Spin-Lattice Relaxation Times


**Purpose** Use the inversion recovery method to measure the $T_1$ relaxation times for the different chemical environments in a molecule. The $T_1$ values will be used to assess motion in different parts of the molecule.

**Introduction**

Spin-Lattice Relaxation When molecules absorb light they are transferred from lower energy states to higher energy states. This leaves the system out of equilibrium, and the system must undergo transitions from the upper states to lower states to get back to equilibrium. This process is illustrated in Figure 1. For nuclear magnetic resonance of protons, the energy level diagram has two energy levels, the lower state with protons "spin up", or aligned with the external field, and the higher state with protons "spin down", or aligned against the external field. Light in the radiofrequency region of the spectrum causes protons to "flip", or jump to higher energy levels. The process whereby the system returns to equilibrium involves spin flips to return to the lower energy state, and the excess energy is lost to the surroundings in the form of heat. The surroundings in magnetic resonance experiments is called the "lattice", therefore the name spin-lattice relaxation. The characteristic life-time of a spin in the upper state is called the spin-lattice relaxation time $T_1$. $T_1$ is the average length of time that a proton remains in the same energy level. The spin-lattice relaxation time is also called the longitudinal relaxation time.

![Figure 1. Absorption and spin-lattice relaxation](image)

An easy way to visualize the process is shown in Figure 2, which illustrates an older method for measuring $T_1$'s called "saturation recovery." At left the molecules start at equilibrium. The radiofrequency source is then turned on, which causes transitions between the two levels. After the source has been on a short time the populations (number of spins in a given level) of the two levels are equalized. With equal populations, the two levels are said to be saturated and will no longer give a resonance signal. The source is then turned off, which allows the molecules to return to equilibrium some time later. A good rule of thumb is that it takes about $5xT_1$ for the system to return to equilibrium.
The NMR signal is caused by the net magnetization of the spins $M_z$, which is proportional to the difference in the populations of the two levels:

$$M_z \propto \text{(# spins in lower state)} - \text{(# spins in upper state)}$$  \hspace{1cm} (1)

Spin-lattice relaxation is a first order kinetic process:

$$\frac{dM_z}{dt} = -\frac{1}{T_1} \ (M_z - M_o)$$  \hspace{1cm} (2)

where $M_z$ is the magnetization at time $t$ and $M_o$ is the magnetization at equilibrium. Integrating equation 2 for the recovery from saturation gives:

$$M_z = M_o \ e^{-t/T_1}$$  \hspace{1cm} (3)

The Effect of Motion on $T_1$  
Relaxation is caused by fluctuating magnetic fields in the sample. There are a number of sources of fluctuating magnetic fields. One example comes from paramagnetic substances in solution. Molecular oxygen is paramagnetic and greatly enhances spin-lattice relaxation; the effect of dissolved oxygen is so strong that oxygen must be removed by a process called "degassing" in order to study other sources of relaxation. Another source of fluctuating magnetic fields that is always present is the dipole-dipole coupling.

Nuclear spins interact through space. We often picture nuclear spins as small bar magnets; assume that we have two bar magnets situated as shown in Figure 3.

![Figure 3. Dipole-dipole coupling of two nuclear spins](image-url)
These bar magnets influence each other over a range of distances, this interaction is called the dipole-dipole coupling. The energy of the interaction is given by:

$$E = \frac{A}{r^3} (1 - 3 \cos^2 \theta)$$  

(4)

where \(r\) is the distance between the two spins and \(\theta\) is the angle between the external field and the vector joining the two spins. The constant \(A\) depends on the magnetic moment of the two coupled spins and their spin orientations, up or down. When a molecule tumbling in solution the angle \(\theta\) changes rapidly and at random causing a fluctuating magnetic field at each nucleus. This fluctuating magnetic field can bring about relaxation.

Not any fluctuating magnetic field will be efficient at bringing about relaxation. The fluctuations must have a large frequency component at the frequency of the transition in order to be efficient. In Figure 4, the transition frequency that corresponds to the magnetic resonance transition is shown at the top, the frequency is given as an angular frequency \(\omega_0 = 2\pi \nu_0\). In our instrument, for protons, this frequency is 500MHz.

![Figure 4. Effect of motion on relaxation. (a) When motions are slow relaxation is inefficient and \(T_1\) is long. (b) When the frequency of the motions match the transition frequency relaxation is efficient and \(T_1\) is short. (c) When motions are too fast, relaxation is once again inefficient. Small molecule reorientations are in this category. The fast motion regime is called “extreme narrowing.”](image)

In (a) we take as an example a large molecule. Large molecules reorient slowly in solution, therefore the angle \(\theta\) also changes slowly. The fluctuating magnetic field caused by the slow...
tumbling of large molecules changes too slowly to be efficient at relaxation at the transition frequency, which causes a long $T_1$. In (b) an intermediately sized molecule tumbles in solution at a rate that produces fluctuations that have frequency components that match the transition frequency. This motion will be efficient at causing relaxation and will give rise to a short $T_1$. In (c) a small molecule is seen to tumble too rapidly causing fluctuating magnetic fields that have components at too high a frequency to cause efficient relaxation. Just as in (a) the $T_1$ will be long. Therefore, the motion of a molecule can be either too fast or too slow to cause efficient relaxation. Only when the motion of the molecule is matched to the transition frequency will relaxation be efficient and $T_1$ short.

The motion of a molecule can be characterized by a correlation time, $\tau_c$. The correlation time is roughly the average time that a molecule spends in a given orientation. Large molecules tumble slowly and therefore have long $\tau_c$. Small molecules tumble very rapidly and therefore have short $\tau_c$. Only when the motion has $\tau_c$ about equal to $1/\omega_0$ will the relaxation be efficient.

Molecular tumbling is not the only motion that can cause relaxation. Internal motion within a molecule can be very effective at causing relaxation. Most importantly, different parts of a molecule can have different frequencies of motion, because of rotation around single bonds. Decyl bromide is a good example of a molecule that has different motional regimes. The carbon-13 relaxation times for the carbons in decyl bromide are shown below.

<table>
<thead>
<tr>
<th>Carbon Location</th>
<th>Relaxation Time (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br – CH$_2$ – CH$_2$ – CH$_2$ – CH$_2$ – CH$_2$ – CH$_2$ – CH$_2$ – CH$_2$ – CH$_3$</td>
<td>2.8 2.7 1.9 2.0 2.1 2.2 3.1 3.9 5.3</td>
</tr>
</tbody>
</table>

At the methyl end of the chain the motions are too fast to provide an efficient mechanism for relaxation. Because the bromine is massive, the motions at the bromine end of the chain are slower and are therefore more efficient at relaxation. Notice that the minimum relaxation time occurs not next to the bromine, but three carbons away. This could mean that the motion at the methylenes adjacent to the bromine is too slow for the most efficient relaxation.

Diffusion, rotation, and molecular motions within the molecule all act essentially independently to change the relaxation time. For example, methyl group rotations (i.e. around the C-C bond leading to the methyl group) are always too fast to provide effective relaxation. However, the C-C bond may be wagging around because the methyl group is attached to a ring. This wagging motion may have the correct frequency components to provide efficient relaxation, leading to a short $T_1$. Therefore, you need to consider all the motions that a particular nucleus may experience. Some may be too slow, some may be too fast, but if some are just at the right frequency the relaxation will be efficient leading to short $T_1$’s.

In summary, spin-lattice relaxation times are a sensitive probe of molecular motion within a molecule. Analysis of relaxation times can tell us whether a given chemical environment is in a rigid portion of the molecule or in a flexible part of the molecule. Assessing the motions within a molecule is the goal of this experiment.

However, caution must be exercised when interpreting relaxation times. For carbon-13, the biggest effect on relaxation is the number of attached protons for a given carbon-13. In decyl bromide for example, all motions being equal, the methyl carbon would be expected to relax more rapidly than the methylene carbons. A simple solution to this problem is to compare carbons with the same number of attached protons. For proton relaxation, between-molecule relaxation, as well as within molecule relaxation, is important. For this reason, carbon-13 relaxation is preferable for careful studies. In this lab experiment, however, we will use proton...
relaxation times in part to save time, but more importantly to provide information needed for next week’s experiment on nuclear Overhauser effects (nOe).

**Inversion Recovery Method**  The saturation-recovery method for $T_1$ determination was discussed above. A better method, available in pulsed spectrometers, is the inversion recovery pulse sequence. This sequence is discussed in detail in Atkins (see reading assignment). In essence, the inversion recovery sequence causes all the "up" spins to become down spins, and visa-versa. This creates a population inversion where there are more spins in the higher state than in the lower state. The spectrum of the system after inversion contains all negative going peaks. After inversion a period of time, $\tau$, is chosen and then the spectrum is acquired. For short $\tau$ values, peaks in the spectrum are negative. For long $\tau$ values, the system is able to reach equilibrium and the spectrum appears normal. The spectra for various $\tau$ values are plotted on the same chart, giving the so-called partially relaxed spectra for the system, as shown in Figure 5. The $T_1$ value for each chemical environment can be obtained by fitting the peak intensity of each peak to the equation:

$$\ln(M_0-M_z) = \ln(2M_o) - \frac{\tau}{T_1}$$

(5)

Notice that taking the ln of both sides of Equation (3) would give $\ln(M_z)=\ln(M_o)-\tau/T_1$, the difference here is that the signal for small $\tau$ is negative. The point where a peak changes from negative to positive is a rough measure of the $T_1$, and is $\tau = T_1 \ln 2$.

![Figure 5. Partially relaxed spectra for different delay times (this is a simulated spectrum where all of the chemical environments have the same $T_1$, which is not generally the case).](image)
**Procedure**

You will be given a small organic compound to study. Beta-ionone is an example of such a compound. Dilute around 5mg in deuterated DMSO. Transfer the sample into a valved NMR sample tube and attach to the vacuum line. Degas the sample to remove oxygen using three freeze-pump-thaw cycles. A freeze-pump-thaw cycle is:

1. Freeze the sample with ice water (the melting point of DMSO is just below room temperature.).
2. Open the sample tube valve to the vacuum line and evacuate the tube for 20 sec.
3. Allow the sample to thaw. When the sample begins to liquify close the sample tube valve.
4. Thaw completely and return to step 1, above.

**T₁ Determination by Inversion Recovery**

Varian VNMR 500 MHz NMR

The T₁ inversion recovery experiment can be run completely under automation from the Routine NMR mode of the spectrometer. You just run the experiment as you would any routine spectrum, you just choose the “INVREC” experiment rather than the regular Proton experiment following the instructions below.

First: Run a standard Proton experiment with locking, shimming, and do auto-tune the probe (click on the Tune check box in the Start tab). Observe the chemical shift range of the sample.

1. Cancel the current queue by clicking of the cancel button, if visible above the queue listing.
2. Under the Start tab go to the Spin/Temp dialog and make sure that the probe temperature is regulated at 25°C. The line under the temperature bar should read: “Current: 25°C Regulated.”
3. Pull down the Automation menu, slide right on Automation Run, and choose New Automation Run. Make sure the Carousel Position selector is showing in the data window (nine positions in a circle). If it is not, click on the small ҈ button at the upper-left of the spectrum display screen.
4. If the queue listing does not list “New Sample”, click on the New Sample button at the bottom-left of the sample queue window.
5. Click on the Carousel position for your sample. The position should be highlighted by a dashed circle.
6. Type in your sample name and select the solvent for your sample from the Solvent buttons or pull down menu (DMSO is used in this experiment). Select the Autoplot check box to have the spectra plotted out.
7. Sign your sample into the log book.
8. Since you are already locked, to save time, in the “Before first EXP (day/night)” dialog box, make sure the Lock option is set to “No (alock = n).” Since you have also shimmed, to save time, make sure the Shim checkbox is not selected. Since you have also tuned the probe, to save time, make sure the Tune checkbox is not selected.
9. Double click on the black T1_MEASURE entry on the bottom of the Queue list. Make sure that the T1_MEASURE entry is highlighted and the carousel position for your sample has the “halo” outlining it.

10. The Acquire tab will open. Under the Default dialog:
   a. Enter the spectra width of 0.5 to 8.5 ppm, to get better resolution of the peaks.
   b. Set the Relaxation delay to 20. sec, which will allow the system to return to equilibrium after every free induction decay, FID.
   c. Set the Min T1 to 0.5 and the Max T1 to 5. sec. Click on the Array Relaxation Data button.
   d. You shouldn’t need to change, but verify none-the-less the following settings:
      Decoupling Mode: Coupled – NOE (i.e. no decoupling)
      T1 Mode: Inversion Recovery
   e. Make sure the AutoGain checkbox is cleared and input a Gain of 10.

Under the Acquisition dialog:
   f. Set the number of scans to 2, which will save some time.

11. Click on the Show Time button to check the total acquisition time. The time should be 10 minutes or less.

12. Now click on the Submit button to run the experiment. The spectral results will be printed.

**T1 Processing Instructions**

1. Double click on the INVREC_01 entry in the Queue list to observe the spectrum.

2. Click on the “Process” tab and then “T1 Analysis” in the dialog list. Click on the Display Last Spectrum button.

3. The first step is to do peak-picking to determine the lines that you want to analyze: Click on the threshold icon. This icon has a yellow horizontal line. Drag with the left mouse button, on the spectrum display, to adjust the threshold so that it is just lower than the least intense peak that you want to analyze.

4. Click on the Do T1 Analysis button. The results are displayed in the text window. Use the scrollbar to find the relaxation times and their uncertainties for each line. Also note the list of chemical shifts for each analyzed line.

5. Click on the Display All Fits button. Check to see if all the fits are valid. To see an individual fit, enter the number of the line in the Display Selected Fit dialog box and press Enter.

6. Click on the Display Fit Summary button. To print the fit results: highlight and copy the contents of the text box onto the clipboard. Include just through the data for the first line fit. On the top Linux menu bar drag right on the Accessories menu and choose gedit Text Editor. Paste the results into gedit and print the results to the HP_LserJet-3005.

**Report**

A formal introduction and theory section are not necessary. Include the following information.

(1) Assign all of the peaks in the spectrum.

(2) Draw the structure, but replace each H with its relaxation time, as shown below.
(3) Decide which parts of the molecule have greater flexibility (faster motions) and which parts of the molecule have hindered motion (slower). Ring structures are good examples of hindered motion.

(4) Choose one of the following statements and explain your choice.
   (a) The motion of the molecule, on the whole, is too fast, so that hindered motion provides more efficient relaxation.
   (b) The motion of the molecule, on the whole, is too slow, so that freer motion provides more efficient relaxation.

(5) Using the intensity data from the first line fit listing, do the linearized version of the fit to find the corresponding relaxation time using Eq. 5. Compare to the automatic results (which use non-linear curve fitting).