A Novel Method for the Determination of Ionization Sites in Polyfunctional Acids and Bases by NMR Relaxation Rate Measurements†

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A method which enables one to determine the site of ionization in polyfunctional acids and bases is presented. The method is based on the changes in NMR longitudinal relaxation time (T_1) of all the nuclei potentially involved, because addition or removal of a proton from a given nuclear site will affect its T_1 in a predictable way. This approach is shown to be effective even under conditions of fast exchange of the acidic proton on a range of monofunctional models (Me_3N and $MeNH_2$, studied by ^{14}N and ^{15}N NMR spectroscopy) and some polyfunctional bases ($MeCONMe_2$, $MeCSNMe_2$, 4-aminopyridine, by ^{14}N and ^{17}O NMR spectroscopy).

We report on a new general method to determine the site of ionization in polyfunctional acids and bases in a given medium, which relies on the changes in the NMR spin–lattice relaxation rate (T_1) and NOE of the nuclei in question, and is not limited to slow proton-exchange spectra.

This problem has been investigated by studying UV or NMR spectral changes (which are suited only for quantitative study), substituent effects (generally based on assumptions),[1] NMR spectra in superacids[2] or theoretical calculations (both referring to unusual conditions).

Examples of compounds studied are: (*a*) strained amides, like quinuclidones, in which *N*-protonation is believed to occur,[1] contrary to the behaviour of normal amides.[1,3] (*b*) Sulfonamides,[4] sulfinamides,[5] sulfenamides,[6] *N*-nitrosoamines,[7] phosphoramides,[8] for which data are too scarce to decide whether they protonate on the acyl group or on the nitrogen atom. (*c*) Hydrazines and various nitrogen heterocycles, possessing multiple sites with small basicity differences.[9] (*d*) Hydroxamic acids, which are *N*-acids in DMSO and the gas phase, but possibly *O*-acids in water or alcohols.[10]

The method we propose consists of measuring the changes in the T_1 of all the possible nuclear sites, because addition or removal of a proton will substantially and predictably influence it, as detailed below.

The dipole–dipole contribution to $1/T_1$ for a non-quadrupolar ($I = 1/2$) nucleus X (*e.g.* ^{13}C, ^{15}N, ^{31}P, ^{77}Se) deriving from n protons at a distance r_{XH} is given by eqn. (1),[11] where γ_X and γ_H are the respective magnetogyric ratios and τ_c the molecular correlation time. This can be determined from the NOE factor η as in eqn. (1), where $\eta_{max} = \gamma_H/2\gamma_X$. If the

$$1/T_1^{DD} = n\hbar^2\gamma_X^2\gamma_H^2\tau_c/r_{XH}^6 = (1/T_1)\eta/\eta_{max} \quad (1)$$

DD contribution is dominant, owing to the sixth-power dependence on the X–H distance the nucleus will be very sensitive to the presence of a directly bonded proton. Therefore, we expect protonation to lead to a shorter T_1, and *vice versa* for deprotonation.

In the case of quadrupolar ($I > 1/2$) nuclei (*e.g.* ^{14}N, ^{17}O, ^{33}S) the relaxation is generally dominated by the quadrupole–electric field gradient mechanism, for which eqn. (2) applies,[11]

$$1/T_1 = R_2^{QF} = R_1^{QF} = (3/40)(2I + 3)/[I^2(2I - 1)]\chi^2(1 + \varepsilon^2/3)\tau_c \quad (2)$$

where $\chi = e^2Qq/\hbar$ is the nuclear quadrupolar coupling constant (product of the nuclear quadrupole moment Q and the electric field gradient q), and ε the asymmetry parameter. Thus T_1 is sensitive to the symmetry of the electronic environment. For example, protonation at nitrogen is known to decrease χ, thus leading to a decrease of R_1 and line width ($R_2^{QF} = \pi W_{1/2}$).[12]

For one species to dominate over the other, measurements may have to be carried out in media with different viscosity, which also affects relaxation because it alters τ_c;[11,12] therefore viscosity changes must be compensated for. For weaker bases, the protonated form was generated in CF_3SO_3H (2.965 cP)[13] or 37% HCl (2 cP). Correspondingly, the neutral form was generated in various H_2O/Bu^{t}OH mixtures, the viscosity of which is available.[14]

The results presented herein concern monofunctional model compounds, plus some selected polyfunctional ones. T_1 measurements were carried out as follows: ^{15}N with the saturation–recovery sequence (modified with inverse-gated decoupling or INEPT[15] for ^{15}N) using degassed solutions; ^{14}N with an inversion–recovery sequence incorporating acoustic ringing suppression;[16,17] ^{17}O T_1's were estimated from line widths; acoustic ringing suppression[16] was employed. NOE experiments were carried out in non-selective mode.[18] The results are collected in Table 1.

Trimethylamine.—^{15}N-labelled Me_3N undergoes the usual nitrogen deshielding upon protonation,[12] and the T_1 value is *shortened* by a factor of 1.5, which agrees with our predictions. The NOE factors (−4.4 at pH 12 and −4.9 at pH 1) are close to the theoretical maximum (−4.93), which shows that the DD mechanism dominates under all conditions. It is important to note that the change is easily observable despite the fact that under the protonating conditions chosen (pH 1) the NH^+ proton undergoes fast exchange with the solvent. The same experiments with ^{14}N NMR spectroscopy give results again consistent with expectations: T_1 is *lengthened* by a factor of 25, with a corresponding line narrowing. This nucleus is advantageous over ^{15}N because of its higher receptivity.

Methylamine.—The protonation of $MeNH_2$ has been monitored by ^{14}N relaxation at various pH values in order to see whether the observed changes are indeed due to protonation and follow the same pattern. Thus, T_1 values have been determined at pH values between 1 and 14; the results yield a sigmoid curve which parallels the one constructed from

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Table 1 Chemical shifts,[a] relaxation times[b] and line widths[c] of monofunctional models and selected polyfunctional bases

Base/Solvent	Nucleus	δ	T_1	$W_{1/2}$
Me_3N	^{15}N			
pH 12		−363.7	99.5	—
pH 1		−352.2	64.1	—
Me_3N	^{14}N			
pH 12		—	0.45	847
pH 1		—	11	29
$MeCONMe_2$	^{14}N			
14% Bu^{t}OH		−270.7	0.42	747
37% HCl		−244.0	0.52	550
$MeCONMe_2$	^{17}O			
14% Bu^{t}OH		288.4	1.3	238
37% HCl		178.4	0.4	808
$MeCSNMe_2$	^{14}N			
31% Bu^{t}OH		−225.7	0.88	556
54% H_2SO_4		−166.9	1.13	434
4-Aminopyridine[d]	^{14}N			
pH 12		−118.6	0.46	713
		−316.2	0.61	562
pH 3		−219.3	6.00	113
		−291.2	0.82	269

[a] In ppm from external CH_3NO_2 (^{14}N, ^{15}N); H_2O (^{17}O). [b] T_1 in s for ^{15}N; in ms for ^{14}N. For ^{17}O, the T_1 values are $T_2^* = 1/\pi W_{1/2}$ in ms. [d] The signal at lower field is the ring nitrogen.

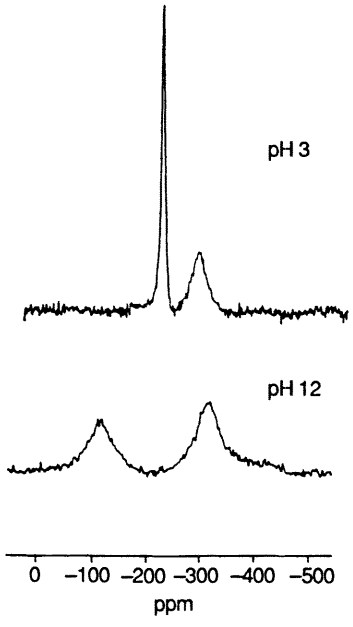

Fig. 1 ^{14}N NMR spectra of 4-aminopyridine at pH 12 and 3. The downfield peak is the ring nitrogen.

chemical shifts. Proton exchange was slow only at pH 1; thus, in general it is not necessary to have slow proton exchange to observe T_1 changes.

N,N-*Dimethyl-acetamide and -thioacetamide.*—In order to avoid interference from the sulfonyl signal in the ^{17}O spectra, we used 37% HCl, whose strength[19] is barely sufficient.[8] Upon protonation, the ^{14}N line undergoes a 20% narrowing (much smaller than the dramatic changes observed before), while the ^{17}O signal is broadened by a factor of three. For the analogous thioamide[20] there is again no substantial change in the ^{14}N spectrum.[21] Both results indicate that protonation does *not* take place on nitrogen, as known. These experiments also set a lower limit for the change to be expected in ^{14}N line widths when nitrogen is not protonated, which may occur because of smaller field gradient changes, due *e.g.* to hydrogen bonding.

4-*Aminopyridine.*—The ^{14}N T_1 of pyridine increases by a factor of 60 upon protonation, again in line with the above considerations. 4-Aminopyridine has a pK of 9.11, and is therefore thought to be protonated at the ring nitrogen.[1] The ^{14}N signals are well separated and assignment is easy;[12] the results clearly show ring protonation because of the marked line narrowing of that peak. The situation is effectively depicted by the spectra of Fig. 1.

Further work on other bases (phosphines, hydrazines, nitrosoamides, nitrogen heterocycles *etc.*) and hydroxamic acids is in progress.[22]

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