ESR peaks do. Thus it is not surprising that they show a similar broadening as T is stepped out.

In conclusion we note that these new 2D-FT-ESR techniques, particularly 2D-FT-ELDOR, are very rich in data on the details of motional dynamics in the slow-motional regime. Further studies utilizing 2D-FT-ELDOR and their detailed theoretical analysis may be expected to resolve a number of the uncertainties that have arisen from previous slow-motional studies with less powerful techniques.

Acknowledgement

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