BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 636—641 (1970)

Ultrasonic Study of Dilute Aqueous Solutions of Urea, Guanidine Hydrochloride and Dioxane

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(Received July 21, 1969)

An ultrasonic study on dilute aqueous solutions of urea, guanidine hydrochloride and dioxane was made by means of a pulse method, with the intention of investigating the influence of these solutes upon the structure of water. Ultrasonic absorption coefficient was measured in the range of frequency from 15 to 45 Mc/sec, and ultrasonic velocity was measured at 5 Mc/sec. Temperature was varied from 10 to 40°C. In urea solutions, the magnitude of α/f^2 (α=absorption coefficient, f=frequency) decreases first very slowly with concentration, then rapidly in the range from about 0.5 to 3M and at about 4—6M reaches a nearly constant value. In guanidine hydrochloride solutions a similar change in the relation α/f^2 *vs.* concentration takes place with a smaller amount than in urea solution. This shows that guanidine hydrochloride has greater ability as a structure breaker in liquid water than urea. In the dioxane-water system, absorption increases with the concentration of dioxane. A nearly flat region in the absorption *vs.* concentration curve is found for a small concentration of dioxane.

This feature in the α/f^2 *vs.* concentration curve, which is found in the small concentration region, is ascribed to the "cooperative breaking effect" upon the "open-packed structure" in water.

In preceding papers[1-3] we have reported results of ultrasonic investigation on aqueous solutions of urea, alkyl-substituted urea and dioxane by means of an ultrasonic pulse technique. These materials are known as denaturing agents for proteins. The main purpose of these studies was the investigation of the influence of solutes upon the structure of water. Along this line, we have further made a detailed investigation and have chosen urea, guanidine hydrochloride and dioxane as solutes in the present study.

Experimental

Apparatus and Procedures for Measurements. The ultrasonic velocity and absorption were measured according to the pulse method developed by Pinkerton.[4] The detailed procedures for the measurements were

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[1] K. Arakawa and N. Takenaka, This Bulletin, **40**, 2739 (1967).

[2] K. Sasaki and K. Arakawa, *ibid.*, **42**, 2485 (1969).

[3] K. Arakawa and N. Takenaka, *ibid.*, **42**, 5 (1969).

[4] J. M. M. Pinkerton, *Proc. Phys. Soc.*, **B62**, 86, 129, 286 (1949).

described in the preceding paper.[5] The sound velocity was measured at a fixed frequency of 5 Mc/sec. The absorption was measured in the frequency range of 15—45 Mc/sec.

Materials. Urea, guanidine hydrochloride and dioxane were obtained from Wako Pure Chemical Industry Co., Ltd. The melting points of the former two materials were 132.5±0.5°C and 175.9±0.5°C, respectively. The urea was the same as that used in the preceding study.[1] The dioxane was used after being treated according to the purification procedure described previously.[3] Its boiling point was 101.1—101.3°C.

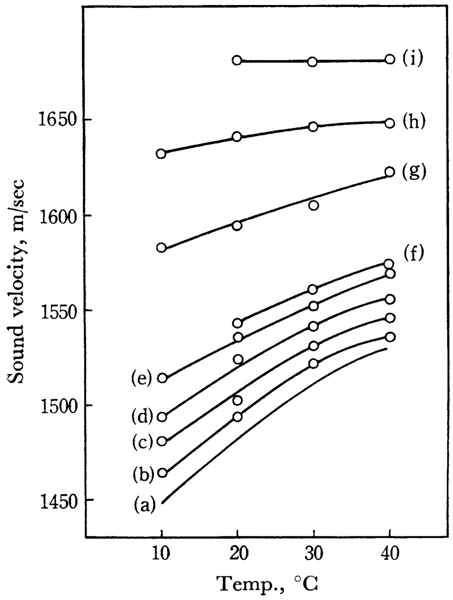

Fig. 1(a). Ultrasonic velocity of aqueous urea solutions.
Concn. (M: mol/*l*) (a) Pure water, (b) 0.5M, (c) 1M, (d) 1.5M, (e) 2M, (f)[3] 2.3_5M (13.6 wt%), (g)[3] 4.4_5M (25 wt%), (h)[3] 6.4M (35 wt%), (i)[3] 8M (42.9 wt%).

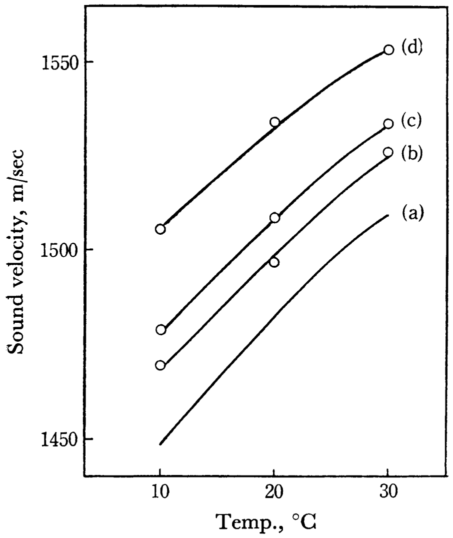

Fig. 1(b). Ultrasonic velocity of aqueous guanidine hydrochloride solutions.
Concn. (M: mol/*l*) (a) Pure water, (b) 0.3M, (c) 0.5M, (d) 1M

Results

Sound Velocity. The ultrasonic velocity at 5 Mc/sec obtained for dilute aqueous solutions of urea and guanidine hydrochloride is plotted against temperature in Figs. 1 (a) and (b). The velocity *vs.* concentration curves obtained for the dilute solutions of dioxane are given in Fig. 2, and those for the entire range composition, including earlier results,[3] are given in Fig. 3.

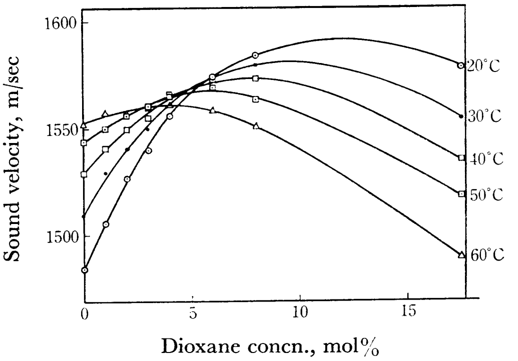

Fig. 2. Ultrasonic velocity of dioxane-water system.

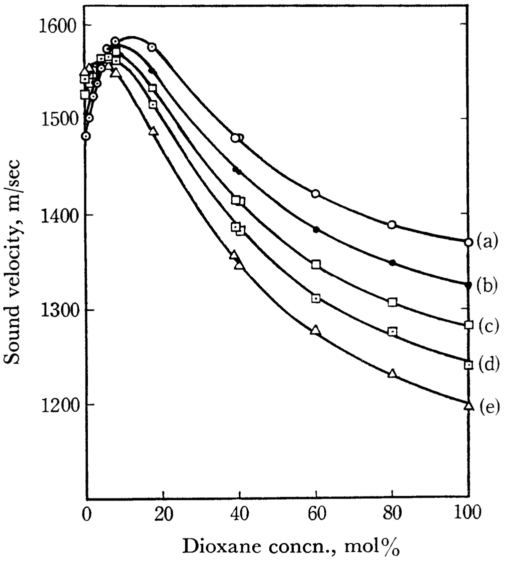

Fig. 3. Concentration dependence of ultrasonic velocity for dioxane-water system.
Temp.: (a) 20°C, (b) 30°C, (c) 40°C, (d) 50°C, (e) 60°C

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[5] K. Arakawa and N. Takenaka, This Bulletin, **40**, 2063 (1967).

We see from Fig. 2, that all curves cross at about 4 mol% of dioxane (about 2M of dioxane), but not at one point. The velocity *vs.* temperature curves for urea and guanidine hydrochloride solutions resemble each other. The accuracy of the data of sound velocity was within 2 m/sec.

Ultrasonic Absorption. The absorption coefficient, α, was measured for solutions of urea, guanidine hydrochloride and dioxane. The accuracy of α is within $\pm 2\%$. No relaxation frequency is observed for urea and guanidine hydrochloride solutions, as seen in Fig. 4(a) and (b). For the dioxane-water system, the α/f^2*vs.* $\log f$ curves given in Fig. 4(c) decrease gradually, but a relaxation frequency can not be determined.[3]

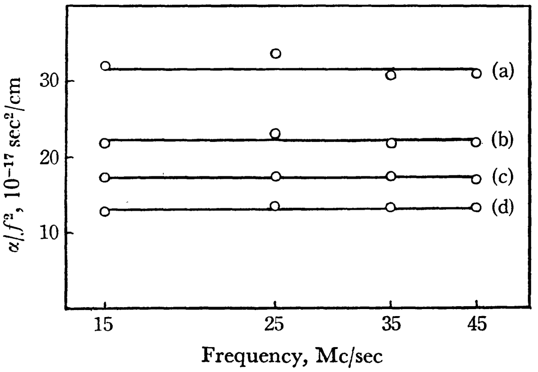

Fig. 4(a). Ultrasonic absorption of aqueous urea solutions. (1.5M urea)
Temp.: (a) 10°C, (b) 20°C, (c) 30°C, (d) 40°C

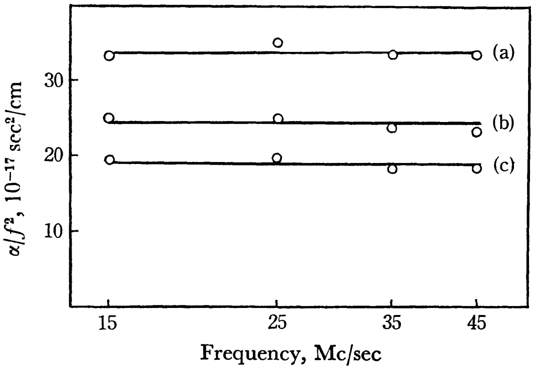

Fig. 4(b). Ultrasonic absorption of aqueous guanidine hydrochloride solutions. (0.5M guanidine hydrochloride)
Temp.: (a) 10°C, (b) 20°C, (c) 30°C

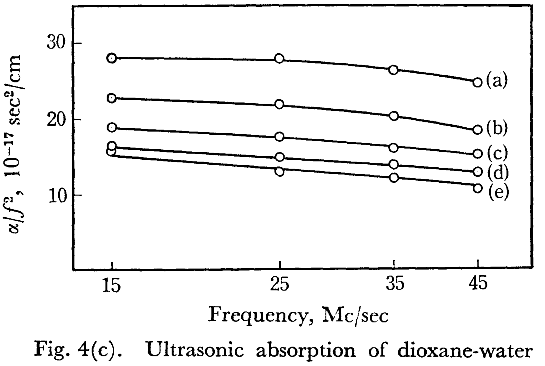

Fig. 4(c). Ultrasonic absorption of dioxane-water system. (4 mol% of dioxane)
Temp.: (a)20°C, (b) 30°C, (c) 40°C, (d) 50°C, (e) 60°C

Concentration Dependence of Ultrasonic Absorption. For urea and guanidine hydrochloride solutions, values of α/f^2 obtained are plotted against concentration by vol% in Figs. 5(a) and (b). In Fig. 6(a) the α/f^2 *vs.* concentration curves for urea solutions are plotted, including the previous data obtained for concentrated solutions,[1] those by Hammes and Schimmel[6] and those by Beauregard and Barrett.[7] In the range of concentration greater than 2M urea (about 9 vol%), the agreement of these data is very good. However, for a smaller concentration a noticeable difference is

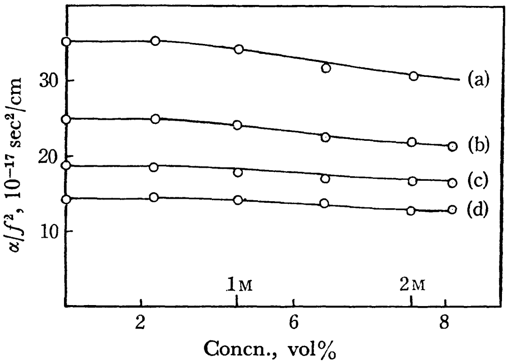

Fig. 5(a). Concentration dependence of ultrasonic absorption for urea solutions.
Temp.: (a) 10°C, (b) 20°C, (c) 30°C, (d) 40°C

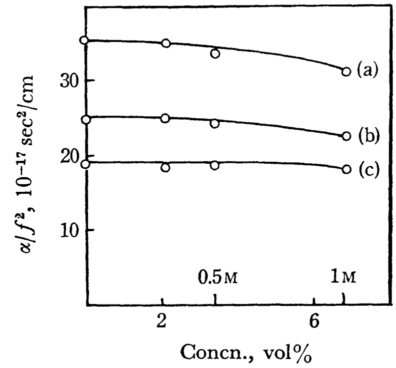

Fig. 5(b). Concentration dependence of ultrasonic absorption for guanidine hydrochloride solution.
Temp. (a) 10°C, (b) 20°C, (c) 30°C

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[6] G. G. Hammes and P. R. Schimmel, *J. Amer. Chem. Soc.*, **89**, 442 (1967).

[7] D. V. Beauregard and R. F. Barrett, *J. Chem. Phys.*, **49**, 5241 (1968).

observed between our present data and those of Hammes and Schimmel.

As seen in Fig. 6(a), the value of α/f^2 decreases first very slowly with concentration. At a concentration greater than 0.5M urea (about 2—3 vol%), it decreases more rapidly, and at about 4—6M becomes nearly constant again.

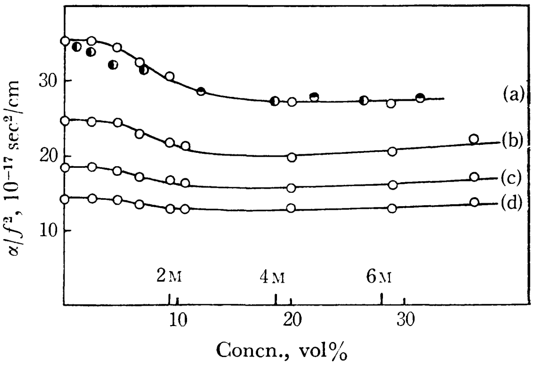

Fig. 6(a). Concentration dependence of ultrasonic absorption for aqueous solution of urea.
Temp.: (a) 10°C, (b) 20°C, (c) 30°C, (d) 40°C
○ Authors, ◐ Hammes and Schimmel,⁶⁾
◓ Beauregard and Barrett⁷⁾

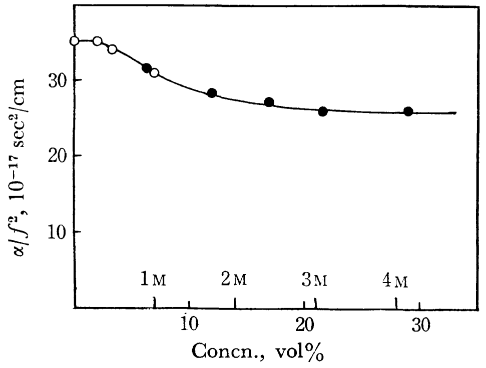

Fig. 6(b). Concentration dependence of ultrasonic absorption for guanidine hydrochloride solution at 10°C.
○ Authors, ● Hammes and Swann⁸⁾

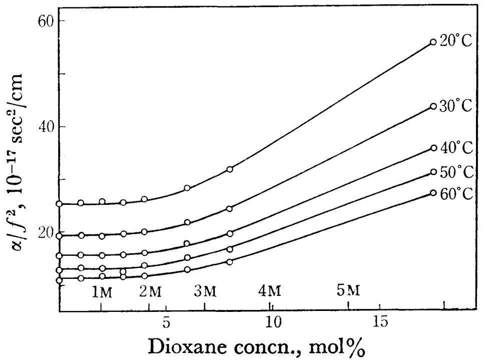

Fig. 7. Concentration dependence of ultrasonic absorption for dioxane-water system at 35 Mc.

In Fig. 6(b) the α/f^2 *vs.* concentration curve of guanidine hydrochloride solution at 10°C is given, including the data reported by Hammes and Swann.⁸⁾ In the curve, a tendency similar to that of urea solution is observed, though the decreasing rate of α/f^2 is greater than in urea solution.

For the dioxane-water system concentration dependence of α/f^2 is given in Fig. 7 for a smaller concentration than 17.5 mol% of dioxane. The results for the entire range of composition is given in Fig. 8, including earlier results.³⁾ As seen in Figs. 7 and 8, the α/f^2 *vs.* concentration curve is nearly flat in the concentration range smaller than about 4 mol% (about 17 vol%; about 2M in molarity). The magnitude of α/f^2 begins to increase rapidly at about 6 mol% of dioxane.

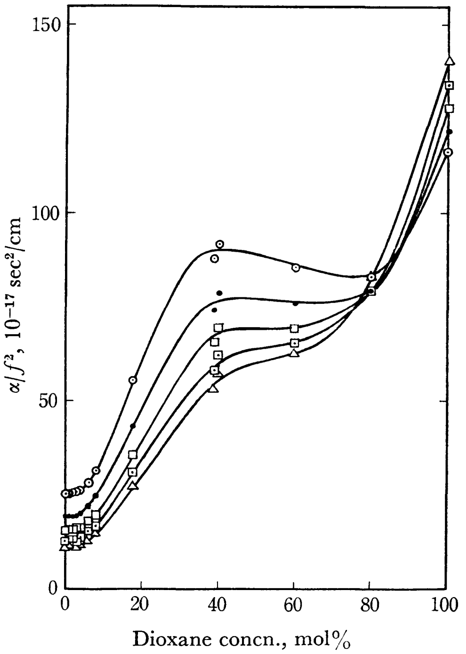

Fig. 8. Concentration dependence of ultrasonic absorption for dioxane-water system at 35 Mc.
Temp.: ⊙ 20°C, ● 30°C, □ 40°C, ⊡ 50°C, △ 60°C

Discussion

The Structure-Breaking Effect of Urea and Guanidine Hydrochloride. Studies have been made on the effect of denaturing agents of urea-guanidium class⁹⁻¹⁶⁾ upon the conformation of

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8) G. G. Hammes and J. C. Swann, *Biochemistry*, **6**, 1591 (1967).

9) W. Kauzmann, *Advan. Protein Chem.*, **14**, 1 (1959).

10) J. A. Gordon and W. Jencks, *Biochemistry*, **2**, 47 (1963).

protein molecules, and also on the effect of organic solvents such as dioxane,[13,17] ethanol. The effect of denaturing agents seems to be due to two factors: direct interaction between the agent and protein molecules,[10,11] and influence on conformation of protein molecules resulting from the change of the structure of water caused by the agent.[1,6,9,18]

Studies on the effect of denaturing agents upon the structure of water have been made recently by the authors,[1,2,18] Hammes *et al.*[6,8] and Beauregard and Barrett.[7] Concerning the behavior of urea in water, there are ambiguities.[19,20] However, from the results of ultrasonic studies, it has been concluded that urea behaves as a structure breaker for the clusters in water. Recently a statistical mechanical explanation of the behavior of urea as a breaker has been given by Frank and Franks.[21]

The data agree in the range of concentration greater than about 2M,[6,7] as seen in Fig. 6(a). However, for a concentration smaller than 2M, our data are greater than those of Hammes and Schimmel, and the curves by the authors are found to be nearly flat in the range of concentration smaller than about 0.5M. This tendency is seen at temperatures from 10°C to 40°C,[*1] as seen in Fig. 6(a). A similar tendency is observed in guanidine hydrochloride solution.

The α/f^2 *vs.* concentration curves for solutions of urea and guanidine hydrochloride are compared in Fig. 9. The magnitude of α/f^2 for guanidine hydrochloride solutions decreases more rapidly than for urea solutions. This supports the idea that guanidine has a greater ability as a structure breaker than urea. It is also seen in Fig. 9 that the constant value of α/f^2 at about 20—30 vol% (4—6M urea) for the urea solution is nearly equal to that for the guanidine solution at about the same volume concentration (3—4M guanidine). This suggests that the "open-packed structure" in water is nearly completely disrupted with addition of 4—6M urea, and nearly the same situation occurs with addition of 3—4M of guanidine hydrochloride.

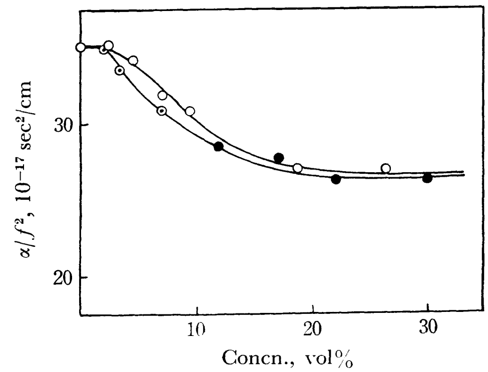

Fig. 9. Comparison between ultrasonic absorption for urea solutions and that for guanidine hydrochloride solution at 10°C.
○ Present data for urea solution, ⊙ Present data for guanidine hydrochloride solution, ● Hammes and Swann's data[8]

Cooperative Breaking[22] of Open-Packed Structure in Water through Addition of Solutes. Frank and Wen[22] have proposed the cooperative formation and breaking of clusters in water. A statistical theory based on the flickering cluster model has been developed by Némethy and Scheraga.[23] We have presented recently a two state theory based on the model proposed by Hall.[24,25]

The source of ultrasonic absorption is attributed to the presence of the "open-packed structure" or "flickering clusters" in water.[*2] This "structure" is in equilibrium with a high density region in water as represented by:

$$\text{open-packed structure} \rightleftarrows \text{close-packed structure,}$$

or

$$\text{flickering clusters} \rightleftarrows \text{unbonded monomers.} \qquad (1)$$

The lifetime of clusters is believed to be of the order of 10^{-11}—10^{-10} sec,[22,23] and the formation

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11) D. R. Robinson and W. Jencks, *J. Amer. Chem. Soc.*, **87**, 2462 (1965).

12) C. Tanford, *ibid.*, **86**, 2050 (1964).

13) C. Tanford, and P. K. De, *J. Biol. Chem.*, **236**, 1711 (1961).

14) C. Tandord, K. Kawahara and S. Lapanje, *ibid.*, **241**, 1921 (1966).

15) P. H. Hippel and K.-Y. Wong, *ibid.*, **240**, 3909 (1965).

16) K. Hamaguchi and A. Kurono, *J. Biochem.* (Tokyo), **54**, 111 (1963).

17) K. Hamaguchi and A. Kurono, *ibid.*, **54**, 497 (1963).

18) M. Watase and K. Arakawa, *Nippon Kagaku Zasshi*, **89**, 383 (1968).

19) M. Abu-Hamdiyyah, *J. Phys. Chem.*, **69**, 2720 (1965).

20) A. Holtzer and M. F. Emerson, *ibid.*, **73**, 26 (1969).

21) H. S. Frank and F. Franks, *J. Chem. Phys.*, **48**, 4746 (1968).

[*1] According to Hammes and Schimmel,[6] a shoulder was observed in the curve at 25°C in dilute solutions (Fig. 1), but it disappears at 10°C.

22) H. S. Frank and W. Y. Wen, *Discuss. Faraday Soc.*, **24**, 133 (1957).

23) G. Némethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3382 (1962).

24) L. Hall, *Phys. Rev.*, **73**, 775 (1948).

25) K. Arakawa and K. Sasaki, This Bulletin, **42**, 303 (1969).

[*2] The situation is different in the case of a uniform state theory,[26] but this theoy can not explain the origin of ultrasonic absorption.

26) M. Falk and T. A. Ford, *Can. J. Chem.*, **44**, 1699 (1966); E. U. Franck and K. Roth, *Discuss. Faraday Soc.*, **43**, 108 (1967).

and breaking of them is supposed to proceed "cooperatively," as proposed first by Frank and Wen. As seen in Fig. 10, the magnitude of α/f^2 for urea solutions is greater than that given by the dotted line in an extremely small concentration. The dotted line represents calculated magnitude of absorption attributed to only water as a solvent under the assumption that no structural change takes place in water.[1,2] The α/f^2 *vs.* concentration curve at 10°C is seen to cross the line at about 1 M, and the magnitude of α/f^2 decreases further with concentration. It is concluded, therefore, that the effect of urea as a structure breaker is not appreciable in an extremely dilute solution, and that the effect becomes appreciable with the increase of urea concentration (about 0.5—1 M urea). This trend in the concentration dependence of α/f^2 for a small concentration seems to be ascribed to the cooperative nature of cluster formation and breaking.

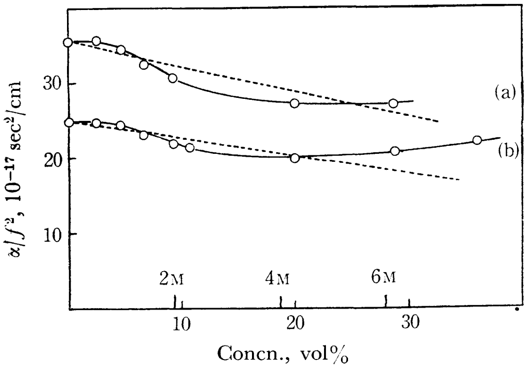

Fig. 10. Concentration dependence of ultrasonic absorption for urea solutions.
Temp. (a) 10°C, (b) 20°C
Dotted line; absorption due to water as solvent.

When a very small amount of urea is added to water, the influence of urea molecules upon the structure of water is isolating, and does not affect the cooperative formation of clusters. Phenomenologically, urea molecules enter the empty space among water molecules with no appreciable influence upon the structure. When the amount of urea becomes greater, the influence becomes appreciable, and urea affects the clusters cooperatively to disrupt them into unbonded monomers. Therefore, for a greater concentration, ultrasonic absorption decreases rapidly with concentration.

The situation is similar in guanidine hydrochloride solutions, the influence being greater than that of urea.

As for the dioxane-water system, the over-all increasing trend of α/f^2 *vs.* concentration curves becomes pronounced because of a large absorption of pure dioxane,[3] but a similar flat region appears for a small concentration. As seen in Figs. 7 and 8, α/f^2 *vs.* concentration curves are nearly flat for a concentration of dioxane less than 4 mol%. This behavior is also attributed to the cooperative nature of cluster formation and breaking. However, the concentration range of nearly flat region is clearly broader than that in urea and guanidine solutions. This is ascribed to the fact that in a dioxane molecule the structure-breaking ability owing to two ether oxygens is diminished in part by the structure-forming ability[27,28] owing to two ($-C_2H_4-$) groups.

Summary and Conclusion

An ultrasonic study on dilute aqueous solutions of urea, guanidine hydrochloride and dioxane has been made by means of a pulse technique in order to investigate the influence of solutes upon the structure of water.

The following conclusions have been obtained.

1) Urea behaves as a structure breaker in water.

2) Guanidine hydrochloride has a greater ability as a breaker than urea.

3) For a small concentration, the structure-breaking effect is not appreciable. This is ascribed to the cooperative nature of cluster formation and breaking in aqueous solutions.

4) In the case of the dioxane-water system, the ability of dioxane as a breaker is diminished in part by the structure-forming effect owing to two ($-C_2H_4-$) groups within a dioxane molecule.

The authors wish to thank Miss Yuko Endo for her cooperation in carrying out this study.

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27) G. Némethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3401 (1962).

28) D. B. Wetlaufer, S. K. Malik, L. Stoller and R. L. Coffin, *J. Amer. Chem. Soc.*, **86**, 508 (1964).