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Effect of Urea on Biomimetic Systems: Neither Water 3-D Structure Rupture nor Direct Mechanism, Simply a More “Polar Water”

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Analysis of a variety of properties of supramolecular aggregates in aqueous urea supports an explanation for the urea effect that differs from traditional explanations based on the direct mechanism of urea–water solvation or the indirect mechanism via rupture of the three-dimensional (3-D) structure of water. The urea-induced effects investigated are increases in amphiphile critical micelle concentrations, ionization degrees (α), and aggregation numbers; decreases in percolation thresholds of reversed micelles; expansion of minimum areas of monolayers; increases in the radii of gyration of polyelectrolytes; changes in morphologies of sodium bis-2-ethylhexylsulfosuccinate thin films on glass substrates; and direct evidence for urea-induced reduction in ion pairing. All of these effects are attributed to an urea-induced enhancement of the hydrophilic properties of water that results in more strongly solvated polar groups and ions and a reduction in ion pair formation. The implications of this analysis for urea effects on protein 3-D structure are briefly highlighted.

Introduction

Urea's effects on a number of surfactant aggregate properties have been studied to obtain insight into the factors controlling aggregate stability and by inference into its well-known effect on globular protein stability.^{1,2} For both surfactant aggregates and proteins, the interpretation generally reduces to a debate on the direct or the indirect mechanisms.¹ In the direct mechanism, favorable H-bonding interactions between urea and water permit urea to replace water in the solvent shell around hydrocarbon, which enhances the solubility of hydrophobic components, such as the hydrocarbon tails of the amphiphiles and the nonpolar residues on proteins.¹ On the other hand, in the indirect mechanism, the rupture of the three-dimensional (3-D) structure of water is postulated

to decrease the energetic cost required to dissolve these same hydrophobic components in bulk solution.²

Recent theoretical analyses show that urea has little effect upon the 3-D structure of water.⁴ Indeed, molecular dynamics simulations by Wallqvist et al.^{4a} show that urea stabilizes the methane–methane contact pair, a renaturation effect, and that urea is preferentially absorbed by a fictitious partially charged methane (M^+ , M^-) ion pair molecule that destabilizes their contacts. The authors concluded that these results are consistent with the concept that a large excess of urea must be added (6–8 M) before adsorption of urea on surface residues of proteins, and not enhanced dissolution of the hydrocarbon components, leads to denaturation. The slightly enhanced solubilization of butane by urea found by D. B. Wetlaufer et al.¹ some years ago and attributed to the altering of

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water structure should be contrasted with recent molecular dynamics simulations of Mountain and Thirumalai.^{6a} Their results show that hydrocarbons from methane to octane undergo fluctuations in their conformations without significantly perturbing local water structure and that the H-bonding network is essentially preserved.^{6a} These results pose serious questions about the viability of the direct or the indirect mechanisms for interpreting the urea effect on protein structure and amphiphile properties.

A plausible alternative is that polar and ionic groups are better solvated by urea–water mixtures than by water alone. For example, micelle formation results in a large increase on the order of 100 fold or greater in the vicinity of the critical micelle concentration (cmc) in the local concentration of surfactant headgroups and counterions.³ Thus, high concentrations of urea at the micellar surface would weaken ionic interactions in the interfacial region and shift the micelle–monomer equilibrium in favor of the monomer. Our group recently used the chemical trapping method to estimate the urea concentration within the interfacial regions of cationic, anionic, and zwitterionic micelles.³ The results show that interfacial molarity of urea is essentially the same as that in bulk solution ($\pm 10\%$) up to 6 M added urea; that is, urea is neither preferentially bound nor excluded from micellar interfaces. Conductivity experiments on NaI in ternary solvents of water, alcohols (3–4 carbons), and urea are consistent with added urea competitively hydrating alcohol clusters and Na^+ and I^- .⁵ The authors concluded that these results are consistent with added alcohols reducing the “structuredness” of sodium dodecyl sulfate (SDS) interfaces and that SDS interfaces are less structured in 3 M urea than in water.^{7d} Briganti et al. showed that added urea also destabilizes nonionic micelles, and the authors concluded that understanding microscopic hydration, including the effects of added urea and increasing temperature, may provide a link between intermicellar and intramicellar repulsions.^{8a} In related results, in 4–8 M urea, the solubility of β -cyclodextrin is orders of magnitude greater than in water, without the inclusion of urea in/on the host cavity,^{6b} and transfer heat capacities of the nucleic acid bases, nucleosides, and nucleotides from water to concentrated aqueous urea solutions suggest stronger hydrophilic–hydrophilic/ionic group interactions with the solvent.^{6c} Thus, stabilization of polar and charged groups and their counterions by added urea should shift the coil–random coil equilibrium of proteins and the micelle–monomer equilibrium of amphiphile aggregates in favor of the random coiled and monomeric forms, respectively, i.e., in the same direction as urea stabilization of hydrophobic components in bulk solution.

The effect of added urea on several different aggregation phenomena is reported, including the effect of added urea on the (i) cmc values and degrees of ionization (α) of various amphiphiles and micellar size and shape; (ii) percolation

Table 1. cmc and Dissociation Degree (α) of Various Amphiphiles as a Function of Urea Concentration ($T \approx 25^\circ\text{C}$)

amphiphile	[urea] (M)	cmc (mM)	α^a
CTACl ^b	0	1.60	0.33
	0.5	1.64	0.36
	1.0	1.68	0.37
	3.0	2.31	0.42
	5.0	3.03	0.44
CTABr	0	0.95	0.26
	3.0	1.57	0.29
	5.0	2.43	0.33
TTABr	0	3.82	0.23
	3.0	6.60	0.38
	5.0	9.48	0.31
SDS	0	8.33	0.36
	3.0	10.0	0.55
C ₁₂ E ₆ ^c	0	1.20×10^{-3}	
	2	2.10×10^{-3}	
	4	3.50×10^{-3}	
TX-100 ^c	0	0.33	
	1	0.41	
	2	0.50	
	3	0.72	
	4	1.00	

^a Alpha values were obtained from the ratio from the slopes of the lines before and after the cmc according to Sepulveda, L.; Cortez, J. *J. Phys. Chem.* **1985**, *89*, 5322. ^b Conductometric data. ^c Data from ref 8a. ^d Data from ref 7h.

thresholds of anionic- and cationic-reversed micelles; (iii) minimum area of cationic, anionic, zwitterionic, and neutral insoluble monolayers; (iv) radii of gyration and second virial coefficients of various polyelectrolytes; (v) thin film formation from sodium bis-2-ethylhexylsulfosuccinate (AOT)-reversed micelles on to hydrophobic glass substrates; and (vi) direct evidence for urea-induced reduction of ion pairing.

The urea-induced changes in aggregation state of all these systems are consistent with added urea increasing the hydrophilicity of the bulk phase and stabilizing polar groups and free ions. The application of this approach to urea effects on globular proteins and other macromolecules stability is straightforward.

Experimental Section

Materials. Urea came from two sources. Urea from Herga, Brasil was recrystallized several times from hot methanol, and the conductance of a 10 M aqueous solution was less than $6 \mu\text{S cm}^{-1}$, ensuring the absence of ionic contaminants. Alternatively, urea (SigmaUltra) from Sigma-Aldrich gave the same low conductance and was used without further purification. Cetyltrimethylammonium chloride (CTACl; Eastman-Kodak) was recrystallized twice from methanol–acetone (85:15). Cetyltrimethylammonium bromide (CTABr; SigmaUltra, Sigma-Aldrich), SDS (SigmaUltra, Sigma-Aldrich), and tetradecyltrimethylammonium bromide (TTABr; SigmaUltra, Sigma-Aldrich) were used without further purification. The cmc values of these amphiphiles were measured by conductivity, Table 1, and are consistent with literature values. Stearic acid (Aldrich, 99%), mp 64°C , was kindly provided by Dr. O. N. Oliveira, Jr. at the Institute of Physics, University of São Paulo at São Carlos, Brasil. Didodecyltrimethylammonium bromide (Eastman-Kodak) was triply recrystallized from ethanol–acetone (85:15). Analysis by Hoffman degradation showed that the alkyl chains were $>95\%$ C₁₈H₃₇. Dipalmitoylphosphatidylcholine (DPPC; Sigma) was used as received. Didodecylmethylammoniumpropane sulfonate (DDAPS) was a generous gift from Dr. D. Zanette, Department of Chemistry, University of Santa Catarina, Brasil. Hyaluronic acid (HA) from *Streptococcus zooepidemicus* (Aldrich), 20% solutions of high and low molecular weight (MW) polyallyl amines (Sigma-Aldrich), and sodium polystyrenesulfonate (PSS; Pressure Chemicals) (MW = 10,000 g/mole) were used as received. Dodecyltrimethylbutylammonium bromide (DDBABr) was synthesized by a

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published procedure,¹³ and its elemental and ¹H NMR analyses were consistent with its structure. AOT (Aldrich) was decolorized with activated charcoal in excess methanol and triply recrystallized from methanol. Water was twice distilled from an all glass apparatus and purified further by passing it through a Millipore Milli-Q system. All other reagents were of the best grade available. Solvents were purified by standard procedures followed by low-pressure distillation.

Methods. Conductivity measurements were carried out using platinum electrodes in a thermostated chamber using an Orion model 160 (Orion) conductivity meter. Thin AOT films were obtained by slowly withdrawing glass plates (BK-7) from the AOT/hexane/water or AOT/hexane/water-urea reverse micelles solutions that were filtered through a Nylon (Millipore) 0.1 μ m membrane. The glass plates were cleaned with "Piranha solution" (70/30 H₂SO₄/H₂O₂, 30%) followed by washing with Milli-Q water and dried under a stream of filtered N₂. Hydrophobic surfaces were prepared by exposing the cleaned plates to 1,1,1,3,3,3-hexamethylsilazane (Aldrich) vapor for ~24 h. Scanning electron micrographs were obtained at the Instituto de Pesquisa em Energia Nuclear-IPEN, São Paulo, Brasil using a Phillips XL30 scanning electron microscope fitted with an energy dispersion X-ray analyzer. The plates were dried for 2 days in a desiccator, and the films were fixed onto the plates by gold sputtering and then examined with the electron microscope.

Monolayer studies were conducted in a dust free room (class 1000) with a KSV-5000 automated Langmuir trough (Finland). The surface of the aqueous phase was cleaned several times by gentle aspiration before the amphiphile monolayers were deposited. The time between deposition and starting compression was typically around 20–30 min. The solvents for deposition were hexane, chloroform, or carbon tetrachloride. Amphiphile films were deposited by using calibrated Hamilton microsyringes, and pH measurements were done directly in the trough subphase in the region outside the compression barriers.

Static light scattering determinations were carried out using a Dawn-DSP or F (Wyatt) instrument in the batch mode. Data were analyzed by using the software developed by Professor W. Reed. The reference solvent for system calibration was toluene (R_0 at 632.8 nm = 1.407×10^{-5} cm²), and latex spheres, diameter = 0.19 nm (Duke), were used for system normalization. The refractivity index increment with concentration was determined with a Phoenix Precision differential refractometer.

Results and Discussion

The effects of urea on the properties of the six different association phenomena are presented later. In the past, the question about how to best interpret these results would have been,¹¹ "Which is the better interpretation for the enhanced solubilization of the hydrophobic components in bulk solution: the direct mechanism by a urea-water microdomain formation or the indirect mechanism by urea-induced rupture of the 3-D water structure?" The question we are asking is, "Does better solvation of the hydrophilic groups and ionic headgroups and counterions provide an unique and correct interpretation of these phenomena?" Stabilization of ions and hydrophilic groups by addition of urea to amphiphilic aggregates, polyelectrolytes, and protein solutions should enhance the stability of the monomeric and random coiled forms and shift the

organized-disorganized equilibria in favor of the disorganized state. Present results support the validity of this new interpretation.

Micellar cmc Values, Alpha Values, Size, and Shape. Table 1 lists the cmc values of selected amphiphiles in the presence and absence of urea. The cmc values compare well with literature values.⁷ Urea increases the cmc of amphiphiles, and the effects are generally greater on cationic than on anionic amphiphiles, but they are also observed with nonionic amphiphiles, e.g., C₁₂E₆⁸ and zwitterionic amphiphiles.^{7b} Added urea increases the dissociation degree (α) of ionic surfactants,⁵ and this increase is accompanied by a loss of counterion selectivity by the micellar interface in cationic, cetyltrimethylammonium halide (CTAX, X = Cl, Br), anionic, methyl dodecyl sulfate (MeDS, Me = Tl, Na), zwitterionic hexadecyldimethylammoniumpropane sulfonate (HPS) micelles, and also cationic vesicles.^{6,10} Added urea, as expected, also decreases micellar aggregation numbers.⁸ The effect of added urea on ionic micelles is consistent with urea-enhanced stabilization of free ions (hindering of ion pair formation). The effect of urea on nonionic amphiphiles can be understood in terms of an increase in solvent polarity leading to a better solvation of the ethylene oxide residues, in a similar fashion as calculated for poly(ethylene oxide).⁹ Added urea also reduces colloidal aggregation of nonionic pranthocyanidin polymers without apparently disordering water structure.⁹ Briganti et al.^{9a} showed that addition of urea to nonionic micelles of hexaethyleneglycol mono-*n*-dodecylether, C₁₂E₆, increases the cmc, decreases micelle size, and shifts the sphere-to-rod transition to higher temperatures. Similar effects on micellar shape and size are observed for ionic surfactants indicating that urea affects both nonionic and ionic surfactants in a similar fashion. Nguyen and Bertrand^{8b} found that added urea shifts the sphere-to-rod transition of SDS micelles to higher temperatures, and we recently found that the NaBr-induced sphere-to-rod transition of cationic TTABr micelles shifts to significantly higher concentrations in the presence of 3 M urea.^{3,18}

Percolation Thresholds of Reversed Micelles. X-ray, static, and dynamic light scattering data, and conductivity measurements show that added urea induces a large increase in the hydrodynamic radii of AOT-reversed micelles due to cluster formation.¹² For comparison, cationic-reversed micelles of DDBABr in chlorobenzene, which also show a typical percolation threshold¹³ as a function of the droplet volume fraction, were investigated. Conductance measurements were done at 50 °C because DDBABr reverse micelles with large volume fractions and in the presence of urea phase separated near room temperature. The electrical conductivity of 0.1 M DDBABr in chlorobenzene solutions ($W_0 = 15$) at three different urea molarities as a function of the volume of the hydrophilic phase is presented in Figure 1. The sharp increases in conductance with added urea and the lower threshold in 4 M urea strongly suggest urea-induced percolation. The lower percolation threshold of the cationic DDBABr as well as its lower stability limits as compared to the anionic AOT micelles¹² is consistent with a stronger

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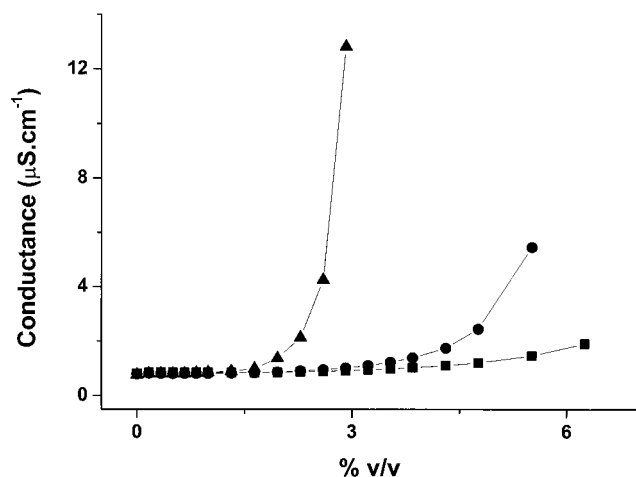
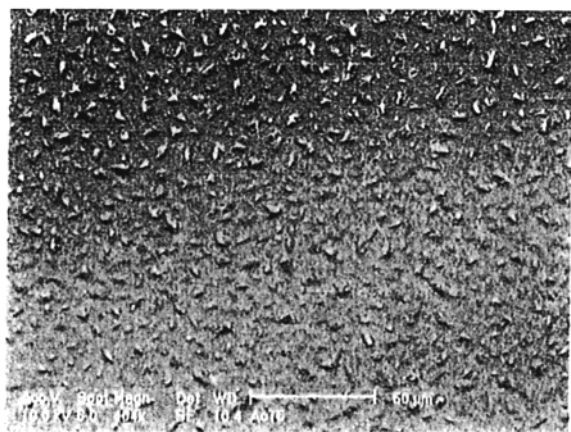
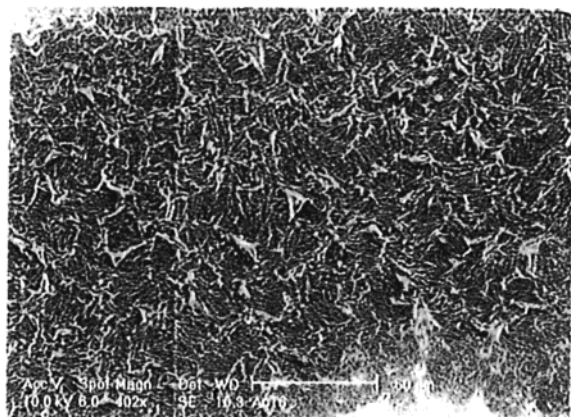


Figure 1. Electrical conductance of 0.1 M DDBABr in chlorobenzene as a function of the volume fraction, % v/v of the hydrophilic phase: water (■), 1 M urea (●), 4 M urea (▲). $T = 50\text{ }^{\circ}\text{C}$.



a



b

Figure 2. Micrographs on hydrophobic glass (see Experimental Section) of thin films of (a) AOT/isooctane/water and (b) AOT/isooctane/3 M urea. Magnification 400 \times . [AOT] = 0.1 M in aqueous phase and the hydrophilic volume fraction = 0.06.

urea effect on cationic interfaces. Monte Carlo¹⁴ simulations suggested that charge fluctuations play an important role in the clustering of reversed micelles. The differences between cationic and anionic reverse micelles are consistent with the greater polarizability of halide anions as

Table 2. A_0 Values for Several Amphiphiles as a Function of Salt Type in the Presence and Absence of Urea ($T = 25\text{ }^{\circ}\text{C}$; Ionic Strength, $\mu = 0.01\text{ M}$)

amphiphile	salt	[urea] (M)	A_0 ($\text{\AA}^2/\text{molecule}$)	subphase pH
DODA	NaCl		58.6	6.8
DODA	NaCl	1.0	82.0	6.8
DPPC	NaCl		63.4	6.8
DPPC	NaCl	1.0	88.1	6.8
DDAPS	NaCl		73.0	6.8
DDAPS	NaCl	1.0	78.5	6.8
stearate	LiCl		19.9	11.5
stearate	LiCl	1.0	25.4	11.5
stearate	NaCl		32.6	11.5
stearate	NaCl	1.0	53.2	11.5
stearate	KCl		36.8	11.5
stearate	KCl	1.0	38.9	11.5
stearic acid	NaCl		24.9	6.8
stearic acid	NaCl	1.0	28.1	6.8
stearic acid			23.4	6.8
stearic acid	KCl		23.5	6.8
stearic acid	KCl	1.0	23.6	6.8

compared to alkali metal cations leading to a longer droplet–droplet contact interactions.¹⁴

AOT Thin Films. Electron micrographs of AOT/hexane/water and AOT/hexane/water/urea thin films adsorbed onto a glass plate¹⁵ are shown in Figure 2. The films were adsorbed on hydrophobic (hexamethylsilazane-treated) glass plates by withdrawing the plates from the reversed micellar solutions and fixed with gold sputtering. Droplets formed in the absence of urea are nearly spherical, but droplets formed in its presence merged into tubular-like structures. These images show how an event at the molecular level propagates into the micrometric domain; that is, long-lived clusters formed during percolation¹² fuse into an interconnected system in the presence of urea. As with aqueous micelles (see previous), added urea should increase the counterion dissociation degree in the micelle droplets producing a stickier droplet–droplet boundary.¹⁴ Thus, urea-induced increases in micellar charge enhance the contact time of the droplets causing them to grow. This effect is also responsible for the reduction in stability of the reversed micelles in the presence of urea.¹²

Minimum Areas of Monolayers. The amphiphiles studied were stearic acid, RCOOH , stearate, RCOO^- , DPPC, a twin-tail sulfobetaine dioctadecylmethylammoniumpropane sulfonate (DDAPS), and dimethyldioctadecylammonium (DODA). Only the minimum monomer area, A_0 , in the presence and in the absence of urea will be discussed. Additional details will be presented elsewhere. As previously shown,¹⁶ the addition of urea into the subphase of DODA monolayers leads to a large increase in A_0 as well as a loss of ionic selectivity as observed with cationic micelles.¹⁰ Table 2 contains a list of the A_0 values for the amphiphiles previously mentioned. Note that the isotherm for stearate monolayers in the presence of NaCl included an intermediate phase transition that prevented precise A_0 determination. The effectiveness of added urea on the expansion of A_0 for each monolayer is obtained from the relative increase in A_0 and follows the order:



with a relatively small effect on electrically neutral stearic acid monolayers.

This order can be understood in terms of two effects, an urea-induced increase in the dielectric constant of the subphase that increases the extent of counterion dissociation and the specific effect on ion pairing.^{16,17} The increase in the dielectric constant is a general medium

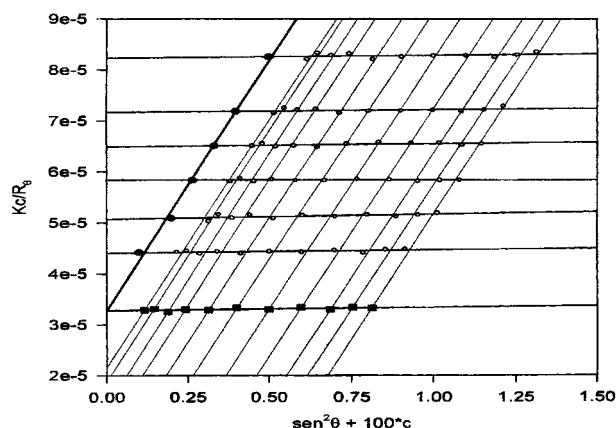


Figure 3. Zimm plot of PAA (MW ~ 30 000 g/mole) in aqueous solution containing 3 M urea and 0.1 M NaCl.

effect and because the monolayer's properties are in equilibrium with the subphase, urea's effects must be mainly local in monolayers. The urea-induced changes in A_0 are thus related to increased solvation of the headgroups and counterions.⁷ Recall³ that the interfacial molarity of urea in SDS and CTAX micelles is about the same as the bulk phase concentration; that is, interfacial urea is not in excess and urea increases α and reduces the selective binding of counterions to monolayers¹⁶ and micelles.¹² Added urea reduces selective counterion binding by reducing ion pairing of headgroups and counterions and increases α by spreading the monolayer and reducing the surface charge density. The effect of added urea on zwitterionic amphiphiles is probably greater than that of anionic amphiphiles because zwitterionic headgroups also bind anions selectively and added urea is reducing the density of anions more than cations at the monolayer surface.^{10,12,16}

Radii of Gyration and Second Virial Coefficients of Polyelectrolytes. The polyelectrolytes investigated were low and high MW poly(allylamine hydrochloride) (PAA), poly(diallyldimethylammonium chloride) (PDDA), and HA. Static light scattering studies for their solutions were carried out to determine their second virial coefficients (A_2) and their gyration radii (R_g). Relevant refractive index increments (dn/dc) for these determinations at various salt concentrations and in the presence of urea were measured at several polyelectrolyte concentrations and several wavelengths and then extrapolated to 632.8 nm, the wavelength used for MW, A_2 , and R_g determinations.¹⁸ Figure 3 shows a typical Zimm projection for a ~30 000 g/mole PAA in the presence of 3 M urea and 0.1 M NaCl. Table 3 collects the parameters obtained for PAA and HA under a variety of conditions. At the investigated NaCl concentration, A_2 values remain essentially constant in the presence and absence of urea, but R_g values consistently increase with added urea. Figure 4 shows A_2 values in the presence and absence of 3 M urea as a function of NaCl for low MW PAA. The invariance in A_2 in water and 3 M urea is clear. Comparison of the trends in A_2 and R_g for HA and PAA shows that they are the same for stiff HA and more flexible PAA. The same trends are observed (results not shown) with a stiffer cationic system of low and high MW of PDDA.

A_2 values are sensitive to changes in interpolymer chain interactions whereas shifts in R_g reflect changes in intramolecular conformations. The invariance of A_2 with added urea within the concentration range studied and at polyelectrolyte concentrations below their overlap concentrations¹⁹ shows that urea does not change the

Table 3. Weight-Averaged MW, Excluded Volumes (A_2), and Gyration Radii (R_g) of PSS and HA

[NaCl] (M)	[urea] (M)	MW $\times 10^{-4}$ (g/mol)	$A_2 \times 10^3$ (mol cm ³ /g)	R_g (Å)
PAA				
0.100		2.90	4.80	
0.100	3	3.04	4.90	
0.010		11.1	12.10	231
0.025		12.5	6.85	251
0.050		11.2	3.58	230
0.100		10.4	2.00	216
0.010	3	13.2	11.30	309
0.025	3	12.5	5.60	330
0.050	3	12.3	3.15	284
0.100	3	11.5	1.97	244
HA				
0.010		158.0	10.40	2080
0.050		139.0	3.20	1370
0.200		141.0	2.39	1440
0.010	3	156.0	9.98	2140
0.050	3	114.0	3.55	1520
0.200	3	150.0	2.27	1450

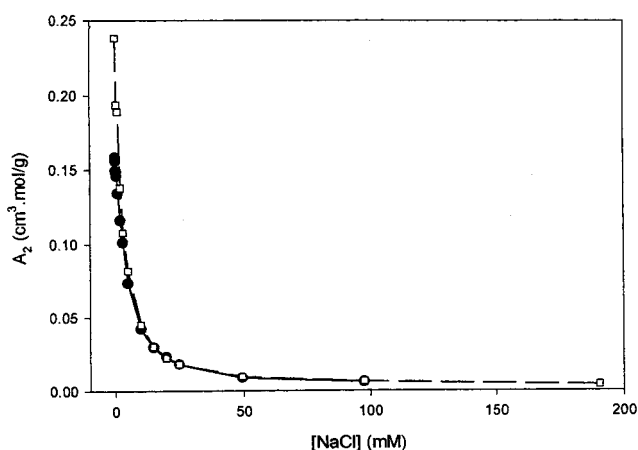


Figure 4. Excluded volumes (A_2) of PAA (MW ~ 30 000 g/mole) as a function of [NaCl] in the absence (●) and in the presence (□) of 3.0 M urea.

electrostatic screening that influences interpolymer chain interactions.²⁰ However, R_g increases with urea for PAA, but the increase is less evident for stiffer HA. These results are consistent with urea–water mixtures that enhance polyelectrolyte headgroup and counterion solvation and conversely hinder their ion pairing but have little effect on the bulk properties of the medium.

Direct Evidence for Urea-Induced Reduction of Ion Pairing. The effects of added urea on the aggregation of an organic nickel complex and changes in the precipitation threshold of oppositely charged polyelectrolyte–amphiphile mixture were studied. Dimerization of nickel tetrasulfophthalocyanine (NiPCS₄) macrocycles is a result of partial charge interaction between adjacent macrocycles.^{21,22} Dimerization constants (K_D) and derived enthalpies and entropies of dimerization as a function of temperature and urea concentration are listed in Table 4. Addition of urea decreases K_D by orders of magnitude, and the change is primarily enthalpic. These results are consistent with an earlier study of the effect of added urea on dye association, such as the dimerization of methylene

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Table 4. Dimerization Constants (K_D), Enthalpies (ΔH), and Entropies (ΔS) for NIPCS₄ in Water and Urea at Various Temperatures²¹

[urea] (M)	K_D (35) ^a $\times 10^7$	K_D (45) ^a $\times 10^7$	K_D (55) ^a $\times 10^7$	K_D (65) ^a $\times 10^7$	ΔH (kcal/mol)	ΔS (kcal/°C mol)
0	5.47	2.14	0.86	0.79	0.219	0.018
3	0.43	0.41	0.22	0.18	0.12	0.017
5	0.0084	0.0070	0.0064	0.0067	0.028	0.017

^a Numbers in parentheses are the temperature in Celsius.

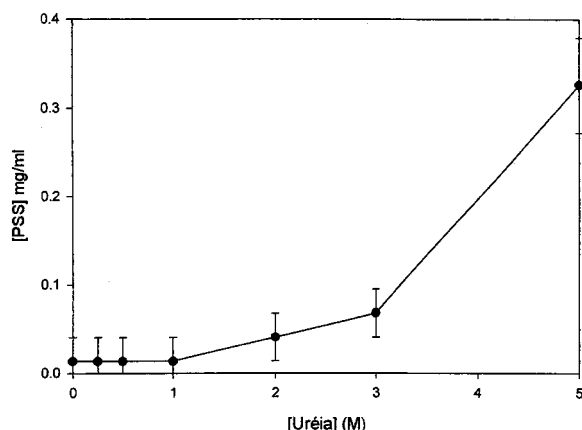


Figure 5. Precipitation threshold of PSS/CTABr solutions as a function of urea observed by the increase in the scattering intensity at 90°. Filled circles represent the arithmetic mean between a solution that was homogeneous for at least 72 h (lower bar) and a precipitated one (upper value) for a given urea concentration and varying PSS between the values presented by the bars ($T = 30^\circ\text{C}$).

blue in water or its association with perchlorate.²³ Enhanced solvation of ions also explains qualitatively why added urea reduces the rate of nickel complexation with *trans*-pyridine-2-azo-*p*-dimethylaniline in sodium decyl sulfate, micelles,^{25a} and the alkaline hydrolysis of cobalt complexes in SDS micelles.^{25b} Urea also increases the solubility of organic salts such as potassium picrate, potassium tetraphenyl borate, tetraphenyl arsonium picrate,²⁶ and inorganic salts such as NaCl, NaI, KCl, KBr, and CsCl.²⁷

Figure 5 shows the phase separation threshold for the interaction of PSS, with CTABr in the presence of urea (for details, see Figure 5 caption¹⁸). Added urea increases the stability limit of the complex.^{24,28} Interactions between amphiphiles and polymers of opposite charge, although relatively complex,²⁴ still depend on the strength of the electrostatic interactions, which are hindered by urea and are consistent with better solvation by urea–water mixtures of the ionic groups and probably a reduction in ion pair formation.^{3,25–28}

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(28) A detailed study on the PSS–CTAX interaction in the presence of urea is underway in our laboratory.

Conclusions

The effect of added urea on the properties of aqueous and reverse micelles, monolayers, polyelectrolytes thin films, and complex formation is all consistent with urea enhancing the stability of free ions and polar groups in solution. Better solvation also hinders ion pair formation, and an urea-induced increase in the bulk dielectric constant reduces ion condensation and local counterion concentrations at the surfaces of amphiphile assemblies, proteins, and other supramolecular assemblies.^{3–5} Recent evidence shows that added urea has little effect on the 3-D structure of water and that even at high concentrations urea does not aggregate but spreads uniformly throughout the solution.^{5,29} Chemical trapping results³ demonstrate that urea molarities in the interfacial regions cationic, anionic, and zwitterionic micelles are essentially the same as in the surrounding bulk solution showing that the uniformity of urea distribution includes the interfacial regions of micellar aggregates and, by interference, the interfaces of protein and other supramolecular aggregates. Finally, dynamic properties such as molecular diffusion and ion conductance of water are barely affected by the addition of urea.^{12,30} The results presented here for a variety of supramolecular aggregates show that enhanced solvation of ionic and polar groups and a reduction in ion pairing at aggregate surfaces shift equilibria in the same direction as the direct and indirect mechanisms for hydrophobic solvation but that urea's effect on hydrophilic interactions is the origin of the effect. The same logic applies to protein denaturation and urea interactions with polar groups at the surface of the coiled protein such that added urea shifts the balance of forces resulting in the net stabilization of its uncoiled, denatured form. Current studies in our group are aimed toward data collecting and revision of available data on ion pair formation in order to properly set the basis for calculations of the “amount” of hydrophilic enhancement and to what extent ion pairing is hindered. These studies would also allow one to estimate the HLB parameter to a condition of enhanced wetting. Applications can be envisaged in detergent technology as well as in other areas whenever an enhanced activity of the hydrophilic component is desirable.

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