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Proton CRAMPS of NH_3^+ Groups: Line Broadening and Molecular Dynamics

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Increased ^1H combined rotation and multiple pulse spectroscopy (CRAMPS) linewidths for protons within NH_3^+ groups in the solid state have been investigated by variable-temperature experiments. Linewidth variation with temperature can be correlated with specific molecular dynamic processes. With L-[^{15}N]-alanine, both the NH_3^+ and CH_3 group reorientations give rise to specific broadening of the respective CRAMPS signals as the temperature is lowered. Such dynamic processes may be more important than ^{14}N-^1H quadrupolar-modified dipolar broadening in determining NH_3^+ proton lineshapes.

Advances in NMR, applying CRAMPS to the study of hydrogen bonding in the solid state, have allowed the correlation of observed ^1H chemical shifts with hydrogen-bond distances (both O–O and O–H) determined by X-ray and neutron crystallography. In particular, good correlations were observed[1] in two cases: a large series of carboxylic acid derivatives and a smaller series of phosphoric acids. In each case, relatively sharp signals could be detected in the ^1H CRAMP spectra, with multiple peaks (though often overlapping to some extent) where hydrogen bonds of differing distances and geometries were present.

Establishing good correlation in the case where O···H···N hydrogen bonds are present would have obvious implications in the biological, biochemical and chemical fields. Earlier ^1H CRAMPS work on model chemical compounds by Scheler *et al.*[2] found a broad line for the only O···H···N hydrogen-bonded protons reported, those in L-alanine. It has also been found[3] that the signals from the protons in both the NH_2 and the NH_3^+ groups present in modification I of 2-aminobenzoic acid (2ABA.I) were broadened, not allowing the observation of the expected five spectral lines from the five separate hydrogen bonds. In both cases, separate sharp signals are observed in the spectra due to the protons in other parts of the molecule. These initial studies indicated that broadened signals would make shift–distance correlations impossible in the O···H···N case. Scheler *et al.*[2] attributed such line broadening to the influence of the quadrupolar ^{14}N nucleus. Line splitting and broadening are often found in ^{13}C solid-state spectra where carbon is directly bonded to ^{14}N, and are due to incompletely averaged quadrupolar-modified dipolar coupling. These effects were further investigated with ^1H–^{14}N systems by Naito *et al.*,[4] with good agreement found between experimental lineshapes obtained from solid amino acids and theoretical modelling. On the other hand, the work on 2ABA.I suggested that dynamic effects may, to some extent, be additionally responsible for line broadening.

As part of a more general solid-state NMR study on hydrogen bonding,[5] CRAMPS experiments have been performed on a series of compounds containing NH_3^+ groups. The work reported herein summarises the results of these experiments, and details further investigations into line-broadening and dynamics.

Experimental

Materials

All samples were obtained from Aldrich and were used without further recrystallisation, with the following exceptions; 2-, 3- and 4-aminobenzoic acid hydrochlorides were prepared by dissolving the appropriate aminobenzoic acid in warm water and mixing (1 : 1) with warm, concentrated hydrochloric acid solutions. The samples were then precipitated by slow cooling, followed by drying and further recrystallisation from water. Different modifications of 2ABA were prepared as described in an earlier publication.[3] $(4ABA)_3 \cdot H_3PO_4$ was prepared by reaction of warm aqueous 4ABA with an excess of concentrated phosphoric acid, with subsequent cooling, precipitation and recrystallisation from water. Structures were confirmed by high-resolution, solid-state ^{13}C CP MAS NMR. All samples were finely powdered before the NMR experiments were performed.

NMR Measurements

Proton CRAMP spectra were recorded at 200.13 MHz using a Bruker CXP-200 NMR spectrometer equipped with standard solid-state hardware. Spectra were recorded using two different CRAMPS probeheads: (a) a home-built, room-temperature system, as described elsewhere,[6] capable of spinning samples at rates up to 6 kHz using 'broomstick' rotors with spherically shaped sample compartments and (b) a commercial CRAMPS probe, manufactured by Doty Scientific, capable of spinning samples at 5 kHz using 5 mm cylindrical rotors, designed for variable-temperature operation. Temperature measurement was performed using an integral thermocouple with estimated errors <1 K. Improved CRAMPS resolution could be obtained with the commercial probe by sandwiching samples at the centre of the rotor using powdered KBr (80% KBr, 20% sample), thus maximising the rf field homogeneity across the sample. Adequate signal-to-noise ratios were easily obtained with 4–32 transients co-added, despite the reduced sample size.

The spectrometer was adjusted to give maximum resolution using a standard CRAMPS tune-up procedure.[6] This involves fine tuning of the quadrature rf pulse amplitudes and phases, transmitter tuning to minimise phase tran-

sient distortions, and setting of the magic angle using a powdered sample of potassium hydrogen sulfate. The MREV-8 dipolar decoupling sequence was used, with a 90°-pulse duration of 2 μs and MREV-8 cycle times between 48–60 μs. Spinning rates between 3–4 kHz were used, with no synchronisation between the pulse cycle time and rotor period. Signals were sampled twice every multiple-pulse cycle, without quadrature detection. Spectral scaling factors were determined by co-adding signals from adamantane obtained with different resonance offsets. Spectra are referenced to the signal from a small amount of adamantane ($\delta_H = 1.74$) added after recording the spectrum of the pure sample. Phase alternation of the preparation pulse was employed to minimise baseline offset artefacts which cause distortions in the data after Fourier transformation. No line broadening or resolution enhancement has been used to produce the results reported here.

Variable-temperature ^1H relaxation data were obtained using a home-built wide-line spectrometer operating at 40 MHz. A saturation-recovery sequence was used to measure T_1, with 100 equally spaced points acquired to define the magnetisation recovery curve. $T_{1\rho}$ data were similarly recorded using two different rf effective spin-locking field strengths: 100 and 130 kHz. For both T_1 and $T_{1\rho}$ measurements, the 90°-pulse duration was adjusted to 1 μs. Samples were held in 10 mm diameter PTFE static sample tubes, placed inside the variable-temperature chamber of a home-built probe. The temperature was measured using a commercial thermocouple accurate to within 1 K.

CRAMPS Results

The results of our ^1H CRAMPS investigations are summarised in Table 1, together with abbreviated codes used to represent individual materials. Considering Table 1, the most striking feature is the variation in linewidth found for protons in NH_3^+ groups. In general, signals from NH_3^+ groups, centred around 9.5 ppm, are significantly broader than other signals in the same spectrum, with linewidths varying between 350 Hz for glycine to 3200 for L-valine. In the cases reported here, there is insufficient resolution to allow measurement of different chemical shifts arising from different hydrogen-bond geometries. For example, in 2ABA.I there are five separate hydrogen bonds with N···H···O distances ranging from 0.2543 to 0.2873 nm.[7] Similar changes in hydrogen-bond distances in O···H···O systems leads to changes in chemical shift of over 10 ppm.[1]

One other potential source of line broadening in these cases is unresolved ^{14}N–^1H J-coupling. It is possible, in favourable cases, to obtain extremely narrow spectra from NH_4^+ groups, which can reorient rapidly at room temperature. In such cases, residual line broadening is sufficiently small to allow the observation in the solid state of a 1 : 1 : 1 triplet, obtained because ^{14}N is a spin-1 nucleus, centred around 7.5 ppm. This has been confirmed by isotopic substitution of spin-$\frac{1}{2}$ ^{15}N, resulting in a 1 : 1 doublet, as shown in the case of ammonium nitrate, NH_4NO_3, in Fig. 1. The magnitude of expected ^{14}N–^1H coupling constants (<100 Hz) cannot fully account for the increased linewidths observed from NH_3^+ groups.

In agreement with the above data obtained at 200 MHz, Scheler *et al.*[2] have also reported a broad line for the NH_3^+ protons in L-alanine, observed under CRAMPS conditions on a home-built spectrometer system operating at 270 MHz. The linewidth observed was attributed to the quadrupolar modification of the ^{14}N–^1H dipolar interaction, making spinning at the magic angle less effective, resulting in line splitting and broadening. This effect is common[8] in high-resolution spectra with ^{13}C (or other spin-$\frac{1}{2}$ nuclei) bonded to quadrupolar nuclei such as ^{14}N or ^{35}Cl. From ^{13}C results, splittings in spectra are greater for carbons bonded to NH_2 than to NH_3^+ groups, owing to the lower symmetry around nitrogen in the former case. The calculations of Naito *et al.*[4] also show this to be the case for ^1H–^{14}N systems, with the NH_3^+-group in glycine significantly narrower and more symmetric than those in peptide and imino functionalities, with the lineshapes calculated for the glycine amino protons assuming a fast rotation about the C_3 axis. The results

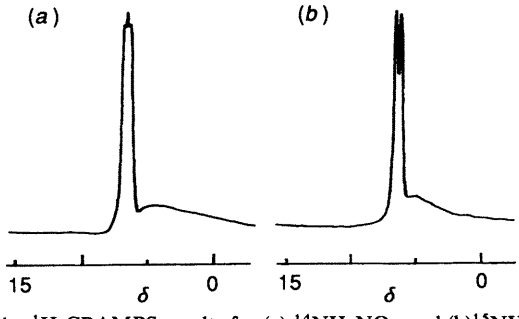

Fig. 1 ^1H CRAMPS results for (*a*) $^{14}NH_4NO_3$ and (b) $^{15}NH_4NO_3$, showing N—H J-coupling. Broad baseline distortions are due to an instrumental artefact during acquisition.

Table 1 CRAMPS results from samples containing NH_3^+ groups

sample	abbreviation	$\delta_H/(NH_3^+)^a$	linewidth/Hz[b]
2-aminobenzoic acid (modification I)[c]	2ABA·I	9.0	2000
2-aminobenzoic acid hydrochloride	2ABA·HCl	9.0	2000
3-aminobenzoic acid (modification II)[c]	3ABA·II	9.5	1200
3-aminobenzoic acid hydrochloride	3ABA·HCl	10.0	1000
4-aminobenzoic acid hydrochloride	4ABA·HCl	9.5	700
4-aminobenzoic acid phosphoric acid salt	4ABA·H_3PO_4	9.0	1000
3-amino-4-methyl-benzoic acid	3A4MBA	9.0	1500
glycine	GLY	9.4	350
D-(−)-α-phenylglycine	DPGLY	8.6	1600
L-alanine	LALA	8.6	2200
L-[^{15}N]-alanine	[^{15}N]-LALA	8.6	2400
L-valine	LVAL	8.1	3200
L-cystine hydrochloride	LCYS·HCl	7.8	850
DL-lysine hydrochloride	DLLYS·HCl	8.5	550
L-aspartic acid	LASP	8.5	1600
L-glutamic acid	LGLU	7.8	1100

[a] Measured relative to TMS using the internal standard adamantane ($\delta_H = 1.74$ ppm). [b] Full width at half height. Where overlapping peaks are present, linewidths are estimated. [c] See ref. 3.

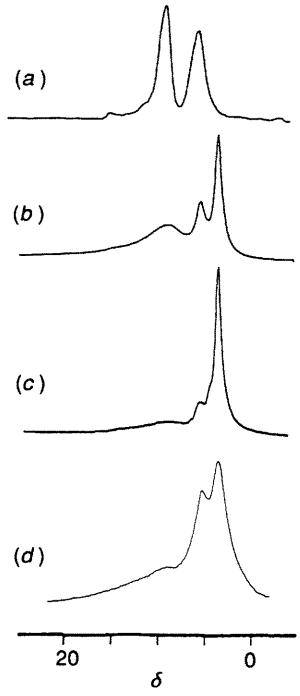

Fig. 2 ^{1}H CRAMPS results for (*a*) glycine, (*b*) L-alanine, (*c*) L-valine and (*d*) L-[^{15}N]-alanine. Note the wide variation in the linewidth of the NH_3^+-proton signals, centred at *ca.* 9 ppm.

obtained in this work, however, show that NH_3^+ CRAMPS signals are invariably broader than expected, with considerable variations. Also, signals from NH_4^+ protons are significantly narrowed with respect to other signals. Given that there is potential mobility in the case of both NH_3^+ and NH_4^+ moieties, it is likely that dynamic processes are largely responsible for the variations in linewidths observed.

More evidence for the importance of dynamic effects was obtained by consideration of the ^{1}H CRAMP spectra of L-alanine and its ^{15}N-labelled analogue (LALA and [^{15}N]-LALA). It is clear from Fig. 2 that substitution of the ^{15}N spin-$\frac{1}{2}$ isotope does not reduce the linewidth; rather a marginal increase is found, from 2200 to 2400 Hz. The spinning rates used (>3 kHz) would be sufficient to remove additional effects from ^{15}N–^{1}H dipolar coupling. The other spectra shown in Fig. 2 obtained from the other amino acids further illustrate the range of linewidths observed. In order to characterise more fully the nature of the observed CRAMPS line broadening, a more detailed study has been made of the isotopically enriched [^{15}N]-LALA.

Variable-temperature CRAMPS Study of L-[^{15}N]-Alanine

^{1}H CRAMPS results were recorded between 210 and 350 K at 10 K intervals, allowing at least 10 min equilibration in the probe before the experiment. Dry nitrogen was used as the spinning drive and bearing gas, with spinning at 2 kHz at all temperatures. Low temperatures were achieved by passing the nitrogen gas through a copper heat exchanger submerged in a dry ice–methanol mixture. The probe was tuned and matched carefully at each temperature to maintain the efficiency of the MREV-8 multiple-pulse sequence. Fig. 3 shows a selection of the spectra obtained, and all the results are summarised in Table 2. The signal from the NH_3^+ group a linewidth (FWHH) variation from 560 Hz at 350 K to 3640 Hz at 250 K. It is difficult to measure the linewidth at temperatures below 250 K. Furthermore, the linewidth of the

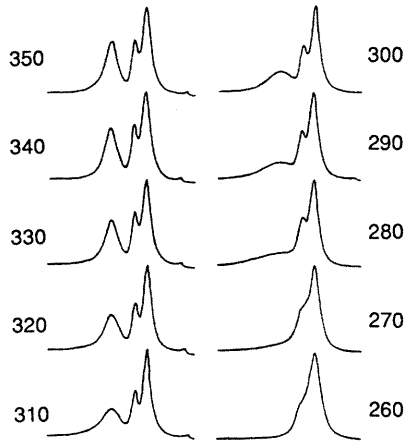

Fig. 3 Variable-temperature CRAMP spectra of L-[^{15}N]-alanine. The operating temperatures (K) are indicated alongside each spectrum.

methyl signal also begins to increase as the temperature is decreased below room temperature. Above 310 K, the methyl band has a constant linewidth of *ca.* 400 Hz which increases to 810 Hz at 210 K. These observations can be accounted for by more detailed consideration of the molecular dynamic processes present in the solid.

Variable-temperature Relaxation Study of L-[^{15}N]-Alanine

Relaxation measurements (T_1) have previously been performed on a series of biomolecules, including L-alanine, by Andrew *et al.*[9] Two distinct molecular motions were found, corresponding to reorientation of the CH_3 and NH_3^+ groups about their respective three-fold axes. The NH_3^+ orientation was found to have an activation energy of 38.6 kJ mol^{-1}. The methyl group reorientation was found to have a significantly lower activation energy, 22.4 kJ mol^{-1}, reflecting the influence of hydrogen bonding on the reorientation of the NH_3^+ group. The two motions were unambiguously distinguished by deuterium exchange of the amino protons. The deuteromethyl-group dynamics have also been studied in L-[$^2H_3$]-alanine by wide-line deuterium NMR.[10] The activation energy was found to be 27 kJ mol^{-1} above a suspected

Table 2 CRAMPS linewidths measured for L-[^{15}N]-alanine at temperatures from 210–350 K

	linewidth/Hz[a]	
T/K	NH_3^+	CH_3
210	>4000	810
220	>4000	780
230	>4000	720
240	>4000	680
250	3640	660
260	3560	610
270	2860	540
280	2320	460
290	1840	440
300	1520	440
310	1080	440
320	740	400
330	660	400
340	580	390
350	560	390

[a] Full width at half height. Where overlapping peaks are present, linewidths are estimated.

phase transition at *ca.* 170 K, and 17 kJ mol^{-1} below this temperature.

T_1 and $T_{1\rho}$ data were obtained on a static sample of the [^{15}N]-LALA used for the CRAMPS study at temperatures between 369 and 139 K, using liquid-nitrogen evaporation at low temperatures, heating the gas flow to maintain temperature control. The results of the relaxation study are shown in Fig. 4. The curve for T_1 is broadly in agreement with that obtained by Andrew *et al.*,[9] though our study did not extend to the same high-temperature range. It is also interesting to note that there is evidence for a phase transition at 178 K in the T_1 and $T_{1\rho}$ results, in agreement with the earlier wide-line deuterium NMR study,[10] given the sudden changes in slope of the relaxation curves.

The solid curves shown in Fig. 4 were computed using the standard equations:[11]

$$T_1^{-1} = \sum_i C_i/3[\tau_i(1 + \omega^2\tau_i^2)^{-1} + 4\tau_i(1 + 4\omega^2\tau_i^2)^{-1}] \quad (1)$$

and

$$T_{1\rho}^{-1} = \sum_i C_i/2[3\tau_i(1 + 4\omega_1^2\tau_i^2)^{-1} + 5\tau_i(1 + \omega^2\tau_i^2)^{-1} + 2\tau_i(1 + 4\omega^2\tau_i^2)^{-1}] \quad (2)$$

with $i = CH_3$ and NH_3^+. In both the above equations, τ_i is the correlation time for three-fold reorientation, ω is the angular resonant frequency in the $\boldsymbol{B}_\circ$ field and C_i is a relaxation constant. In eqn. (2), ω_1 is the angular frequency corresponding to the intensity of the spin-locking rf field (*i.e.* $\omega_1 = \gamma\boldsymbol{B}_1$). Furthermore, the correlation times are assumed to obey the simple activation energy equation:

$$\tau_i = \tau_{oi} \exp(E_i/RT) \quad (3)$$

where E_i is the energy for the three-fold reorientation. Appropriate parameters were used for operation at 40 MHz using spin-locking fields of 100 and 130 kHz. A computer program was written to optimise the values of R_i, C_i and τ_{oi}, fitting the results to the data points obtained at temperatures above the phase transition. The results of the fitting process are summarised in Table 3. The activation energies obtained are in good agreement with previous results, confirming the observation that the methyl group reorientation in L-alanine has a relatively high activation energy (26.5 kJ mol^{-1}) compared with other molecules.

If values of CRAMPS T_2 (derived from the linewidth) obtained from the variable-temperature CRAMPS experiment are now plotted alongside the wideline relaxation data (see Fig. 4), the relationship between the increases in CRAMPS linewidth and molecular motion can be observed, as has been briefly mentioned in the proceedings of a NATO Advanced Study Institute Meeting.[12] The dynamic processes responsible for both T_1 and $T_{1\rho}$ relaxation are clearly causing line broadening of the CRAMP spectra. There is an offset between CRAMPS, T_1 and $T_{1\rho}$ data since the measurement techniques probe motions on different timescales. However, because the CRAMPS measurements are based on spectra, they are selective, distinguishing between CH_3 and NH_3^+ motions in a manner not realised in the static relaxation study. Under multiple-pulse conditions, the relationship between pulse spacing and molecular correlation time is important. For a four-pulse sequence,[11]

$$T_2^{-1} = 2M_2\tau/3\alpha\{1\text{-}[(5\cosh\alpha\text{-}2)\sinh^2\alpha](\alpha\sinh 3\alpha)^{-1}\} \quad (4)$$

where $\alpha = \tau/\tau_i$ and τ is the pulse spacing in the multiple-pulse sequence. Hence with typical pulse spacings of 1–3 μs, CRAMPS linewidths are sensitive to motions in the 100s of kHz to 1 MHz range. Above room temperature, both the CH_3 and NH_3^+ motions are fast compared to this characteristic experimental timescale, and the lines are motionally narrowed. As the temperature is lowered, the reorientation is slowed, and the lines broaden. It follows from eqn. (4) that the minimum in T_2 (maximum linewidth) occurs when $\tau_i = 4\tau/\pi$. Therefore, in order to obtain CRAMP spectra free from line broadening (to obtain chemical shifts from distinct hydrogen-bonded protons of the NH_3^+ group), temperatures below 200 K would have to be used, with lines only sharpening as the temperature is lowered sufficiently to enter the slow rotation regime. Such low temperatures were not available with the equipment used for CRAMPS in this work.

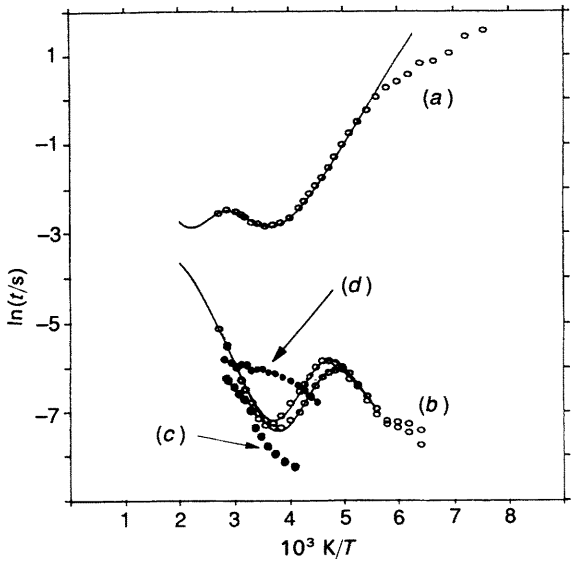

Fig. 4 T_1, $T_{1\rho}$ and CRAMPS T_2 relaxation-time dependence on temperature for L-[^{15}N]-alanine. (*a*, ○) T_1 relaxation obtained at 40 MHz. (*b*, ○) $T_{1\rho}$ relaxation obtained at 40 MHz with spin-locking fields of 100 and 130 kHz. Solid lines are best-fit curves calculated using the parameters in Table 3. (●) CRAMPS T_2 data points calculated from linewidths for (*c*) NH_3^+ and (*d*) CH_3 groups.

Conclusions

The results presented in this paper clearly demonstrate the important influence of dynamic processes in ^{1}H CRAMP spectra. For many NH_3^+ systems, dynamic broadening would appear to be more important than the expected quad-

Table 3 Best-fit results of the computer fit to the ^{1}H variable-temperature relaxation data obtained on L-[^{15}N]-alanine.

	NH_3^+			CH_3		
	E/kJ mol^{-1}	$C/10^8$ s^{-2}	$\tau_\circ/10^{-14}$ s	E/kJ mol^{-1}	$C/10^8$ s^{-2}	$\tau_\circ/10^{-14}$ s
T_1	33.6[a]	28.0[a]	5.0	26.5	30	170.0
$T_{1\rho}$ [b]	33.6	28.0	17.0	26.4	42	27.0

[a] Fixed value obtained from the $T_{1\rho}$ fit. [b] Fitted simultaneously to the data obtained with 100 and 130 kHz spin-locking fields.

rupolar effects. Molecular motions on the 100s of kHz to 1 MHz timescale lead to broadened CRAMPS signals, which cannot be better narrowed by optimisation or improvement of the multiple-pulse experiments used. It is expected that dynamic line broadening will also be important in cases other than those considered here, such as polymer chain reorientation in amorphous phases and where exchange or reorientation of strongly bound water occurs, *e.g.* in zeolites and minerals.

Dynamic line broadening unfortunately precludes the use of CRAMPS at room temperature to study many O···H···N hydrogen-bonding systems, particularly where NH_3^+ groups are concerned. It is likely that temperatures well below 200 K would be required to study such systems in the slow reorientation regime, in order to understand better the effects of hydrogen-bond distance on chemical shift. Whilst such work should be possible, given the availability of variable-temperature NMR probeheads, CRAMPS experiments are extremely sensitive to probe tuning and matching variations leading to degradation of multiple-pulse performance. Care would have to be taken both to maintain performance and compensate for any changes in the multiple-pulse scaling factor. In addition, temperature-dependent phase transitions, as found in [^{15}N]-LALA, may further complicate such studies. Because of these motional effects, the contribution to CRAMPS linewidths due to ^{14}N quadrupolar interactions may also be better studied at low temperatures, eliminating dynamic broadening.

Finally, the importance of temperature control at 'room temperature' is highlighted. The apparent difference in linewidth between the NH_3^+ signal from [^{14}N]-LALA and [^{15}N]-LALA may be accounted for by a small change in temperature during data collection, rather than by the more drastic isotopic substitution.

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