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# Chemical Shift Standards in High-Resolution Solid-State NMR (2) $^{15}$N Nuclei

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**Synopsis.** $^{15}$N NMR spectra of the shift standard compound, pure nitromethane, have been measured with high resolution by spinning the liquid sample at the magic angle. Using the precise resonance position of nitromethane, the chemical shifts of the solid second-standard materials such as ammonium salts and glycine have been measured with great precision.

High-resolution solid-state NMR has been widely used recently. Many of them are concerned with $^{13}$C and $^{29}$Si nuclei, while measurements of $^{15}$N nuclei are rather limited.[1] One of the reasons is that the sensitivity of $^{15}$N nuclei is very low because of the low natural abundance and the low gyromagnetic ratio. However, the $^{15}$N nuclei is important for the studies of biosubstances such as proteins, polypeptides, and amino acids.

The chemical shift can be expressed as the deviation of the resonance frequency from that of a standard material. In solution $^{15}$N NMR, pure nitromethane is widely used as the first standard.[1] On the other hand, various materials have been used for the first standard in high-resolution solid-state $^{15}$N NMR. $NH_4Cl$[2] and glycine[3] have been used as the solid shift standards, and various liquid samples used in solution NMR have also been used as the first standard; for example, $HNO_3$ aqueous solution, $NH_4NO_3$ saturated aqueous solution, and liquid $NH_3$.

In our previous paper,[4] we have reported precise chemical shift values of several second standard materials in $^{13}$C, $^{29}$Si, and $^{1}$H NMR determined with respect to pure tetramethylsilane. In the present work, we have extended the previous work to $^{15}$N NMR. The key point is how we measure the peak position of the first standard precisely.

## Experimental

Materials were available commercially; $CH_3NO_2$ from Tokyokasei, $HCONH_2$ and $NH_4H_2PO_4$ from Kokusankagaku, $^{15}NH_4Cl$, $NH_4Cl$ (10at.% $^{15}$N), $^{15}NH_4NO_3$, and Glycine-$^{15}$N from Aldrich, $NH_4Cl$ from Mitsubishikasei, $NH_4NO_3$ and $(NH_4)_2SO_4$ from Nacalai, and glycine from Wako. They were used as supplied.

The instrument used for the NMR measurements was a Bruker MSL400 pulsed spectrometer, whose magnetic field strength was 9.4 T (the resonance frequency, 40.561 MHz). The detail of the hardware has been described previously.[4] The rotor was spun at spinning rates of about 3 kHz and 0.5—1.0 kHz for solid and liquid samples, respectively. Temperature was 294 K, unless otherwise stated.

INEPT pulse sequences with and without $^{1}$H decoupling[5] were used for liquid samples as well as the ordinary single pulse sequence. Two kinds of pulse sequences were used for solid samples; single pulse with $^{1}$H decoupling only during the data acquisition, and cross polarization (CP) from $^{1}$H to $^{15}$N with $^{1}$H decoupling during acquisition. CP was always used for the unenriched solid samples, in which the contact time was 3 ms, the recycle time was 8—10 s, and the number of scans was 100—200.

## Results and Discussion

**$^{15}$N Spectra of Nitromethane and Formamide.** Nitromethane is the first standard, and formamide is used as the liquid second standard. Table 1 summarizes the chemical shifts and linewidths obtained in this work. The resonance frequency of pure nitromethane measured without decoupling and with MAS is assumed to be 0 ppm throughout this work, the reason of which is described below.

Figure 1 shows the spectra of pure nitromethane without and with $^{1}$H decoupling. Linewidths less than 0.010 ppm have been obtained by the initial careful shimming. Figure 1A shows $XH_3$ pattern, in which the coupling constant between X and H is 2.33±0.01 Hz. This value agrees excellently with that of Strelenko et al.[6] Figure 1B shows the decoupled spectrum, and only one sharp resonance line can be observed. However, note that the resonance position of the decoupled spectrum does not

Table 1. Results of Pure Nitromethane and Formamide

| Shape | Spinning | $^{1}$H Coupling | $\delta^{a)}$ ppm | FWHM$^{b)}$ ppm |
|---|---|---|---|---|
| [Nitromethane] | | | | |
| Cylinder | MAS | Coupled | $0^{c)}$ | 0.007 |
| | | Decoupled | −0.03 (0.01) | 0.008 |
| Sphere | Static | Coupled | 0.04 (0.05) | 0.20 |
| Cylinder | Static | Coupled | 0.00 (0.02) | 0.20 |
| [Formamide] | | | | |
| Cylinder | MAS | Coupled | −266.712 (0.006) | 0.018 |
| | | Decoupled | −267.02 (0.10) | 0.082 |

a) Higher frequecy side is expressed as positive. Values in the parentheses are experimental errors.
b) Full width at half maximum. c) Assumed to be 0 ppm.

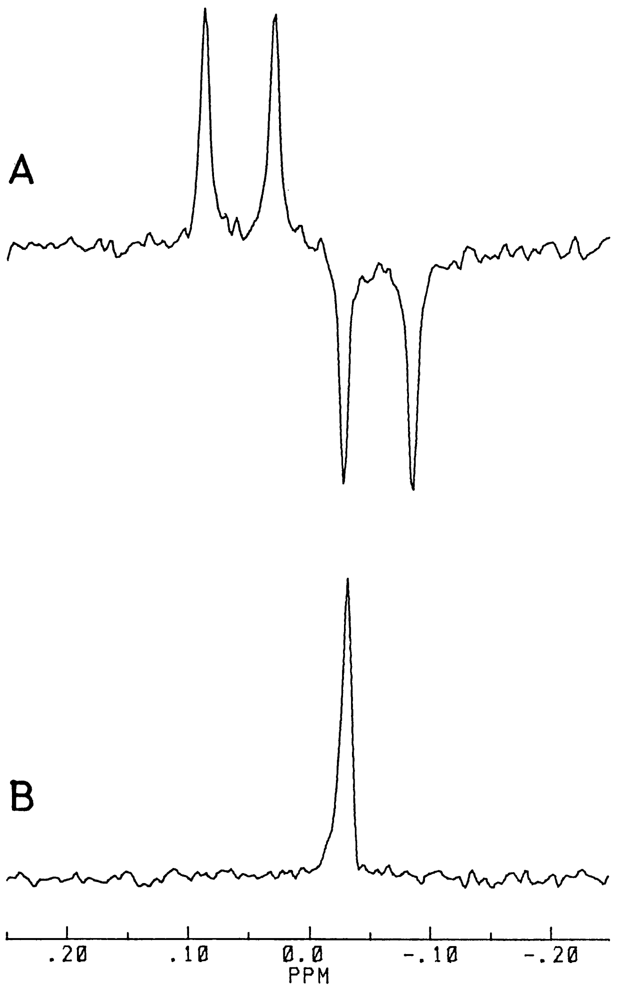

Fig. 1. $^{15}N$ MAS NMR spectra of neat nitromethane. (A) INEPT without decoupling, and (B) refocused INEPT with $^{1}H$ decoupling. Experimental parameters are as follows; 100 scans, $\tau$ (=1/4$J$) 109 msec, spectral width 1250 Hz, data size 32 kwords, and line broadening 0.1 Hz.

agree to the center of the coupled spectrum. We have repeated these measurements several times, including the single-pulse-sequence measurements with and without decoupling, and conclude that the deviation is not the experimental error. The deviation ranges from 0.026 to 0.037 ppm, depending on the experiment.

Which spectrum is independent of the measurement, the decoupled or coupled spectrum? The answer comes from the similar measurements on formamide, $HCONH_2$. The coupled spectrum shows $XH_2$ pattern accompanied by a long-range splitting. The coupling constants are $^{1}J(^{15}N-^{1}H)=89.21\pm0.02$ Hz and $^{2}J(^{15}N-^{1}H)=14.03\pm0.02$ Hz. The decoupling induces a shift of $-0.30\pm0.10$ ppm. This deviation is much larger than that observed in nitromethane, and the fluctuation from experiment to experiment is also larger. We have compared the spectra of nitromethane with those of formamide. The difference in the resonance frequency between the coupled spectra of the two compounds are always constant, while the difference between the decoupled specta fluctuates up to 0.20 ppm. Consequently, the coupled spectrum is independent of the experiment. Thus, the coupled spectrum should be taken as the shift standard. In the present work, the center of the coupled spectrum of pure nitromethane (Fig. 1A) is assumed to be 0 ppm.

The origin of the decoupling induced shift is discussed briefly. The most possible mechanism is that the decoupling induces temperature increase in the sample, resulting in the resonance frequency shift. Nitromethane and formamide have large dielectric constants,[7] and thus the decoupling pulse loses its energy, which converts into heat. The heating effect is largely dependent on the experimental conditions, and the shift in the decoupled spectrum fluctuates from experiment to experiment. To check the above hypothesis, we have measured the temperature dependence of the $^{15}N$ chemical shifts in nitromethane and formamide under the MAS condition. The temperature range studied is between 294 and 304 K. The resonance lines shift towards lower frequency as the temperature increases, which direction is the same as the decoupling induced shift. The magnitudes of the tempearture dependence are $-0.0045$ and $-0.0089$ ppm $K^{-1}$ for nitromethane and formamide, respectively. A simple calculation indicates that the decoupling increases the temperature of the sample by 6—8 K and 23—45 K in nitromethane and formamide, respectively. The specific inductive capacities of the two compounds are 35.8 (30 °C) and 111.0 (20 °C).[7] Since the dielectric constant of formamide is markedly large, the decoupler heating is very efficient in this compound. The linewidth in the decoupled spectrum of formamide (0.082 ppm) is much broader than in the coupled spectrum (0.018 ppm). The decoupler heating might produce chemical shift dispersion in the sample.

Anderson and Freeman[8] have proposed a mechanism of the decoupling induced shift, called "Bloch-Siegert shift." However, this mechanism cannot be applied to the present case, since the estimated shift is

Table 2. $^{15}N$ Chemical Shifts in Solids[a)]

| Compounds | $\delta$[b)] | FWHM[c)] |
|---|---|---|
| | ppm | ppm |
| $^{15}NH_4Cl$ | −341.168 (0.011) | 0.077 |
| $NH_4Cl$ (10at.% $^{15}N$) | −341.168 (0.011) | 0.085 |
| $NH_4Cl$ | −341.15 (0.02) | 0.14 |
| $^{15}NH_4NO_3$ | −358.45 (0.01) | 0.30 |
| $NH_4NO_3$ | −4.98 (0.06) | 0.50 |
| | −358.43 (0.03) | 0.27 |
| $NH_4H_2PO_4$ | −356.88 (0.02) | 0.20 |
| $(NH_4)_2SO_4$ | −355.70 (0.01) | 0.13 |
| | −355.98 (0.01) | 0.12 |
| Glycine-$^{15}N$ | −347.54 (0.03) | 0.57 |
| Glycine | −347.58 (0.05) | 0.70 |

a) Measured at $\nu_0$=40.561 MHz with MAS. b) With respect to the resonance frequency of pure nitromethane measured under MAS without $^{1}H$ decoupling. The values in the parentheses are experimental errors. c) Full width at half maximum.

much smaller than the experimental shift.

Next the effect of the bulk magnetic susceptibility is checked and discussed for pure nitromethane. The procedure is the same as the previous work,[4] which results are summarized in Table 1. Although the experimental error is large for the static samples, the observed values demonstrate that the effect of the bulk magnetic susceptibility is negligible within the experimental error in MAS NMR. Furthermore, the susceptibility effect is negligible in the static cylinder whose cylindrical axis is inclined at the magic angle.

**Second Shift Standards.** Practically, solid materials are adequate in order to set up the instrument. We have measured $^{15}$N CP/MAS NMR spectra of various ammonium salts and glycine, and have determined their chemical shifts with respect to pure nitromethane. Table 2 summarizes the obtained chemical shifts and linewidths. Cross polarization is not always necessary for the $^{15}$N-enriched compounds, while it is indispensable for the compounds with the natural-abundance $^{15}$N. Although $^{15}NH_4NO_3$ has two kinds of N atoms, only the chemical shift of $^{15}NH_4$ is listed in the table, since $NO_3$ was not observed in a few scans. $(NH_4)_2SO_4$ has two peaks originated from crystallographycally inequivalent sites.[9] Since this work delas with the shift standard, detailed discussion on the correlation between the chemical shifts and the structure will be published elsewhere. For routine use, we recommend to use $^{15}NH_4Cl$ and $NH_4Cl$ (10 at.%$^{15}$N) to set up the instrument and as the second shift standard. The linewidths are smallest among the solid materials measured.

## References

1) M. Witanowski, L. Stefaniak, and G. A. Webb, *Ann. Rep. NMR Spectrosc.*, **18**, 1 (1986).

2) C. I. Ratcliffe, J. A. Ripmmester, and J. S. Tse, *Chem. Phys. Lett.*, **99**, 177 (1983).

3) L. J. Mathias, D. G. Powell, J.-P. Autran, and R. S. Porter, *Macromolecules*, **23**, 963 (1990).

4) S. Hayashi and K. Hayamizu, *Bull. Chem. Soc. Jpn.*, **64**, 685 (1991).

5) J. K. M. Sanders and B. K. Hunter, "Modern NMR Spectroscopy," Oxford University, Oxford (1987), p. 81.

6) Yu. A. Strelenko, V. N. Torocheshnikov, and N. M. Sergeyev, *J. Magn. Reson.*, **89**, 123 (1990).

7) The Chemical Society of Japan, "Kagakubinran," 3rd ed, Maruzen, Tokyo (1984), p. II-501.

8) W. A. Anderson and R. Freeman, *J. Chem. Phys.*, **37**, 85 (1962).

9) M. L. H. Gruwel, M. S. McKinnon, and R. E. Wasylishen, *Chem. Phys. Lett.*, **139**, 321 (1987).