Spin Echo Dephasing

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General Comments Concerning Relaxation

Every person who has put a sample into an EPR spectrometer has performed a relaxation time experiment. You assume that the sample comes to equilibrium by the time that you start collecting data. A wise spectroscopist checks that the microwave power is selected to be in the linear response regime.

Any process that takes a spin from one resonant field to another may contribute to a measured relaxation curve. A true $T_1$ requires coupling of electron spins to lattice thermal energies.
What can you learn by measuring spin echo dephasing in solids?

- Feasibility of doing pulse experiments that depend upon echo detection
- Local spin concentration
- Librational motion
- Proton spin concentration
- Methyl group types and concentrations
- Dynamic processes that average inequivalent nuclei
- Enhancement of echo dephasing by neighboring fast relaxing spin
A Spins and B Spins

The spins that are excited in an experiment are called the A spins. All other spins are designated as B spins. As a function of time A spins become B spins due to spectral diffusion, which decreases the number of A spins that can be observed.
Factors to consider in designing an experiment to measure dephasing

Want low resonator Q to minimize dead time

\[ Q = 2\pi \nu \tau \]

where \( \nu \) is microwave frequency
\( \tau \) is the cavity ring-down time

To decrease excitation of echo modulation use longer pulses such as 40 and 80 ns

To decrease the effects of instantaneous diffusion use turning angles less than 90\(^\circ\)

Measure temperature dependence to characterize dynamic processes.
Analysis of Spin Echo Decays

1. Fit to a stretched exponential

\[ E(2\tau) = \exp \left[ -\left( \frac{2\tau}{T_m} \right)^x \right] \quad \text{Eq. (1)} \]

- \( \tau \) is the time between pulses
- \( T_m \) is the dephasing time constant
- \( x \) is a phenomenological parameter that depends on dephasing mechanism
- Values of \( x \) are between 0.5 and about 2.5.

For nuclear spin diffusion, \( x > 2 \)
For a process that averages inequivalent environments, \( x \) varies from \( \sim 2 \) to 0.5 to 1 as the rate of the process increases.
For instantaneous diffusion \( x = 1 \)
If dephasing is \( T_1 \) driven, \( x = 1 \)
Locations of Spin Labels on Human Carbonic Anhydrase II

The single native cysteine in HCAII was mutated to serine. Cysteines at the sites selected for spin labeling were introduced by site-directed mutagenesis.

Comparison of Ways to Account for Modulation

Example: deuterium modulation in echo decays for spin-labeled carbonic anhydrase in deuterated solvent

Buried spin label, near many methyl groups.  

Surface spin label, near few methyl groups.

Decay obtained by fitting peaks was subtracted from data.
Spin-labeled HCA II in Deuterated Solvent

Method 1: Fit eq. (1) to maxima in echo decay. This gives shorter $T_m$ and smaller $x$.
Method 2: Divide experimental data by simplified modulation function and fit eq. (1) to the resulting decay. A more accurate modulation function should give the most accurate results.
Method 3: Fit eq. (1) to experimental data at long $\tau$ values

<table>
<thead>
<tr>
<th></th>
<th>Method 1</th>
<th>Method 2</th>
<th>Method 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_{\chi}$ [$\mu$s]</td>
<td>$x$</td>
<td>$T_{\chi}$ [$\mu$s]</td>
</tr>
<tr>
<td>L79C</td>
<td>1.4</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>W97C</td>
<td>2.4</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>C206</td>
<td>4.2</td>
<td>1.0</td>
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<tr>
<td>F176C</td>
<td>4.3</td>
<td>0.9</td>
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<tr>
<td>W245C</td>
<td>6.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>W16C</td>
<td>9.1</td>
<td>0.9</td>
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</table>

Analysis of Spin Echo Decays

2. Fit to a model appropriate for a particular dephasing mechanism
   
a. Protons or other nuclear spins that contribute to nuclear spin diffusion
b. Dynamic averaging of inequivalent nuclei
c. Dipolar interaction with rapidly relaxing metal
Instantaneous Diffusion

• A spin contributes to two-pulse echo formation if the precession frequency for the spin remains constant during the time $2\tau$.
• If an event changes the precession frequency of a spin during the interval $2\tau$, then the second pulse does not exactly reverse the precession that occurred during the first time interval $\tau$ and the spin does not contribute to echo formation.
• If the second pulse flips both the spin that we are observing and a neighboring spin that is dipolar coupled to the observed spin, then the pulse changes the field at the observed (A) spin and hence the precession frequency of the A spin and causes it to not contribute to the echo. This effect is called instantaneous diffusion.
• Instantaneous diffusion increases with spin concentration and with the pulse turning angle and for the same spin concentration is more important for narrower spectra.

Instantaneous Diffusion

Protons in the solvent dominate dephasing

When protons are replaced by deuterons, instantaneous diffusion makes a larger contribution.

Instantaneous diffusion is decreased by making turning angle smaller.

Note the difference in x-axis scales for the 3 plots.

Instantaneous Diffusion

E' center in irradiated SiO$_2$ at room temperature

E(2$\tau$) = (constant) $\exp(-2b\tau)$

$b = \frac{1}{T_m} + \gamma \left( \frac{4\pi^2 g\beta C}{9\sqrt{3}} \right) \sin^2 \left( \frac{\theta}{2} \right)$

$\tau$ is the time between pulses
$\theta/2$ is the turning angle for the first pulse
C is [spin] in spins/cm$^3$

Electron-electron Dipolar Interaction

In a sample of irradiated glassy SiO$_2$ the value of $1/T_m$ extrapolated to low turning angle was 25 $\mu$s.

Based on the slope of the plot, the spin concentration was $\sim 6 \times 10^{17}$ spins/cm$^3$.

Bloembergen et. al (1948) estimated that $T_2$ due to spin-spin dipolar interaction was given by

$$T_2 = \frac{\frac{hr^3}{2\pi\mu^2}}{2(3.1416) \left( \frac{9.28 \times 10^{-21} \text{ erg G}^{-1}}{118 \times 10^{-8} \text{ cm}} \right)^2}$$

$\mu$ is the electron magnetic moment

$T_2 = 2 \times 10^{-5}$ sec ($= 20$ $\mu$s), which is in reasonable agreement with experiment.

Nuclear Spin Diffusion

Frequently, an unpaired electron is dipolar coupled to many surrounding nuclear spins. If one of these nuclear spins flips, the dipolar coupling to the unpaired electron is changed, which changes the precession frequency for the unpaired electron. Although the spin flip rate for an individual nuclear spin is relatively slow, the probability of some nuclear spin flipping is large, because of the large number of nuclear spins in typical organic materials. The most common nuclear spin flip process is a flip-flop, $I_1^+I_2^-$. The rate of this process increases proportional to the nuclear spin concentration. Dipolar coupling is proportional to $\gamma$ so proton spin diffusion is a more effective dephasing process than deuteron spin diffusion.

In the absence of methyl groups, $1/T_m$ increases monotonically with proton concentration.

The effects of methyl groups on dephasing depend upon the type of methyl group.
Effects of Methyl Groups

Echo decay curves are substantially different for tempone in these 3 solvents with approximately the same concentration of methyl protons. Because the barrier to rotation is lower, the aromatic methyl groups in xylene are less effective in echo dephasing than the methyls in n-propanol or 2,5-Me₂-THF.
T_M of Spin Labeled HCA II at 40 K and Comparison with Information Concerning Probe Location

<table>
<thead>
<tr>
<th>residue</th>
<th>L79C</th>
<th>W97C</th>
<th>C206</th>
<th>F176C</th>
<th>W245C</th>
<th>W16C</th>
</tr>
</thead>
<tbody>
<tr>
<td>location</td>
<td>buried</td>
<td>buried</td>
<td>buried</td>
<td>at/near surface</td>
<td>surface/intermed</td>
<td>surface</td>
</tr>
<tr>
<td>Probe mobility, CW, RT</td>
<td>rigid</td>
<td>rigid</td>
<td>rigid</td>
<td>mobile</td>
<td>rigid/mobile</td>
<td>mobile</td>
</tr>
<tr>
<td>number of CH_3 in 5 to 10 Å shell</td>
<td>24</td>
<td>15</td>
<td>11</td>
<td>13</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>T_M [µs] in H_2O/glycerol</td>
<td>1.6</td>
<td>2.7</td>
<td>3.8</td>
<td>3.7</td>
<td>4.7</td>
<td>4.3</td>
</tr>
</tbody>
</table>
Spin Lattice Relaxation

As temperature increases, $1/T_1$ increases and eventually becomes the dominant contribution to $1/T_m$. The contribution from nuclear spin diffusion is smaller in deuterated solvent so $1/T_1$ dominates echo dephasing at lower temperature in deuterated solvent than in natural abundance solvent.

- Saturation recovery
- Inversion recovery

Vanadyl ion in 1:1 water:glycerol

Librations and Anisotropy in $T_m$

Vanadyl porphyrin in 9:1 toluene:THF

The change in resonance field per degree of rotational reorientation is largest at intermediate orientations of the molecule.

$1/T_m$ is largest at intermediate orientations and smallest along the principal axes.

Field-swept echo detected spectrum at 50 K
First-derivative CW spectrum at 20 K

Dephasing due to averaging of equivalent couplings

When rate of averaging is slow relative to inequivalence
   CW spectrum shows inequivalent resonances
   $T_m$ is long
When rate of averaging is comparable to inequivalence
   CW spectrum shows one broad signal
   $T_m$ is short
When rate of averaging is fast relative to inequivalence
   CW spectrum shows averaged resonance
   $T_m$ is long
Methyl Group Rotation – Nitroxyls

$1/T_m$ for Fremy’s salt is approximately independent of temperature below about 120 K. The temperature dependence of $1/T_m$ for tempone is due to rotation of the ring methyl groups at rates comparable to the difference between the hyperfine splittings that are averaged by the rotation. The hyperfine coupling to the methyl protons is too small to resolve in CW lineshapes. The activation energy is 2.0 kcal/mole.


Methyl Group Rotation – Cu$^{2+}$ Complexes

The effects of methyl rotation at a rate comparable to differences between the hyperfine interactions can also be seen in Cu$^{2+}$ complexes such as bis(diethyldithiocarbamato)copper, Cu(dtc)$_2$.

Interaction with Low Spin Fe(III) - Calculated

Relative echo intensity calculated for a nitroxyl spin label interacting with low-spin met myoglobin Fe(III)-imidazole at \( \tau = 500 \) ns for a range of interspin distances.
Interaction with Low Spin Fe(III) – Experimental

Relative intensity of two-pulse spin echoes for three spin-labeled variants of metmyoglobin. Values of Fe(III) relaxation rates as a function of temperature were used to estimate the $\tau_C$. 
Correlation between minimum echo intensity and Fe-nitrooxyl interspin distance
Summary of Dynamic Processes

The two-pulse spin echo decay time, $T_m$, provides information concerning a range of dynamic processes including:

- Libration of molecules in glasses
- Averaging of inequivalent nuclei
- Averaging of dipolar interaction with metal ion
Acknowledgements

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The names of colleagues at other Universities and of our students who performed the studies that provided many of our examples are given in the literature cited.

Instrumentation development and support were provided by Prof. George Rinard and Mr. Richard Quine.

Further citations of the literature, from which we have learned much of what we share with you today, are included in S. S. Eaton and G. R. Eaton, *Biol. Magn. Reson.* 19, 29 (2000).