An Investigation of Water–Urea and Water–Urea–Polyethylene Glycol Interactions[1]

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Abstract: Ultrasonic attenuation measurements in aqueous urea solutions have been made in the presence and absence of a synthetic polymer, polyethylene glycol. No relaxation process is observed in aqueous urea solutions (0–8 *m* urea) over the frequency range investigated (18–175 Mc/sec at 10 and 25°). The quantity α/f^2, where α is the pressure amplitude absorption coefficient and f is the frequency, changes markedly between 0 and 4 *m* urea, and is then independent of urea concentration. The velocity of sound also depends markedly on urea concentration. A detailed analysis of the data suggests that a cooperative change in the solvent structure is primarily responsible for the decreased attenuation and increased sound velocity caused by urea addition to water. Ultrasonic attenuation measurements in aqueous polyethylene glycol solutions have been made at 10° over the molecular weight range 600–20,000. A single relaxation process is observed for all molecular weights in the frequency range of 14–175 Mc/sec. The relaxation time increases markedly with molecular weight up to a molecular weight of approximately 7000 and is then independent of molecular weight; no concentration dependence of the relaxation time is observed over the entire range of molecular weights. This relaxation process is attributed to a cooperative change in the local water and hydrophobic structure associated with the polymer. The relaxation time for the polymer of molecular weight 20,000 has been studied at 10° as a function of urea concentration. This relaxation time decreases sharply between 2.0 and 4.0 *m* urea; it is independent of urea concentration above 4.0 *m* (up to 8.0 *m*) and below 2.0 *m*. The sharp change is attributed to a cooperative breakdown in the local water structure around the polymer which is probably accompanied by an increased solvation and loosening of the polymer structure. Intrinsic viscosity measurements support these conclusions. The relationship of these findings to the interpretation of studies of protein denaturation in aqueous urea solutions is discussed.

Although urea is commonly employed to denature proteins and nucleic acids, the mechanism of denaturation by urea is still not well understood. Several studies have shown that the denaturation process is cooperative,[3-5] *i.e.*, spectral changes accompanying denaturation occur over a relatively small range in urea concentration. Specific interactions of urea with the macromolecule and/or changes in the solvent structure may be responsible for the denaturation process, but the relative importance of each of these processes is not known. An understanding of this mechanism should provide information about forces involved in determining the structure of macromolecules in aqueous solutions.

Considerable evidence exists which indicates that the solvent plays a major role in determining the configuration of macromolecules in solution. For example, Tanford and his associates have shown that β-lactoglobulin[6] and ribonuclease[7] undergo marked configurational changes when organic solvents are added to aqueous solutions of the respective proteins. The helix–coil transition of polybenzyl glutamate is brought about by small variations in the solvent composition.[8] Results from theoretical and experimental investigations[9-11] of the structure of water have suggested that water possesses a high degree of order arising from the cooperative formation of water clusters or "ice-like" structures. These structures are important determinants of the thermodynamic properties of aqueous solutions of hydrocarbons[12-15] and of the stability of the "hydrophobic bond" in proteins.[14]

Urea is known to increase the solubility of aliphatic and aromatic amino acids[16] and nucleotides.[17] This may be due to specific interactions with urea or may be due to the effect of urea on the structure of water. The suggestion has been made that addition of urea causes a breakdown and/or alteration in the water structure.[14,18] The alteration of the water structure could cause disruption of hydrophobic bonds in proteins and result in denaturation.[14,18] A small amount of evidence has been presented which supports the idea that urea disrupts the water structure,[18] but an argument to the contrary has also been presented.[19]

We present here the results of a study of ultrasonic absorption in aqueous urea solutions in the presence and absence of a synthetic polymer, polyethylene glycol. The measurement of ultrasonic attenuation is useful for probing the microscopic solvent structure[20-22] and may be used to study relaxation processes

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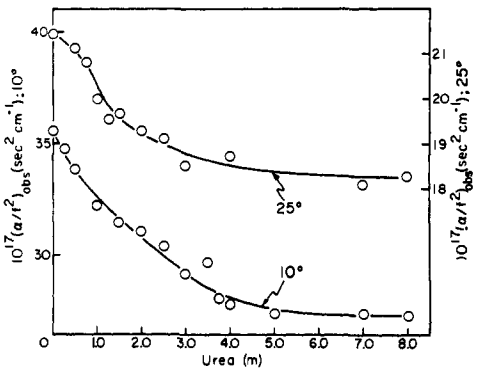

Figure 1. $(\alpha/f^2)_{obsd}$ *vs.* molality of urea at 10 and 25°.

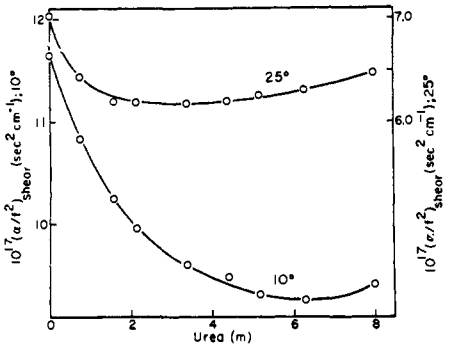

Figure 2. $(\alpha/f^2)_{shear}$ *vs.* molality of urea at 10 and 25°.

with characteristic time constants of 10^{-7}–10^{-10} sec.[23,24] The results obtained strongly support the hypothesis that urea causes a marked change in the water structure. Urea also causes a change in the local water structure around the polymer. The changes in the local water structure occur at higher urea concentrations than the changes in the bulk solution. Furthermore, urea appears to cause an increased solvation of the polymer which may be accompanied by a loosening of the polymer structure.

Experimental Section

The ultrasonic absorption apparatus and the technique for obtaining data have been previously described.[20,21,23,24]

Fresh urea solutions (Baker analyzed reagent) were prepared daily and for each concentration investigated. Values of α/f^2 (α is the pressure amplitude absorption coefficient; f is the frequency of the ultrasonic wave) and v, the sound velocity, were determined at two or more frequencies for each urea concentration at 10 and 25°. The error in the values of α/f^2 and velocities is estimated to be ±2%.

Samples of sharp molecular weight fractions of polyethylene glycol were generously donated by Dow Chemical Co., Midland, Mich. Values of α/f^2 for various polymer solutions were determined at 11–15 frequencies spaced between 10 and 175 Mc/sec. No dispersion in the velocities was observed.

Densities and viscosities of aqueous urea solutions, aqueous urea–polymer solutions, and aqueous polymer solutions were determined with Fisher pycnometers and Cannon-Fenske viscometers, respectively. The error in the densities and viscosities is estimated as ±0.1 and ±1.0%, respectively.

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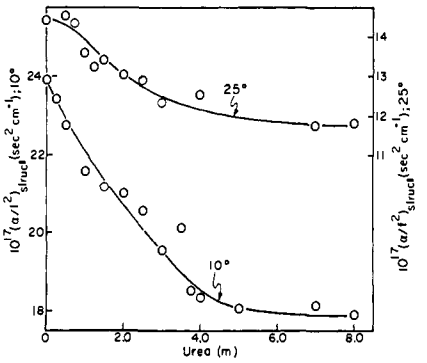

Figure 3. $(\alpha/f^2)_{structural}$ *vs.* molality of urea at 10 and 25°.

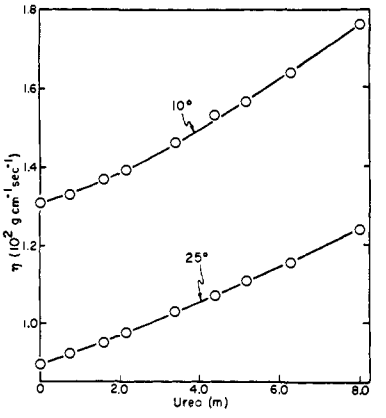

Figure 4. Viscosity *vs.* molality of urea at 10 and 25°.

Results and Treatment of Data

Plots of α/f^2 *vs.* urea concentration at 10 and 25° are given in Figure 1. No relaxation process was observed in the frequency range investigated (18–175 Mc/sec). The experimentally observed values of α/f^2, $(\alpha/f^2)_{obsd}$, can be split into two parts.

$$(\alpha/f^2)_{obsd} = (\alpha/f^2)_{shear} + (\alpha/f^2)_{structural} \quad (1)$$

The first term is due to shear viscosity and the second to structural effects. For an isotropic continuum fluid[23,24]

$$(\alpha/f^2)_{shear} = (8\pi^2/3v^3\rho)\eta \quad (2)$$

where η is the viscosity and ρ is the density of the liquid. The contribution of the shear and structural effects to $(\alpha/f^2)_{obsd}$ are given in Figures 2 and 3; the shear viscosities are displayed in Figure 4.

The adiabatic compressibility, κ_s, may be calculated from the equation[23,24]

$$\kappa_s = 1/\rho v^2 \quad (3)$$

Values of ρ and v are given in Table I and κ_s is displayed in Figure 5.

In all polyethylene glycol solutions investigated, the frequency dependence of α/f^2 could be accurately represented by a single relaxation time. Theoretical curves of α/f^2 *vs.* log f were constructed to determine relaxation times according to the equation[24]

$$\alpha/f^2 = \frac{A\tau}{1 + (\omega\tau)^2} + B \quad (4)$$

where $\omega = 2\pi f$ and A and B are constants.

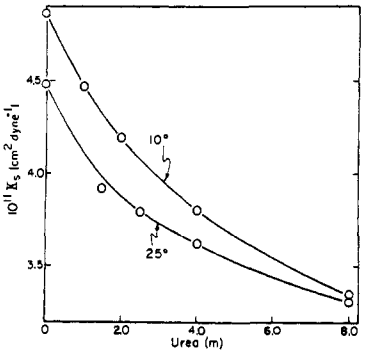

Figure 5. Adiabatic compressibility, κ_s, *vs.* molality of urea at 10 and 25°.

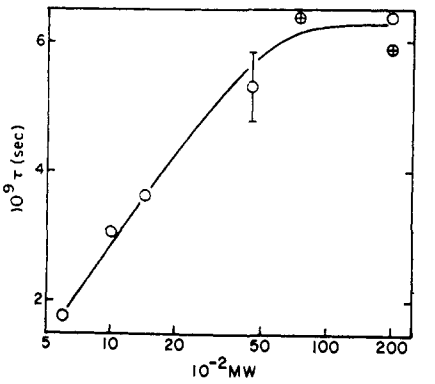

Figure 6. Relaxation time, τ, *vs.* molecular weight; ⊕, data from ref 21; O, this study. The error bracket corresponds to a 10% error in the relaxation time.

Relaxation times of polyethylene glycol–water solutions were determined as a function of concentration over the molecular weight range of 600–20,000. The ultrasonic parameters at various concentrations and molecular weights are given in Table II. (Higher concentrations were employed at lower molecular weights because $A\tau$ was much smaller than for higher molecular weight polymers.) The relaxation time is independent of concentration (Table II) but is dependent on molecular weight (see Figure 6).

Table I. Densities and Ultrasonic Velocities for Water–Urea Solutions

Urea, *m*	Density, g/cc[a] 10°	25°	$10^{-5}v$, cm/sec 10°	25°
0.0	0.9997	0.9970	1.44	1.50
1.0	1.016		1.49	
1.5		1.020		1.58
2.0	1.030		1.52	
2.5		1.032		1.60
4.0	1.055	1.050	1.58	1.62
8.0	1.094	1.086	1.65	1.67

[a] Values taken from a curve of density *vs.* molality of urea with experimental points at 0.0, 1.58, 3.39, 6.00, and 8.00 *m*.

Relaxation times for solutions of polyethylene glycol, mole w 20,000, were determined as a function of urea concentration at constant mole fraction, 0.035, of the ethylene oxide monomer. The ratio of polymer monomer unit to solvent molecules was, therefore, constant for all experiments rather than the molal concentration of polymer monomer units. Since the relaxation time is independent of polymer concentration, the differences in polymer molalities (~2.0 *m* in pure H_2O and ~1.5 *m* in 8 *m* urea) is inconsequential. Intrinsic viscosities in aqueous solution at 10° were determined over the molecular weight range of 1000–20,000. The intrinsic viscosity $[\eta]$ is accurately represented by the equation

$$[\eta] = 10^{-3.24}M^{0.66} \quad (5)$$

where M is the molecular weight. Intrinsic viscosities of the M = 20,000 species were also determined in aqueous urea solutions at 10° (Figure 7).

Discussion

The observed absorption, $(\alpha/f^2)_{obsd}$, displayed in Figure 1 is made up of the two parts shown in Figures 2 and 3. The sound absorption (or energy losses) given by eq 2, $(\alpha/f^2)_{shear}$, represents the sound absorption due to the viscous shear stresses which occur when a pressure wave is propagated through the medium at a frequency well below the viscosity relaxation frequency of the liquid (~10^{12} cps for H_2O). The contribution of the structural properties of the liquid is given by $(\alpha/f^2)_{structural}$. When a pressure (or sound) wave is propagated through the medium, the liquid structure is always at equilibrium with respect to the oscillating pressure at frequencies below the structural relaxation frequency (~10^{11}–10^{12} cps for H_2O). Energy losses occur because the ultrasonic wave perturbs the liquid

Table II. Ultrasonic Parameters at 10°

Polymer mol wt	Polymer concn, *m*	10^{-5} *v*, cm/sec	$10^8A/m$, sec cm^{-1} *m*$^{-1}$	$10^{17}B$, sec^2 cm^{-1}	$10^9\tau$, sec
600	2.0	1.51	1.4	40.0	1.8 ± 0.4
	4.0	1.57	1.2	45.5	1.9 ± 0.3
1,000	2.0	1.52	1.3	40.65	3.1 ± 0.5
	4.0	1.57	1.3	48.0	2.7 ± 0.3
1,450	2.0	1.53	1.5	41.0	3.6 ± 0.4
4,500	2.0	1.53	1.7	41.5	5.3 ± 0.5
7,500[a]	1.20	1.48	1.3	39.8	6.9
	1.98	1.51	1.2	43.2	6.4
20,000[a]	1.20	1.48	1.2	40.1	7.6
	1.98	1.50	1.4	42.6	5.9
20,000	2.0	1.54	1.4	42.0	6.4 ± 0.7

[a] Data from ref 21.

structure. In a hypothetical completely structureless liquid, $(\alpha/f^2)_{structural}$ would be zero and sound absorption arises only from $(\alpha/f^2)_{shear}$. This situation is encountered in monatomic gases.[24]

In pure water, the ratio of the structural to the shear contribution of $(\alpha/f^2)_{obsd}$ is 2.05 at both 25 and 10°. Addition of urea to water causes a marked decrease in both the structural and viscous contributions to the absorption (Figures 2 and 3), although $(\alpha/f^2)_{shear}$ begins to increase at high urea concentrations. This increase is due to the larger viscosities of aqueous urea solutions at high urea concentrations (Figure 4). In 8 *m* urea the ratio of $(\alpha/f^2)_{structural}$ to $(\alpha/f^2)_{shear}$ is 1.83 and 1.90 at 25 and 10°, respectively. Thus, the relative contribution of $(\alpha/f^2)_{structural}$ to the total absorption is somewhat less in 8 *m* urea than in pure water.

The large and sharp decrease in $(\alpha/f^2)_{structural}$ strongly suggests that addition of urea to water causes a change in the solvent structure. Furthermore, the large decrease in the adiabatic compressibility (Figure 5) and increase in density (Table I) is consistent with the viewpoint that a much more closely packed structure is formed as urea is added to water. (In aqueous solutions, there is usually little numerical difference between the adiabatic and isothermal compressibilities at the temperatures of interest here.) This may be due to an increase in the population of unbonded water molecules which pack more tightly than the molecules which are hydrogen bonded in the water clusters.[11] Since the entropy of dilution of urea solutions is positive, the water structure is also probably less ordered in aqueous urea solutions than in the pure liquid.[18] The temperature dependence of the viscosity varies little with increasing urea concentrations. The Arrhenius activation energies for pure water and 8 *m* urea are 4.2 and 3.9 kcal/mole, respectively. Apparently, changes in this activation energy are not a very sensitive measure of structural changes in aqueous urea solutions.

The nonideality of aqueous urea solutions has been quantitatively accounted for by the assumption that urea may self-polymerize through hydrogen bonding.[25] The estimated association constant for dimerization at 25° is 0.041 M^{-1}, and a volume change of 3 cc/mole has been calculated.[14,25] Because of this large volume change, the excess ultrasonic absorption due to this equilibrium should be relatively large and easily detectable.[23] However, no relaxation process is observed in the frequency range of 18–175 Mc/sec. It is unlikely, though not impossible, that this equilibrium is relaxing at higher frequencies because the observed absorption is less than that of the pure solvent; the converse would be expected if a chemical reaction were occurring with a characteristic relaxation frequency above 200 Mc/sec. If the dimerization relaxation frequency is above 200 Mc/sec, then $(\alpha/f^2)_{structural}$ of urea solutions is even smaller than calculated (Figure 3). The possibility also exists that the relaxation frequency is well below 10 Mc/sec but this is also very unlikely since hydrogen bonding reactions are usually quite rapid.[22,26,27] Therefore, any significant self-association of urea in a chemical sense in aqueous urea solutions is doubtful. A similar conclusion has been reached previously.[28]

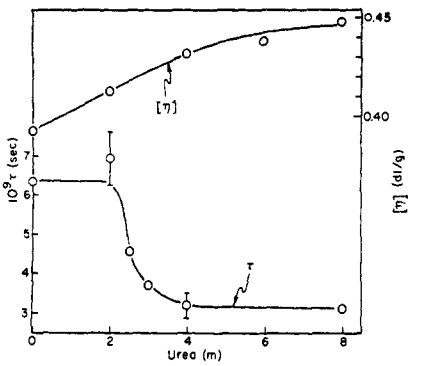

Figure 7. Relaxation time, τ, and intrinsic viscosity, $[\eta]$, *vs.* molality of urea. The error bracket corresponds to a 10% error in the relaxation time.

The relaxation process observed in polyethylene glycol solutions will now be considered. The fact that the relaxation time is independent of concentration (Table II) makes it improbable that the relaxation process involves polymer–polymer interactions. Several facts suggest that the process observed is not a viscosity relaxation of the polymer. Viscosity relaxation times may be calculated from the theory of Zimm.[29,30] The *k*th relaxation time is

$$\tau_k = M\eta[\eta]/0.586RT\lambda_k \qquad (6)$$

where M is the molecular weight, η is the solvent viscosity, $[\eta]$ is the intrinsic viscosity, R is the gas constant, T is the temperature, and the λ_k's are tabulated constants.[30] Regardless of the value of λ_k chosen, the data (Figure 6) cannot be even approximately described by eq 6. Furthermore, urea addition causes both η and $[\eta]$ to increase (Figures 4 and 7) but the relaxation time decreases (Figure 7), which is contrary to the predicted behavior (eq 6). Finally, only a single relaxation time is observed whereas a spectrum of times is expected for viscosity relaxation.[29] The relaxation time is also probably not solely due to hydrogen bonding of water to the oxygen atoms of the polymer chain since such a process would not be expected to show a large dependence of the relaxation time on molecular weight (Figure 6).

A cooperative type of solvent–polymer interaction is most likely responsible for the observed relaxation time. The local water structure around the polymer consists of water molecules hydrogen bonded to the oxygen atoms of the chain and the hydrophobic water structure around the CH_2CH_2 groups; also CH_2CH_2 groups probably overlap to form "hydrophobic bonds." The single relaxation time is probably due to the overall cooperative formation and breakdown of the local water and hydrophobic structure; the spectrum of relaxation times associated with the elementary steps of cooperative hydrogen bond formation between water molecules are too fast to be observed in the frequency range investigated here. Undoubtedly, some changes in the configuration of the polymer occur concurrently

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with changes in the local water structure. The quantity A/m is a complex *thermodynamic* function of the relaxation process involved.[23] It is relatively constant over the entire range of molecular weight (Table II); this suggests that (on the average) the over-all change in local water structure around each monomer unit is independent of molecular weight.

The relaxation time increases with increasing molecular weight up to a molecular weight of approximately 7000, whereupon it is relatively independent of molecular weight (Figure 6). The cooperative water structure surrounding the polymer will vary in size according to the size of the polymer chain. A maximum or cooperative unit of the polymer–solvent structure may be reached at a polymer molecular weight of 7000, thus accounting for the relative insensitivity of the relaxation time to molecular weights above 7000. The relaxation time is shorter at low molecular weights because of the smaller size of the polymer and of the cooperative water structure around the low molecular weight species.

The relaxation time of the polymer of mol w 20,000 undergoes a sharp decrease between 2 and 4 *m* urea (Figure 7). This decrease is not due to a simple polymer–urea interaction since the relaxation time is unchanged between 0–2 and 4–8 *m* urea. The sharp transition is probably due to a cooperative change in the local solvent structure which may result in a loosening of the polymer structure and give rise to an increased solvation and shorter relaxation time. This interpretation is supported by the observation of Schick who reports that addition of urea increases the critical micelle concentration values of polyoxyethylene-alkanols.[31] He suggests that urea addition causes an increased hydration of the polyethylene oxide chain by reducing the cooperative structure of water. Since the mole fraction of polymer monomer units is constant for all experiments, the amplitude parameter, A, is determined by thermodynamic variables only, and it displays a sharp change from 2.2×10^{-8} sec cm^{-1} in 2 *m* urea to 4.3×10^{-8} sec cm^{-1} in 3 *m* urea. Above 3 *m* it is independent of urea concentration (A is 4.0×10^{-8} sec cm^{-1} in 8 *m* urea). This is further evidence that a cooperative change in the local solvent structure is caused by urea.

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The change in local solvent structure could involve a cooperative binding of urea to the polymer. Presumably, this interaction would only occur, however, if the local cooperative water structure around the polymer was concurrently destroyed, thus allowing urea to interact with the polymer. Moreover, if *specific* binding occurred, additional relaxation processes would be expected.

Addition of urea causes a slight increase in intrinsic viscosity (Figure 7), although a sharp transition similar to the behavior of the relaxation time is not observed. This is not surprising since the intrinsic viscosity and relaxation time reflect different (but possibly related) processes.

For many systems the high frequency limiting value of the absorption parameter B (*cf.* eq 4) is that of the pure solvent. In all cases, B exceeds the value for the pure solvent (B decreases about linearly from 42.0 to 36.0×10^{-17} sec^2 cm^{-1} between 0 and 4 *m* urea and then only decreases to 35.5×10^{-17} sec^2 cm^{-1} in 8 *m* urea). This excess absorption may be due to the altered solvent structure in the presence of polymer and/or additional "unrelaxed" reactions. For polymer solutions, B cannot be split into two parts as was done for the aqueous urea solutions since the main contribution of the polymer to the viscosity of the medium has probably relaxed at lower frequencies.[21]

The results presented here suggest that urea causes a change in both the bulk water structure and the local water structure around the synthetic polymer. Changes in local solvent structure are probably accompanied by an unfolding of the polymer. Thus the effect of urea on polyethylene glycol appears to mimic protein denaturation. The effect of urea on the polymer solvent structure is probably not due to specific polymer–urea interactions. Structural change of the solvent may play a major role in the mechanism of protein and nucleic acid denaturation by urea. These changes in solvent structure may disrupt hydrophobic bonds.[14] However, the specific binding of urea to the macromolecule may also be a major factor responsible for the denaturation process. The relative importance of each of these possibilities remains to be assessed. Ultrasonic investigations of both aspects of this problem are underway.