

ARTICLES

Effect of Urea Addition on Micellization and the Related Phenomena

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Data on the critical micelle concentration (cmc) of sodium dodecyl sulfate (SDS) at 30 °C at lower urea concentration (≤ 0.25 M) presented in this article differ from the earlier literature reports (i.e., increase of cmc). Evidence of the cmc lowering effect of urea is supported by (i) the results of SANS studies performed on 0.3 M SDS + tetra-*n*-butylammonium bromide (Bu_4NBr) with and without added urea, (ii) the observance of shifts in the sphere-to-rod transition in a typical system (3.5% SDS + 0.28 M NaCl + 1-pentanol; Lindemuth, P. M.; Bertrand, G. L. *J. Phys. Chem.* **1993**, 97, 7769), and (iii) CP measurements on TX-100 solutions. All of these effects are attributed to a urea-induced micellization occurring at lower concentrations of the additive. The implications of these results of the lower [urea] regime may be useful in protein research.

1. Introduction

To understand the microscopic basis of the role of urea as a denaturant, a debate on the direct (favorable H bonding between urea and water) versus indirect (rupture of the 3D structure of water) mechanism has continued for quite a long time.^{1–4} Taking note of fairly recent theoretical analyses^{5,6} and on the basis of their own as well as other results⁷ of urea's effect on supramolecular/amphiphile properties, Politi et al.⁸ have proposed a third alternative in which "neither water 3D structure rupture nor direct mechanism, simply a more *polar water*" is formed with enhanced hydrophilicity, as a consequence of which better solvation of polar or ionic headgroups takes place.

Urea continues to be considered a potent protein denaturant. A report on protein renaturation taking place in the presence of urea at low concentrations, however, appeared in 2002.⁹ In most of the studies, urea has been added from moderate (1 M) to high concentrations (6–8 M). The stabilization of the methane–methane contact pair by urea, a renaturation effect, has also been shown using molecular dynamics simulations.¹⁰ In view of the above, the pertinent question is "Does urea act as a denaturant at all concentrations"? The observations suggest that the interpretation of the urea effect in surfactant/protein research is still an open problem that demands additional investigation. To address the problem, we devised experiments to determine the effect of added urea (lower to moderate concentrations) on (a) the critical micelle concentration (cmc) and degree of counterion dissociation (α) of sodium dodecyl sulfate (SDS), (b) the 1-pentanol concentration required to bring about a sphere-to-rod ($s \rightarrow r$) transition in 3.5% SDS + 0.28 M NaCl system,¹¹ and (c) the cloud point (CP) variation of poly(ethylene glycol) *t*-octylphenyl ether (TX-100). A few small-angle neutron scattering (SANS) measurements were also made to obtain the micellar parameters (aggregation number (n_s) and fractional charge (β)) of 0.3 M SDS + 0.2 M tetra-*n*-butylammonium bromide (Bu_4NBr) + urea systems.

2. Experimental Section

SDS (>99%, Fluka), TX-100 (product no. 93420, Fluka), 1-pentanol (99%, Fluka), urea (99%, BDH), Bu_4NBr ($\geq 99\%$, Fluka), and NaCl (99%, Merck) were used as received. Demineralized double-distilled water was used throughout.

The conductivities were measured with an ELICO-type CM 82T bridge equipped with platinized electrodes (cell constant 1.02 cm^{-1}). Freshly prepared stock solutions of SDS were used. The conductivity runs were carried out by adding increasing amounts of the concentrated SDS solution to the thermostated (at 30 ± 0.1 °C) solvent (water or water + urea). It is well known¹² that specific conductivity is linearly related to the [surfactant] in both the premicellar and postmicellar regions and that the intersection point between the two straight lines provides the cmc, whereas the ratio of the slopes of the postmicellar (S_2) to that of the premicellar region (S_1) provides the degree of counterion dissociation (α).

SANS measurements were performed using a spectrometer with the following details:¹³

mean wavelength (λ) of the BeO filtered beam = 5.2 \AA
angular divergence of the incident neutron beam = $\pm 0.5^\circ$
beam size at the sample position = $1.5 \text{ cm} \times 1.0 \text{ cm}$.

accessible wave-vector transfer ($Q = 4\pi \sin \theta / \lambda$, where 2θ is the scattering angle) range = $0.018\text{--}0.32 \text{ \AA}^{-1}$.

To have good contrast between the micelles and the solvent, we prepared samples for SANS measurements in D_2O . The scattered neutrons were detected in an angular range of $1\text{--}15^\circ$ using a linear He^3 position-sensitive gas detector (PSD) that allowed for the simultaneous recording of data over the full Q range. The samples were held in a 0.5-cm path-length quartz cell. The cell was properly stoppered and thermostated at 30 ± 0.1 °C. All of the samples contained 0.3 M SDS + 0.2 M Bu_4NBr with or without urea added (0.25 or 0.5 M). The raw data were corrected for the background, empty-cell scattering and sample transmission. The corrected intensities were normalized to absolute cross-section units, and thus, the coherent differential scattering cross section, $d\Sigma/d\Omega$, versus Q was obtained.

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Viscosity measurements under Newtonian flow conditions were performed using an Ubbelohde viscometer (thermostated at 30 ± 0.1 °C) as described elsewhere.¹⁴

The CP measurements were taken in solutions with different concentrations of TX-100 in the presence of varying amounts of urea. Stock solutions of TX-100 (with or without urea) were used to obtain various sample solutions. The samples were taken in Pyrex glass tubes, which were then immersed in a thermostat and heated at a uniform rate of 0.1 °C/min. The procedure of recording the CP has been detailed elsewhere.¹⁵

SANS Analysis. The relevant model to analyze the SANS data is summarized below. For monodisperse interacting micelles of volume V_m present at a number density n_m and of scattering-length density ρ_m dispersed in a medium of scattering length density ρ_s , $d\Sigma/d\Omega$ may be written as^{16,17}

$$\frac{d\Sigma}{d\Omega} = n_m V_m^2 (\rho_m - \rho_s)^2 \{ \langle F^2(Q) \rangle + \langle F(Q) \rangle^2 [S(Q) - 1] \} + B \quad (1)$$

The same expression for noninteracting micelles (i.e., $S(Q) \approx 1$) is given by

$$\frac{d\Sigma}{d\Omega} = n_m V_m^2 (\rho_m - \rho_s)^2 \langle F^2(Q) \rangle + B \quad (2)$$

Here $F(Q)$ is the single particle form factor, $S(Q)$ is the interparticle structure factor, and B is a constant term that represents the incoherent scattering, which is mainly due to hydrogen in the sample. The aggregation number n_s of the micelle is related to the micellar volume V_m by the relation $V_m = n_s v$, where v is the volume of a surfactant monomer obtained by Tanford's formula.¹⁸ For an ellipsoidal micelle

$$\langle F^2(Q) \rangle = \int_0^1 [F(Q, \mu)]^2 d\mu \quad (3)$$

$$\langle F(Q) \rangle^2 = \left\{ \int_0^1 [F(Q, \mu)] d\mu \right\}^2 \quad (4)$$

$$F(Q, \mu) = \frac{3(\sin x - x \cos x)}{x^3} \quad (5)$$

$$x = Q[a^2 \mu^2 + b^2(1 - \mu^2)]^{1/2} \quad (6)$$

where a and b are, respectively, the semiminor and semimajor axes of the ellipsoid and μ is the cosine of the angle between the axis of revolution and Q .

For a rod-shaped micelle¹⁹ of length $L = 2l$ and radius R ($= a$),

$$\langle F^2(Q) \rangle = \int_0^{\pi/2} \frac{\sin^2(Ql \cos \phi)}{Q^2 l^2 \cos^2 \phi} \times \frac{4J_1^2(QR \sin \phi)}{Q^2 R^2 \sin^2 \phi} \sin \phi d\phi$$

(ϕ is the angle between the axis of the rod and bisectrix, and J_1 is the Bessel function of order unity.) In this analysis, the rod is fit with a fixed radius that is equal to the length of surfactant monomer ($= a$), varying the other dimension.

$S(Q)$ specifies the correlation between the centers of different micelles and is the Fourier transform of the radial distribution function $g(r)$ for the centers of mass of the micelles. In the analysis, $S(Q)$ has been calculated using the mean spherical approximation.¹⁶ The fractional charge β ($= Z/n_s$, where Z is the micellar charge) is an additional parameter in the calculation of $S(Q)$.

In this analysis, the only unknown parameters needed to calculate $d\Sigma/d\Omega$ are the β and n_s ($= 4\pi a^2 b/3v$).

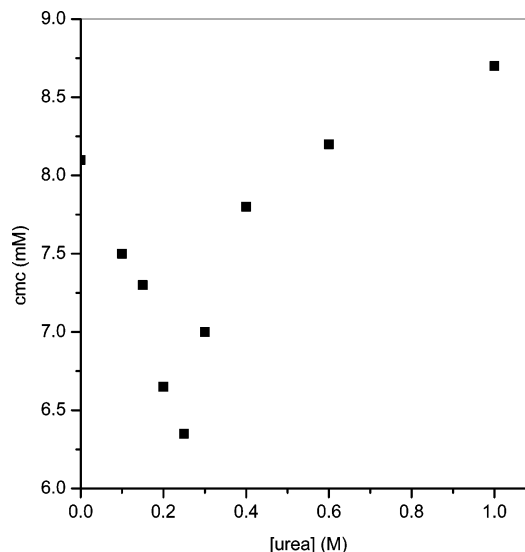


Figure 1. Variation of the cmc of SDS with added [urea] at 30 °C.

The data (corresponding to 0.3 M SDS + 0.2 M Bu₄NBr with and without urea) were analyzed using the above method. The minor axis a ($= 16.7$ Å), which is the length of the extended SDS monomer, was obtained from Tanford's formula.¹⁸ n_s and β were taken as parameters of the fit.

3. Results and Discussions

Before presenting our results of urea's effects on the properties of surfactant solutions, it seems appropriate to mention that even after the appearance of a report on the cmc decrease of SDS in the presence of butylurea up to a certain concentration (~ 0.25 M),²⁰ surprisingly, no attempts have been made to extend the studies to a low [urea] regime. The results to be described warrant more studies to be performed in a low concentration regime with urea and other members of the family.

Figure 1 shows the variation of the cmc of SDS with added urea at 30 °C. As the [urea] increases, the cmc decreases, reaches a minimum, and then starts to increase again. It can be seen that a well-defined minimum occurs at ~ 0.25 M urea. This shows that urea addition up to a certain concentration facilitates micellization. A similar trend was observed earlier with the addition of butylurea²⁰ and discussed in terms of interstitial/substitutional dissolution(s) of the additive. It has been observed that urea increases the dielectric constant of water.^{8,21,22} Additionally, in an independent study on micelle formation in polar solvents,²³ it was observed that the cmc decreases for the same surfactant according to the increase of the dielectric constant. Singh et al.²³ concluded that micelle formation in these solvents could be a combined effect of the dielectric constant of the medium, the nature of hydrogen bonding, and the dispersion forces among the alkyl chains of the surfactant ions. Thus, the dielectric constant of the solvent medium seems to be an important factor (among others) to affect the micellization of ionic surfactants. Consequently, urea can influence the state of solvation of both the polar and nonpolar portions of the SDS molecule. Urea has different hydrogen bonding sites and can form bonds with water molecules, which more than compensate for the disturbance of the bonds existing in pure water. No such compensation occurs with nonpolar groups, and their solution in water is accordingly resisted. In the present situation (at low [urea]), the solvation of the dodecyl chain in dodecyl sulfate (DS⁻) would be decreased. Hence, increased hydrogen bonding in solvent (urea + water) and decreased solvation of dodecyl

TABLE 1: Slopes of Specific Conductance–[SDS] Plots in Water (or Water + Urea) below (S_1) and above (S_2) the cmc and Degree of Counterion Dissociation (α) at 30 °C

[urea] (M)	$S_1(= (dK/dC)_{C < \text{cmc}})$ (S·cm ⁻¹ /mM)	$S_2(= (dK/dC)_{C > \text{cmc}})$ (S·cm ⁻¹ /mM)	α
0	0.73	0.26	0.36
0.10	0.75	0.36	0.48
0.15	0.62	0.28	0.45
0.20	0.79	0.42	0.53
0.25	0.83	0.46	0.55
0.30	0.74	0.36	0.49
0.40	0.73	0.34	0.47
0.60	0.71	0.27	0.38
1.00	0.70	0.30	0.43

chains together may contribute to the increase in the hydrophobic effect and the decrease in the cmc of SDS at low [urea] (≤ 0.25 M). Alternatively, a certain number of surfactant monomers can exist in the solution, and any further addition of the monomers will transform them into micelles. The cmc in water is settled in this way. Now, the presence of urea + water (instead of pure water) causes an increase in the dielectric constant of the urea + water system (solvent). It has been reported that the aqueous urea solution is in a single phase and that urea molecules are spread homogeneously throughout.⁸ In pure water, there are Na⁺ and DS⁻ ions at the experimental temperature. According to Coulomb's law, the force between the two charges is inversely proportional to the dielectric constant of the medium. Consequently, the latter will influence two types of the ions in the following ways:

(1) the attraction between Na⁺ and DS⁻ would decrease (i.e., diminished ion association),

(2) the Na⁺–Na⁺ repulsion would decrease, and

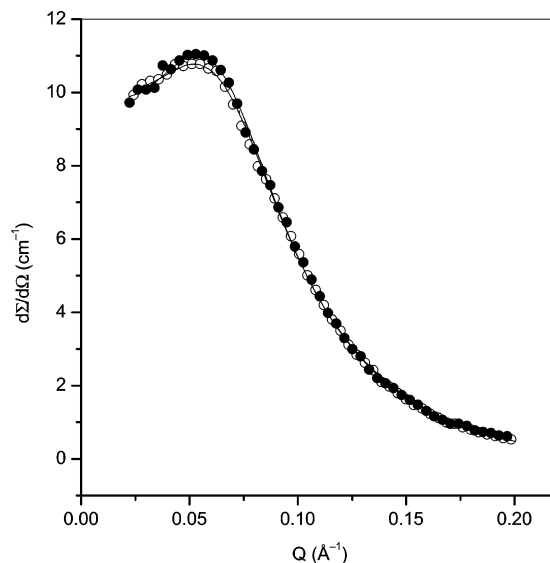
(3) the DS⁻–DS⁻ repulsion would decrease

The decrease in electrostatic repulsion between DS⁻–DS⁻ would allow micellization to take place at lower [SDS] because one opposing factor of the phenomenon is now weaker and hence the hydrophobic interactions may dominate. This mechanism probably operates in the lower [urea] regime (< 0.25 M). This also explains the observed increase in α due to urea addition (Table 1). Thus, urea appears to enhance hydrophobic interactions and the aggregation tendency at low urea concentrations.

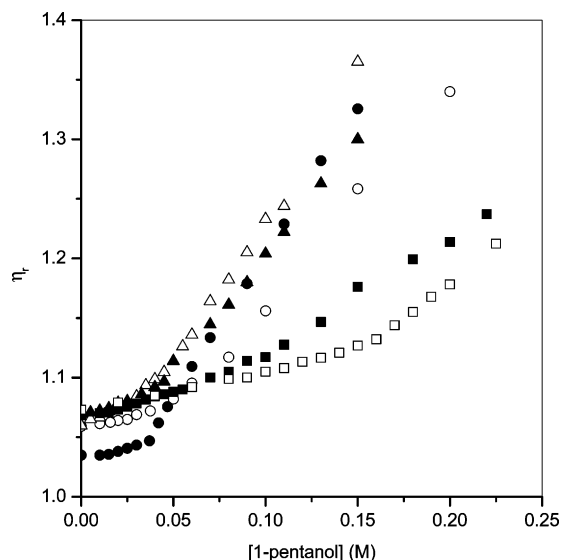
The ability of large amounts of urea to dissolve in water is a consequence of the minimum disruption of the overall hydrogen bonding of the aqueous solution. At moderately higher [urea], the additive molecules get preferentially adsorbed onto charged hydrophilic residues (DS⁻)¹⁰ and lead to repulsion between DS⁻–DS⁻ with the resultant increase in cmc. This is indeed observed (Figure 1). It is worth noting that the effect shows up at comparatively much lower [urea] than in the case of proteins, which may be due to the size difference between DS⁻ and a typical protein molecule; obviously, a smaller DS⁻ would require less urea content for the DS⁻–DS⁻ repulsion effect to become operative.

The values of S_1 , S_2 , and α are summarized in Table 1. The α value (0.36) obtained in pure water is in agreement with the literature value.²⁰ Table 1 also shows that α increases with increasing [urea], up to 0.25 M, and then decreases at higher concentrations. This again shows different urea effects in low and high concentration regimes. Such behavior has been experimentally observed.^{20,24,25}

To provide additional evidence regarding the special urea effect, we carried out SANS measurements on 0.3 M SDS + 0.2 M Bu₄NBr systems. A representative $d\Sigma/d\Omega$ versus Q spectrum together with the theoretical fit is given in Figure 2. Table 2 contains the n_s , β , semiminor (a), and semimajor (b)

**Figure 2.** SANS distributions from 0.3 M SDS + 0.2 M Bu₄NBr with (●) and without (○) 0.5 M urea at 30 °C. The solid lines are theoretical fits, and the marks (●, ○) are experimental data points.**TABLE 2: SANS Data for the 0.3 M SDS + 0.2 M Bu₄NBr System in the Presence of Urea at 30 °C**

[urea] (M)	n_s	β	a (Å)	b (Å)
0	177	0.13	16.7	80.44
0.25	189	0.14	16.7	85.79
0.50	184	0.14	16.7	83.50

**Figure 3.** Variation of η_r of the 3.5% (w/v) SDS + 0.28 M NaCl system with 1-pentanol addition at various fixed concentrations of urea at 30 °C: ○, 0.0; ●, 0.05; △, 0.1; ▲, 0.2; ■, 0.3; □, 0.5 M urea. (All [urea] are not shown to avoid overlapping of the curves.)

axes values for the systems containing urea (0, 0.25, or 0.50 M). A perusal of the data shows that the n_s of the system at 0.25 M urea increases in comparison to the case when urea is absent (parent system). As the [urea] increased to 0.5 M, the n_s value decreased. The trend in the variation of n_s and b values is sufficient to demonstrate that at low concentration added urea increases the aggregation tendency in the micellar system, which substantiates the observations of cmc measurements.

The interpretation advanced above for the low concentration regime of urea finds additional support on the basis of following two different types of studies: (i) Viscometric measurements were performed on a well-defined surfactant system (3.5% (w/

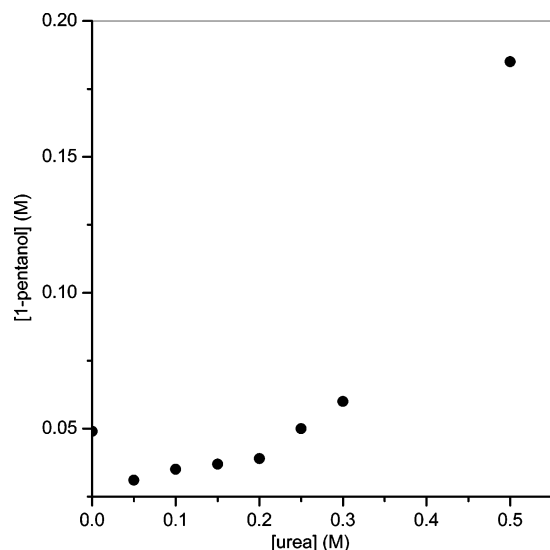


Figure 4. Variation of [1-pentanol] needed for sphere-to-rod ($s \rightarrow r$) transition with [urea] at 30 °C. (System: 3.5% (w/v) SDS + 0.28 M NaCl.)

v) SDS + 0.28 M NaCl) to find the 1-pentanol concentration needed to bring about the $s \rightarrow r$ transition.¹¹ Surfactant solutions containing spherical micelles are isotropic and of low viscosity.²⁶ The presence of anisotropic micelles (e.g., rod-shaped) in the solution causes a distinct rise in viscosity.^{27,28} Viscosity can, therefore, be used to study such transitions in solutions.^{26,29,30} We chose the system of ref 11, and the effect of the addition of 1-pentanol on the viscosity behavior was seen (Figure 3). Viscosity increases in the plot of relative viscosity (η_r) versus [1-pentanol] at ~ 0.048 M (at 30 °C), which is closer to the concentration (0.04 M at 25 °C) obtained by incremental calorimetric titration.¹¹ (As we know, temperature retards the $s \rightarrow r$ transition, our value of the [1-pentanol] at 30 °C seems acceptable.) [1-Pentanol] for each [urea] was obtained from the η_r versus [1-pentanol] plots (Figure 3), and the effect of urea addition on the [1-pentanol] needed for the $s \rightarrow r$ transition is shown in Figure 4. We can see that the required 1-pentanol concentration first decreases and then increases with the added urea. The results indicate that the $s \rightarrow r$ transition is facilitated by low [urea] in the system, which supports the viewpoint that urea acts as an ameliorator for micellization up to a certain concentration (as inferred from the cmc data). (ii) Variation of the CP of TX-100 solutions with added urea is illustrated in Figure 5. The CP first decreases, followed by an increase with progressive urea addition. The initial CP decrease indicates that the dehydration of the TX-100 micelle has taken place by the addition of urea (in a low-concentration regime). The decrease in the CP is not remarkable but enough to show that urea does influence the amphiphile properties, which, in turn, depend on the [urea] itself. Because urea increases the dielectric constant of water, the strength of attractive van der Waals interactions would also increase in the presence of urea at the experimental temperature. Another factor to be taken into consideration is the size of the urea molecule, which is significantly larger than water. The addition of urea, therefore, results in a thicker hydration shell, and the steric repulsion between the micelles would increase with an increase in the [urea]. The data in Figure 5 show that both of the effects are operating and that their dominance is dependent upon the [urea] present in the system. Initially (at low [urea]), the former effect dominates, whereas at moderate [urea], the steric effect becomes predominant. At intermediate concentrations, both of the effects are present, and

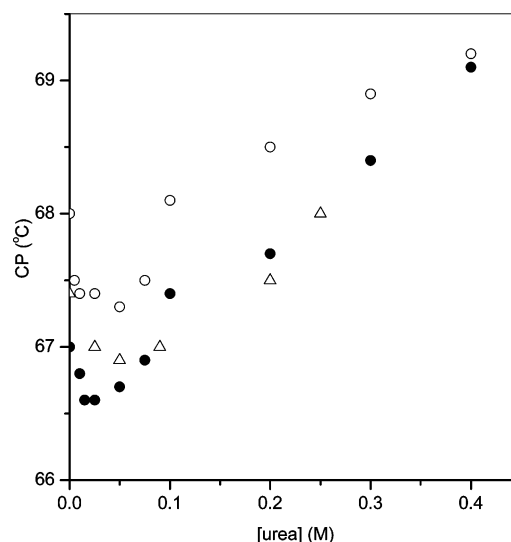


Figure 5. Variation of CP of TX-100 solutions with the addition of urea: ○, 5 mM; △, 10 mM; ●, 25 mM TX-100.

when they are balanced, a minimum appears in the CP–[urea] profile (Figure 5).

4. Summary

In conclusion, we can say that the phenomenon of micellization is remarkably influenced by urea addition and that the effect is dependent upon whether the urea concentration is low (<0.25 M) or high (>1 M) in the surfactant solution. In the lower [urea] regime, micellization is influenced by an increase in the dielectric constant of the solvent medium (cmc decreases). Micellization is predominantly influenced by the adsorption of urea molecules on DS^- at higher [urea], which delays micelle formation (cmc increases). The same logic applies to protein denaturation, and it is not unlikely that urea can renature the protein structure at low concentrations.

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