Studies of the Aqueous Solutions of Guanidinium Salts. VI. Complexes of Guanidinium Halides and Symmetrical Tetraalkylammonium Halides

Koichiro Miyajima, Hiromitsu Yoshida, and Masayuki Nakagaki

Faculty of Pharmaceutical Sciences, Kyoto University, Yoshidashimoadachi-cho, Sakyo-ku, Kyoto 606

(Received March 22, 1977)

Guanidinium halides (bromide and chloride) form crystalline complexes with symmetrical tetraalkylammonium bromides (tetraethyl-, tetrapropyl-, and tetrabutylammonium bromides and tetrabutylammonium chloride) in aqueous solutions at room temperature. These complexes were analyzed and found to be either binary complexes of two kinds of salts or ternary complexes with water as a third component. Each complex showed complicated peaks in the DSC measurements, which indicated the phase transition in the solid state. The solubility of each salt in ternary systems was measured, and phase diagrams were constructed for some of the ternary systems. A mutual salting- in effect was observed in all the ternary systems, and this effect was discussed in terms of complex formation and the structual change of water.

The guanidinium ion has a strong resemblance to urea in its shape, size, and functional group except for a charge which is distributed around the periphery of the disc-shaped ion. Even in aqueous solutions, the guanidinium ion and urea have a similar effect toward hydrophobic solutes when they are mixed with hydrocarbon gas[1] and symmetrical tetraalkylammonium salts.[2,3] These properties of urea and guanidinium salts are regarded as the main factor in the denaturation of protein in their aqueous solutions.[4] In 1966 Saito *et al.*[5] reported the complex formation of urea with various tetraalkylammonium salts in aqueous solutions. Therefore, it seems worthwhile to investigate the interaction between a hydrophobic solute and a guanidinium salt in an aqueous solution, especially at a high concentration.

From these points of view, we studied the mutual solubility of a guanidinium halide and a symmetrical tetraalkylammonium halide in aqueous solutions and isolated crystalline complexes containing each salt from aqueous ternary solutions. In this paper, we wish to report the preparation and characterization of various crystalline complexes formed by guanidinium halides and tetraalkylammonium halides in aqueous solutions.

In addition, the solubilities of these salts were measured in ternary solutions, the total concentrations and the mole ratio being changed. From these data, phase diagrams were constructed for some of the ternary systems.

Experimental

Materials. Symmetrical tetraalkylammonium halides were obtained from the Nakarai Chemical Co., and were purified by recrystalization from appropriate solvents described elsewhere.[6] The guanidinium halides used were the same as those of the previous paper.[7] Reagent-grade urea obtained from Wako Chemical Co., Ltd., was used without further purification after being dried *in vacuo*.

Preperation of Complex. A guanidinium halide and a tetraalkylammonium halide were mixed at an appropriate mole ratio, after which a desired quantity of water was added to dissolve the solid. The solution was then subjected to natural evaporation in an open beaker at room tempetature. The crystals thus formed were collected by filtration, cut to small pieces, and pressed between filter papers to remove the mother liquid. These crystals were powdered and dried for several hours at a relative humidity of 30—50% at room temperature and used for the direct analysis of complexes.

Method of Analysis. For each of the crystalline complexes, wet residues, and saturated solutions, the halide ion was determined by Mohr's method, and the tetraalkylammonium ion, by colorimetry. The latter method is based on the fact that the tetraalkylammonium ion and Metyl Orange form a complex which is slightly soluble in water and soluble in organic solvents, such as chloroform and 1,2-dichloroethane. Therefore, the contents of the tetraalkylammonium ions were determined by measuring the optical densities of extracted solutions of the tetraalkylammonium–Methyl Orange complex at 425 nm. This method was useful in the presence of guanidinium salts. The difference between the contents of the halide and tetraalkylammonium ions correspond to the content of the guanidinium ion. The water content was also deternined from these values and the total weight of the sample.

The phase diagrams were obtained by Schreinmakers' "wet residue" method.[8] In this method, the solid components were dissolved in water at various concentrations, and solutions were agitated in a thermostat until equilibrium was reached. Then the liquid phase and the wet residue were separated, and both were weighed and determined. The compositions obtained by the above procedure are plotted on a triangular diagram in Fig. 1. Let the points a, b, and c represent the compositions of the saturated solutions, and the points a′, b′, and c′, the compositions of the corresponding wet residues. Then, the tie-line a-a′, b-b′, and c-c′, must pass, on the extention past a′, b′, and c′, through the composition of the solid complex P.

Results

Crystalline Complexes and Phase Diagrams. Tetrabutylammonium Bromide [(n-Bu)$_4$NBr]–Guanidinium Bromide [GuBr]–Water. By applying Scheinmakers' wet residue method, the triangular diagram shown in Fig. 1 was obtained for the $(n\text{-Bu})_4\text{NBr}$–GuBr–$\text{H}_2\text{O}$ system. In this diagram, A and B are the solubilities of $(n\text{-Bu})_4$NBr and GuBr respectively in water at 25 °C. Curve ACDB shows the compositions of saturated solutions containing both $(n\text{-Bu})_4$NBr and GuBr. As is shown in the plots, Scheinmakers' wet residue method indicated the presence of the P complex, which contains equimolar amounts of $(n\text{-Bu})_4$NBr, GuBr, and H_2O in a crystal. This conclusion was confirmed by the direct analysis of the crystal obtained from mixed solutions containing both salts in different mole ratios. The crystal form

TABLE 1. SOME CHARACTERISTICS OF THE CRYSTALLINE COMPLEXES FORMED BY GUANIDINIUM HALIDES AND SYMMETRICAL TETRAALKYLAMMONIUM HALIDES

System	Crystal form of complex	Stoichiometric composition in mole ratio	Mp of complex (°C)	Mp of pure R_4NBr (°C)
$(n\text{-Bu})_4NBr\text{-}GuBr\text{-}H_2O$	Prism	1 : 1 : 1	65— 66	119
$(n\text{-Pr})_4NBr\text{-}GuBr\text{-}H_2O$	Plate	2 : 1 : 0	153—155	250
	Flat needle	3 : 5 : 0	108—110	250
$(Et)_4NBr\text{-}GuBr\text{-}H_2O$	Flat needle	1 : 1 : 0	147—149	250
	Needle	1 : 2 : 1	160—161	250
$(n\text{-Bu})_4NBr\text{-}GuBr\text{-}H_2O$	Prism	1 : 1 : 1	60— 63	119
$[(n\text{-Bu})_4NCl\text{-}GuBr\text{-}H_2O]$	Prism	1 : 1 : 1	60— 63	75

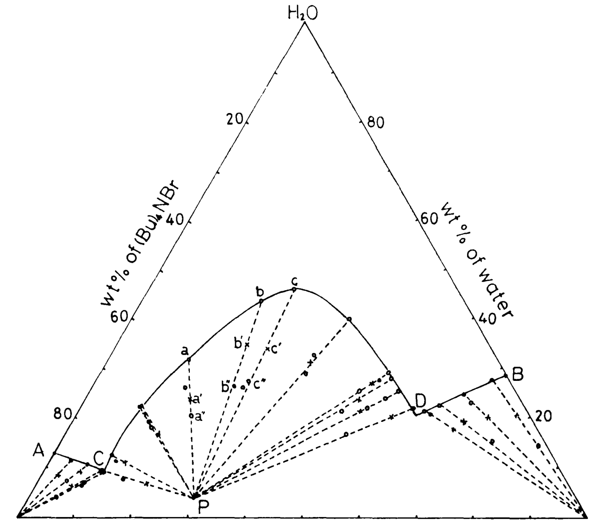

Fig. 1. Triangular diagram for ternary system $(n\text{-Bu})_4NBr\text{-}GuBr\text{-}H_2O$ at 25 °C by Schreinmakers' wet residue method. Complex P $(n\text{-Bu})_4NBr : GuBr : H_2O = 1 : 1 : 1$.

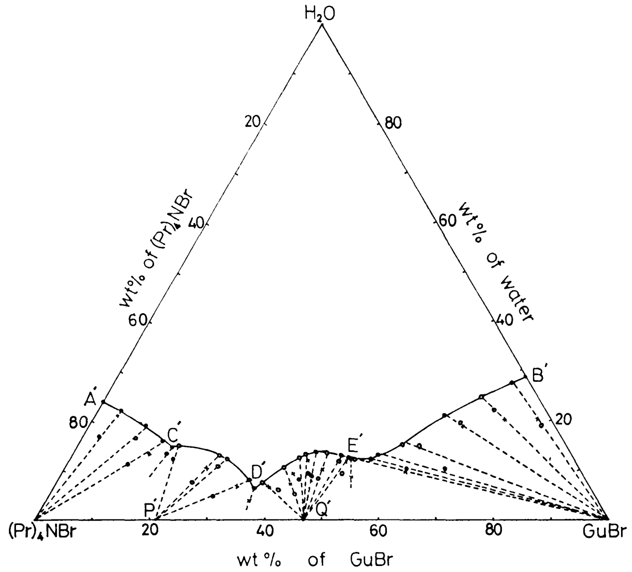

Fig. 2. Triangular diagram for ternary system $(n\text{-Pr})_4NBr\text{-}GuBr\text{-}H_2O$ at 25 °C by Schreinmakers' wet residue method.
Complex P' $(n\text{-Pr})_4NBr : GuBr = 2 : 1$. Complex Q' $(n\text{-Pr})_4NBr : GuBr = 3 : 5$.

and the melting point of the complex are shown in Table 1. The crystal of the P complex is stable in an atmosphere at room temperature, but when it was allowed to stand for several hours *in vacuo* at room temperature, it melted by losing water. The amount of water lost corresponded almost exactly to the amount obtained by the direct analysis of the crystal. Tetrabutylammonium bromide forms a clathlate hydrate, $(n\text{-Bu})_4NBr\cdot 32.8H_2O$ at low temperature.[9] Therefore, the same experiment as was done at room temperature was carried out again at 3 °C, no clathlate hydrate was found to form when enough guanidinium bromide was dissolved in the solution. In the $(n\text{-Bu})_4NBr\text{–}GuCl\text{–}H_2O$ or $(n\text{-Bu})_4NCl\text{–}GuBr\text{–}H_2O$ system, a similar crystalline complex was isolated from the mixed solutions, but the half mole of the bromide ion was replaced by the chloride ion in the crystal.

Tetrapropylammonium Bromide $[(n\text{-}Pr)_4NBr]\text{–}GuBr\text{–}H_2O$. As is shown in Fig. 2, the wet residue method indicated the presence of two kinds of complexes, P' and Q'. These complexes did not contain water, and the mole ratio of $(n\text{-Pr})_4NBr$ and GuBr was 2 to 1 and 3 to 5 for the P' and Q' complexes respectively. The crystal forms and melting points are shown in Table 1.

Tetraetylammonium Bromide $[(Et)_4NBr]\text{–}GuBr\text{–}H_2O$. Tetraetylammonium bromide also formed two kinds of complexes with GuBr in aqueous solutions. As is shown in Fig. 3, the P" complex contained equimolar amounts of the two salts, and no water, while the Q" complex contained $(Et)_4NBr$, GuBr, and H_2O in the mole ratio of 1 : 2 : 1. The crystal forms and melting points are shown in Table 1.

We did not study ternary systems containing the salts with only a chloride or iodide ion as a common anion, but we may predict with a high possibility of success the formation of crystalline complexes in these systems, judging from the formation of a crystalline complex in the $(n\text{-Bu})_4NBr\text{–}GuCl\text{–}H_2O$ system.

Thermochemical Properties of the Complexes. For the elucidation of the thermochemical properties of these complexes, DSC was employed. Some typical DSC charts and heats of fusion are shown in Fig. 4. and Table 2 respectively. The heats of fusion were determined by comparing the peak area with that of urea. As may be seen in Fig. 4, these DSC charts may be classified into three typical groups. A peak with a shoulder is observed for Type A, a doublet peak for Type C, and two isolated peaks for Type B. No complex shows

TABLE 2. SOME THERMOCHEMICAL PROPERTIES OF THE CRYSTALLINE COMPLEXES

Complex	Type of chart	Transition temp by DSC (°C)	Heat of transition (kcal/mol of complex)
$[(n\text{-Bu})_4NBr][GuBr][H_2O]$	A	59.6 (shoulder) 63.7 (peak)	2.68
$[(n\text{-Pr})_4NBr]_2[GuBr]$	B	135.4 (peak) 155.2 (peak)	1.43 1.59
$[(n\text{-Pr})_4NBr]_3[GuBr]_5$	C	103.1 (peak) 106.9 (peak)	2.17
$[(Et)_4NBr][GuBr]$	A	147.2 (shoulder) 149.6 (peak)	0.99
$[(Et)_4NBr][GuBr]_2[H_2O]$	C	154.3 (peak) 159.2 (peak)	2.17
$[(n\text{-Bu})_4NBr][GuCl][H_2O]$	A	58.7 (peak) 61.0 (peak)	2.02

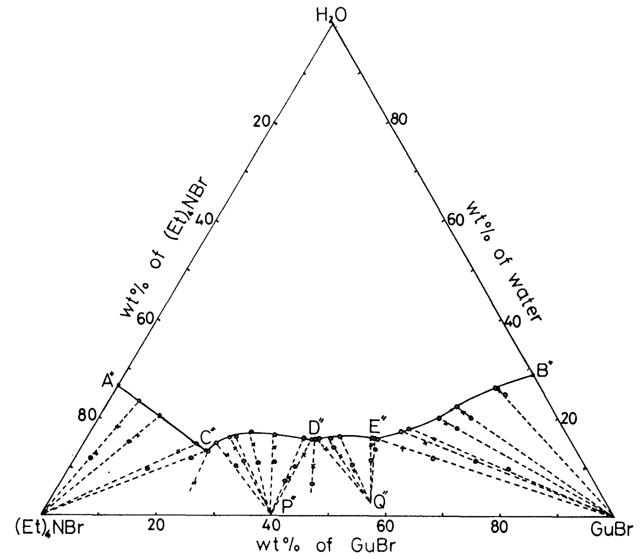

Fig. 3. Triangular diagram for ternary system $(Et)_4$-NBr-GuBr-H_2O at 25 °C by Schreinmakers' wet residue method.
Complex P'' $(Et)_4NBr : GuBr = 1 : 1$. Complex Q'' $(Et)_4NBr : GuBr : H_2O = 1 : 2 : 1$.

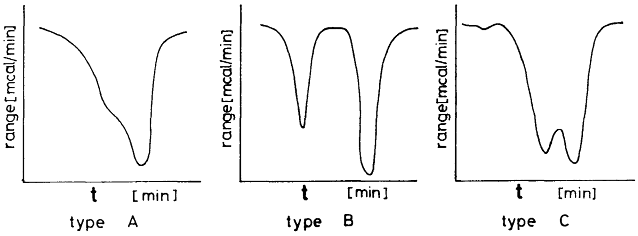

Fig. 4. Typical DSC charts for the crystalline complexes. Range: 8 mcal/min. Temperature-programing rate: 5 °C/min.

a single peak. These phenomena seem to suggest the presence of polymorphism in each complex. As may be seen in Table 2, the melting points, as determined by the usual method, correspond to the temperature of the second peaks in Fig. 4. Therefore, the shoulders of the first peaks in Fig. 4 may arise from the phase change in the solid states. The structure of these complexes are now being studied by means of X-ray diffraction analysis.

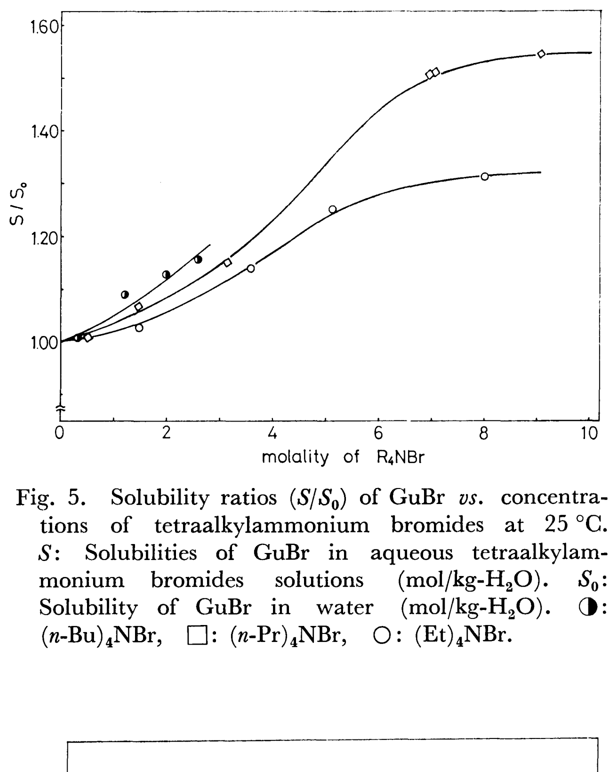

Fig. 5. Solubility ratios (S/S_0) of GuBr *vs.* concentrations of tetraalkylammonium bromides at 25 °C. S: Solubilities of GuBr in aqueous tetraalkylammonium bromides solutions (mol/kg-H_2O). S_0: Solubility of GuBr in water (mol/kg-H_2O). ◑: $(n\text{-Bu})_4NBr$, □: $(n\text{-Pr})_4NBr$, ○: $(Et)_4NBr$.

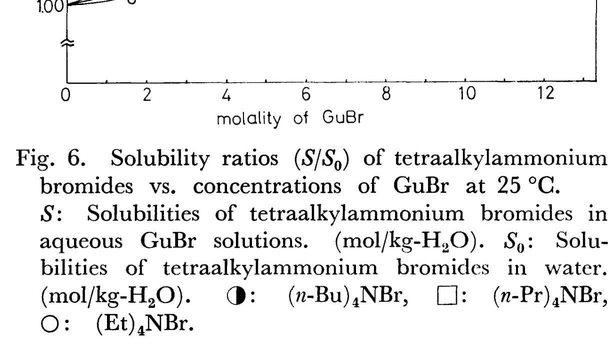

Fig. 6. Solubility ratios (S/S_0) of tetraalkylammonium bromides vs. concentrations of GuBr at 25 °C.
S: Solubilities of tetraalkylammonium bromides in aqueous GuBr solutions. (mol/kg-H_2O). S_0: Solubilities of tetraalkylammonium bromides in water. (mol/kg-H_2O). ◑: $(n\text{-Bu})_4NBr$, □: $(n\text{-Pr})_4NBr$, ○: $(Et)_4NBr$.

Solubility Measurement. As is shown in Fig. 1, 2, and 3, the solubility of each salt in a ternary solution increased as compared with that in pure water. The effect of tetraalkylammonium bromide on the solubility of GuBr is shown in Fig. 5. Each tetraalkylammonium bromide has a salting-in effect toward GuBr in aqueous solutions, $(n\text{-Bu})_4\text{NBr}$ having the strongest effect among them. As is shown in Fig. 6, GuBr also has a salting-in effect toward tetraalkylammonium bromide and is most effective toward $(n\text{-Bu})_4\text{NBr}$. These phenomena constitute the so-called "mutual salting-in" effect. These salting-in effects of GuBr toward tetraalkylammonium bromides are greater than those of urea.[5] This fact is consistent with the effectiveness of GuBr and urea for protein denaturation in aqueous solutions.

Discussion

Guanidinium halides form complexes with symmetrical tetraalkylammonium halides in aqueous solutions just as urea does, though the compositions of complexes are not the same. However, the fact that urea, thiourea,[5] and guanidinium halides form complexes with symmetrical tetraalkylammonium halides suggests that the shape, the size, and the functional group play important roles in the reaction of the complex formation, although there is a quite significant difference (electrolyte and nonelectrolyte) between urea and guanidinium halides.

$(n\text{-Bu})_4\text{NBr}$ does not form a clathlate hydrate, but does form complexes with guanidinium halides in the presence of enough guanidinium halide, even at low temperatures. This fact indicates the presence of a stronger interaction between guanidinium bromide and $(n\text{-Bu})_4\text{NBr}$ than between water and $(n\text{-Bu})_4\text{NBr}$. As for the structures of crystalline complexes, we must await a detailed X-ray analysis. However, the following things may be deduced. $(n\text{-Bu})_4\text{NBr}$ forms the clathlate hydrate in which the tetrabutylammonium ion is surrounded by a cage formed by water molecules and bromide ions.[9] In contrast with the clathlate hydrate, the amount of guanidinium bromide and water in the crystalline complex seems to be too small to form a cage. On the other hand, the difference of ionic size between the guanidinium ion and the tetrabutylammonium ion is too much to form a mixed crystal as in the case of alkali halide. Therefore, the crystal structures of these tetraalkylammonium halide-guanidinium halide complexes may be different from those of either a clathlate hydrate or a mixed crystal.

We can postulate two kinds of mechanisms for the mutual salting-in effect in the ternary systems. One is the salting-in due to the formation of a water-soluble complex in the ternary solutions (Mechanism A). If the water-soluble complex is a 1-to-1 complex of GuBr and tetraalkylammonium bromide, and if the specific hydration of the complex is not taken into account, the solubility ratio (S/S_0) must be proportional to the concentration of the solubilizing salt (m) and the slope must correspond to $KS/(1+KS_0)$, where K is the formation constant of the water-soluble complex. As has been stated in the experimental section, tetrabutylammonium bromide and tetraethylammonium bromide form a crystalline complex in which guanidinium bromide and tetraalkylammonium bromide are in a mole ratio of 1-to-1. However, as may be seen in Figs. 5 and 6, a linear relation between (S/S_0) and m was not obtained in either case. This fact suggests that even if the water-soluble complexes were formed in an mixed aqueous solution, the compositions of water-soluble complexes would be different from those of crystalline complexes.

The other mechanism is the salting-in due to the interaction between the guanidinium ion and the tetraalkylammonium ion through the alteration of the water structure (Mechanism B). The guanidinium ion is a structure breaker, while the tetraalkylammonium ions are hydrophobic structure makers. Since an aqueous solutions of tetraalkylammonium bromide is more structured than pure water, the guanidinium ion breaks the water structure in the aqueous solution of tetraalkylammonium bromide more clearly than that in pure water. This effect decreases the activity coefficient of guanidinium bromide. On the other hand, when tetraalkylammonium bromide is added to an aqueous solution of guanidinium bromide, which is less structured than pure water, the ability of the tetraalkylammonium ion to form a water structure will decrease as compared with that in pure water. Thus, the effect of the addition of guanidinium bromide on the structure of water is similar to that of the elevation of the temperature. This effect decreases the activity coefficient of tetraalkylammonium bromide.

We can say that Mechanism A is based on the contribution of the enthalpy change, while Mechanism B is based on that of the entropy change. The thermodynamic way of determining which of these two mechanisms is really working is to measure the free energy and the heat change on mixing these two salts in an aqueous solution. When the free energy change on mixing tetrabutylammonium bromide and guanidinium bromide in an aqueous solution was investigated, large negative free energy changes were obtained.[3] Therefore, a promising avenue to the elucidation of the mutual salting-in effect may be to measure the heat of mixing tetraalkylammonium and guanidinium bromides in aqueous solutions.

References

1) D. B. Wetlaufer, S. K. Malik, L. Stoller, and R. L. Coffine, *J. Am. Chem. Soc.*, **86**, 508 (1966).

2) W-Y. Wen and M. Lee, *J. Phys. Chem.*, **73**, 2895 (1969).

3) K. Miyajima, H. Yoshida, and M. Nakagaki, Abstr. No. 2M 11, 34th National Meeting of the Chemical Society of Japan, Hiratsuka, April 1976.

4) P. H. von Hippel and T. Sheich, "Structure and Stability of Macromolecules," ed by Tismasheff and G. Fasman, Marcel Dekker, Vol. 2, New York (1969), p. 417.

5) S. Saito, M. Lee, and W-Y. Wen, *J. Am. Chem. Soc.* **88**, 5107 (1966).

6) B. E. Conway, R. E. Verrall, and J. E. Desnoyers, *Trans. Faraday Soc.*, **62**, 2738 (1966).

7) K. Miyajima, H. Yoshida, and M. Nakagaki, *Nippon Kagaku Zasshi*, **1976**, 366.

8) S. H. Maron and C. H. Prutton, "Principles of Physical Chemistry," 3rd ed, Macmillan Co., New York (1958), p. 417.

9) W-Y. Wen, "Water and Aqueous Solution," ed by R. A. Horne, Wiley Interscience, New York (1972), p. 614.