LECTURE NOTES CHEM 781

PART 9: Relaxation and Dynamics

December 3, 2008

9.1. Longitudinal relaxation (T_1) and distance

NOE experiments measure distances (typically H-H) trough dipolar *cross relaxation* between the two nuclei. That will only be possible if the two nuclei have different chemical shift. In the case of equivalent nuclei measurement of *overall dipolar relaxation* may still allow for a measure of distance in some cases.

By definition, longitudinal relaxation is the recovery of z magnetization towards equilibrium. For two spins I^A and I^B coupled by dipolar coupling it is

$$dI_z^A/dt = (2W_1^A + W_0 + W_2)(I_0^A - I_z^A) + (W_2 - W_0)(I_0^B - I_z^B) \qquad \textbf{(9.1)}$$

If $I^A \neq I^B$ (for example ^{13}C-^1H) and we decouple I^B (^1H) longitudinal relaxation depends only on the first term (I_z^B = constant = 0 throughout the experiment). As already mentioned in part one of the lecture we get an mono exponential recovery of z-magnetization:

$$\vec{\mathbf{M}}_z = M_0\vec{\mathbf{I}}_z[1 - e^{-\frac{t}{T_1}}] \qquad \textbf{(1.24)}$$

The above equation assumes $M_z(0) = 0$ (90° pulse or saturation). The second term in **9.1** only influences the equilibrium value M_0 by accounting for the NOE.

The rate of recovery or relaxation time ($R_1 = 1/T_1$) is then given by

$$1/T_1^A = (2W_1^A + W_0^{AB} + W_2) = {}^1/_{10}D^2_{AB}[J(\omega_A-\omega_B) + 3J(\omega_A) + 6J(\omega_A+\omega_B)] \text{ (heteronuclear) } \textbf{(9.2a)}$$

In the case of two equivalent spins ($I^A = I^B$) the two terms in **(9.1)** combine to

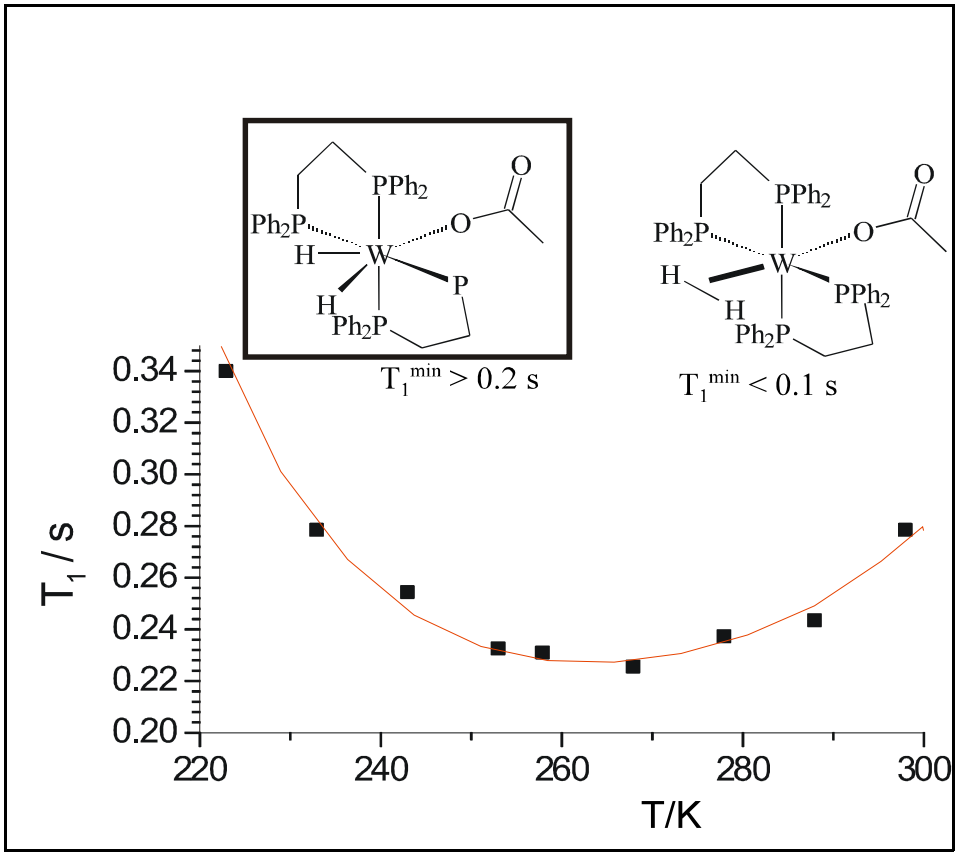
$$1/T_1^A = (2W_1^A+2W_2^{AB}) = {}^3/_{10}D^2_{AB}[J(\omega_A) + 4J(2\omega_A)] \qquad \text{(homonuclear)} \qquad \textbf{(9.2b)}$$

D_{AB} is the dipolar coupling constant $D_{AB} = (\gamma_A\gamma_B\hbar^2)/r_{AB}^3$ containing the distance information and the spectral densities $J(\omega) = \dfrac{\tau_c}{1+\omega^2\tau_c^2}$ depend on the correlation time (motion).

The dipolar relaxation will thus depend on both the distance and the molecular motion. If the molecular tumbling rate (and thus τ_C) can be determined independently **(9.2)** will be useful in determining distances. That is usually not the case. However, measurement of the temperature dependence of T_1 can typically achieve that goal: Relaxation will be most efficient around $\tau_C \approx 1/\omega_0$ and T_1 will reach a minimum at that point, increasing for longer τ_C (slower motion, lower T) and shorter τ_C (faster motion, higher T).

Exact analysis of **(9.2b)** yields the minimum T_1 for the correlation time $\tau_C = \sqrt{2/5}/\omega_0$ which can then be used to relate H-H distance to T_1:

$$1/T_1^{min} = 615/910 \cdot \sqrt{2/5}\ \gamma_A^4 \hbar^4/(\omega_0 r^6_{AB}) \qquad \textbf{(9.3)}$$

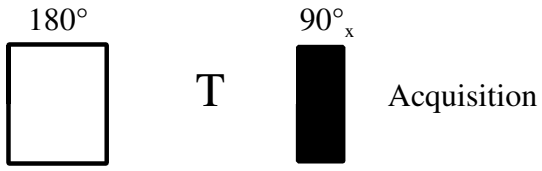


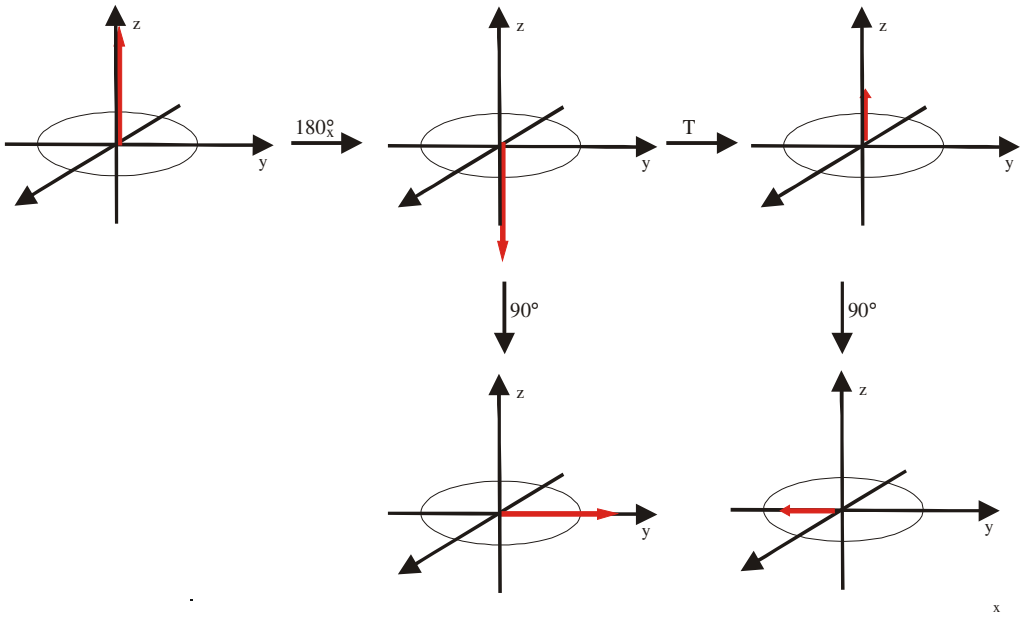
Use of T_1 measurement to distinguish two possible structures: The hydride H-H distance in the dihydride complex is considerably longer than in the dihydrogen complex, resulting in different predicted values for T_1^{min}. The experimental $T_1^{min} = 0.23$ s and thus indicates the presence of the dihydride complex.

For two isolated spins **(9.3)** would allow the exact calculation of the H-H distance. However in the example above additional relaxation may take place from dipolar coupling to the other protons in the molecule and alternate relaxation mechanisms. All this could cause experimentally determined T_1 to be shorter than calculated from **(9.3)**. However that does not change the qualitative argument made in the example above as any of the additional relaxation pathways would shorten T_1.

9.2. Measurement of longitudinal relaxation time

The longitudinal relaxation time T_1 is measured with the inversion recovery experiment which is similar to the 1D transient NOE experiment, but uses a regular (non selective) 180° pulse:



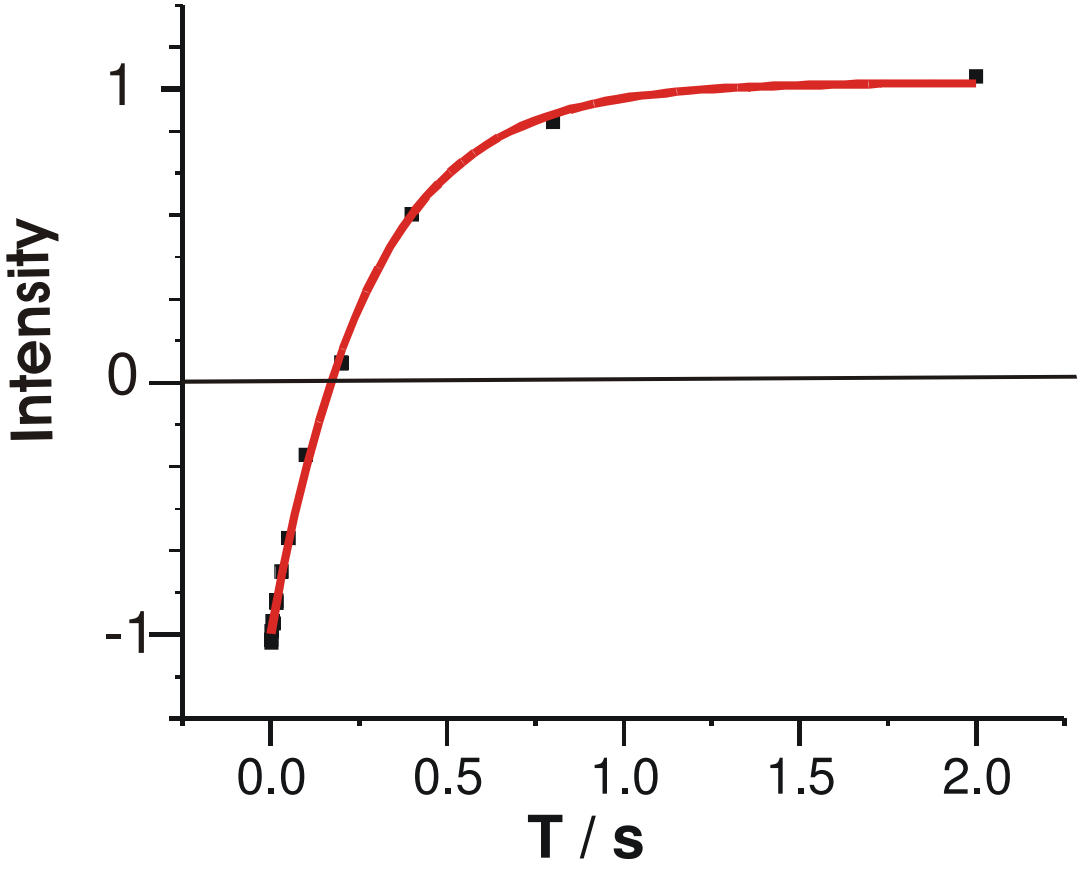


The 180° pulse place the magnetization along the -z axis. During the waiting time T the magnetization will relax back towards its equilibrium value. The 90° pulse will then convert the

current z magnetization into observable y magnetization, with the signal intensity reflecting the z-magnetization present before the pulse. Repeating the experiment with different waiting times allows to measure the exponential recovery curve, from which T_1 can be extracted:

$$M_z(T) = M_z^0 \, [1\text{-}2 \, exp(-T/T_1)]$$

Note that this is slightly different from the situation after a 90° pulse due to the different initial value of M_z. For T = 0 an inverted signal is observed, and for $T >> T_1$ the equilibrium signal is observed. For $T = \ln 2 \cdot T_1$ zero signal is observed. An exponential fit to the data gives T_1



9.3. Relaxation and motion

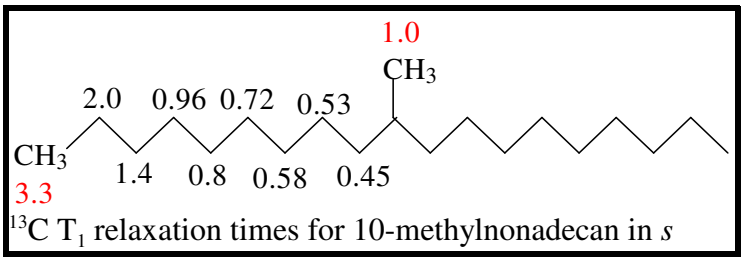
As mentioned earlier, relaxation depends both on the strength of the interaction (here dipolar coupling) and a motion. That also implies that if the magnitude of the interaction (i.e. distance) is known, the relaxation times can be used to probe for molecular motion. That is the case for many ^{13}C-H bonds. Due to its low natural abundance, and the r^{-6} dependence on the distance, relaxation of the ^{13}C nucleus of a C-H group usually solely depends on dipolar relaxation to the directly attached hydrogen. As there are many data on C-H bond lenghts ^{13}C relaxation measurements can be used to probe for molecular motion.

In the case of rapid tumbling ($\tau_c\omega_0 \ll 1$, small molecules) **(9.2a)** the relaxation rate is proportional to the correlation time or the inverse of the diffusion coefficient:

$$\frac{1}{T_1} = \frac{\gamma_C^2\gamma_H^2\hbar^2}{r_{CH}^6}\tau_C = \frac{\gamma_C^2\gamma_H^2\hbar^2}{r_{CH}^6}\frac{1}{6D_{diff}} \quad (9.4)$$

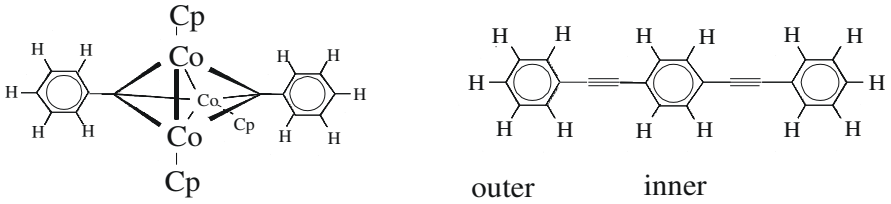
The above equation is valid for a rigid spherical molecule. In real molecules, fast internal rotation about single bonds will contribute to the diffusion coefficient, resulting in different correlation times for rigid and flexible parts of the molecule. Relaxation measurements thus can reveal rotations in the MHz range.

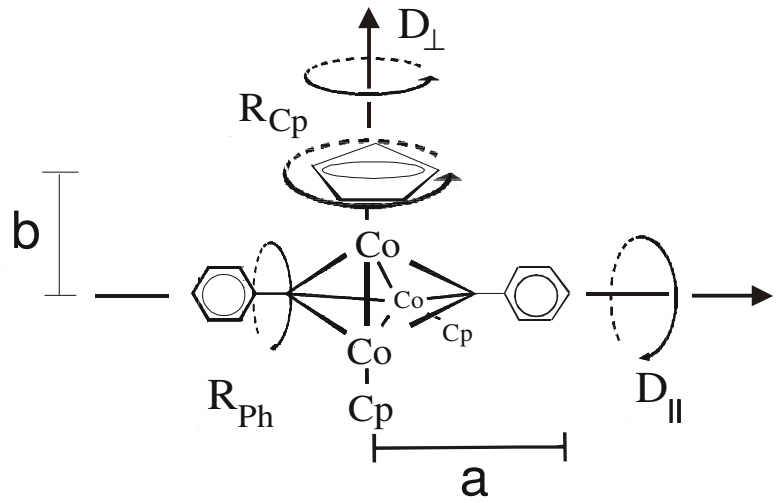
For example in 10-methylnonadecan the ^{13}C relaxation times increase toward the end of the chain indicative of increased flexibility of the outer part of the chain:



^{13}C T_1 relaxation times for 10-methylnonadecan in *s*

In addition to internal rotation molecules are rarely spherical. More often the overall tumbling of the molecule is anisotropic, i.e. tumbling about different axes takes place at different rates. Bistolane and the shown cobalt cluster complex are cigar shaped and the relaxation of the phenyl carbons depends on three diffusion coefficients: tumbling perpendicular to the main axis





($D_\perp$), tumbling parallel to the main axis ($D_\parallel$) and internal rotation R_{Ph}. Quantitative analysis of such data can become very tedious:

$$\frac{1}{T_1} = \frac{\gamma_C^2 \gamma_H^2 \hbar^2}{r_{CH}^6} \left(\frac{A}{6D_\perp} + \frac{B}{5D_\perp + D_\parallel + R} + \frac{C}{2D_\perp + 4(D_\parallel + R)} \right) \qquad (9.5)$$

where $A = (3\cos^2\Theta - 1)/4$, $B = 3\sin^2\Theta\cos^2\Theta$ and $C = (3\sin^4\Theta)/4$ with Θ the angle between the C-H bond and the rotational axis.

However, qualitatively one can see that for C_{para} the C-H bond is part of the rotation axis for both internal rotation and $D_\parallel$. Dipolar coupling for this for this group is thus only modulated by $D_\perp$ which is considerably smaller and thus relaxation times for these carbons are much shorter.

	$T_{1,para}$/s	$T_{1,ortho}$/s	$T_{1,meta}$/s	$T_{1,inner}$
$Cp_3Co_3(CPh)_2$	1.3(1)	4.0(2)	4.1(2)	-
Bistolane	0.83	4.8	4.6	3.9

9.4. Other relaxation mechanisms:

So far it was assumed that dipolar coupling is the only mechanism contributing to relaxation. While cross relaxation (and thus NOE) is solely determined by dipolar coupling, other mechanisms can contribute additive to the overall longitudinal relaxation rate:

$$1/T_1^{total} = 1/T_1^{dipol} + 1/T_1^{CSA} + 1/T_1^{Quad} + 1/T_1^{SR} + 1/T_1^{paramagnetic} \ldots$$

(that also means we started with the most complicated example of relaxation)

For protons and C-H, N-H usually dipolar coupling is dominant.

9.4.1. Relaxation by chemical shift anisotropy (CSA)

As mentioned before, chemical shielding can depend on the orientation of the molecule with respect to the field. While in solution only an average isotropic shift is observed, the nucleus actually experiences an fluctuating local field. For axial symmetry the magnitude of the field fluctuation is given by the difference between the orientations with maximum and minimum shielding ($\sigma_\parallel$ and $\sigma_\perp$):

$$\Delta B_0^{CSA} = {}^1/_3\ \gamma\ B_0\ (\sigma_\parallel - \sigma_\perp) \qquad \textbf{(9.6)}$$

and obtains the relaxation time

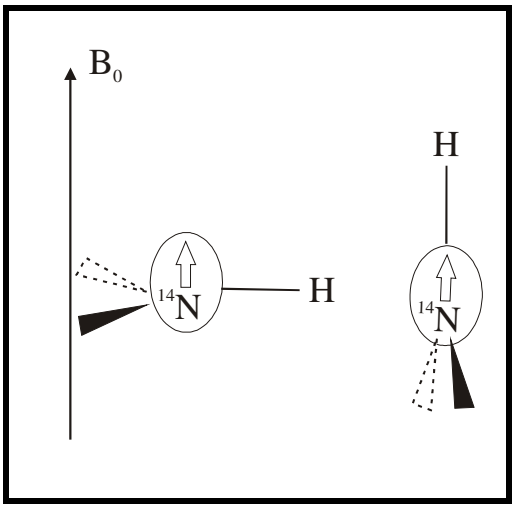
$$\frac{1}{T_1^{CSA}} = \frac{2}{15}\gamma^2 B_0^2 (\sigma_\parallel - \sigma_\perp)^2 \frac{\tau_c}{1+\omega_0^2\tau_c^2} \qquad \textbf{(9.7)}$$

- CSA is an important relaxation mechanism for tertiary C=O and C≡ groups as there are no protons nearby and the anisotropy of the shielding is particulary large.
- also important for heavy I = ½ nuclei (^{103}Rh, ^{183}W, ...) As they also exhibit large chemical shift ranges.
- note the dependence on B_0^2 for $1/T_1^{CSA}$. At higher fields this relaxation mechanism becomes more important. That is good news for metal NMR as repetition times are reduced, but may cause less than maximum NOE's for C-H groups at very high field.

Measurement of T_1 at different fields allows to separate T_1^{CSA} from T_1^{DD} and other

9.4.2. Relaxation from Quadrupol interaction

This mechanism occurs only for I > ½ as those nuclei are not spherical but shaped like an ellipsoid. Thus in a non symmetric environment different orientations of the nucleus relative to the environment will have different energies (interaction with electric field gradient).



As this energy can be quite large (several MHz) it is the dominant relaxation mechanism for all I > ½ nuclei and results in often extremely short relaxation times for these nuclei.

Consequences:

- I > ½ nuclei usually have very broad lines except when in very symmetric environments (octahedral or tetrahedral) and/or for nuclei with very small quadrupolar coupling constant (^{2}D, ^{11}B, ^{7}Li)
- a very small repetition delay d1 (0.1s) and acquisition time (td = 4k) can be used in many cases
- almost never is NOE observed for any of those nuclei (^{7}Li NMR is one exception)
- for neighboring nuclei the fast relaxation acts like decoupling (if $1/T_1 > J$) and couplings are often not observed: that is why we don't see ^{14}N-H coupling (^{14}N > 99%). Only very large couplings or to couplings to low QC nuclei is observed. In some cases, neighboring coupling partners will appear broadened

9.4.3. Other relaxation mechanisms

- Interaction with unpaired electrons (spin) will be discussed with NMR of paramagnetic compounds. Note that O_2 is paramagnetic
- Spin Rotation: currents induced by rotation of molecule, only important for very small

molecules and in gas phase, sometimes methyl groups

9.5. Tranversal relaxation time T_2

While T_1 describes relaxation of z-magnetization, T_2 describes relaxation of x,y-magnetization. In short every process contributing to T_1 will also reduce the alignment of spins and thus contribute to T_2. However in addition spins can get out of alignment without inducing transitions and therefore T_2 can be shorter, but never longer than T_1:

$$T_2 \leq T_1$$

The concept of this additional mechanism is related to chemical exchange. As mentioned above spins will experience different local fields (and thus shifts) depending on their orientation. When the motion is sufficiently fast only one average value is observed (δ in case of chemical shift, 0 for dipolar coupling). However as the tumbling gets slower, the averaging will become less efficient and the nuclei will experience several frequencies. As the tumbling is random, the spins will get out of alignment and spread out, causing a decay of the NMR signal or a broadening of the line in the frequency domain.

So while T_1 goes through a minimum and increases as the tumbling gets slower beyond the minimum, T_2 will continue to get shorter for slower tumbling.

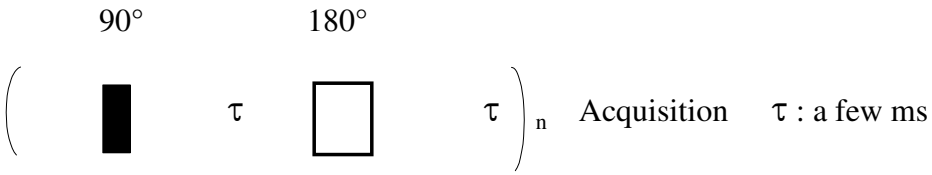
- Only for small molecules ($\tau_c \omega_0 << 1$) is $T_2 = T_1$ (most organic molecules though)
- in the slow motion regime ($\tau_C \omega_0 >> 1$) $T_2 < T_1$. That becomes a problem for large molecule NMR as the lines keep on getting broader

9.5.2. Measurement of T_2:

For very short T_2 (quadrupolar nuclei, large molecules) one can assume $T_2 = T_2^*$ and T_2 can be obtained directly from the linewidth :

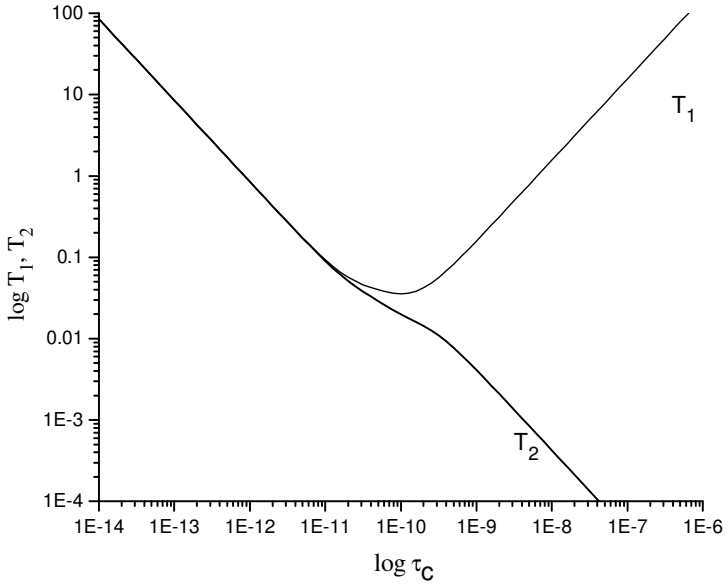
$T_2 = 2/(\pi \Delta\nu_{1/2})$

However for small molecules T_2 is usually very small and the inhomogeneity broadening will dominate the line width. As the inhomogeneity will behave like different chemical shifts it can be refocused by a spin echo:



As for T_1 measurements several spectra with different delay times are obtained and the dependence of the signal intensity on the delay time is monitored. In this case one varies the number of cycles n and the total delay time is $T = 2n\tau$. As inhomogeneity is refocused the signal decay depends only on T_2:

$$M_{x,y} = \exp(-T/T_2)$$



9.6. NMR and molecular dynamics

As seen above, NMR can be used to probe for the rate of molecular reorientation taking place on a MHz scale. Other NMR experiments can be uses to measure dynamics of lower speed, each method sensitive to a particular range of rate constants:

Technique	limiting condition for rate constant	approx. range of k / s^{-1}	typical example
conventional 1D spectra (change in integration)	$k \ll 1/AQ$ slow enough to sample several spectra during reaction issues of sample mixing, and shimming time	< 0.1	All Slow chemical reactions not at equilibrium, Protein folding, slow deuterium exchange
magnetization transfer (NOESY etc)	$\Delta\nu \gtrsim k \lesssim 1/T_1$ slow enough to see separate lines, but faster than relaxation of sample	0.01 - 10	ligand interconversion and mobility,
line broadening and coalescence	$k \approx \Delta\nu_{AB}$, $k \gtrsim 1/T_2$ Intermediate between two separate signals and one average signal. Exchange rate is order of magnitude of peak separation, and larger than T_2 relaxation rate so broadening is larger than natural line width	1 - 10^3	hindered rotations, ring inversions ligand rearrangements proton exchange
T_1,ρ relaxation times	$k \approx \gamma B_1/2\pi$ order of spin lock B_1 field strength	10^3 - 10^4	ligand rotation
T_1, T_2 relaxation times	$k \approx \omega_0$ order of Lamor frequency	10^8 - 10^{10}	Internal rotation with low activation barrier (methyl groups, Cp rings, Phe groups)

9.6.1 Chemical exchange and line broadening

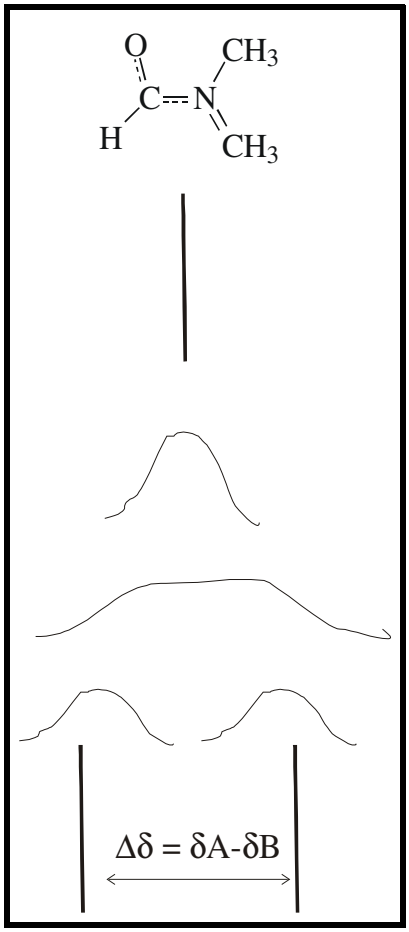
An additional process which causes line broadening in a similar manner is chemical exchange. I t can cause broad lines even for small molecules. It can be very qualitatively understood by the following consideration:

So far we always considered either a static molecule or we assumed that we saw an average between several conformations in rapid exchange. So we considered always one of two extremes. Now we have to ask how does the spectrum look like somewhere between those extremes which occurs when the rate of interchange k_{AB} becomes similar to the separation of the peaks. Clearly there has to be a smooth transition.

Example N,N-dimethylformamide:

The rotation about the partial double bond is slow enough that at room temperature that the two methyl groups have different chemical shift. However at 120° C one signal is observed as the exchange is now fast enough to give one average signal.

In the temperature range in between the two peaks will broaden with rising temperature, move into one and then gradually sharpen to give one sharp signal. The temperature where the signal is at its broadest is called the *coalescence point*. It occurs when the two signals just merge together. That is when the exchange rate k_{AB} (here the rotation about the C-N bond) reaches the magnitude of the separation between the peaks (measured in Hz) or more precise



$$k_{coal} = \pi/\sqrt{2}\,(\nu_A - \nu_B) = \pi/\sqrt{2}\cdot B_0\,\Delta\delta_{AB}$$

– This is a very quick method to determine the rate constant of the interchange. But note that you will only get one value for one specific temperature. Also as chemical shift depends on field it will depend on the magnet used. Need to determine $\Delta\delta$ from a low temperature spectrum

- At temperatures *below* the coalescence point (slow exchange limit) the line width can be used to approximately determine k_{AB}.

$$k_{AB} = \pi \cdot \Delta \nu_{ex} \quad \text{(Slow exchange limit)}$$

where $\Delta\nu_{ex}$ is the broadening due to exchange. It is determined from the observed line width by subtracting the "natural" line width determined from a spectrum without exchange broadening: $\Delta\nu_{ex} = \Delta\nu_{obs} - \Delta\nu_0$

- in a similar fashion the following approximation can be used at temperatures above the coalescence point:

$$k_{AB} = \pi(\nu_A - \nu_B)^2/(2\Delta\nu_{ex}) \quad \text{(Fast exchange limit)}$$

- exchange can involve more than two species or can be intermolecular involving different populations of the isomers (equilibrium constant K_C): the broadening pattern will become more complex and the above approximations will not be applicable. But simulation of line shape possible with a computer to determine rate constants (line shape analysis)

9.6.2 Exchange and magnetization transfer

As mentioned before chemical exchange can have the same effect on NOE spectra as relaxation via W_0 . In order to be effective two conditions have to be fulfilled:

- one has to observe two separate signals
- the rate of exchange has not to be much slower than longitudinal relaxation

Thus the condition for magnetization transfer via exchange is $(\nu_A - \nu_B) > k_{AB} > 1/T_1$

- exchange too slow to give broadening still can give magnetization transfer
- experiments used are 2D NOESY, ROESY or 1D inversion transfer (1D NOE experiment). In the literature one often refers to EXCSY when NOESY is applied to exchange, but it is still NOESY
- behaves like negative NOE for all molecular sizes or temperatures: Exchange peaks come positive in both NOESY and ROESY. NOESY distinguishes exchange peaks and NOE peaks by sign only for small molecules

- for qualitative results (what exchanges with what) one single NOESY is enough, for quantitative analysis a series of 1D or 2D NOE spectra with different mixing time is acquired, followed by a painful non linear curve fitting to obtain k_{AB}
- sometimes two exchanging protons also show NOE to each other complicating the extraction of the exchange rate

9.6.3 Rate constants and activation barriers

Rate constants can be related to the activation barrier of the process. That is usually done using the Eyring equation (I know this is not a P-Chem lecture, but ...):

$$k_{AB} = \frac{kT}{h} e^{-\frac{\Delta G^{\neq}}{RT}}$$

Note that k_{AB} is the rate constant of exchange and k the Boltzmann constant

Typically one measures k_{AB} for a range of temperatures (the larger the better) and then does a logarithmic plot of rate constant versus inverse temperature, ore more precise a logarithmic form derived from the Eyring equation:

$$\ln \frac{k_{AB}h}{kT} = -\frac{\Delta G^{\neq}}{R} = \underbrace{\frac{\Delta H^{\neq}}{R}}_{slope} \frac{1}{T} + \underbrace{\frac{\Delta S^{\neq}}{R}}_{\text{int}ercept}$$

That allows to determine both enthalpy and entropy of activation, but note that the accuracy of $\Delta S^{\ddagger}$ strongly depends on a large temperature range being sampled.

9.7. Fast Exchange and Binding Constants