The NMR Time Scale

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It is common jargon to say that some process is fast or slow on the "NMR time scale" implying that there is in fact some precise time scale that is particularly appropriate to NMR and which is understood by all. While there may have been some validity in this imprecise jargon when relaxation time measurements were ignored, and when NMR was confined to protons, the truly multinuclear character of NMR spectroscopy and the ready access to NMR relaxation rates provided by commercial instrumentation today makes any blanket statements about the "NMR time scale" imprecise at best and more often than not completely meaningless.

What are the time scales associated with NMR? A convenient way to look at this question is in terms of the rates of physically or chemically significant processes that can be extracted from a study of NMR spectra. The primary features that are easily available in any spectrum are the intensity, the resonance frequencies or the chemical shifts, the fine structure or the scalar coupling patterns, and the relaxation rates associated with the lines. Each leads to a somewhat different "NMR time scale" because different rates or rate constants may be extracted from study of these different aspects of the spectrum. The intensity of a line may be used trivially to measure concentration as a function of time as in any form of spectroscopy and, since this application provides no confusion about the time scale for the reactions or the rates involved, it will not be discussed further.

Fundamental Background

The fundamental basis for extracting kinetic information from changes induced in an NMR spectrum by a chemical exchange event is the uncertainty principle. If a nucleus may sample more than one magnetic environment in a chemical exchange process, then the uncertainty in the energy or equivalently the resonant frequency is related to the lifetime, τ, by the familiar relation

$$\Delta\nu = \mathrm{h}/(2\pi\tau) \tag{1}$$

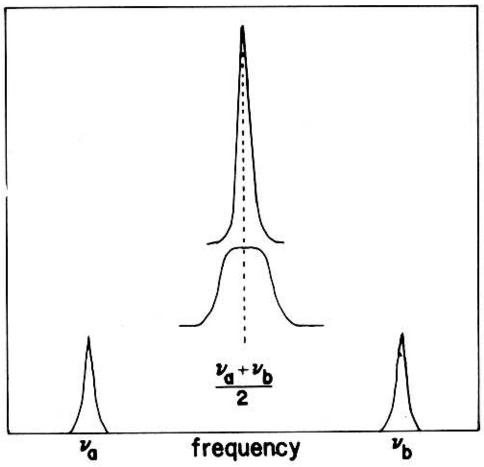

Figure 1. The idealized NMR spectra for a pair of exchanging resonances separated by a frequency $\Delta\nu = \nu_a - \nu_b$ shown schematically at the extremes of slow and fast exchange.

That is, the frequency of the resonance is precisely measurable as long as the lifetime in that state is long, but as this lifetime decreases the uncertainty in the energy or resonance frequency increases and one observes a lifetime broadening. In the simplest case that the observed nucleus exchanges between two environments with equal populations, the spectrum collapses to a single line at the average resonance position when the exchange rate, $1/\tau$, is large compared with the frequency difference between the two lines (*1, 2*). A pair of lines will just merge to become one at the point,

$$\tau_{\text{coalescense}} = (\sqrt{2}\,\pi\Delta\nu)^{-1} = k^{-1} \tag{2}$$

where here $\Delta\nu$ is the energy difference between the two lines expressed in Hz. An idealized representative situation is sketched in Figure 1. This relation is useful for making more precise statements about NMR time scales. More often than not one hears that some process is fast or slow on the NMR time scale. If it is slow, resonances are well resolved for the interchanging species, while if it is fast, resonances are averaged and only a single line is resolved.

The Chemical Shift

When considering the time scales appropriate to averaging two resonance lines separated by a chemical shift difference, eqn. (2) shows that the two lines will be resolved if the exchange rate is small compared with the chemical shift difference in Hz, $\Delta\nu$, between the two lines. Only a single resonance will be observed if the exchange rate is large compared to the shift difference. Thus, to know the time scale appropriate to the averaging process that causes coalescence of the NMR

Representative Chemical Shift Ranges, Coupling Constants, and Time Scales

Nucleus	Approximate Shift Range, ppm	Shifts at 1.4T Field or 60 MHz for ^{1}H, kHz	Time Scale Range[a] for 1.4T shifts	Shifts at 7T Field or 300 MHz for ^{1}H, kHz	Time Scale Range[a] for 7T shifts	Scalar Coupling Consts, Hz	Scalar Coupling Time Scale[b]
^{1}H	0– 10	0– 0.6	0.2 s–0.4 ms	0– 3	0.2 s–75 μs	$^2J_{HH}$ ~10	~22 ms
^{13}C	0– 200	0– 3	0.2 s–75 μs	0– 15	0.2 s–15 μs	$^1J_{CH}$ ~150	~1.5 ms
^{15}N	0– 900	0– 5.4	0.2 s–40 μs	0– 27	0.2 s– 8 μs	$^1J_{NH}$ ~50	~4.5 ms
^{19}F	0– 300	0– 17	0.2 s–13 μs	0– 85	0.2 s– 3 μs	$^2J_{HF}$ ~50	~4.5 ms
^{31}P	0– 700	0– 17	0.2 s–13 μs	0– 85	0.2 s– 3 μs	$^2J_{P-P}$ ~20	~11 ms
^{59}Co	0–15,000	0–214	0.2 s–1μs	0–1,070	0.2 s– 0.2 μs	$^1J_{Co-N}$ ~50	~4.5 ms
^{199}Hg	0– 3,000	0– 32	0.2 s–7 μs	0– 160	0.2 s– 1.4 μs	$^1J_{^{13}C-^{199}Hg}$ ~ 2,500	~90 μs

[a] Taken as the coalescence lifetime given by eqn. 2 for a maximum shift; e.g., 10 ppm for ^{1}H, and a minimum resolvable shift of 1 Hz.
[b] Taken as the coalescence lifetime obtained by substitution of J for $\Delta\nu$ in eqn. 2.

lines, one must know the chemical shift difference at least approximately. Here additional care is required about blanket statements because chemical shifts may differ greatly from one nucleus to another as shown in the table.

Inspection of column 2 of the table shows that the chemical shift ranges differ considerably from one nucleus to another. It must be appreciated that the chemical shifts actually averaged by an exchange event generally will be some fraction of the approximate total ranges indicated. Nevertheless, the conditions for the averaging of two resonances will clearly be very different when one is observing protons compared with nuclei like fluorine or cobalt, which have much larger chemical shifts. Comparison of columns 3 and 4 representing the chemical shift ranges at two common proton resonance frequencies, 60 and 300 MHz, points out an additional problem. Because the chemical shift difference between two resonances increases linearly with the strength of the applied dc magnetic field, the exchange rate between two averaging resonances must be 5 times larger at 300 MHz than at 60 MHz to achieve a coalesced spectrum. Thus, to make spectra look identical on the ppm chemical shift scale, the temperature of the compound studied would have to be somewhat higher on the 300 MHz spectrometer than on the 60 MHz spectrometer because the time scale for averaging has shifted by a factor of 5. To know which time scale of chemical lifetimes is appropriate for averaging NMR chemical shifts, it is clearly necessary to know the magnitude, in Hz, of the chemical shift being averaged.

Scalar Coupling

Essentially identical arguments apply to the averaging of scalar couplings, and it is sufficient for present purposes to replace $\Delta\nu$ by J in eqn. (2) to provide the same sort of basis for discussion of the time scales that will lead to averaging or collapse of the spectrum. If the lifetime of the nucleus in a particular environment is short compared with the reciprocal of the scalar coupling constant in this case, then the coupling will not be directly observable, and only a single, coalesced line will appear. Inspection of the table indicates that, while the scalar coupling constants do not span as wide a range of values as the chemical shifts and the scalar coupling constants are independent of field, there may be considerable variation from one nucleus to another. Since these splittings in the spectrum may range from roughly one Hz to several kHz, there is not a unique time scale appropriate to the averaging of the scalar coupling effects in the spectrum. To put meaning into a statement that a process is slow or fast on this time scale, clearly one needs to know the magnitude of the splitting constant. Of course these constants will vary with environment and distance between the coupling nuclei.

Relaxation

The averaging of even large shifts generally still limits the accessible rates of processes conveniently studied to times on the order of microseconds or longer. However, NMR relaxation spectroscopy extends this range considerably. There is a great variety of NMR relaxation rates that may be measured which all differ somewhat in the instrumental and theoretical framework required. However, the range of rates that may be studied is adequately represented by consideration of the relaxation time that is most easily understood and measured, the longitudinal NMR relaxation time, T_1, or the rate, $1/T_1$. $1/T_1$ is the first-order rate constant that characterizes the return of nuclear magnetization to an equilibrium magnitude in the direction of the applied field strength following a perturbation. The perturbation may be as simple as dropping the sample into the field in the first place and monitoring the growth of magnetization or monitoring the return to equilibrium after the magnetization has been driven away from equilibrium by a strong r.f. pulse (*3*).

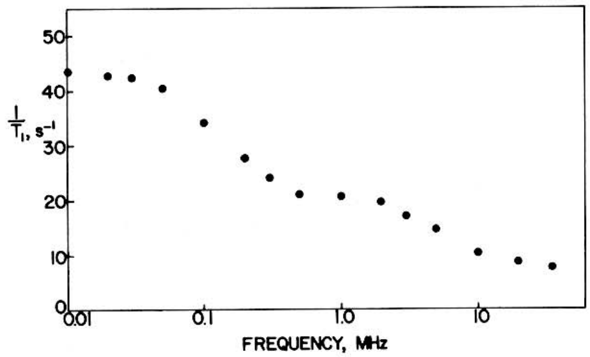

Figure 2. Proton longitudinal relaxation rate as a function of frequency for a 0.80 m*M* aqueous $MnCl_2$ solution at 286 K measured from 0.01 to 35 MHz using a field cycling technique (*4*).

The longitudinal NMR relaxation rate is driven by fluctuations in the magnetic interactions that modulate the energy levels of the nuclear spins in the sample. However, since orientation of a spin in a magnetic field requires energy, the fluctuation spectrum must have components at the resonance or Larmor frequency of the observed nucleus for the fluctuations to be effective. The relaxation equation for the isotropic intramolecular dipole-dipole interaction is

$$\frac{1}{T_1} = \frac{3}{10}\frac{\gamma^4\hbar^2}{\mathbf{r}^6}\left[\frac{\tau_c}{1+\omega^2\tau_c^2} + \frac{4\tau_c}{1+4\omega^2\tau_c^2}\right] \quad (3)$$

where γ is the magnetogyric ratio, $\hbar$ Planck's constant divided by 2π, $\mathbf{r}$ the intermoment distance, ω the resonance frequency, and τ_c the correlation time characterizing the fluctuations in $\mathbf{r}$. The resonance frequency, of course, depends on the strength of the applied dc magnetic field, H, and on the magnetogyric ratio, γ, of the nucleus observed according to the relation

$$\nu_{\text{reson}} = \gamma H/2\pi \quad (4)$$

Depending on the nucleus observed and the field strength, the practical resonance frequencies presently range from several MHz to a maximum of 600 MHz for protons. The usual experiment is to measure the relaxation rate at fixed frequency as a function of temperature. Equation (3) predicts that the rate will pass through a maximum when $\omega\tau_c$ is close to unity (0.6158 to be more precise). If the structure of the molecule is known, the internuclear distances are determined so that the only unknown in eqn. (3) at this point is the correlation time characterizing reorientation of the intermoment vector, $\mathbf{r}$. The time scale of molecular events that can be monitored by the longitudinal relaxation rates are thus on the order of the reciprocal of the resonance frequency, $(2\pi\gamma)^{-1}$ and with present day spectrometers may practically include times from about 10^{-8} to 10^{-10} s. It is to be noted that the relaxation rates actually measured are usually on the order of seconds but are determined by much faster molecular motions near or above the Larmor or resonance frequency.

What do these times correspond to physically that may be of importance to a chemist? Any motion that modulates the magnetic interaction may contribute. These times characterize such processes as very rapid chemical interconversions, like proton transfers or translational or rotational motions. The longitudinal relaxation rate provides a very direct way to measure the correlation time for rotational reorientation of a protein molecule for example (*4*).

If nuclear relaxation is driven by interaction with an electron magnetic moment, as in a paramagnetic metal ion complex, then the time scale of events that may be monitored by relaxation is pushed to very short times. The relaxation of water protons by manganese(II) ion serves as an example. The longitudinal relaxation equation in this case becomes

$$\frac{1}{T_1} = \frac{2}{15}\frac{\gamma_I^2\gamma_e^2\hbar^2 S(S+1)}{\mathbf{r}^6}\left[\frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{7\tau_c}{1+\omega_s^2\tau_c^2}\right] + \frac{2}{3}S(S+1)\left(\frac{A}{\hbar}\right)^2\left[\frac{\tau_e}{1+\omega_s^2\tau_e^2}\right] \quad (5)$$

where the correlation times τ_c and τ_e may have several contributions (5–7). For the aqueous solution of the aquo complex, the correlation time for the first term in brackets is the rotational correlation time of the metal complex, while that for the second term is the electron relaxation time, T_{1e}. A is the nuclear electric hyperfine coupling constant which is analogous to the scalar or J coupling between nuclei, except that in the present case an unpaired electron spin is involved. ω_I and ω_s are the Larmor precession frequencies for the proton and the electron, respectively. The frequency dependence of the relaxation rate for the water protons permits resolution of the different contributions as shown Figure 2. The inflection at low frequency is caused by the last term in eqn. (5) and therefore reports the electron relaxation time, T_{1e}. The higher frequency inflection results from the ω_s part of the first term in eqn. (5) and reports the rotational correlation time of the metal complex. If we take the inflection point as 5.5 MHz and write the electron Larmor frequency ω_s as $(\gamma_e/\gamma_H)\omega_I = 658\ \omega_I$, then the rotational correlation time of the metal complex is the reciprocal of this inflection frequency, $\tau_{rot} \simeq 1/(658 \times 2\pi\nu_I) = 44$ ps. Though this is a limited example, it demonstrates clearly that the study of nuclear relaxation extends the time scale accessible to investigation by nuclear magnetic resonance measurements well into the picosecond range.

Summary

In summary the "NMR time scale" spans the range from days to picoseconds. I suggest that much of the ambiguity and confusion about the NMR time scale may be eliminated if the interaction that is modulated by a dynamic process is always included in a statement about the spectrum or relaxation involved. Thus, instead of suggesting that some process is fast on the "NMR time scale," which is ambiguous, indicating that the process is apparently fast or slow compared with, for example, the chemical shift difference between the two resonances which is expected to be on the order of xxx Hz (not ppm) at the fields used, will minimize ambiguity.

Acknowledgment

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Literature Cited

(1) See for example Bovey, F. A., "Nuclear Magnetic Resonance Spectroscopy," Academic Press, New York, **1969**, Ch. VII.
(2) Kaplan, J. I., and Fraenkel, G., "NMR of Chemically Exchanging Systems," Academic Press, New York, **1980**.
(3) Farrar, T. C., and Becker, E. D., "Pulse and Fourier Transform NMR," Academic Press, New York, **1971**.
(4) Hallenga, K., and Koenig, S. H., *Biochemistry*, **15**, 4255 (1976).
(5) Swift, T. J., *in* "NMR of Paramagnetic Molecules," (*Editors:* LaMar, G. N., Honocks, Jr., W. DeW., Holm, R. H.), Academic Press, New York, **1973**, Ch. 2.
(6) Dwek, R. A., "Nuclear Magnetic Resonance in Biochemistry: Applications to Enzyme Systems," The Clarendon Press, Oxford, **1973**.