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# Studies on the Aqueous Solutions of Guanidinium Salts. XIII. NMR Study of the Interactions between Guanidinium Salt and Tetraalkylammonium Salts in Water

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The quadrupole relaxation of $^{14}N$ nucleus of guanidinium bromide has been measured in aqueous ternary solutions containing symmetrical tetraalkylammonium salts by the $^{1}H$ NMR method. By means of Pople's line shape analysis and the Debye-Einstein equation, it has been confirmed that no complex formation takes place in the aqueous ternary solution containing symmetrical tetraalkylammonium salts. The rotational correlation time of the guanidinium ion is linearly related to the absolute viscosity of the solutions containing the homologous series of alkylammonium salts. A similar relationship was obtained for the ternary solutions with sugars or polyethylene glycols. This suggests that the correlation time depends on the microviscosity around the guanidinium ion.

In a previous work, the free energy change on mixing aqueous guanidinium bromide [GuBr] and tetrabutylammonium bromide [$(n\text{-Bu})_4NBr$] solutions was determined and the mutual salting-in effect was observed.[1] The result has been interpreted in terms of the following: (a) complex formation between two kinds of solutes even in an aqueous ternary solution, and (b) alteration of the water structure, *viz.* structural salting-in. (a) is based on the fact that the guanidinium halides form crystalline complexes with symmetrical tetraalkylammonium halides in concentrated ternary solutions,[2] and (b) on the data of enthalpy of mixing of $Bu_4NBr$ and guanidinium chloride in aqueous solution reported by Chawla *et al.*[3] The transfer of $Bu_4NBr$ from water to guanidinium chloride solution is accompanied by a decrease in the excess partial molar heat capacity, indicating that the hydrophobic hydration around tetraalkylammonium ion is reduced in the aqueous guanidinium salt solution. Recent investigation of the volume of mixing of $GuBr$–$Bu_4NBr$–$H_2O$ system has revealed that the reduction of the hydrophobic hydration caused by the structural breaking action of the guanidinium ion gives rise to increase in solution volume.[4] It seemed of interest to confirm whether the large salting-in effect is due to the complex formation or the structural change of water. For this purpose we measured the $^{1}H$ NMR spectra of GuBr in the aqueous ternary solution with various alkylammonium bromides, sugars or polyethylene glycol, with attention focussed on the change of shapes of amino proton signals. The results analyzed by means of Pople's theory[5] and the Debye-Einstein equation[6] support interpretation (b), the mutual salting-in effect due to the structural change of water.

## Experimental

*Materials.* Symmetrical tetraalkylammonium bromides (Wako Chemical Co., Ltd.) were recrystallized at least twice from an appropriate solvent.[7] The other alkylammonium salts $C_iH_{2i+1}N(CH_3)_3Br$, ($i=2$—$5$), $C_6H_5N(CH_3)_3Br$, $C_6H_5N(C_2H_5)_3Cl$ (reagent grade, Tokyo Kasei Chemical Co., Ltd.) were recrystallized from ethanol several times. Sucrose, D-glucose and D-fructose of reagent grade were used without further purification. Polyethylene glycols (EG) with molecular weight 200 and 300 (Nakarai Chemical Co., Ltd.) were used without further purification. The solid solutes were dried *in vacuo* over phosphorus pentoxide before use. All solutes were dissolved in redistilled and deionized water. GuBr used was the same compound as reported previously.[8] The concentration of GuBr was 2 mol/kg·water throughout the experiments. All samples were not degassed. The pH values of these solutions were maintained in the range 4.2—4.6 with 0.01 mol dm$^{-3}$ hydrobromic acid solution.

*Method for NMR Measurement.* The $^{1}H$ NMR spectra were measured on a Varian HA-100 D NMR spectrometer operating at 100 MHz. Cyclosilane-$d_{18}$ sealed in a co-axial capillary tube was used for an internal lock signal and as an external reference for chemical shift. The temperature variation was made with a temperature variable accessory unit (V-6040) calibrated by ethylene glycol. Regulation of temperature was accurate to $\pm 2$ °C throughout the NMR measurement.

*Viscosity Measurement.* The absolute viscosity of these ternary solutions, $\eta$, was measured with an Ostwald type viscometer. Temperature of the thermostat bath was controlled within $\pm 0.03$ °C. The kinetic energy correction was made for determination of viscosity by the equation,

$$\eta/d = At - B/t,\ A = 2.628\times 10^{-3}\ (\mathrm{cm^2/s^2}),$$
$$B = 15.00\ (\mathrm{cm^2}),$$

where $t$ is the flow time and $d$ the density of the solution, measured with Seiko SDM 421 and 422 vibration densimeters.[4]

## Results and Discussion

Figure 1 shows the $^{1}H$ NMR spectra of a 2 mol/kg·water GuBr solution and a mixed aqueous solution of GuBr and $R_4NBr$ molalities of which are 2 mol/kg·water, respectively. The broad signal at *ca.* 7—7.5 ppm is due to the NH absorption of the guanidinium ion, the sharp signal at about 5.15 ppm to the absorption of water proton. The chemical shifts are given in Table 1. The NH resonance shows a narrowing and slight shift to down field on addition of $R_4NBr$, the resonance of water proton remaining almost unchanged. The extent of the narrowing and shift progresses with increase in the chain lengths of the alkyl group. The sharpening accompanied by the addition of $Bu_4NBr$ also depends on the concentration of the added salt. The down field shift seems to arise from the increase in the hydrogen bond of the guanidinium ion with water molecules. However, the absolute values of

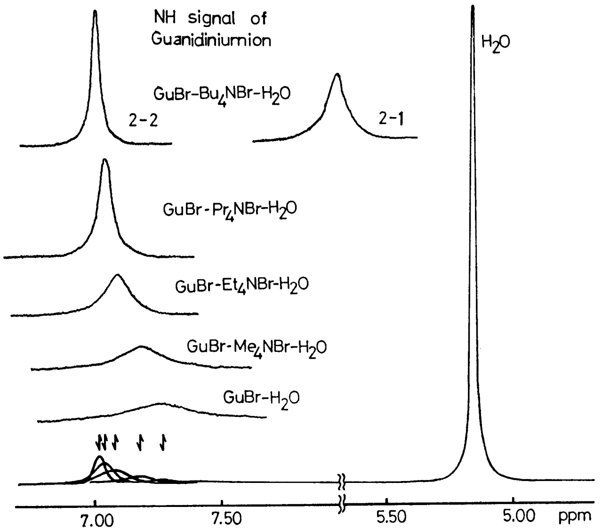

Fig. 1. $^1$H NMR spectra of 2 mol/kg·water GuBr solution and the mixed aqueous solutions of GuBr and $R_4NBr$.

the shift could not be estimated since correction of the bulk susceptibility was not available. Two explanations can be considered for the narrowing: (a) change of the exchange rate of NH protons with the water proton, and (b) change of the extent of broadening by quadrupole relaxation of the $^{14}$N nucleus. NMR studies of the aqueous guanidinium solutions show that the NH proton signals change depending on the pH of the solutions, the corresponding water proton signal varying simultaneously.[9] However, in the present case, the line width of the water proton is little affected in spite of the sharpening of NH signal of the guanidinium ion. Thus (a) can be eliminated and we will further consider explanation (b). The narrowing phenomenon can be treated by the equations derived by Pople for the NMR line shape of spin 1/2 nucleus relaxing by quadrupole mechanism.[5] In the following, we will discuss the change of line shapes by means of this theory.

The theoretical line shape $I(x)$ for the spectra of nuclei of spin 1/2 ($^1$H) coupled to a nucleus of spin 1 ($^{14}$N) is expressed by the parameter $\lambda$ which is a product of spin-lattice relaxation time $T_1$ of the $^{14}$N nucleus and the NH coupling constant $^1J_{\mathrm{NH}}$:

$$I(x) = D\frac{45+\lambda^2(5x^2+1)}{225x^2+\lambda^2(34x^4-2x^2+4)+\lambda^4(x^6-2x^4+x^2)} \quad (1)$$

$$x = \Delta\nu/^1J_{\mathrm{NH}} \quad (2)$$

$$\lambda = 10T_1\,{}^1J_{\mathrm{NH}}, \quad (3)$$

where $\Delta\nu$ is the frequency relative to the center and $D$ is a constant concerning the peak area. When we determine the parameter which can reproduce the best fit curve to the observed line shape, we simply take into account the half height width $\Delta\nu_{1/2}$, instead of the total line shape of the observed signal. Thus we get from Eqs. 1—3

$$I(x_0) = I(0)/2 = (45+\lambda^2)/8\lambda^2, \quad (4)$$

where $x_0$ is $\Delta\nu_{1/2}/2\cdot{}^1J_{\mathrm{NH}}$. Thus $\lambda$ can be so determined as to satisfy Eq. 4. This simplified treatment may be justified since the observed NH resonance is simply a single peak, suggesting that the parameter $\lambda$ is approximately less than 5 (Fig. 1). In analyzing the observed line width by Eq. 4, the "natural" line width at half height in the absence of quadrupole relaxation should be substracted.[10] However, this approximation would cause no large error to the results. The theoretical curve of NH proton resonance for the GuBr–H$_2$O and GuBr–Bu$_4$NBr–H$_2$O systems were calculated by assuming the $^1J_{\mathrm{NH}}$ value for both systems as 63 Hz, calculated from $^{15}$NH coupling constant of urea by taking into account the difference of gyromagnetic ratio between $^{14}$N and $^{15}$N. The theoretical curves are shown in Fig. 2 together with the observed ones. The peak height and area of the calculated signal largely depend on the assumed magnitude of $\lambda$. Accordingly, the peak height for the GuBr–Bu$_4$NBr–H$_2$O system was adjusted to that of the observed one, and the peak height for the GuBr–H$_2$O system was so determined as to give the same area of signal as that of the GuBr–Bu$_4$NBr–H$_2$O system. In spite of the assumption of the $^1J_{\mathrm{NH}}$ value and approximate treatment for the line shape analysis, the calculated curve almost entirely fits the observed one.

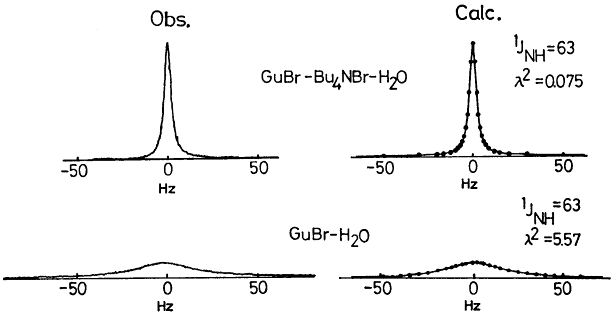

Fig. 2. Observed and calculated line shape of NH signals for the GuBr–Bu$_4$NBr–H$_2$O and GuBr–H$_2$O systems.

From the adjusted parameters $\lambda$ and by assuming $^1J_{\mathrm{NH}}$ as 63 Hz, the remaining physical constant $T_1$, spin-lattice relaxation time of the $^{14}$N nucleus can be calculated by means of Eq. 3. In the case of extreme narrowing condition ($w_0\tau_c \ll 1$, where $w_0$ is the Larmor frequency of $^{14}$N nucleus), usually satisfied in our cases, the spin-lattice relaxation time is also related to the molecular rotational correlation time $\tau_c$ by means of the relation[11]

$$\frac{1}{T_1} = \frac{3}{2}\pi^2\left(1+\frac{\theta^2}{3}\right)\left(\frac{e^2qQ}{h}\right)^2\tau_c, \quad (5)$$

where $\theta$ is the asymmetry parameter of the field gradient tensor and $e^2qQ/h$ the quadrupole coupling constant. $T_1$ and $\tau_c$ calculated for each system from the values of $^1J_{\mathrm{NH}}$ are given in Table 1, $\theta$ and $e^2qQ/h$ being given in the footnote. The value of $T_1$ obtained for the GuBr–H$_2$O system is comparable with those obtained by a different method, *i.e.* 0.932 ms for guanidine and 1.44 ms for urea,[13] which indicates that our assumption is reasonable. The spin-lattice relaxation times of mixed solutions are shortened with the increase of alkyl chain length of the added tetraalkylammonium bromide, *e.g.*,

TABLE 1. THE NMR DATA OF GUANIDINIUM SALT SOLUTIONS CONTAINING TETRAALKYLAMMONIUM SALTS

| System | Chemical shift (ppm) | $\Delta\nu_{1/2}$ (Hz) | $\lambda^2$ | $T_1$ (ms) | $\tau_c \times 10^{12}$ (s) |
|---|---|---|---|---|---|
| GuBr | 7.21 | 39.0±1.0 | 5.57±0.33 | 1.19±0.03 | 4.38 |
| GuBr–$Me_4NBr$ | 7.29 | 26.0±1.0 | 2.38±0.20 | 0.78±0.03 | 6.69 |
| GuBr–$Et_4NBr$ | 7.38 | 15.6±0.5 | 0.86±0.06 | 0.47±0.02 | 11.1 |
| GuBr–$Pr_4NBr$ | 7.41 | 7.6±0.2 | 0.20±0.01 | 0.23±0.01 | 22.7 |
| GuBr–(*n*-Bu)$_4$NBr | 7.44 | 4.6±0.2 | 0.07±0.01 | 0.14±0.01 | 37.3 |

$^1J_{NH}=63$ Hz, $\theta=0.412$, $e^2gQ/h=3500$(kHz).[12]

even an order of magnitude in the case of $R=-C_4H_9$. Since the quadrupole coupling constant would not be much influenced by the addition of $R_4NBr$, the relation in Eq. 5 indicates that this shortening is attributable to the increase of the rotational correlation time, $\tau_C$, *i.e.*, slowing down of the rotational motion of the guanidinium ion. The relationship between diffusing particle size and correlation time can be expressed by the Debye-Einstein equation[6]

$$\tau_c = \frac{4\pi a^3}{3kT}\cdot\eta, \tag{6}$$

where $k$ is the Boltzmam constant, $T$ absolute temperature, $\eta$ the viscosity of the solution, and $a$ the radius of the solute molecule.

In the ternary system tetraalkylammonium bromide–GuBr–$H_2O$, the crystalline complexes with various mole ratios were isolated from highly concentrated solutions. The excess free energy of mixing obtained from the ternary system GuBr–$Bu_4NBr$–$H_2O$ at relatively high concentration where the crystalline complexes were not isolated, were negative and large in magnitude. Considerably large salting-in effect was observed in the same system even at low concentration regions. It is of interest to confirm whether the complexes with the same composition as the crystalline complexes actually exist in the ternary solution or not. If no water soluble complexes exist in aqueous ternary solution, the complex formation is not the cause of large salting-in. Equation 6 is useful for our purpose. In order to calculate the correlation time $\tau_C$ by means of this equation, we should determine the radii of the solutes, $a$. By assuming that the 1-1 complexes are formed between GuBr and $R_4NBr$ in aqueous solution, the radii of complex, $a'$, were obtained by spherical approximation of the sum of the partial molar volumes of infinite dilution, and by using these radii and the observed viscosities of the solutions, the correlation times, $\tau_C^{calc}(a')$ at 30 °C were calculated. The results are given in Table 2, together with values of $a$ calculated from observed $\tau_C$ and $\eta$. Disagreement of the $\tau_C$ values with the corresponding observed values is remarkable. The volume ratio of guanidinium ion in water and aqueous tetrabutylammonium bromide solution obtained from the $a$ values is 1.87. On the assumption that a 1 to 1 complex is formed between GuBr and $Bu_4NBr$, the ratio is calculated to be 9.52 with the $a'$ values. This indicates that no complex between guanidinium bromide and tetraalkylammonium bromide would be formed in ternary aqueous solution.

From the thermodynamic studies of the aqueous solutions of guanidinium salts and urea, it has been shown that guanidinium halides and urea have very similar physicochemical properties in aqueous solutions.[14] The NH resonance of aqueous urea solutions

TABLE 2. COMPARISON BETWEEN THE OBSERVED AND CALCULATED VALUES OF $\tau_c$

| System | $\tau_c^{obsd}\times 10^{12}$ (s) | $a'$ (Å) | $\tau_c^{calcd}(a')\times 10^{12}$ a) (s) | $a$ (Å) | $\eta$ (c.p.) |
|---|---|---|---|---|---|
| GuBr | 4.38 | 2.51 | 13.4 | 1.73 | 0.848 |
| GuBr–$Me_4NBr$ | 6.69 | 4.22 | 81.0 | 1.84 | 1.077 |
| GuBr–$Et_4NBr$ | 11.1 | 4.63 | 154.0 | 1.93 | 1.551 |
| GuBr–$Pr_4NBr$ | 22.7 | 5.00 | 322.0 | 2.06 | 2.570 |
| GuBr–(*n*-Bu)$_4$NBr | 37.3 | 5.32 | 579.1 | 2.13 | 3.863 |
| Urea | 2.88$^{b)}$ | 2.61 | 15.6$^{b)}$ | 1.54 | 0.784 |
| Urea–$Me_4NBr$ | 6.30 | 3.99 | 70.1 | 1.79 | 1.101 |
| Urea–$Et_4NBr$ | 10.3 | 4.44 | 139.0 | 1.86 | 1.585 |
| Urea–$Pr_4NBr$ | 16.2 | 4.84 | 295.0 | 1.84 | 2.597 |
| Urea–(*n*-Bu)$_4$NBr | 26.4 | 5.17 | 540.0 | 1.89 | 3.900 |

a) $\tau_c^{calcd}(a')$ is calculated by means of Eq. 6 with $a'$ value on the assumption that a 1 to 1 complex is formed between tetraalkylammonium bromides and GuBr or Urea. b) The calculation of the observed $\tau_c$ in urea solution was carried out using the following values: $^1J_{NH}=63$ Hz, $e^2qQ/h=3510$ kHz, $\theta=0{,}323$.[13]

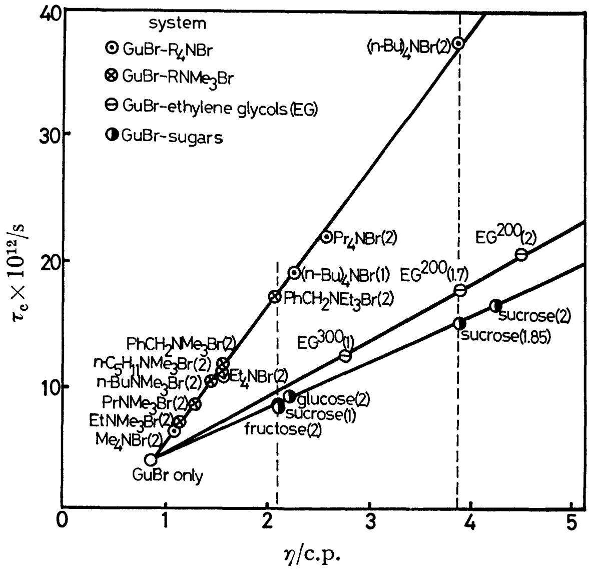

Fig. 3. Realtionships between the observed $\tau_c$'s and $\eta$'s. The molalities of the second solutes are shown in parentheses.

might therefore be also expected to show narorwing to an extent similar to that of the GuBr solutions on addition of $R_4NBr$. This was actually observed. In the case of urea (Table 2), complex formation with tetraalkylammonium salts in the aqueous solution[15] should be excluded as above.

With regard to the viscosity dependence of $\tau_C$, it has been argued that microviscosity should be employed instead of macroviscosity.[16–19] The relationship between the observed $\tau_C$ of the guanidinium ion and the measured viscosity of the ternary solutions has been investigated. $\tau_C$ values are plotted against $\eta$ of various ternary solutions containing alkylammonium salts, sugars or polyethylene glycols as the second solute in Fig. 3. We see that linear relationship exists between $\tau_C$'s and $\eta$ values of the ternary solutions of the homologous series, irrespective of the concentration of the second solutes. The effect of the second solute on solution viscosity immediately influences $\tau_C$. However, the $\tau_C$ values of the guanidinium ion have different values in the ternary solutions, even when the macroviscosity of the solutions is the same. This seems to indicate that $\tau_C$ is related to the quantity proportional to microviscosity around the guanidinium ion. The $\tau_C$ values seem to depend closely on the microstructure of the solution surrounding the guanidinium ion. The type of hydration of a series of alkylammonium salts was assumed to be of cage-like structure and that of a series of sugars to be purely hydrogen bonding with a definite orientation.[20] The type of hydration of polyethylene glycols is considered to be a mixed one in view of the chemical structure. Since the influence of the second solute on the water structure differs and each effect is reflected in the microviscosity surrounding the guanidinium ion, $\tau_C$ may have different values in the solutions where the macroviscosity is the same. Thus we might expect that $\tau_C$ would also influence the activation energy for the rotational motion of the guanidinium ion, $E_{\tau_C}$. The values of $\tau_C$ at various temperatures were calculated from the line shape of NH proton (Fig. 4). $E_{\tau_c}$ values for various ternary solutions with the same viscosity (3.86 c.p.), and binary guanidinium bromide solution were determined by the temperature dependence of $\tau_C$ by means of the equation

$$\tau_c = \tau_c^0 \exp(E_{\tau_c}/RT). \tag{7}$$

The slope of $\ln \tau_C$ *vs.* $1/T$ curves determined by least squares fit gives the following activation energies: 5.3±0.3 kcal/mol for $GuBr-H_2O$ system, 7.9±0.2 kcal/mol for $GuBr-Bu_4NBr-H_2O$ system, 7.1±0.2 kcal/mol for $GuBr-EO_{200}-H_2O$ system and 7.0±0.2 kcal/mol for $GuBr$–sucrose–$H_2O$ system.

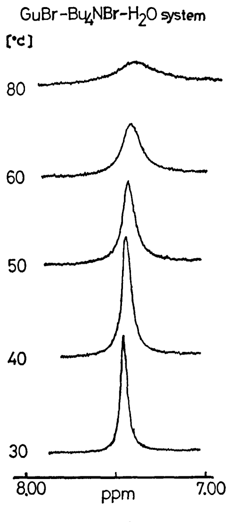

Fig. 4. Temperature dependence of the line shape of the NH proton for the ternary system $GuBr-Bu_4NBr-H_2O$.

The activation energy of viscous flow $E_\eta$ was also obtained by means of

$$E_\eta = \frac{RT\,\mathrm{d}\ln\eta}{\mathrm{d}\left(\frac{1}{T}\right)}. \tag{8}$$

Linear relationship holds between $E_{\tau_c}$ and $E_\eta$, indicating that the activation energy of macroviscous flow is proportional to the activation energy of the microviscous flow on the assumption that $\tau_C$ is another expression of microviscosity (Fig. 5).

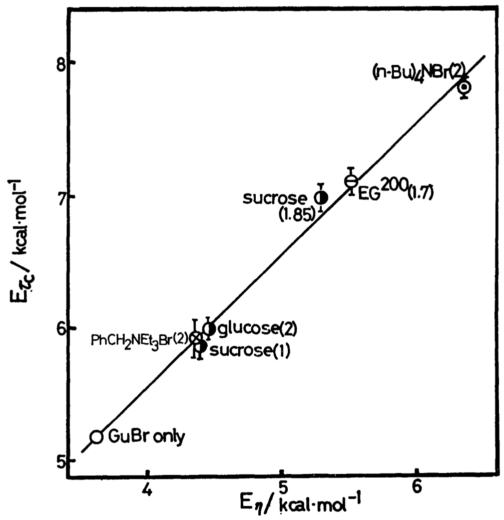

Fig. 5. Relationship between $E_{\tau_c}$ and $E_\eta$.
The molalities of the second solutes are shown in parentheses.

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