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Solvent Effects on the Spin-Lattice Relaxation Times and Chemical Shifts of *N*-Methylacetamide and *N*,*N*-Dimethylacetamide

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The spin-lattice relaxation time T_1 and chemical shift of *N*-methylacetamide (NMA) and *N*,*N*-dimethylacetamide (DMA) were measured in D_2O and CCl_4 solutions. In D_2O solution, it has been found that the weak interamide hydrogen bonding of NMA does not affect the spin-lattice relaxation entirely, and the molecular motion of NMA and DMA is almost identical. However, quite different results were obtained for CCl_4 solution. The molecular association of NMA in CCl_4 solution is strong enough to exhibit appreciable effects on spin-lattice relaxation. The life time of the associated molecules has been estimated to be longer than 10^{-10}—10^{-11} sec. The association of DMA at high concentration region in CCl_4 could be verified from the concentration dependence of the spin-lattice relaxation rate as well as chemical shift.

The contribution of the peptide bond character and the interamide hydrogen bonding to the conformational stability of the synthetic polypeptides and proteins in solutions have long been studied.[1] Quantitative information about model compounds which contain peptide bonds is expected to provide basic insight into the complexity of macromolecules.

So far, the interamide interaction, the double bond character and the hindered internal rotation of various amide compounds in solutions have been studied quantitatively, mainly by using IR, high resolution NMR and thermodynamic technique. IR studies of *N*-methylacetamide in various solvents by Klotz, Fransen, and Fornham[2,3] led us to conclude that in aqueous solution the interamide hydrogen bonding has no intrinsic strength, but is moderately strong in apolar solvent. Recently, Rabinovitz and Pines[4,5] and Neuman *et al.*[6] have studied the proton chemical shift of *N*,*N*-dimethylformamide, *N*,*N*-dimethylacetamide in CCl_4 and from the observed concentration dependence of chemical shift they argued the dimerization of amides. Equilibrium properties have been studied extensively, and quantitative features have been obtained and discussed. However, non-equilibrium properties such as microscopic molecular motion or relaxation process have not been studied in detail.

In this study, the proton spin-lattice relaxation time and chemical shift were measured on *N*-methylacetamide (NMA) and *N*,*N*-dimethylacetamide (DMA) in D_2O (aqueous environment) and CCl_4 (apolar environment). As is well known, the proton spin-lattice relaxation in diamagnetic liquid is mainly due to the dipole-dipole interactions between the nuclear magnetic moments both in the same molecule and in the neighboring molecules.

For most molecular liquids, the observed spin-lattice relaxation time T_1 can be expressed as[7,8]

$$(1/T_1) = (1/T_1)_{\text{rot.}} + (1/T_1)_{\text{transl.}} \tag{1}$$

$$(1/T_1)_{\text{rot.}} = \frac{3}{2}\hbar^2\gamma^4\sum_j\langle r_{ij}^{-6}\rangle\cdot\tau_c \tag{2}$$

$$(1/T_1)_{\text{transl.}} = 3\pi^2\hbar^2\gamma\eta N/kT \tag{3}$$

where $(^1/T_1)_{\text{rot.}}$ represents the intramolecular contribution depending on the rotational motion of the molecule, and $(^1/T_1)_{\text{transl.}}$ represents the intermolecular contribution which depends on the relative translational motions of the molecules. r_{ij} is the distance between ith and jth nucleus; τ_c is the rotational correlation time for molecule (including the effect of internal rotation in certain circumstances): η and N are solution viscosity and spin density, respectively, and all other notations have their usual meanings.

We measured T_1's and the chemical shifts at various solute concentrations, and obtained the intramolecular spin-lattice relaxation time $(T_1)_{\text{rot.}}$ by the dilution procedure.[9,10] We estimated τ_c values taking molecular geometry into consideration. Based on the results obtained on τ_c values, the concentration dependence of T_1 and the chemical shifts we would like to discuss the molecular motion, intramolecular interaction and the peptide bond character in aqueous and apolar environments.

Information on molecular motion or the relaxation process should help us understand the properties of these molecules in detail in relation to the information from equilibrium measurements.

Experimental

Materials. NMA and DMA were reagent grade samples from Tokyo Kasei Co., Ltd. and used without further purification, because their NMR spectra did not show any observable impurity signals. Heavy water and carbon tetrachloride were provided by E. Merck AG, Darmstadt and

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[1] For example S. Mizushima, "Structure of Molecules and Internal Rotation," Academic Press, New York (1954), Chap V 1, p. 117.

[2] I. M. Klotz and J. S. Fransen, *J. Amer. Chem. Soc.*, **84**, 3461 (1962).

[3] I. M. Klotz and S. B. Farnham, *Biochemistry*, **7**, 3879 (1968).

[4] M. Rabinovitz and A. Pines, *J. Chem. Soc.*, *B*, **1968**, 1110.

[5] M. Rabinovitz and A. Pines, *J. Amer. Chem. Soc.*, **91**, 1585 (1969).

[6] R. C. Neuman, Jr., W. R. Woolfenden, and V. Jonas. *J. Phys. Chem.*, **73**, 3177 (1969).

[7] N. Bloembergen, E. M. Purcell, and R. V. Pound, *Phys. Rev.*, **73**, 679 (1948).

[8] A. Abragam, "The Principle of Nuclear Magnetism," Oxford (1961).

[9] G. Bonera and Rigamonti, *J. Chem. Phys.*, **42**, 171 (1965).

[10] J. W. Emsley, J. Feeny, and L. H. Sutcliffe, "Progress in Nuclear Magnetic Resonance Spectroscopy," Vol. 3, Pergamon Press, London (1967), Chap. 5.

Tokyo Kasei Co., Ltd., respectively. The atmospheric oxygen dissolved in the sample was removed carefully by several freeze-pump-thaw cycles in NMR tube (ϕ=0.5 cm), and the sample tube then was sealed off in a vacuum (10^{-5}—10^{-6} mmHg). After sealing, the sample tube was immediately subjected to experiment.

NMR Measurements. A JNM-C-60H spectrometer of Japan Electron Optics Lab. operated at 60 MHz was used. Measurements of T_1 were carried out at 25±1°C in almost all cases by the adiabatic rapid passage and saturation recovery methods.

Experimental errors were smaller than ±3% for T_1 values longer than about 3 sec. For T_1 smaller than 3 sec, errors were smaller than ±5%. The signal of *N*-methyl protons of NMA splits into doublet due to the indirect spin-spin coupling with N-H proton. However, because of the small coupling constant ($J \simeq 4.8$ Hz), only one recovery curve with a broad line width was observed.

trans- and *cis*-*N*-Methyl groups with respect to the carbonyl group of DMA undergo different shielding effects due to the double bond character of the central C–N bond. Thus, *N*-methyl proton resonance reveals a doublet ($\Delta\nu \simeq$ 9—10 Hz). Two distinct recovery curves were then obtained, but almost all T_1 values calculated from these were equal within experimental errors. Thus we adopted the average value as T_1 for both *N*-methyl protons. The chemical shift was measured at 25±1°C by the usual side-band-technique using Tetramethylsilane (TMS) as an internal reference.

Results and Discussion

The concentration dependence of the relaxation rate $1/T_1$ for methyl protons of NMA and DMA in D_2O and CCl_4 solutions is shown in Figs. 1 and 2, respectively. T_1's of *C*-methyl and *N*-methyl protons in D_2O solutions of NMA or DMA and CCl_4 solution of NMA are equal within experimental errors, but not in CCl_4 solution of DMA. From the observed linear relation between $1/T_1$ and concentration, it is possible to estimate $(1/T_1)_{\text{rot.}}$ of the solute molecule for each solution, except for DMA in CCl_4 solution. However, at the concentration range above and below about 3 mol/*l*, linearity, appears again, and an estimation of $(1/T_1)_{\text{rot.}}$ or τ_c values is possible by extrapolating to infinite dilution. The results of $(T_1)_{\text{rot.}}$ thus obtained and τ_c

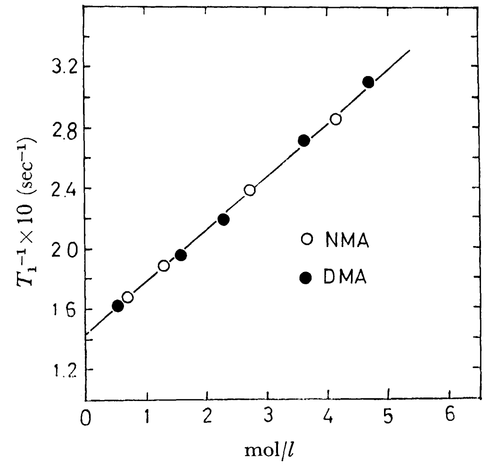

Fig. 1. Relaxation rate *vs.* solute concentration for MNA and DMA in D_2O solutions at 25°C.

T_1's of *C*- and *N*-methyl protons are equal within the experimental errors, so expressed by the same experimental points.

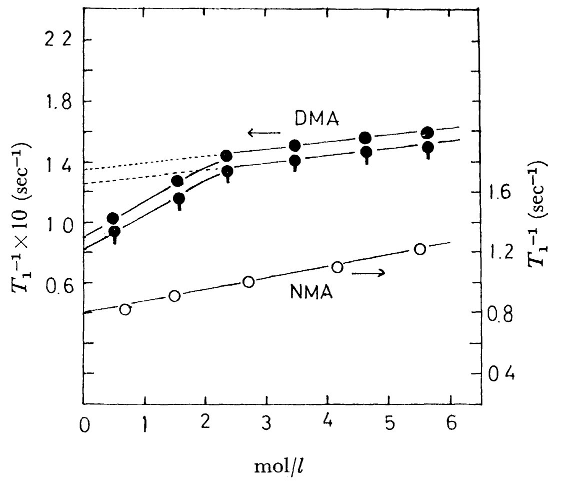

Fig 2. Relaxation rate *vs.* solute concentration for NMA and DMA in CCl_4 solutions at 25°C.

T_1's of *C*- and *N*-methyl protons of NMA are equal and expressed by the same experimental points (open circles). ●—T_1's of *C*-methyl protons of DMA. ⧫—T_1's of *N*-methyl protons of DMA.

Table 1. T_1 and τ_c values of NMA and DMA in D_2O, CCl_4 solution at 25°C

Solvent	Solute					
	NMA			DMA		
	Observed protons	$(T_1)_{\text{rot.}}$	$\tau_c\times 10^{12}$	Observed protons	$(T_1)_{\text{rot.}}$	$\tau_c\times 10^{12}$
D_2O	$-\overset{\overset{O}{\|}}{C}-CH_3$	6.90 sec	2.58 sec	$-\overset{\overset{O}{\|}}{C}-CH_3$	6.90 sec	2.5_4 sec
	$-\overset{\|}{N}-CH_3$	6.90	2.50	$-\overset{\|}{N}(CH_3)_2$	6.90	2.4_8
CCl_4	$-\overset{\overset{O}{\|}}{C}-CH_3$	1.25	14.4	$-\overset{\overset{O}{\|}}{C}-CH_3$	11.1 7.40	τ_{c1}: 1.5_9 τ_{c2}: 2.4_0
	$-\overset{\|}{N}-CH_3$	1.2_5	$14._0$	$-\overset{\|}{N}(CH_3)_2$	12.2 7.8_0	τ_{c1}: 1.4_1 τ_{c2}: 2.2_0

τ_{c1} and τ_{c2} represent the correlation times estimated by extrapolating to infinite dilution from the low and high concentration regions, respectively.

estimated by using Eq. (2) are shown in Table 1. It was found that the relaxation process of NMA molecule in D_2O solution is very similar to that of DMA. Corresponding results and $\tau_c = 1.6 \times 10^{-12}$ sec were also obtained at 45°C. However, in CCl_4 solution a quite different relaxation tendency resulting from aqueous solution was observed for both NMA and DMA molecules.

In order to calculate the interprotonic distance, we adopted the molecular geometry of NMA proposed by Pauling.[11] We have assumed that the geometry of DMA is the same as that of NMA except for the substituted *N*-methyl proton. Moreover, internal rotation of methyl groups is assumed to be almost free. We adopted 1.78 Å for the interprotonic distance in methyl group.

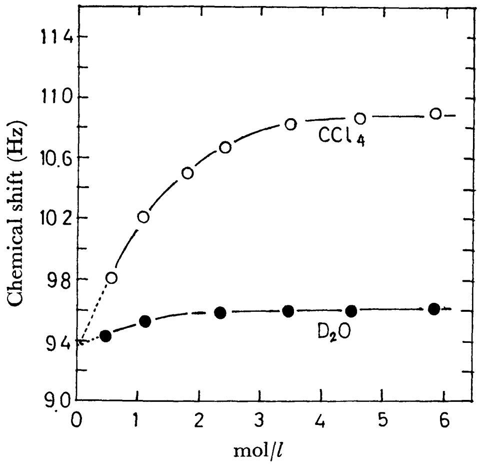

Fig. 3. Concentration dependence of the chemical shift between the two *N*-methyl resonance signals of DMA in CCl_4 (open circles) and D_2O (solid circles) solutions at 25°C.

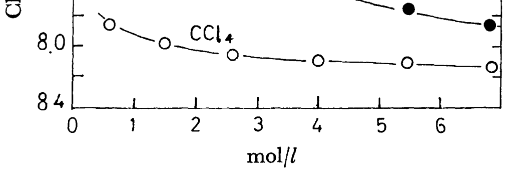

Fig. 4. Concentration dependence of the chemical shift (measured from internal TMS) of N–H protons of NMA in CCl_4 (open circlds) and $CDCl_3$ (solid circles) solutions at 25°C.

The concentration dependence of the chemical shift of *N*-methyl protons of DMA both in D_2O and CCl_4 solutions at 25°C is shown in Fig. 3. The behavior in CCl_4 solution agrees qualitatively with the results of Neuman *et al.*[6] The concentration dependence of the chemical shift seems to be similar in its linearity to the concentration dependence of $1/T_1$. The concentration dependence of N–H proton chemical shift of NMA in CCl_4 solution is shown in Fig. 4 with the additional results[12] in $CDCl_3$ solution. It is easily seen that in CCl_4 solution the chemical shift of N–H proton appears at a lower field than that in $CDCl_3$ solution, and depends on the concentration slightly. Considering that the behavior of N–H proton chemical shift of NMA is mainly due to the interamide hydrogen bond and the proton participating in the hydrogen bonding resonates at a lower field, it is suggested that in CCl_4 solution this molecule could form relatively strong intermolecular hydrogen bonding at about 0.5 mol/*l*. This is in accordance with the conclusion of Klotz and Fransen[2] by IR method. They concluded that almost all the molecules (NMA) associate through the hydrogen bonding at 0.5 mol/*l* in CCl_4 solution. Therefore it may be considered that the extrapolated $(T_1)_{\text{rot.}}$ of NMA in CCl_4 solution represents the rotational correlation time of the molecule which is restricted in the associated form but not disturbed by the intermolecular dipole-dipole interactions.

Since τ_c values as well as the concentration dependence of T_1 for NMA and DMA in aqueous solution are nearly equal and the chemical shift between the two *N*-methyl groups is almost independent of the solute concentration, the intermolecular interactions between solute molecules do not seem to be appreciable as discussed later. This leads us to the consideration that the estimated τ_c values may be related to the isolated molecule free from solute-solute interactions. Klotz and Fransen[2] also concluded that in aqueous solution the degree of association of NMA molecule is smaller than 10% even at moderately high concentration of about 5 mol/*l*. Their conclusion is in accordance with our present NMR results.

It is clear from Fig. 3 that the chemical shift, $\Delta\nu$, between the *N*-methyl protons of DMA in aqueous solution tends to coincide with that in CCl_4 solution at infinite dilution. This suggests that DMA molecules in dilute CCl_4 solution do not interact with each other as strongly as to affect the spin-lattice relaxation process. Neuman *et al.*,[6] and Rabinovitz and Pines[4,5] concluded from the observed dependence of the chemical shift of *N*-methyl doublet that DMA and *N,N*-dimethylformamide molecules dimerize at moderate concentration in CCl_4 solution. We also assume the behavior of $1/T_1$ at the moderate concentration region to be due to the dimerization of solute molecules. Thus it is considered that τ_c extrapolated from high concentration region may be associated with the DMA molecule weakly influenced by the molecular dimerization and not disturbed by the intermolecular dipole-dipole interactions.

Let us discuss the solvent effects in both aqueous and apolar solutions as follows.

Aqueous Solution. For both NMA and DMA molecules τ_c values of *C*-methyl and *N*-methyl groups are nearly equal. The fact that τ_c values of different methyl groups in the same molecule are equal means

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[11] L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca (1960), Chap, 8, p. 281.

[12] K. Sato and A. Nishioka, unpublished data.

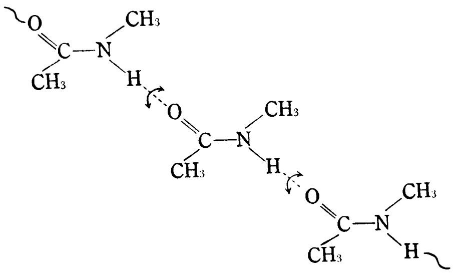
that the molecular rotational motion may be isotropic with respect to both methyl groups, provided that the internal rotation of methyl groups are almost in the same degree. τ_c values of NMA and DMA are about 2.5×10^{-12} sec, and the concentration dependence of T_1 or the contribution from intermolecular relaxation factors is equal. It can be concluded that the molecular rotational and translational motions are almost the same for NMA and DMA molecule in aqueous solution.

Recently, Anderson and Fryer[13,14] discussed the effect of the molecular association on the molecular rotational motion or the spin-lattice relaxation process, and concluded that if the lifetime of the associated molecules moving as a unit is longer than the rates of chemical exchange between the associated molecules, the effect of association is pronounced, whereas the effect is small if the lifetime of the molecular association is shorter. Thus it may be concluded that in aqueous solution the interamide hydrogen bonding is very weak, and if the molecular association is formed through the hydrogen bonding its lifetime is shorter than 10^{-11}—10^{-12} sec.

Thus the peptide bond character and the interamide interaction are not appreciable in aqueous solution, and do not affect entirely the microscopic molecular motion. From the concentration dependence of the chemical shift of *N*-dimethyl doublet of DMA solution, we conclude that the double bond character of the central C–N bond through the solute-solute interactions is not stabilized by the increase of solute concentration. This means implicitly that in contrast to CCl_4 solution the intermolecular interaction or the molecular association does not exist between DMA molecules in aqueous solution over the range of concentration studied.

Apolar Solution. The rotational correlation times of *C*-methyl and *N*-methyl groups of NMA are equal; $\tau_c=1.4\times10^{-11}$ sec. The concentration dependence of $1/T_1$ is also in the same order of magnitude. It is reasonable to consider that the molecular rotational and translational motions are isotropic as in the case of aqueous solution. τ_c of NMA seems to be about five to ten times longer than the values observed in ordinary low molecular weight compounds.[10] This provides another verification that τ_c estimated in the present study is related to the rotational correlation time of NMA molecule restricted in the associated form of chain. We might conclude that the interamide association through the hydrogen bonding is strong enough to affect the spin-lattice relaxation, and that the life time of the association is longer than 10^{-10}—10^{-11} sec. Considering the fact that the interamide association is strong, and the molecular rotational motion is isotropic with respect to both methyl groups, we conclude that the rotational motion is predominant around the associated chain.

(See below, the *trans* configuration of NMA has been verified and well known.[1])



According to Neuman and Rabinovitz, we may ascribe the peculiar concentration dependence of the chemical shift and T_1 to molecular dimerization. Another feature in CCl_4 solution differing from that in aqueous solution may be the fact that T_1 values of *C*-methyl and *N*-methyl protons are not equal over the experimental range of concentrations. This probably means that the molecular motion of DMA in CCl_4 is anisotropic with respect to both methyl groups. But the mechanism of this anisotropy is not clear. We might discuss from the effect of dimerization the reason for the concentration depedence of the relaxation rate in the dilute concentration region differing from that in the moderate concentration region. We consider that in dilute solutions the free DMA molecules are dominant and the fraction of the dimerized molecule increases linearly with increasing concentration (above about 3 mol/*l*). However, almost all DMA molecules dimerize and produce apparently different relaxation contributions from those at dilute solution region. τ_c values (denoted by τ_{c1}) obtained by extrapolating into infinite dilution from lower concentration region is plausible for most low molecular weight compounds. The value may correspond to the rotational correlation time of the isolated DMA molecule. τ_c (denoted by τ_{c2}) estimated from moderately concentration region is smaller than twice τ_{c1} of the isolated DMA.

The BPP theory[7] predicts $\tau_c=4\pi\eta a^3/3kT$ for the rigid molecule, where η and a represent solution viscosity and the radius of spherical molecule, respectively. If the dimerized molecule with a long lifetime is rigid enough to affect the spin-lattice relaxation process considerably, τ_{c2} must be roughly eight times greater than τ_{c1} according to BPP. Therefore we conclude that although the effect of association on spin-lattice relaxation is appreciable, it is not so significant, and that life time of association may be at most 10^{-11}—10^{-12} sec. It seems that both characters of monomer and dimer influence the relaxation process. This is also verified from the fact that T_1 values of *C*-methyl protons differ from those of *N*-methyl protons to the same degree as in dilute concentration region in moderately high concentrations where the fraction of associated molecule is expected to be larger.

τ_c of the isolated DMA is shorter in CCl_4 than in aqueous solution. The reasons might be as follows.

(1) The solution viscosity of aqueous solution at 25°C is larger than that of CCl_4 solution by factor about 1.5.[12] (2) The effect of solvation[10] of water molecules around the DMA molecules is expected to reduce molecular mobilities.

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[13] J. E. Anderson and P. A. Fryer, *J. Chem. Phys.* **50**, 3784 (1969).

[14] J. E. Anderson, *ibid.*, **51**, 3578 (1969).