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The Ultrasonic Study of Aqueous Solutions of Urea

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An ultrasonic investigation of aqueous solutions of urea was made by means of the pulse technique. The ultrasonic velocity and absorption were measured on solutions of about 14—43 wt% in the frequency range from 5 to 45 Mc/sec. In an 8 M urea solution the velocity *vs.* the temperature curve was found to be horizontal in the temperature range from 20 to 40°C. The α/f^2 *vs.* $\log f$ curves (α, absorption coefficient; f, frequency) showed a very small frequency dependence throughout this experiment. The magnitude of α/f^2 first decreases upon the addition of urea; at about 4—6 M of urea it reaches a shallow minimum, thereafter slowly increasing. At about 8 M of urea the contribution to the ultrasonic relaxation from the open-packed structure in water completely disappears, and the contribution from the water-urea structure as well as that from the association of urea is small.

It has been well known that urea is a good reagent for the denaturation of protein; it is generally believed that the denaturation is due to the unfolding of the protein molecules with the higher-order structure. The important roles of the urea molecules in this process are thought to be the breaking of hydrogen bonds and that of hydrophobic interactions, by which means the higher-order structures are formed.¹⁾ The situation is very complicated, and no decisive conclusions concerning the behavior of urea have been obtained.

Therefore, it is important to investigate the behavior of urea molecules in aqueous solutions. There is yet some ambiguity concerning the behavior of urea.²⁾ We have now, then, studied some properties of aqueous solutions of urea by measuring the ultrasonic velocity and absorption by means of the pulse method. The results will be reported in this paper.

Experimental

An ultrasonic pulse method was used for the measurements of the sound velocity and of the absorption. The apparatus used and the procedure for the measurements have been described in a previous paper.³⁾ The ultrasonic absorption was determined over the frequency range of 15—45 Mc/sec and at temperatures from 10 to 40°C. The sound velocity was measured at 5 Mc/sec. The temperature was held constant to within ±0.1°C. The sample of urea used was Wako's guaranteed reagent in the crystal form.[*1] Aqueous solutions of urea with four different concentrations within the range of about 14—43 wt% were prepared.

Results

Ultrasonic Velocity. The accuracy of the data of sound velocity was within 2 m/sec. The results obtained are plotted against the temperature in Fig. 1, where the sound velocity of pure

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1) R. B. Simpson and W. Kauzmann, *J. Am. Chem. Soc.*, **75**, 5139 (1953); G. Némethy, *Angew. Chem., international edit.*, **6**, 195 (1967).

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

3) K. Arakawa and N. Takenaka, This Bulletin, **39**, 447 (1966).

[*1] Wako Pure Chemical Industry Co., Ltd., melting point: 132.5±0.5°C.

water obtained by Greenspan *et al.*[4] is also given. As may clearly be seen in Fig. 1, the initial slope of the sound velocity with respect to the temperature decreases upon the increase in the concentration of urea. At the urea concentration of 8 M, which is often used in the study of the denaturation of proteins, the ultrasonic velocity becomes independent of the temperature at temperatures from 20°C to 40°C.

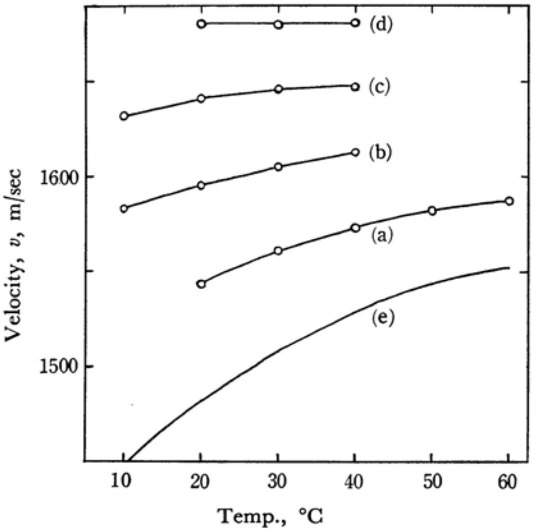

Fig. 1. Ultrasonic velocity of aqueous solutions of urea.
(a) 13.6 wt%, (b) 25 wt%, (c) 35 wt%, (d) 8 mol/*l* (42.9 wt%), (e) Pure water

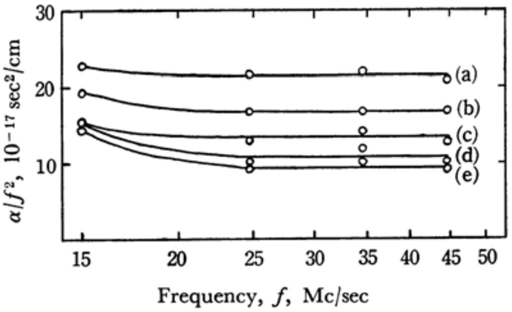

Fig. 2(a). Ultrasonic absorption of aqueous solutions of urea.
Urea: 13.6 wt%
(a) 20°C, (b) 30°C, (c) 40°C, (d) 50°C, (e) 60°C

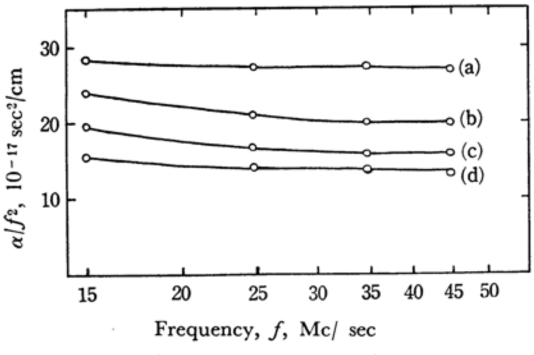

Fig. 2(b). Ultrasonic absorption of aqueous solutions of urea.
Urea: 25 wt%
(a) 10°C, (b) 20°C, (c) 30°C, (d) 40°C

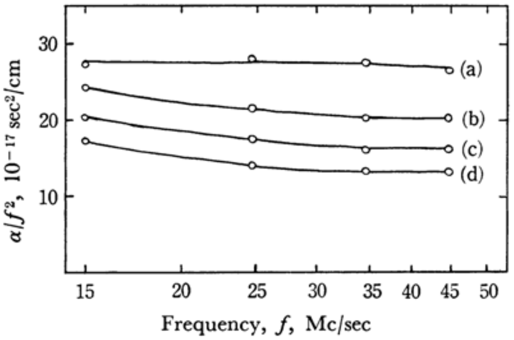

Fig. 2(c). Ultranonic absorption of aqueous solutions of urea.
Urea: 35 wt%
(a) 10°C, (b) 20°C, (c) 30°C, (d) 40°C

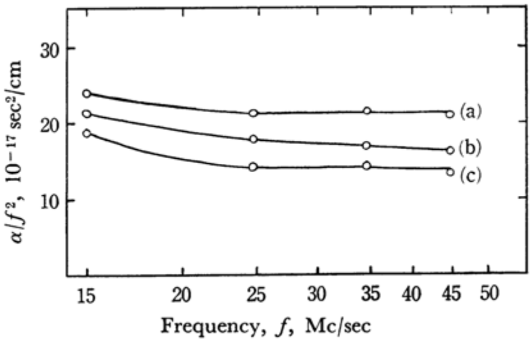

Fig. 2(d). Ultrasonic absorption of aqueous solutions of urea.
Urea: 8 mol/*l* (42.9 wt%)
(a) 20°C, (b) 30°C, (c) 40°C

Ultrasonic Absorption. The absorption coefficient, α, was measured for solutions of 13.6, 25, 35 wt% and 8 M of urea (42.9 wt%) over the frequency range from 15 to 45 Mc/sec. The accuracy of α obtained was within $\pm 2\%$. In Figs. 2(a)—2(d) α/f^2 is plotted against $\log f$. The α/f^2 values of these solutions are smaller than that of pure water and are nearly constant over the frequency range of 25—45 Mc/sec. In Fig. 3 these constant values of α/f^2 are plotted against the concentration of urea at each temperature. In Fig. 3a shallow minimum is seen at about 25—35 wt%. Over the concentration range of about 20—40 wt% the α/f^2 values are practically constant. The dotted curves given in Fig. 3 represent

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[4] M. Greenspan and C. E. Tschiegg, *J. Res. Natl. Bur. Stds.*, **58**, 249 (1957), *J. Acoust. Soc. Am.*, **31**, 75 (1959).

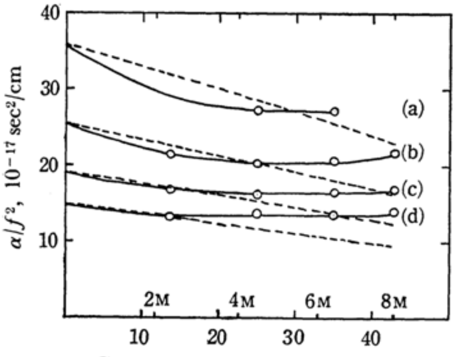

Fig. 3. Concentration dependence of ultranonic absorption for aqueous solutions of urea.
(a) 10°C, (b) 20°C, (c) 30°C, (d) 40°C

the calculated magnitude of absorption at each temperature, with reference only to water according to its volume fraction.

Discussion

It has been widely believed that urea acts as a hydrogen bond breaker. However, the true situation is not so simple. Eliassaf *et al.* have reported that urea behaves as a hydrogen-bond breaker in aqueous solutions of polyacrylic acid, while in aqueous solutions of polymethacrylic acid it does not.[5)]

According to Kauzmann and his coworkers,[1)] the rupture of hydrogen bonds is only one of several processes which may control the rate of the unfolding of the ovalbumin molecule in a urea solution. Maeda and his coworkers have concluded, from the results of their viscometric study[6)] of aqueous solutions of polyvinyl alcohol, that urea behaves as a breaker of the intramolecular hydrogen bonds in the solutions, and also that its ability as a breaker is not as great as that of sodium hydroxide.

On the other hand, Levy *et al.* have recently concluded, from the titration data on some dicarboxylic acids,[7)] that urea does not have any great effect on the intramolecular hydrogen bonds. However, the situation is quite different in the studiy of Levy *et al.* compared with that in the earlier studies described above, because their study was concerned with the intramolecular hydrogen bonds within small molecules, where the specific molecular structure has definitely some effect.

As has been stated above, the behavior of urea as the addend in some aqueous solutions is not simple. However, it is certain that urea has a great effect upon hydrogen bonds, hydrophobic bonds, *etc.* Especially, urea may be supposed to have an effect upon the structure of liquid water, to increase the activity of water as a solvent, and, thereafter, to have an effect upon the state of solute molecules in aqueous solutions.

As may be seen in Fig. 3, the ultrasonic absorption in urea solutions is lower than that in the water component only (shown by dotted curves), which has been calculated according to the volume fraction from the absorption of pure water. From this fact it can surely be said that urea molecules destroy the "open-packed structure" or the "flickering clusters" in liquid water,[8-10)] which are the main sources of ultrasonic absorption in water in this frequency range. The rupture of the open-packed structure in water results in an increase in the mole fraction of unbonded water molecules. When urea is added to water, it results in the rupture of the open-packed structure and causes a reduction of the ultrasonic absorption. The contributions of the urea-water structure and the association of urea, (urea)$_n$,[*2] to the total ultrasonic relaxation are clearly small, as may be seen in Fig. 3. This is supported by the report of Klotz *et al.* in which they concluded, from the data of infrared spectroscopy, that the fraction of the dimer of urea is very small in its aqueous solution.[11)]

The small increase in the magnitude of α/f^2 after it reaches its minimum at about 4—6 M of urea may be supposed to be the contribution from the structure involving urea, the main portion of which is some urea-water structure.

It has been concluded that the effect of urea on the water structure is the rupture of the open-packed structure. This destruction is nearly completed at the concentration of about 6 M of urea. This conclusion is confirmed by the data on ultrasonic velocity given in Fig. 1. The initial increase in the velocity with respect to the temperature becomes slower when urea is added, and at the concentration of 8 M of urea the plot of velocity *vs.* temperature become horizontal. This result is ascribed to the increase in the "close-packed structure" or the increase in the number of unbonded water molecules.

This behavior of urea solutions is supported by the thermodynamical study of Scatchard *et al.*[12)]

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5) J. Eliassaf and A. Silberberg, *J. Polymer Sci.*, **41**, 33 (1959).

6) H. Maeda, T. Kawai and S. Seki, *Kobunshi Kagaku (Chem. High Polymers)*, **15**, 719 (1958).

7) M. Levy and J. P. Magoulas, *J. Am. Chem. Soc.*, **84**, 345 (1962).

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

9) C. M. Davis and H. A. Litovitz, *J. Chem. Phys.*, **42**, 2563 (1965).

10) G. Némethyl and H. A. Scheraga, *ibid.*, **36**, 3382 (1962).

*2 (urea)$_n$: n-mer of urea.

11) I. M. Klotz and J. S. Franzen, *J. Am. Chem. Soc.*, **84**, 3461 (1962).

12) G. Scatchard, W. J. Hamer and S. E. Wood, *ibid.*, **60**, 3061 (1938).

According to them, the activity of water in urea solutions becomes larger than that in an ideal mixture, and the activity coefficient is at its maximum at about 6—8 M of urea.

Summary and Conclusions

When urea is added to water, the open-packed structure linked with hydrogen bonds is broken up into unbonded water molecules. The rupture is nearly completed at about 6 M of urea. The unbonded water molecules are more active than the associated ones. At the concentration of 8 M of urea, the contribution of the open-packed structure to the ultrasonic relaxation completely disappears. The contributions from the water-urea structure and from the association of urea are very small.