

## Interaction of Bovine Serum Albumin with Cationic Single Chain+Nonionic and Cationic Gemini+Nonionic Binary Surfactant Mixtures

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The interaction of bovine serum albumin (BSA) with cetyltrimethylammonium bromide (CTAB),  $C_{16}C_4C_{16}Br_2$ , Brij58, and their binary mixtures has been studied using tensiometry, spectrofluorometry, and circular dichroism at physiological pH and 25 °C. The tensiometric profiles of CTAB and  $C_{16}C_4C_{16}Br_2$  in the presence of BSA exhibit a single break at a lower surfactant concentration termed as  $C_1$  (concentration corresponding to saturation of the interface) compared to their critical micelle concentration (CMC) in the buffered solution. However, for Brij58, CTAB+Brij58, and  $C_{16}C_4C_{16}Br_2$ +Brij58, two breaks were observed, first at the critical aggregation concentration (CAC), corresponding to onset of interaction with BSA and the second at  $C_1$  corresponding to saturation of the interface. The interaction of CTAB+Brij58 and  $C_{16}C_4C_{16}Br_2$ +Brij58 mixtures with the BSA solution is discussed in terms of competition between surfactant–surfactant and surfactant–BSA interactions. CTAB+Brij58 and  $C_{16}C_4C_{16}Br_2$ +Brij58 mixtures show nonideality with respect to mixed micelle formation, which is reflected in their interaction with the BSA. The interaction of CTAB+Brij58 with BSA decreases with increase in the mole fraction of CTAB in the mixture, whereas in  $C_{16}C_4C_{16}Br_2$ +Brij58 the reverse is the case. The results of the present study may prove fruitful in optimizing the properties of surfactant–protein mixtures relevant for many formulations.

### Introduction

Protein–surfactant interactions have been a subject of extensive studies because of their relevance to the field of pharmaceuticals, paints and coatings, adhesives, oil recovery, and so forth.<sup>1–4</sup> Moreover, such studies can provide insight relevant to the solubilizing and denaturing<sup>1,2,5</sup> /renaturing<sup>6–10</sup> action of surfactants on proteins. Protein–surfactant interactions are usually dependent on the surfactant features. Compared to the anionics, cationic surfactants weakly interact with the proteins as a consequence of smaller relevance of electrostatic interactions at the pH of interest.<sup>11</sup> However, the binding isotherms of both types of surfactants have been found to be similar.<sup>11,12</sup> At low surfactant concentrations, ionic surfactants bind to the oppositely charged sites of proteins, causing them to unfold and expose more binding sites. As the surfactant concentration is increased, the binding becomes cooperative, and ultimately the protein is saturated by the surfactant and its aggregates.<sup>2</sup> Compared to ionics, nonionic surfactants bind weakly to the proteins due to the absence of electrostatic interactions, thus making micelle formation in bulk more favorable.<sup>1,13</sup> For general aspects of interactions between ionic surfactants and water-soluble proteins, sodium dodecyl sulfate (SDS) and bovine serum albumin (BSA) have been often used as a representative system.<sup>14</sup> The interaction of proteins with the surfactant molecules can change the conformation of proteins in the bulk<sup>1,2,15,16</sup> and at the interface.<sup>17–20</sup> Therefore, understanding of interaction between the surfactants and proteins in the bulk and at the interface, formation of protein–surfactant complexes

and displacement of protein molecules from the interface by surfactant molecules is important from scientific as well as practical viewpoints. A number of papers deal with the theoretical and experimental studies on the adsorption behavior of protein surfactant mixtures,<sup>17–38</sup> and different mechanisms for the displacement of protein molecules from the interface by the surfactants have been suggested such as orogenic displacement<sup>35</sup> or competitive adsorption.<sup>38</sup>

Recent investigations<sup>39–44</sup> on the interaction of cationic gemini surfactants with proteins have revealed that such surfactants interact more efficiently with proteins as compared to conventional single chain surfactants because of their unique aggregation properties such as lower critical micelle concentration (CMC) and kraft temperature, special aggregation morphology, strong hydrophobic microdomains, and so forth.<sup>45–48</sup> Cationic surfactants, being antimicrobial, have attracted attention with respect to their interaction with deoxyribonucleic acid (DNA) and lipids.<sup>49</sup> Surfactant mixtures exhibit a wide range of properties and are known to perform better than their individual components<sup>50,51</sup> making them important for technological, pharmaceutical, and biological fields. However, little attention<sup>52</sup> has been paid toward their interaction with proteins. Recently, Lu et al.<sup>52</sup> reported very weak interaction of mixed cationic–anionic (decyltriethylammonium bromide + sodium decylsulfonate) surfactants with the BSA due to the strong synergism in mixed micelle formation between the cationic and anionic surfactants in aqueous solutions.

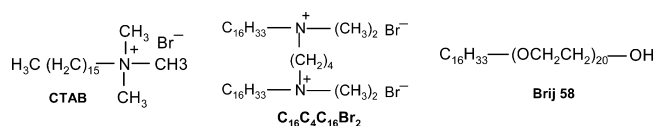
In this paper, we report the results of interaction of BSA with some cationic+nonionic mixed surfactants at 25 °C and physiological pH, employing tensiometry, spectrofluorometry, and circular dichroism (CD). A conventional cationic single chain surfactant, cetyltrimethylammonium bromide (CTAB), its gemini homologue, bis(cetyldimethylammonium)butane dibromide

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## SCHEME 1



( $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ ), nonionic polyoxyethylene (20) cetyl ether (Brij58), and the binary mixtures CTAB+Brij58 and  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ +Brij58 were selected. Mixtures of oppositely charged surfactants were avoided because of their very strong interaction with each other and consequently very weak interaction with the protein.<sup>52</sup> Since the gemini surfactants are known to interact with BSA more efficiently than their conventional homologues,<sup>45–48</sup> it will be interesting to see how their mixtures will interact with each other and with BSA at physiological pH. The main focus of our investigation was to study (1) the effect of binary mixtures of CTAB+Brij58 and  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ +Brij58 on the interfacial and conformational properties of BSA as a function of surfactant composition, and (2) the role of surfactant–surfactant interaction in modifying the interaction parameters of mixed surfactant systems with BSA.

## Experimental Section

**Materials.** BSA (Sigma), CTAB (Sigma) and Brij58, were used as received. The structure of the surfactants is given in Scheme 1. The gemini bis(cetyldimethylammonium)butane dibromide ( $\text{C}_{16}\text{H}_{33}(\text{CH}_3)_2\text{N}^+-\text{CH}_2)_4-\text{N}^+(\text{CH}_3)_2\text{C}_{16}\text{H}_{33}\cdot 2\text{Br}^-$ ) was synthesized and characterized as described elsewhere.<sup>53</sup> All the stock solutions of BSA, CTAB,  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ , and Brij58 were prepared in 60 mM phosphate buffer of pH 7.4 with triple-distilled water and utilized to prepare the samples of desired concentrations. The concentration of the BSA solution was determined by measuring its absorbance at 280 nm on a Hitachi U-1500 spectrophotometer and was kept constant (3  $\mu\text{M}$ ) throughout the study.

**Methods. Tensiometry.** Surface tension measurements were made with a Kruss 9 Tensiometer by the Whilhemmy plate method. Ten milliliters of 3  $\mu\text{M}$  BSA solution in phosphate buffer was taken in the sample vessel, and the surfactant concentration was varied by adding the appropriate aliquots of concentrated surfactant solution using a Hamilton microsyringe so that no more than 1.0 mL of surfactant solution is added to BSA solution to keep the protein concentration practically constant and readings were taken after thorough mixing and temperature equilibration. The temperature was maintained at 25 °C (within 0.1 °C by circulating water from a HAAKE GH thermostat. The accuracy of measurements was within 0.1 dyn  $\text{cm}^{-1}$ . The plate used was washed with acetone followed by triple-distilled water and finally dried over a flame after every measurement. This nullified the effect that might arise due to adsorption of protein on plate.

**Aggregation Studies.** Rayleigh's scattering measurements were performed by observing emission at 350 nm after exciting at the same wavelength on a Hitachi spectrofluorometer (model 2500) equipped with a PC.

**Fluorescence Measurements.** The fluorescence spectra were collected at 25 °C with a 1 cm path length cell using a Hitachi spectrofluorimeter (model 2500) equipped with a PC. The excitation and emission slits were set at 5 nm. The reference sample consisting of the buffer and the detergent did not give any fluorescence signal. The solution was excited at 280 and 295 nm and the emission spectra were recorded in the range of 300–400 nm.

**Circular Dichroism.** CD measurements were carried out with a Jasco spectropolarimeter, model J-720, equipped with a microcomputer. The instrument was calibrated with D-10-camphorsulfonic acid. All the CD measurements were carried out at 25 °C with a thermostatically controlled cell holder attached to a Neslab RTE-110 water bath with an accuracy of  $\pm 0.1$  °C. Far-UV CD spectra were acquired with use of a cell of 1 mm path length over the wavelength range of 200–250 nm. A reference sample containing buffer and detergent was subtracted from the CD signal for all measurements. The high tension voltage for the spectra obtained was found to be less than 600 V. Spectra were collected with a scan speed of 20 nm/min and a response time of 1 s. Each spectrum is the average of four scans. The results were expressed as mean residue ellipticity (MRE) in  $\text{deg cm}^2 \text{dmol}^{-1}$ , defined as

$$\text{MRE} = \frac{\theta_{\text{obs}}}{10 \times n \times C_p \times l}$$

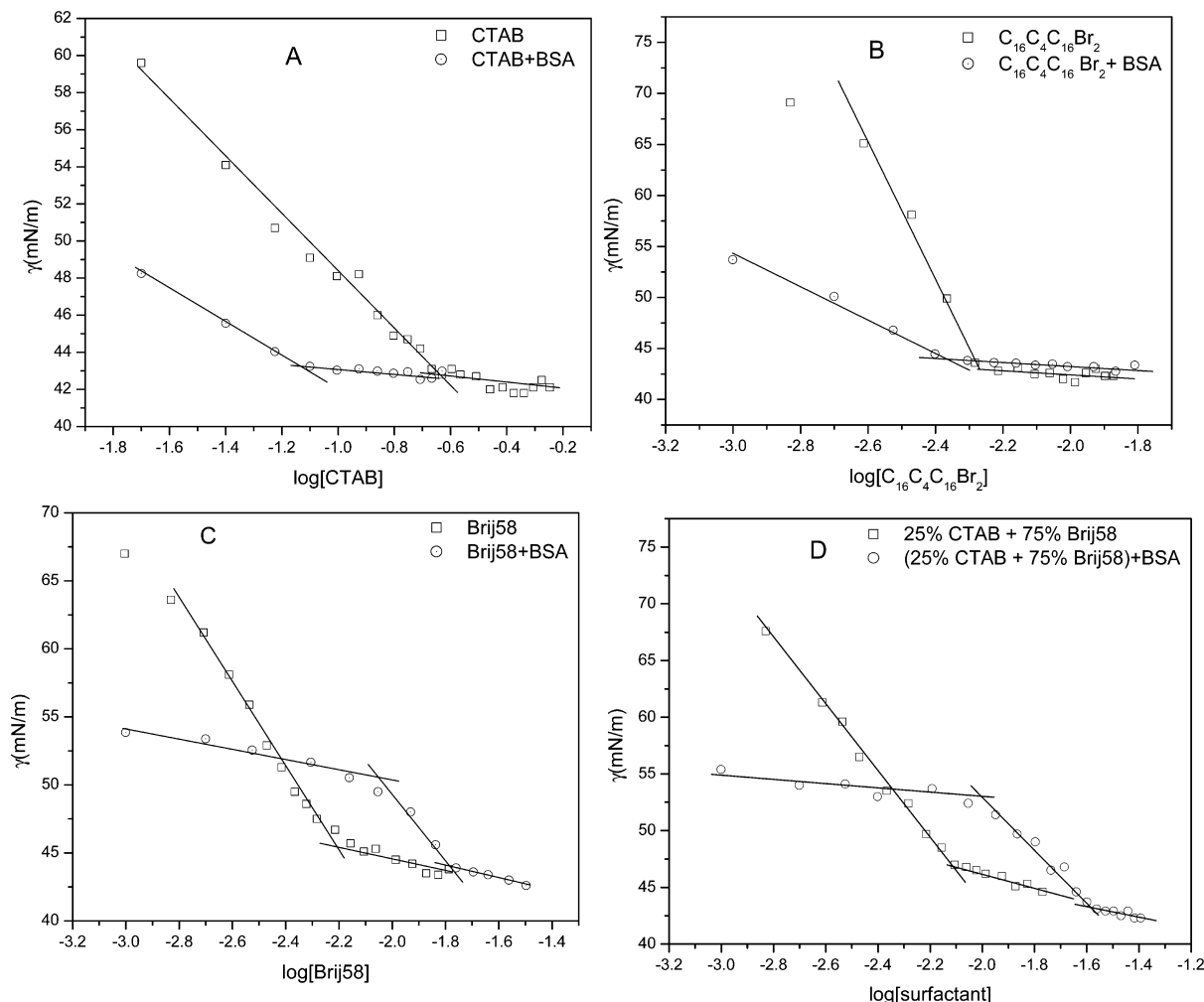
Where  $\theta_{\text{obs}}$  is the CD in millidegrees,  $n$  is the number of amino-acid residues (585),  $l$  is the cell-path length in centimeters, and  $C_p$  is the molarity.

## Results and Discussions

**CMC and Mixed Micelle Formation.** The threshold surfactant concentration required to saturate the air/solution interface called the CMC, corresponds to the break point in the surface tension ( $\gamma$ ) versus  $\log[\text{surfactant}]$  plot. Tensiometric profiles for the addition of CTAB,  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ , and Brij58 to buffer solution under identical experimental conditions are illustrated with squares in Figure 1, and the corresponding CMC values are given in Table 1. While the CMCs of the CTAB and  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$  in buffer solution were lower compared to that in aqueous solution due to decrease in the electrostatic repulsion between the charged head groups at elevated ionic strength,<sup>54,55</sup> that of Brij58 was slightly higher. This slight increase in the latter case can be attributed to possible increase in headgroup size of the Brij58 by the interactions of phosphate buffer ions with the polar oxyethylene groups.

The CMCs of the binary CTAB+Brij58 and  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ +Brij58 mixtures obtained from the tensiometric plots (Figure 1 D, as a prototype) are also included in Table 1. The CMC values of the mixtures show deviation from the ideal CMC values calculated using the Clint equation,<sup>56</sup> indicating antagonism or synergism in mixed micelle formation. The estimate of deviation and hence nonideality has been made in light of Rubingh's model.<sup>57</sup> The interaction parameter,  $\beta$ , and the micellar mole fraction,  $X_1$ , thus obtained are included in Table 1. A negative  $\beta$  implies an attractive interaction (synergism) between the surfactants in the mixed micelles. The results suggest synergism in all the binary mixtures of CTAB and Brij58, a behavior reported for the cationic–nonionic mixed systems<sup>58,59</sup> and attributed to weakening of significant electrostatic self-repulsion of cationics and strong steric self-repulsion of nonionics. Since ionic strength (60 mM phosphate buffer) of the solutions in the present study is very high, the effect of electrostatic interactions may be small. Therefore, the synergism can be largely attributed to the dilution of strong steric self-repulsion between the 20 oxyethylene groups of Brij58 by the incorporation of CTAB monomers in the mixture.

The CMC of the  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ +Brij58 mixtures increase slightly with increase in the mole fraction of  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$  in the mixture and is higher than the corresponding ideal CMC, indicating positive deviation from the ideal behavior. This is



**Figure 1.** Tensiometric profiles in the absence (squares) and presence of 3  $\mu\text{M}$  BSA (circles) for CTAB (A), C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub> (B), Brij58 (C), and 25% CTAB+75%Brij58 (D) at physiological pH and 25 °C.

**TABLE 1: Experimental CMC, CAC,<sup>a</sup> C<sub>1</sub>,<sup>b</sup> Ideal CMC, Interaction Parameter ( $\beta$ ), and Micellar Composition ( $X_1$ ) of Pure and Mixed Surfactant Systems at Various Mole Fractions of Cationic Surfactant ( $\alpha_{\text{cationic}}$ ) at Physiological pH and 25°C**

$\alpha_{\text{cationic}}$	CMC <sub>exp</sub> (mM)	CAC (mM)	C <sub>1</sub> (mM)	CMC <sub>ideal</sub> (mM)	$\beta$	$X_1$
CTAB+Brij58						
0.0	0.0067	0.012	0.0174			
0.25	0.0076	0.0102	0.0275	0.0088	-3.31	0.11
0.5	0.01	0.0107	0.0316	0.013	-3.10	0.17
0.75	0.015	0.0214	0.0380	0.0246	-3.32	0.27
1.0	0.23		0.0891			
C <sub>16</sub> C <sub>4</sub> C <sub>16</sub> Br <sub>2</sub> +Brij58						
0.0	0.0067	0.0120	0.0174			
0.25	0.007	0.0091	0.0417	0.0063	0.55	0.241
0.5	0.0074	0.0072	0.0302	0.0059	0.92	0.595
0.75	0.0076	0.0062	0.0166	0.0055	1.04	0.823
1.0	0.0053		0.0046			

<sup>a</sup> Surfactant concentration corresponding to onset of interaction with BSA. <sup>b</sup> Surfactant concentration corresponding to saturation in interfacial tension in the presence of BSA.

corroborated by the positive  $\beta$  values (Table 1), with a similar change with gemini mole fraction. Rodriguez et al reported<sup>60</sup> that difference in the packing parameters of component surfactants leads to nonideality in their mixed micelle formation. They observed that the addition of a spherical micelle-forming surfactant (dodecyltrimethylammonium bromide (DTAB)) to one forming thread-like micelles (C<sub>12</sub>C<sub>4</sub>C<sub>12</sub>Br<sub>2</sub>) results in

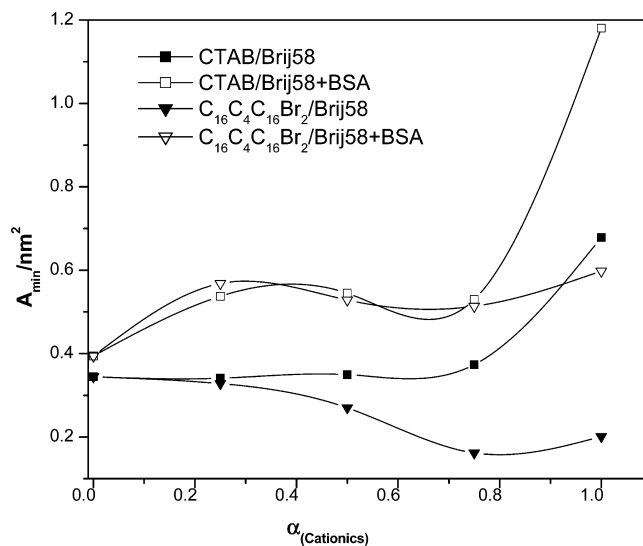
decrease in the packing parameter ( $P$ ). According to Nagarajan and Wang,<sup>61</sup> one contribution to the Gibbs energy of micellization is the deformation Gibbs energy of the surfactant tail. This contribution, which is positive, increases when  $P$  increases for a given hydrophobic chain length. Therefore, a decrease in  $P$  favors micellization. The difference in shape of the micelles affects not only the hydrophobic interaction between the two components within the mixed micelle but also the electrostatic interactions at the surface of the mixed micelle. Keeping in view the weak electrostatic interactions in our system, the nonideality in the mixed micelle formation of C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub> and Brij58 can be largely attributed to the hydrophobic and steric factors. The factors may include (1) large headgroup size of both the components of the mixture leading to greater steric repulsion, (2) the unfavorable hydrophobic environment for the polar oxyethylene groups of Brij58, created by the hydrocarbon spacer of the gemini on the surface of mixed micelles, and (3) the tendency of the components to form different shaped micelles. The increase in magnitude of  $\beta$  with the mole fraction of C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub> in the mixture might be due to the incorporation of C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub> into the Brij58 micelles. This would lead to increase in  $P$  and therefore antagonism in mixed micellization.

The tensiometric profiles of CTAB, C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>, and Brij58 in the presence of BSA are included in Figure 1(circles). The tensiometric profiles of surfactants in the presence of BSA show lower surface tension values than the tensiometric profiles of surfactants in the absence of BSA, due to the surface activity

of BSA ( $\gamma = 56$  mN/m for a 3  $\mu$ M BSA solution). The tensiometric profile for CTAB and  $C_{16}C_4C_{16}Br_2$  in the presence of BSA consists of a single break at a lower concentration compared to their CMC values in the buffer solution. This concentration corresponding to the saturation in interfacial tension is denoted by  $C_1$ . At physiological pH, BSA has an overall negative charge.<sup>62</sup> The interaction between proteins and ionic surfactants at the interface is dominated by the electrostatic interactions.<sup>63,64</sup> With increase in concentration of CTAB and  $C_{16}C_4C_{16}Br_2$ , the available charges in the protein molecules are compensated by the oppositely charged surfactant ions, thus forming electroneutral complexes of enhanced surface activity compared to native protein.<sup>63,64</sup> Lower  $C_1$  values of cationics compared to the CMC values indicate increased hydrophobicity of BSA decorated with the surfactant monomers.<sup>65</sup> The tensiometric profile for Brij58 in the presence of BSA shows two break points: the first one, which is less sharp, corresponds to the onset of interaction of surfactant with the protein/displacement of protein from the interface by the surfactant, usually denoted by the critical aggregation concentration (CAC),<sup>65,66</sup> and the second one corresponds to saturation in interfacial tension/completion of displacement process denoted by  $C_1$ . Up to the CAC,  $\gamma$  remains more or less constant, signifying that Brij58 cannot interact with the BSA adsorbed at the air/solution interface. Therefore, the CAC signifies the onset of interaction/displacement at the air/solution interface. Above the CAC,  $\gamma$  decreases until the interface is saturated or the displacement process is complete to yield  $C_1$ . Decrease in  $\gamma$  can either be due to the specific binding of the alkyl chains of Brij58 to the hydrophobic regions of BSA, thereby increasing its hydrophobicity or competitive adsorption between the BSA and Brij58. Constancy in  $\gamma$  up to the CAC suggests the absence of specific interactions between Brij58 and BSA. CD and aggregation results also indicate the absence of specific interaction between BSA and Brij58, although in the bulk. However, after the CAC, the decrease in  $\gamma$  is indicative of adsorption, which most likely is the main mechanism of nonionic surfactant–protein interaction at the interface.<sup>63,64,67</sup> Competitive adsorption not only depends on the nature of the surfactant but also on the concentration of the surfactant in the bulk. It is apparent from the tensiometric plot that displacement of BSA from the interface (decrease in  $\gamma$ ) occurs only after the Brij58 in the presence of BSA tensiometric plot crosses the tensiometric plot of pure Brij58. This is because, at lower concentration, the surface activity of Brij58 is lower than the surface activity of 3  $\mu$ M BSA, but above the CAC its surface activity becomes higher than that of 3  $\mu$ M BSA and hence gets preferentially adsorbed at the interface, thereby displacing BSA. The preferential adsorption of BSA at the interface below the CAC restrains the adsorption of Brij58; therefore, higher concentration of surfactant ( $C_1$ ) is required to saturate the interface.

Similar to Brij58, the tensiometric profiles for CTAB+Brij58 and  $C_{16}C_4C_{16}Br_2$ +Brij58 mixtures in the presence of BSA consist of two breaks (CAC and  $C_1$ ), showing dominance of Brij58 in deciding the shape of the tensiometric profiles. In the case of the CTAB+Brij58 system, like the CMC, the CAC as well as  $C_1$  increase with increase in mole fraction of CTAB in the mixture. However, unlike the CMC, the CAC as well as  $C_1$  decreases with increase in the mole fraction of  $C_{16}C_4C_{16}Br_2$  in  $C_{16}C_4C_{16}Br_2$ +Brij58 mixtures.

The existence of the CAC in the tensiometric plots of mixtures in the presence of BSA shows that, up to a certain concentration (i.e., the CAC), Brij58 represses the interaction of cationics with BSA. This indicates that below the CAC there is some in-



**Figure 2.** Variation of  $A_{\min}^{\text{CMC}}$  and  $A_{\min}^{\text{Cl}}$  in the absence and presence of BSA, respectively, with the composition of the CTAB+Brij58 and  $C_{16}C_4C_{16}Br_2$ +Brij58 mixtures at physiological pH and 25 °C.

teraction between the surfactant mixture components present in the bulk of the solution, which can affect their interaction with the BSA adsorbed at the interface. Attractive interactions between the mixture components below the CAC can decrease their affinity to interact with BSA. The increase in CAC and  $C_1$  with increase in the mole fraction of CTAB in the CTAB+Brij58 mixture may be due to the higher  $C_1$  value of CTAB and also due to the attractive interaction between the mixture components in the bulk. The attractive interactions between the mixture components below the CAC may be presumed on the basis of synergism in their mixed micelle formation. In the case of  $C_{16}C_4C_{16}Br_2$ +Brij58, the decrease in CAC and  $C_1$  with increase in mole fraction of  $C_{16}C_4C_{16}Br_2$  may be attributed to the lower  $C_1$  value of  $C_{16}C_4C_{16}Br_2$  and also to the unfavorable interactions between the mixture components in the bulk below the CAC expected from the antagonism in the mixed micelle formation.

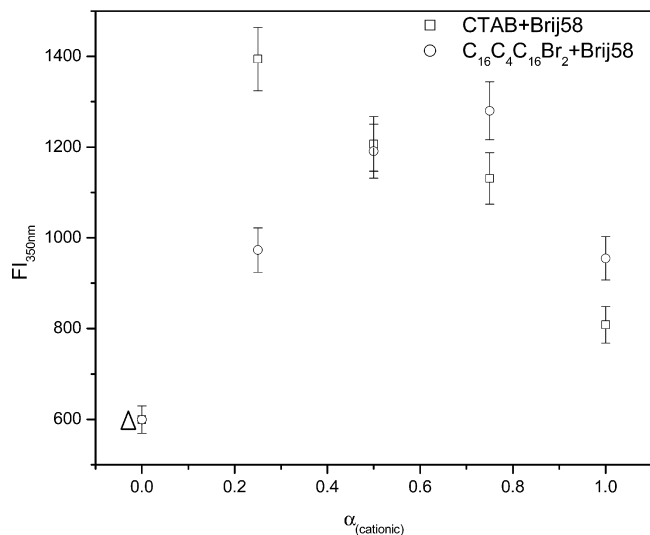
**Interfacial Properties at the Air/Solution Interface.** The surface excess concentration ( $\Gamma_{\max}$ ) in moles per square meter at the CMC and the minimum surface area per molecule ( $A_{\min}$ ) in square nanometers at the air/solution interface for individual surfactants and their binary mixtures both in the absence as well as the presence of BSA were calculated from the following equations:<sup>58</sup>

$$\Gamma_{\max} = -\frac{1}{2.303nRT} \lim_{C \rightarrow \text{CMC}} \left( \frac{d\gamma}{d \log C} \right) \quad (1)$$

$$A_{\min} = \frac{10^{18}}{N\Gamma_{\max}} \quad (2)$$

where  $C$  is the total surfactant concentration,  $N$  is Avogadro's constant, and  $R$  and  $T$  have their usual meanings. As the ions from the buffer have no preference toward interfacial adsorption,  $n$  is assumed to be equal to unity.<sup>62,65</sup> The variation of  $A_{\min}^{\text{CMC}}/A_{\min}^{\text{Cl}}$  with the composition of CTAB+Brij58 and  $C_{16}C_4C_{16}Br_2$ +Brij58 systems is presented in Figure 2. The results reveal that  $A_{\min}^{\text{CMC}}$  values are always lower than the  $A_{\min}^{\text{Cl}}$  values, indicating a less compact monolayer in the presence of BSA, confirming the presence of BSA–surfactant complexes at the air/solution interface.<sup>65</sup> In the

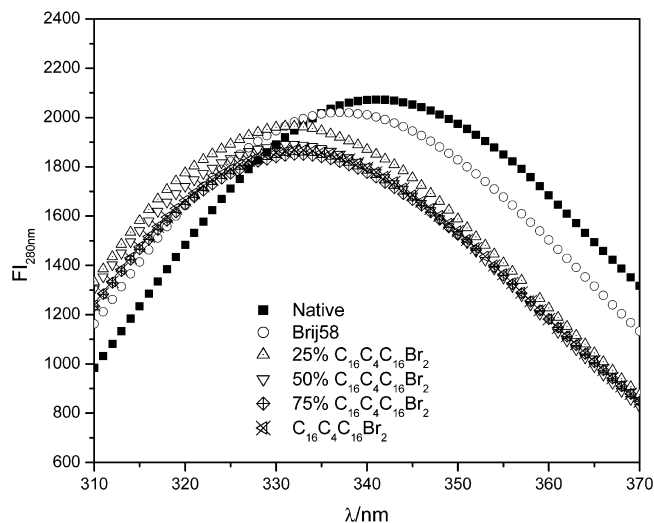
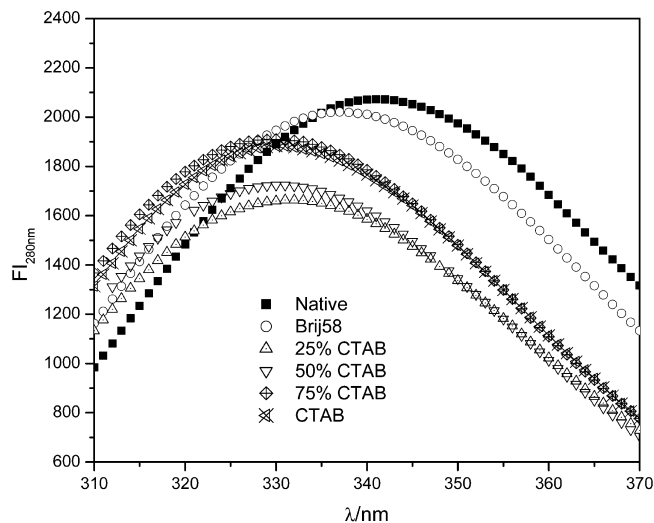




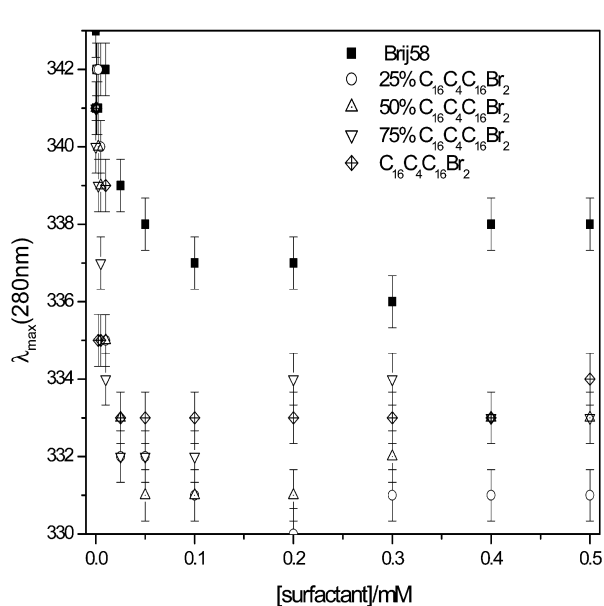
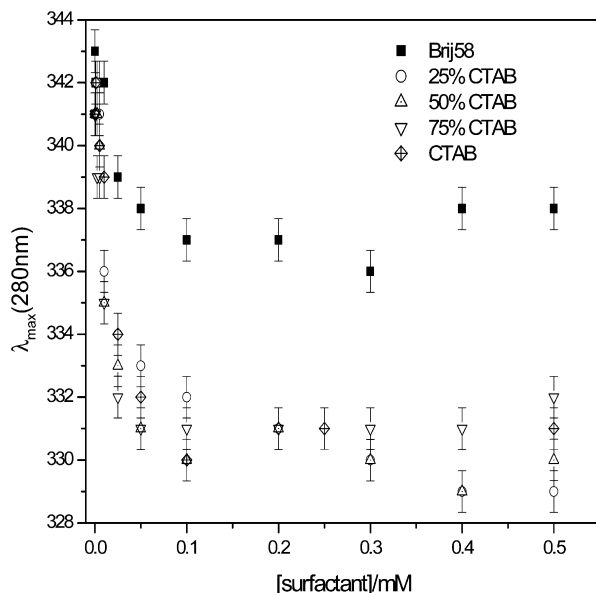
**Figure 3.** Variation of FI maxima of 3  $\mu$ M BSA solution excited at 350 nm ( $FI_{350\text{ nm}}$ ) in the absence ( $\Delta$ ) and presence of 0.1 mM binary mixtures at various mole fractions at physiological pH and 25  $^{\circ}$ C.

case of Brij58, a slight difference in  $A_{\text{min}}^{\text{CMC}}$  and  $A_{\text{min}}^{\text{Cl}}$  (Figure 3) indicates the negligent amount of BSA at the interface (displacement of BSA from the interface by the Brij58) probably due to the absence of specific interactions and the higher surface activity of Brij58 above the CAC than the 3  $\mu$ M BSA solution.

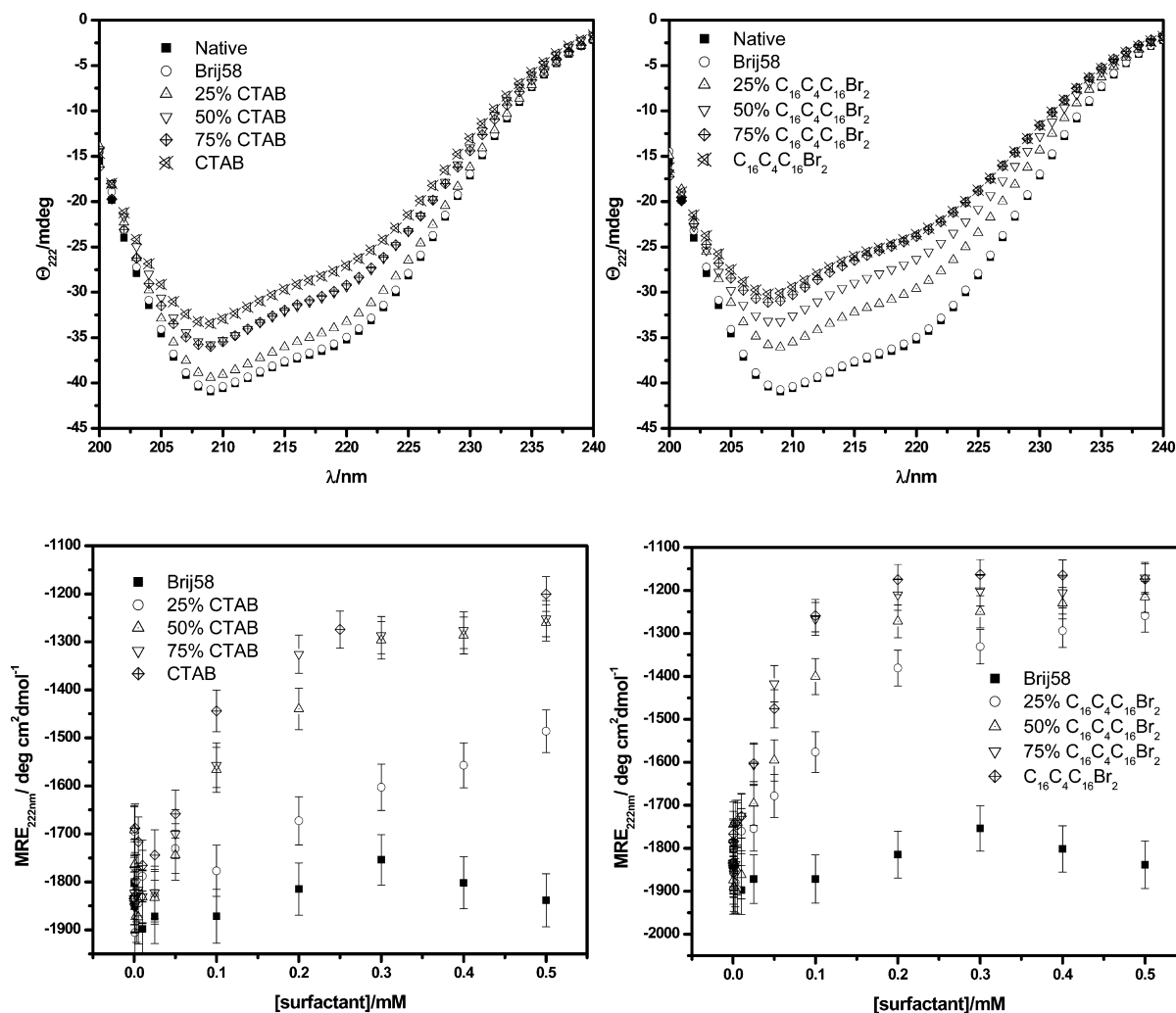
**Aggregation Studies.** The change in the scattering intensities at 350 nm mainly arises from the change in number and/or size of aggregates.<sup>68</sup> Figure 3 illustrates the effect of pure and binary surfactant mixtures on the BSA–surfactant aggregation at a particular surfactant concentration (0.1 mM). It is clear from the figure that Brij58 does not alter the scattering intensity of native BSA, indicating no interaction between the BSA and Brij58 in the bulk. In conformity with the earlier reports,<sup>39,41,44</sup> C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>, because of its unique aggregation properties, interacts more efficiently with BSA than CTAB. The figure also reveals that some cationic+nonionic surfactant mixtures interact more strongly with the BSA than do their individual components as a result of the lower  $C_1$  of the mixture relative to that of the



**Figure 5.** Fluorescence spectra (excited at 280 nm) of 3  $\mu$ M BSA solution in the native state and in the presence of 0.1 mM CTAB, 0.1 mM C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>, 0.1 mM Brij58, and their 0.1 mM binary mixtures at various mole fractions at physiological pH and 25  $^{\circ}$ C.



**Figure 4.** Variation of  $\lambda_{\text{max}}^{280}$  of 3  $\mu$ M BSA solution with the concentration of CTAB, C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>, Brij58, and their binary mixtures at various mole fractions at physiological pH and 25  $^{\circ}$ C.



**Figure 6.** Far-UV CD spectra of native BSA and in the presence of 0.1 mM CTAB, 0.1 mM  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ , 0.1 mM Brij58, and their 0.1 mM binary mixtures (top) and the effect of concentration of pure and binary mixtures at various mole fractions on the ellipticity at 222 nm of BSA (bottom) at physiological pH and 25 °C.

cationic surfactants. The interaction decreases as the mole fraction of CTAB in the CTAB+Brij58 mixtures increases, whereas in  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ +Brij58 mixtures, the reverse is the case. These results are in agreement with the tensiometric results that  $C_1$  increases with increase in mole fraction of CTAB in CTAB+Brij58 but decreases with increase in mole fraction of  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$  in  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ +Brij58 mixtures. Lower  $C_1$  implies more aggregates at a given surfactant concentration available for the interaction.

**Intrinsic Fluorescence.** The change in the wavelength of emission maximum ( $\lambda_{\text{max}}$ ), a parameter sensitive to the protein conformations, and fluorescence intensities (FIs) at 340 nm by excitation at 280 and 295 nm can be used to examine the protein–surfactant interactions.<sup>69</sup> At 295 nm, only two tryptophan residues present in BSA get excited, which are used to probe changes in their respective microenvironments and hence provide a picture of local change in the protein.<sup>70</sup> At 280 nm, however, both tryptophan as well as tyrosine residues get excited, providing a picture of global change in the protein.

The change in the wavelength of maximum emission by excitation at 280 nm ( $\lambda_{\text{max}}^{280}$ ) is illustrated in Figure 4 and indicates that BSA exhibits a blue shift in the presence of the surfactants. The blue shift increases with increase in the concentration of the surfactant and then attains a constant value at higher surfactant concentrations. Decrease in  $\lambda_{\text{max}}^{280}$  with increase in

surfactant concentration indicates a shift of fluorophores toward the more hydrophobic environment.<sup>69</sup> The constancy in  $\lambda_{\text{max}}^{280}$  thereafter means no change in the hydrophobicity around the fluorophores and/or no more binding of surfactant to the BSA, possibly due to the presence of disulfide bonds, which prevents it from complete unfolding. Although the extent of decrease of  $\lambda_{\text{max}}^{280}$  of BSA is more due to CTAB+Brij58 than  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ +Brij58 mixtures, in the presence of the latter mixture, the resulting changes reach the saturation point earlier. This is consistent with the early aggregate formation of gemini and its binary mixtures with BSA. A closer examination of Figure 4 reveals that the extent of this shift in the presence of CTAB+Brij58 mixtures is approximately similar to that in the presence of CTAB alone. However,  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ +Brij58 mixtures result in higher shift values than the  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$  surfactant alone. This shows that the hydrophobic environment around the fluorophores of BSA is more in the presence of  $\text{C}_{16}\text{C}_4\text{C}_{16}\text{Br}_2$ +Brij58 mixtures than gemini surfactant alone at the same concentrations. The variation in  $\lambda_{\text{max}}$  excited at 295 nm was found to be similar to that at 280 nm and hence is not shown. Figure 5 depicts the effect of composition of 0.1 mM surfactant mixtures on the FI of BSA excited at 280 nm ( $\text{FI}_{280\text{nm}}$ ). It is clear from the figure that CTAB+Brij58 mixtures are more effective in decreasing the FI of BSA than their individual components because of their much lower  $C_1$  values relative to those of CTAB. The  $\text{FI}_{280\text{nm}}$

increases as the mole fraction of CTAB in the mixture increases, which is in allegiance with the  $C_1$  changes. However, the decrease in  $FI_{280nm}$  of BSA is less in the presence of C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>+Brij58 mixtures than C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub> because of the lower  $C_1$  value of C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>. In accordance with the  $C_1$  changes, the  $FI_{280nm}$  of BSA decreases with increase in the mole fraction of C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub> in the C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>+Brij58 mixture.  $FI_{280nm}$  changes of BSA therefore follow the  $C_1$  changes faithfully. A lower (higher) value of  $C_1$  implies more (less) micelle-like aggregates, at a given surfactant concentration, generating rich (poor) hydrophobic environment in the vicinity of BSA fluorophores and decreasing (increasing) the  $FI_{280nm}$  of BSA.

**Far-UV CD.** CD can be used to probe transitions in the secondary structure of the proteins. BSA exhibits two negative bands in the far-UV CD spectrum at 208 and 222 nm, characteristic of  $\alpha$ -helical structure.

Alterations in ellipticity at 222 nm ( $\theta_{222}$ )/MRE are used to monitor changes in the denaturation of BSA with varying the nature/amount of the single and binary surfactant mixtures. Figure 6 shows typical far-UV CD spectra of native BSA and BSA in the presence of 0.1 mM CTAB, 0.1 mM C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>, and 0.1 mM Brij58 at physiological pH. It is clear from the figure that CTAB as well as C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub> denature the BSA, but the gemini surfactant is more effective in doing so than CTAB. These results are in conformity with previous results<sup>39,41,44</sup> and have been attributed to the unique characteristics of gemini surfactants. However, the structure of BSA remains almost intact in the presence of Brij58.

Among the binary mixtures of CTAB+Brij58 and C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>+Brij58, the denaturation of BSA increases in the direction of increasing mole fraction of CTAB and C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub> in their respective mixtures. The adsorbed ionic surfactant can locally break the intrachain hydrophobic interaction and provide the electrostatic repulsion favoring the denaturation. Therefore, as the mole fraction of cationics (responsible for denaturation) in their respective mixtures increases, more and more BSA gets denatured. However, the extent of denaturation by C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>+Brij58 mixtures is always found to be more than that by the corresponding CTAB+Brij58 mixtures, showing the higher efficiency of the former compared to the latter in this regard, attributable to unique aggregation properties of C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>.

Figure 6 depicts the variation of denaturation of BSA as a function of concentration of CTAB, C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>, Brij58, and their binary mixtures. BSA denatures more and more with an increase in surfactant concentration and then attains a constant value in most of the cases being consistent with the fluorescence results. The negative and constant MRE values in the presence of higher surfactant concentration suggests retention of the secondary structure of BSA and is contrary to the opinion of Chakraborty et al.<sup>71</sup> that BSA wraps around the SDS micelles because, in that case, complete denaturation is expected. This may be attributed to the rigidity of BSA owing to the presence of 17 disulfide bonds. The binding of surfactant initiates the denaturation and, with increasing surfactant concentration, micelle-like clusters are formed on the protein, which break hydrophobic interactions in the protein and facilitate electrostatic repulsion, thereby denaturing it further. The inability of Brij58 to denature BSA could be due to the lack of charge on it and the absence of specific binding between Brij58 and BSA.

Among the mixed systems, the overall concentration of the surfactant mixtures corresponding to saturation in the denaturation of BSA decreases with increase in the mole fraction of the cationic counterpart in the mixture. Moreover, C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>+Brij58 mixtures denature the BSA at a much lower

concentration than CTAB+Brij58 mixtures, possibly because of lower CAC values of C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>+Brij58 than CTAB+Brij58.

In both kinds of mixtures we observed a common feature that, although the denaturation of BSA increases with increase in mole fraction of cationic surfactant in the binary mixture, irrespective of the variation of CAC and  $C_1$ , it is always less than the denaturation induced by the respective pure cationic surfactant, therefore suggesting electrostatic repulsion as the primary cause responsible for its denaturation.

## Conclusions

The results reveal that the affinity of a mixture to interact with the BSA has an inverse relationship with the interaction between the components of the mixture (nonideality): the more the affinity between the components of the mixtures, the less their interaction with the BSA. Aggregation and fluorescence results indicate that some surfactant mixtures interact more efficiently with the BSA compared to their individual components. Like C<sub>16</sub>C<sub>4</sub>C<sub>16</sub>Br<sub>2</sub>, its mixtures with Brij58 denature the BSA more strongly than CTAB and its mixture with Brij58. Keeping in view the better performance and range of properties displayed by surfactant mixtures and the importance of surfactant–protein mixtures, the results of the present study may prove fruitful in the selection of such mixtures widely used in food processing, cosmetics, pharmaceutical industries, and so forth.

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## References and Notes

- (1) Ananthapadmanabhan, K. P. In *Interactions of Surfactants with Polymers and Proteins*; Goddard, E. D., Ananthapadmanabhan, K. P., Eds.; CRC Press, Inc: London, U.K., 1993; Chapter 8.
- (2) Jones, M. N. *Chem. Soc. Rev.* **1992**, 21, 127.
- (3) Jones, M. N. In *Food Polymers, Gels and Colloids*; Dickenson, E., Ed.; The Royal Society of Chemistry: Cambridge, U.K., 1991; pp 65–80.
- (4) McClements, D. J. *Food Emulsions: Principles, Practice and Techniques*, 2nd ed.; CRC Press: Boca Raton, FL, 2004.
- (5) Tanford, C. *Adv. Protein Chem.* **1968**, 23, 121.
- (6) Moriyama, Y.; Takeda, K. *Langmuir* **1999**, 15, 2003.
- (7) Moriyama, Y.; Sato, Y.; Takeda, K. *J. Colloid Interface Sci.* **1993**, 117, 420.
- (8) Tandon, S.; Horowitz, P. *J. Biol. Chem.* **1986**, 261, 15615.
- (9) Tandon, S.; Horowitz, P. *J. Biol. Chem.* **1987**, 262, 4486.
- (10) Rozema, D.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, 117, 2373.
- (11) Few, A. V.; Ottewill, R. H.; Parreira, H. C. *Biochim. Biophys. Acta* **1955**, 18, 136.
- (12) Nozaki, Y.; Reynolds, J. A.; Tanford, C. *J. Biol. Chem.* **1974**, 249, 4452.
- (13) Moore, P. N.; Puvvada, S.; Blankschtein, D. *Langmuir* **2003**, 19, 1009.
- (14) Takeda, K.; Moriyama, Y.; Hachiya, K. Protein Interactions with Ionic Surfactants Part I: Binding and Induced Conformational Changes. In *Encyclopedia of Surface and Colloid Science*, 2nd ed.; Somasundaran, P., Hubbard, A., Eds.; Taylor and Francis: London, U.K., 2006, and the references therein.
- (15) Gelamo, E. L.; Silva, C. H.; Imasato, H.; Tabak, M. *Biochim. Biophys. Acta* **2002**, 1594, 84.
- (16) Ding, Y.; Shu, Y.; Ge, L.; Guo, R. *Colloids Surf., A* **2007**, 298, 163.
- (17) Kotsmar, Cs.; Pradines, V.; Alahverdijeva, V. S.; Aksenenko, E. V.; Fainerman, V. B.; Kovalchuk, V. I.; Krägel, J.; Leser, M. E.; Noskov, B. A.; Miller, R. *Adv. Colloid Interface Sci.* **2009**, 150, 41.
- (18) Lad, M. D.; Ledger, V. M.; Briggs, B.; Frazier, R. A.; Green, R. J. *Langmuir* **2003**, 19, 5098.

- (19) Kotsmar, Cs.; Grigoriev, D. O.; Makievski, A. V.; Ferri, J. K.; Krägel, J.; Miller, R.; Möhwald, H. *Colloid Polym. Sci.* **2008**, *286*, 1071.
- (20) Kotsmar, Cs.; Arabadzhieva, D.; Khristov, Khr.; Mileva, E.; Grigoriev, D. O.; Miller, R.; Exerowa, D. *Food Hydrocolloids* **2009**, *23*, 1169.
- (21) Fainerman, V. B.; Zholob, S. A.; Leser, M. E.; Michel, M.; Miller, R. *J. Phys. Chem. B* **2004**, *108*, 16780.
- (22) Alahverdijeva, V. S.; Grigoriev, D. O.; Fainerman, V. B.; Aksenenko, E. V.; Miller, R.; Möhwald, H. *J. Phys. Chem. B* **2008**, *112*, 2136.
- (23) Pereira, L. G. C.; Theodoly, Olivier.; Blanch, H. W.; Radke, C. J. *Langmuir* **2003**, *19*, 2349.
- (24) Kotsmar, Cs.; Kragel, J.; Kovalchuk, V. I.; Aksenenko, E. V.; Fainerman, V. B.; Miller, R. *J. Phys. Chem. B* **2009**, *113*, 103.
- (25) Mackie, A. R.; Gunning, A. P.; Wilde, P. J.; Morris, V. J. *Langmuir* **2000**, *16*, 8176.
- (26) Kotsmar, Cs.; Grigoriev, D. O.; Xu, F.; Aksenenko, E. V.; Fainerman, V. B.; Leser, M. E.; Miller, R. *Langmuir* **2008**, *24*, 13977.
- (27) Rampon, V.; Genot, C.; Riaublanc, A.; Anton, M.; Axelos, M. A. V.; McClements, D. J. *J. Agric. Food Chem.* **2003**, *51*, 2482.
- (28) Lin, S. Y.; Wu, T. F.; Tsao, H. K. *Macromolecules* **2003**, *36*, 8786.
- (29) Paul, C.; Krishnan, A.; Fiore, V. F.; Vogler, E. A. *Langmuir* **2008**, *24*, 2553.
- (30) Pradines, V.; Kragel, J.; Fainerman, V. B.; Miller, R. *J. Phys. Chem. B* **2009**, *113*, 745.
- (31) Freer, E. M.; Yim, K. S.; Fuller, G. G.; Radke, C. J. *J. Phys. Chem. B* **2004**, *108*, 3835.
- (32) Wangsakan, A.; Chinachoti, P.; McClements, D. J. *J. Agric. Food Chem.* **2001**, *49*, 5039.
- (33) Fainerman, V. B.; Kovalchuk, V. I.; Aksenenko, E. V.; Michel, M.; Leser, M. E.; Miller, R. *J. Phys. Chem. B* **2004**, *108*, 13700.
- (34) Kotsmar, Cs.; Grigoriev, D. O.; Makievski, A. V.; Ferri, J. K.; Krägel, J.; Miller, R.; Möhwald, H. *Colloid Polym. Sci.* **2008**, *286*, 1071.
- (35) Mackie, A. R.; Gunning, A. P.; Ridout, M. J.; Wilde, P. J.; Morris, V. J. *Langmuir* **2001**, *17*, 6593.
- (36) Latnikova, A. V.; Lin, S. Y.; Loglio, G.; Miller, R.; Noskov, B. A. *J. Phys. Chem. C* **2008**, *112*, 6126.
- (37) Fainerman, V. B.; Leser, M. E.; Michel, M.; Lucassen-Reynders, E. H.; Miller, R. *J. Phys. Chem. B* **2005**, *109*, 9672.
- (38) Miller, R.; Fainerman, V. B.; Leser, M. E.; Michel, M. *Colloids Surf., A* **2004**, *233*, 39.
- (39) Li, Y.; Wang, X.; Wang, Y. *J. Phys. Chem. B* **2006**, *110*, 8499.
- (40) Pi, Y.; Shang, Y.; Peng, C.; Liu, H.; Hu, Y.; Jiang, J. *Biopolymers* **2006**, *83*, 243.
- (41) Wu, D.; Xu, G.; Feng, Y.; Li, Y. *Int. J. Biol. Macromol.* **2007**, *40*, 345.
- (42) Wu, D.; Xu, G.; Sun, Y.; Zhang, H.; Mao, H.; Feng, Y. *Biomacromolecules* **2007**, *8*, 708.
- (43) Gull, N.; Sen, P.; Khan, R. H.; Kabir-ud-Din, J. *Biochem.* **2009**, *145*, 67.
- (44) Gull, N.; Sen, P.; Khan, R. H.; Kabir-ud-Din, *Langmuir* **2009**, *25*, 11686.
- (45) Zana, R.; Xia, J., *Gemini Surfactants*; Eds.; Marcel Dekker: New York, 2003.
- (46) Zana, R. *Adv. Colloid Interface Sci.* **2002**, *97*, 205.
- (47) Siddiqui, U. S.; Ghosh, G.; Kabir-ud-Din. *Langmuir* **2006**, *22*, 9874.
- (48) Wettig, S. D.; Verrall, R. E.; Foldvari, M. *Curr. Gene. Ther.* **2008**, *8*, 9.
- (49) Moulik, S.; Dutta, P.; Chatteraj, D. K.; Moulik, S. P. *Colloids Surf. B* **1998**, *11*, 1.
- (50) Sulthana, S. B.; Rao, P. V. C.; Bhat, S. G. T.; Nakano, T. Y.; Sugihara, G.; Rakshit, A. K. *Langmuir* **2000**, *16*, 980.
- (51) Hill, R. M. In *Mixed Surfactant Systems*; Ogino, K., Abe, M., Eds.; Surfactant Science Series; Dekker: New York, 1993; Vol. 46, Chapter 11.
- (52) Lu, R. C.; Cao, A. N.; Lai, L. H.; Zhu, B. Y.; Zhao, G. X.; Xiao, J. X. *Colloids Surf., B: Biointerfaces* **2005**, *41*, 139.
- (53) Kabir-ud-Din; Fatma, W.; Khan, Z. A.; Dar, A. A. *J. Phys. Chem. B* **2007**, *111*, 8860.
- (54) Kabir-ud-Din; Sheikh, M. S.; Dar, A. A. *J. Colloid Interface Sci.* [Online early access]. DOI: 0.1016/j.jcis.2009.01.041.
- (55) Dar, A. A.; Rather, G. M.; Das, A. R. *J. Phys. Chem. B* **2007**, *111*, 3122.
- (56) Clint, J. H. *J. Chem. Soc., Faraday Trans. 1* **1975**, *71*, 1372.
- (57) Rubingh, D. N. In *Solution Chemistry of Surfactants*; Mittal, K. L., Ed.; Plenum Press: New York, 1979; Vol. 1, p 337.
- (58) Zhou, Q.; Rosen, M. J. *Langmuir* **2003**, *19*, 4555.
- (59) Dar, A. A.; Rather, G. M.; Ghosh, S.; Das, A. R. *J. Colloid Interface Sci.* **2008**, *322*, 572.
- (60) Rodriguez, A.; Graciani, M. M.; Moreno-Vagas, A. J.; Moya, M. L. *J. Phys. Chem. B* **2008**, *112*, 11942.
- (61) Nagarajan, R.; Wang, Ch.-Ch. *Langmuir* **2000**, *16*, 5242.
- (62) Lee, C. T., Jr.; Smith, K. A.; Hatton, T. A. *Biochemistry* **2005**, *44*, 524.
- (63) Miller, R.; Fainerman, V. B.; Makievski, A. V.; Kragel, J.; Grigoriev, D. O.; Kazakov, V. N.; Sinyachenko, O. V. *Adv. Colloid Interface Sci.* **2000**, *86*, 39.
- (64) Fainerman, V. B.; Zholob, S. A.; Leser, M. E.; Michel, M.; Miller, R. *J. Phys. Chem.* **2004**, *108*, 16780.
- (65) Chakraborty, T.; Chakraborty, I.; Moulik, S. P.; Ghosh, S. *Langmuir* **2009**, *25*, 3062.
- (66) Chakraborty, T.; Chakraborty, I.; Moulik, S. P.; Ghosh, S. *Langmuir* **2006**, *22*, 9905.
- (67) Fainerman, V. B.; Zholob, S. A.; Leser, M.; Michel, M.; Miller, R. *J. Colloid Interface Sci.* **2004**, *274*, 496.
- (68) Xia, J.; Zhang, H.; Rigsbee, D. R.; Dubin, P. L.; Shaikh, T. *Macromolecules* **1993**, *26*, 2759.
- (69) Deep, S.; Ahluwalia, J. C. *Phys. Chem. Chem. Phys.* **2001**, *3*, 4583.
- (70) McLachlan, A. D.; Walker, J. E. *J. Mol. Biol.* **1977**, *112*, 543.
- (71) Chakraborty, A.; Seth, D.; Setua, P.; Sarkar, N. *J. Phys. Chem. B* **2006**, *110*, 16607.

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