High Resolution 15N Nuclear Magnetic Resonance in Natural Abundance by Fourier Pulsed Techniques

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Summary High resolution 15N n.m.r. spectra at 21·14 kG have been observed directly by means of large (10 mm) spinning tubes, proton noise decoupling, and Fourier transform pulsed techniques both for samples enriched and with a natural abundance of 15N.

EACH naturally occuring nitrogen isotope, 14N ($I = 1$) and 15N ($I = \frac{1}{2}$), has a very low sensitivity: 1.01×10^{-3} and 1.04×10^{-3} respectively compared with 1.00 for the same number of protons. The high resolution limitation of 14N, which has a high natural abundance (99·63%), is that the n.m.r. lines are commonly broad (>50 Hz) because of the short relaxation times which arise from electric quadrupole effects. Although the narrow lines (<1 Hz) given by the 15N isotope make it more suitable for high resolution work, the low natural abundance (0·36%) gives a formidable sensitivity problem in natural abundance studies.[1] The detection difficulty was overcome by Ray[2] using continuous-wave, rapid-passage techniques but only with considerable loss of accuracy, as shown by double resonance studies on enriched samples.[1]

10 mm spinning tubes and equipped with a Digital PDP-8/I computer and a Fabritek 1074 multichannel analyser.
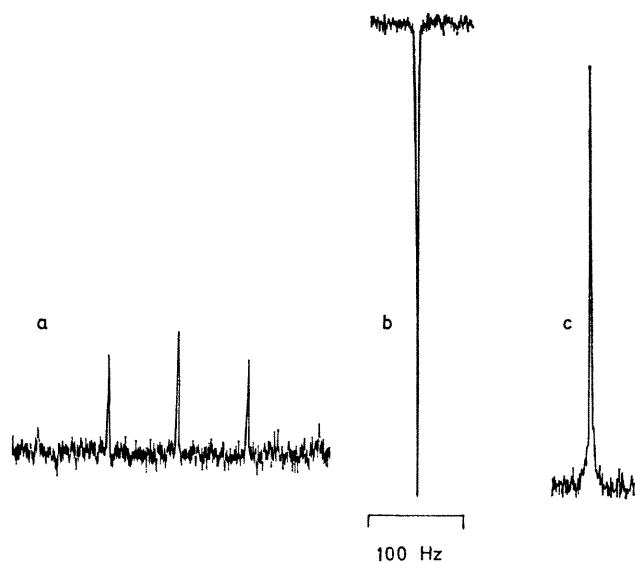

FIGURE 1. 15N Spectra of 5M-NH4Cl in 2N-HCl, 95% enriched in 15N. (a) Single passage continuous wave spectrum, undecoupled. (b) Single passage continuous wave spectrum, wide band noise decoupled, showing negative NOE effect. (c) Single pulse Fourier transform spectrum, wide band noise decoupled.
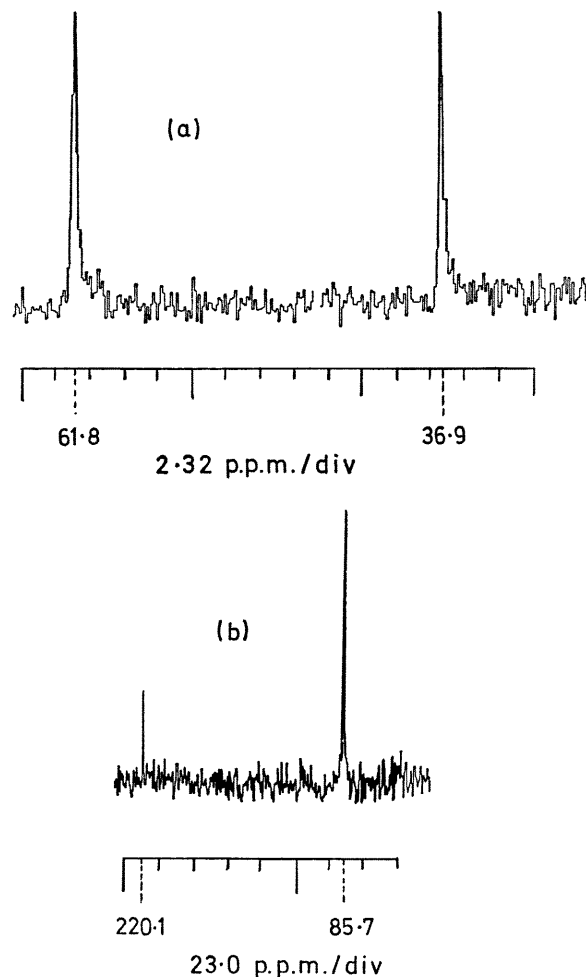
FIGURE 2. 15N Spectra in natural abundance. (a) Phenyl-hydrazine with 10% internal hexafluorobenzene lock. (b) Equimolar mixture of acetonitrile and N-methylformamide with 10% internal hexafluorobenzene lock.

True high resolution work employing accumulation techniques with continuous wave operation has been accomplished by Roberts and his co-workers.[3]

We report the first application of pulsed Fourier techniques and the use of wideband noise decoupling as means of improving the signal-to-noise (S/N) ratio in 15N spectra. The results were obtained on a Bruker HFX-13 spectrometer operating at 21·14 kG with an 19F lock system,

Comparisons of proton noise-decoupled spectra both in continuous-wave and Fourier mode with a single resonance, continuous-wave spectrum are shown in Figure 1 for a 5M solution of [15N]ammonium chloride in 2N-HCl. An enriched sample was used for convenience. Proton noise decoupling [Figure 1(b)] gives an enhanced, inverted signal. The enhancement shown is ca. −2·4 and arises from the nuclear Overhauser effect (NOE). The inversion is a consequence of the difference in sign of the magnetogyric ratios of 1H and 15N. The calculated NOE value for an AX system where X = 15N and A = 1H is −3·94 if the relaxation mechanism is mainly by a one bond dipolar mechanism.[4]

Natural abundance ^{15}N results

Compound*	Lock configuration**	Chemical shift† observed	Chemical shift† lit. value	Total time (10^3 s)	S/N ratio‡
NH_4NO_3[a]	.E	0 ± 0.2	0	3·35	6
$HCONHMe$	I	87.2 ± 0.4	88.2 ± 0.2[7]	6·71	45
$HCONHMe$[b] }	I {	85.7 ± 0.9		3·86	16
CH_3CN }		220.1 ± 0.9		3·86	5
CH_3CN	I	221.2 ± 0.4	221 ± 2[8]	6·71	3
$PhNHNH_2$[c]	I	61.8 ± 0.3		1·68	15
$PhNHNH_2$[c]	I	36.9 ± 0.3		1·68	15
$N_2H_4H_2O$	E	26.7 ± 0.3		1·34	15
NH_4CNS[d]	E	6.7 ± 0.4	9.3 ± 3[9]	12·3	7
NH_4CNS[d]	E	189.1 ± 0.4	187.5 ± 1.5[10]	12·3	3
$HCONH_2$	E	91.3 ± 0.9	92.4 ± 0.2[6]	1·34	14
CH_3NO_2	I	357.4 ± 0.4	358.5 ± 2.5[10]	2·68	17

* Pure liquid unless otherwise stated.
** E denotes external C_6F_6 in 5 mm concentric tube; I denotes 10% by volume internal C_6F_6.
† In p.p.m. downfield.
‡ Mean peak height divided by root mean square noise; 10 mm tube.
[a] $15M$-NH_4NO_3 in $2N$-HCl in this study. Different standards were used in the referenced determinations; consequent errors unknown.
[b] 1 : 1 molar solution.
[c] Preliminary assignment;
[d] *Ca.* $5M$ in $2N$-HCl.

We confirm that the observed NOE is markedly changed by pH owing to proton exchange effects.[3]

Figure 1(c) shows the magnitude spectrum (with arbitrary phase) in 2K points resulting from Fourier transformation of the free induction decay, following a single r.f. pulse of $40 \mu s$ duration, acquired for 1·6 s in 4096 points.

The S/N Fourier gain depends upon the effective spectral range covered. For a 1kHz (*ca.* 100 p.p.m.) range we estimate a gain over continuous-wave work in the same total time of *ca.* 25.

We detected no difference in ^{15}N shifts for the sample in the three cases to within the experimental error of 0·1 p.p.m. The ^{15}N frequency was 9, 120, 343·5 $\pm$ 1·0 Hz and is the sum of the carrier frequency (to ±0.1 Hz) and shift (to ±1.0 Hz), at a field given by the ^{19}F resonance of C_6F_6 at 84, 666, 691·4 $\pm$ 0·1 Hz.

The pulse length for the power conditions in our spectrometer was optimised at $40 \mu s$ for the enriched sample and for 1000 or more decays. The same conditions were then used to obtain ^{15}N spectra in natural abundance for a variety of samples. Optimisation for each case is desirable since each should have different relaxation characteristics but is scarcely convenient because of the long accumulation times.

Figure 2 shows two typical spectra. The phenylhydrazine spectrum [Figure 2(a)] was obtained in 0·5 h. It is notable that the NH and NH_2 groups have the same NOE effect. This is the observed effect for the CH, CH_2, and CH_3 groups in ^{13}C n.m.r., which can be theoretically accounted for provided the direct dipole–dipole mechanism dominates.[4] Figure 2(b) is the spectrum of an equimolar mixture of methyl cyanide and N-methylformamide (with 10% by volume of C_6F_6 as internal lock). The amide signal is 2·9 times larger than the cyanide signal presumably because of a negligible NOE effect for nitrogen atoms not directly attached to protons.

Other results are shown in the Table. The longest experiment was of 3·5 h duration. Each experiment was repeated, sometimes with a different acquisition time for each decay (*i.e.* different effective spectral width). The shift errors refer to the more accurate of the measurements, *i.e.* are for the longer acquisition times. Susceptibility corrections for the various lock configurations used (internal C_6F_6; external C_6F_6 in a concentric 5 mm tube) are less than 0·3 p.p.m. and have not been allowed for.

Excellent agreement with previously determined shifts either for ^{15}N or for ^{14}N (the isotope effects is negligible[5]) has been obtained. This is exemplified by [^{15}N]formamide and N-methyl[^{15}N]formamide for which high precision results (> 1 p.p.m.) were obtained in this laboratory by double-resonance techniques.[6] Small discrepancies exist and are being evaluated in the light of concentration and other differences between sub-standard solutions.

Of particular interest, therefore, are the results for the ammonium ion: a downfield shift of 6·7 $\pm$ 0·6 p.p.m. is observed for a $5M$ solution of the thiocyanate relative to $15M$-nitrate. This is consistent with a 9·3 $\pm$ 3 p.p.m. ^{14}N measurement for saturated aqueous solutions previously reported, but largely ignored.

The facility of the measurements ensures a moderate applicability to problems in natural abundance and a widespread future for labelling work including solvent, concentration and gegenion effects.

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