Physicochemistry of hexadecylammonium bromide and its methyl and ethanolic head group analogues in buffered aqueous and gelatin solution

DEBOLINA MITRA and SATYA P MOULIK*

Centre for Surface Science, Department of Chemistry, Jadavpur University, Kolkata 700 032
e-mail: spmcss@yahoo.com

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Abstract. In this work, the interfacial and bulk behaviour of the amphiphiles hexadecylammonium bromide and its methyl and ethanolic head group analogues in buffered aqueous and gelatin solution has been examined. The analogues are of two categories: the methyl and the combined methyl and ethanolic head group representatives are considered as Group A compounds, and all non-methyl but ethanolic head group species are taken as Group B compounds. Different physical techniques have been employed to ascertain the amphiphilic behaviour in solution. The self-aggregation of these surfactants at different pH has been studied along with pH dependent interfacial activity of gelatin. The interaction of the two categories of the surfactants with gelatin at different pH has been investigated. A scheme for this interaction at various stages of the process has been proposed. The influence of the surfactant head groups on the interaction process has been assessed.

Keywords. Gelatin; surfactant; interaction; pH; head group.

1. Introduction

The changes in the molecular architecture of amphiphiles have an important consequences on their physicochemical properties and diverse applications of their organized assemblies.¹ Increased surfactant alkyl chain length results in a decreased critical micelle concentration (cmc) and the degree of counterion dissociation, and increase both in the aggregation number and the Kraft point.^{1,2} Also by altering the counterion, effective micellar surface charge density changes with a concomitant alteration of micellar effect on reaction rates and equilibria.³ It also plays a controlling role in determining the exothermicity of micelle formation and structural changes in micelles by way of the altered microenvironment in the Stern layer.⁴ Modification of surfactant head group on micellar properties is a less explored field. In aqueous solution, on increasing the hydrophobic character of the head group, the rates of reactions sensitive to change in solvent polarity or steric factors are enhanced.⁵ Enzyme kinetics in microemulsion medium has also a direct

impact on head group variation. Surface active enzymes preferably reside at the oil–water interface in close proximity with substrates if the surfactant head group is large.^{6–8} Thus, large hydrophobic headgroups partially exclude water from the interface resulting in a less polar reaction environment and leave ample spaces to accommodate substrate and enzymes.

The role of both polymer and surfactant in their interaction has been experimentally suggested by Goddard,⁹ Hayakawa and Kwak,¹⁰ and theoretically advanced by Nagarajan (who has proposed several types of possible arrangements of the interacted products).¹¹ The NMR,¹² SANS¹³ and fluorescence techniques¹⁴ have significantly contributed to the revelation of possible structural features of the polymer-surfactant interacting systems. Cationic lipid–protein interaction has been the subject of detailed investigations over the past few decades because of their interest in fundamental science and biotechnological applications.¹⁵ In such cases, modification of either protein or surfactant architecture generates ample scope for designing the process as per need. It has been found that on altering the head group architecture of cationic lipid molecules,

*For correspondence

in vitro transfection efficiency of DNA drastically varies. In presence of hydrophilic moieties like hydroxyethyl group, glucose or polyethylene groups, the efficiency enhances considerably.¹⁶ Dasgupta *et al*⁷ and Chatterjee *et al*¹⁸ have studied the interactional and packing behaviour of DNA with a variety of hydroxylated cationic surfactants.

Gelatin is a protein extracted from collagen that occurs in animal's connective tissues, and widely used as a gelling agent in food, pharmaceutical, photography and cosmetic manufacturing, etc.^{19,20} The tendency of gelatin to form coacervates with other proteins or hydrocolloids makes it useful for precipitating materials that cause haze in wines, juice, vinegar, etc. The coacervate hardens in presence of an aldehyde and is used suitably for encapsulation in pharmaceuticals. Gelatin also forms coacervates with oppositely charged surfactants, and to this end, studies have been mainly made with anionic surfactants.^{21–40} Interaction of gelatin with polyanions like sodium polystyrenesulfonate (NaPSS) or sodium poly(2-acrylamido-2-methylpropanesulfonate)⁴¹ or mixed micelles of SDS and a sugar-based non-ionic surfactant⁴² have been found to depend on the critical mole fraction, charge density, and chain length of the surfactants. But reports on the interaction of gelatin with non-ionic surfactants⁴³ or with cationic surfactants of varied types have been strikingly limited.^{44–48}

We have studied here the interaction of gelatin with the alkyltrimethylammonium bromide surfactants, and in particular the analogues of the C₁₆ variety having modified head groups in detail. The surface and bulk properties have been examined by various techniques and a schematic illustration of the involved interaction processes has been proposed. Physicochemical and interactional properties of this kind have been scarcely explored in the past.^{17,18} The study has been performed at pHs 5.4 and 9.0 without and with gelatin. With reference to the reported gelatin-CTAB interaction profile,⁴⁹ we have illustrated in detail and discussed the effects of the surfactant head group modification and pH on the said interaction process.

2. Experimental

2.1 Materials

Gelatin (average molar mass 38,000 g mol⁻¹ and isoelectric pH (IEP) 4.84) was a product of Sigma

USA (>99% pure)); the sample we used earlier.^{44,45,49,50} Of the surfactants used, CTAB was an AR grade product of Aldrich (USA, >99%). The preparation and characterization of the other surfactants used were reported elsewhere.^{7,8,18,49,51} They are subdivided in two groups as presented in scheme 1. Borax and boric acid were AR grade products of Merck (India). Pyrene (Aldrich, USA) was a gift sample from the Polymer Science Laboratory of IACS, Kolkata, India. It was purified by vacuum sublimation and then crystallized twice from a 3:1 ethanol-water mixture. The water used in the study was doubly distilled water of specific conductance 2–4 $\mu\text{S cm}^{-1}$ at 303 K. The surfactants produced expected cmcs as reported in literature.^{49,51} They did not contain surface active impurities as γ (surface tension) vs. log[surfactant] plots did not produce minima.

2.2 Methods

Measurements were taken either in boric acid–borax buffer medium at pH 9 or in acetic acid–sodium acetate buffer at pH 5.4 and ionic strength, $\mu = 0.005$. The biopolymer solution was prepared by dissolving a desired amount of dried gelatin flakes at room temperature to the aqueous buffer solution and allowing the biopolymer to swell for 30 min. It was then heated to 313 K with constant stirring until complete dissolution. The solution was then thoroughly mixed by stirring, cooled to room temperature and stored for use. The concentration of gelatin used has been expressed in g% (w/v). The maximum [gelatin] used in this study was 0.05%. The temperature for all experiments was 303 K, which is far above the gelation temperature of gelatin.⁴⁹

2.2a Tensiometry: Tensiometric measurements of γ were taken with a calibrated du Nouy tensiometer (Kruss, Germany) by the ring detachment technique. 10 mL gelatin solution of desired concentration (or pure buffer solution for dilution experiments) was taken in a thermostated (accuracy within ± 0.1 K) double walled container at the requisite temperature into which a stock surfactant solution of desired concentration (~ 10 – 16 times cmc) was step-wise added (as required) with a Hamilton microsyringe allowing 20 min equilibration time after each addition followed by the measurement of γ . The detailed procedure can be found in earlier reports.^{18,49} Duplicate measurements were taken to check reproducibility.

bility. The γ values were accurate within $\pm 0.1 \text{ mN m}^{-1}$ and were corrected following the principles of Harkins and Jordan.⁵²

2.2b Conductometry: The conductivity measurements were performed with a Jenway (UK) conductometer using a cell of unit cell constant. The same procedure as in tensiometry of addition of a stock concentrated surfactant solution ($\sim 10\text{--}30$ times cmc) to 10 mL gelatin solution or buffer solution at 303 K (accurate within $\pm 0.1 \text{ K}$) was followed. After ensuring thorough mixing and temperature equilibration, the specific conductance (κ) was measured. The accuracy of the measurements was within $\pm 1\%$. The measurement details can be found in our earlier reports.^{53,54}

2.2c Spectrophotometry: (i) Turbidimetry – Measurements were taken in an UV-visible spectrophotometer (model 1601 of Shimadzu, Japan) operating in dual beam mode using a matched pair of quartz cuvettes of path length 1 cm under thermostated condition at $303 \pm 0.1 \text{ K}$. The buffer solution was used as the control. The amphiphile stock solution (at $\sim 4\text{--}6$ times cmc) was then progressively added with a Hamilton microsyringe in the sample cell (consisting of 2.5 mL gelatin solution of desired strength) as required, and the solution was stirred well for mixing and allowed 5 min for equilibration. The spectral measurements were taken in the percent transmittance mode (%T) in the wavelength range of 200–700 nm. Turbidity index ($100 - \%T$ at 264 nm) was plotted against [surfactant]. At this λ , pure gelatin produced a shoulder in the spectra. The measured absorbance values were corrected for blank absorbance corresponding to the dilution of protein by the buffer solution. The measurement details can be found in our earlier report.⁵⁴

(ii) Fluorimetry – The fluorimetric measurements were taken in Fluoromax-P, Horiba Jobin Yvon (USA) fluorimeter using a fluorescence quartz cuvette of path length 1 cm. Experimental procedure was similar to that of turbidimetry excepting that here both gelatin and the stock surfactant solution contained $1.5 \mu\text{M}$ pyrene. The procedure details can be found in literature.¹⁵ The emission spectra of pyrene was scanned from 350 to 450 nm after excitation at 335 nm allowing an excitation and emission slit width of 5 nm, a wave length increment of 1.0 nm and an integration time of 0.10 s. The ratio

of the fluorescence intensities at the vibrational peaks of I_3 and I_1 in the emission spectra were then plotted against [surfactant].

2.2d Isothermal titration calorimetry (ITC)

The OMEGA ITC microcalorimeter of Microcal, Northampton (MA, USA) was used for thermometric measurements. A concentrated solution of the surfactant taken in the $350 \mu\text{L}$ microsyringe was added for an injection duration of 30 s to 1.325 mL of gelatin solution (taken in the calorimeter cell) at equal intervals of 240 s in multiple steps (32–42 additions) under constant stirring (350 rpm) condition. An identical gelatin solution (1.645 mL) was taken in the reference cell. The heat released at each step of interaction of the surfactant with gelatin was recorded and the enthalpy per mole of surfactant added was calculated with the ITC Microcal Origin 2.9 inbuilt software. The experiment of dilution of buffered amphiphile was also performed with the same injection matrix as that of the interaction experiment, taking aqueous buffer solution both in the reference and titration compartments of the cell. The enthalpy of the dilution (ΔH_d) or net enthalpy of interaction (interaction-dilution, ΔH_{i-d}) process was then plotted against [surfactant]. Each run was duplicated to check reproducibility. Water was circulated within the calorimeter by a NESLAB RTE100 bath at a temperature lower within five degree of the experimented temperature. The temperature in the cell compartment of the calorimeter was automatically scanned up to the desired temperature of measurement and adjusted with an accuracy of $\pm 0.01 \text{ K}$.

3. Results and discussion

3.1 C_{16} chain containing cationic surfactants

The cationic surfactants used are analogues of cetylammmonium bromide (CAB). They resulted either by replacing hydrogen(s) by methyl group(s) or ethanolic ($\text{CH}_2\text{CH}_2\text{OH}$) group(s) or both (scheme 1 categorizes them as Group A and Group B surfactants). Group A consists of the compound *N*-cetyl-*N,N,N*-trimethylammmonium bromide (CM_3AB) and its analogues *N*-cetyl-*N,N*-dimethyl-*N*-ethanoylammmonium bromide (CEM_2AB) and *N*-cetyl-*N,N*-diethanoyl-*N*-methylammmonium bromide (CE_2MAB). The Group B representatives are *N*-cetylammmonium

bromide (CAB), *N*-cetyl-*N*-ethanoylammonium bromide (CEAB), *N*-cetyl-*N,N*-diethanoylammonium bromide (CE₂AB) and *N*-cetyl-*N,N,N*-triethanoylammonium bromide (CE₃AB). The CE₃AB product may thus be synthesized both from CM₃AB and CAB. The surfactants have high solubility in water excepting CAB and CEAB which have shown fairly low solubility. Micellization of these surfactants in non-buffered aqueous solution have been reported earlier.^{49,51}

3.2 Amphiphilic head group behaviours at different pH

There were reports on the effect of varying chain length^{55–58} or counterion^{59,60} on the cmc of surfactants, but studies on the effect of varying head groups on the cmc of surfactants have been of less preponderance.⁶¹ Since in the present experiments, different pHs, 5.4, 6.5 and 9.0 have been used, the effect of pH on the ionization of the hydroxyl groups of the studied surfactants need to be addressed. The Group B surfactants CAB, CEAB and

CE₂AB contain three, two and one hydrogen(s) at the positively charged ammonium head group respectively. At pH 9.0, they act as an acid, discharge H⁺ (in solution) and neutral conjugate base species, which is insoluble at the above said pH. Since such a provision of deprotonation is not possible either for Group A surfactants (since the ammonium head group are all methyl substituted) or CE₃AB (containing three ethanolic groups), the question of insolubility does not arise.^{6–8} The Group B surfactants are, therefore, not studied at pH 9.0. It may be mentioned that for all the amphiphilic species, γ_{cmc} decreases with pH.

The head group dependent surface chemical and self aggregation properties of the two groups of surfactants at all the three pHs 5.4, 6.5 and 9.0 are comprehensively presented and discussed below with reference to table 1. The cmc's of Group A surfactants determined by different methods (surface tension, conductometry and microcalorimetry) are consistent. The observed cmc decreases by the increasing presence of the ethanolic group in the head at all the pHs (depiction at pH 9 in figure 1a). For the Group B, at all pHs the cmc follows the order CE₂AB < CE₃AB < CAB << CEAB. Only tensiometric method is used for the study (not illustrated). The γ_{cmc} values of each group of surfactants are nearly independent of their head group types. Their average magnitude at cmc is greater for Group A than for Group B. Thus the Group B representatives are more surface active than Group A species. The A_{min} and Γ_{max} (complimentary to each

Group A

CM ₃ AB [<i>N</i> -hexadecyl- <i>N,N,N</i> -trimethylammonium bromide]

CEM ₂ AB [<i>N</i> -hexadecyl- <i>N</i> -(2-hydroxyethyl)- <i>N,N</i> -dimethylammonium bromide]	CE ₂ MAB [<i>N</i> -hexadecyl- <i>N,N</i> -bis(2-hydroxyethyl)- <i>N</i> -methylammonium bromide]
Group B

CAB [<i>N</i> -hexadecylammonium bromide]	CEAB [<i>N</i> -hexadecyl- <i>N</i> -(2-hydroxyethyl)ammonium bromide]

CE ₂ AB [<i>N</i> -hexadecyl- <i>N,N</i> -bis(2-hydroxyethyl)ammonium bromide]	CE ₃ AB [<i>N</i> -hexadecyl- <i>N,N,N</i> -tris(2-hydroxyethyl)ammonium bromide]

Scheme 1. Surfactants used in the present work.

Figure 1. Tensiometric and microcalorimetric profiles of pure surfactants at 303 K. (a) tensiometric plots for Group A surfactants at pH 9.0; (b) enthalpograms of CEM₂AB at pH 9.0 and 5.4.

Table 1. Interfacial and bulk parameters of Group A and B surfactants at pH 5.4, 6.5 and 9.0.^a

pH	System	cmc			Interfacial adsorption parameters				Bulk properties		
		ST	Cond	Mcal	γ_{cmc}	$10^7 \Gamma_{\text{max}}$	A_{min}	$-\Delta G_{\text{ads}}^{\circ}$	β	$-\Delta G_{\text{m}}^{\circ}$	$-\Delta H_{\text{m}}$
9.0	CM ₃ AB	0.83	0.89	0.95	33.2	11.9	1.40	74.4	0.68	46.7	8.81
	CEM ₂ AB	0.74	0.74	0.64	33.3	10.8	1.54	81.2	0.60	45.2	13.9
	CE ₂ MAB	0.54	0.61	0.55	33.5	10.2	1.63	81.0	0.49	43.3	15.5
6.5	CM ₃ AB	1.06	0.99	1.09	33.5	21.4	0.78	—	0.67	45.5	7.79
	CEM ₂ AB	—	0.85	0.91	—	—	—	—	0.70	47.3	15.4
	CE ₂ MAB	—	0.78	0.83	—	—	—	—	0.68	46.9	16.8
	CAB	30.2	—	—	27.0	26.8	0.62	52.7	—	36.3	—
	CEAB	98.0	—	—	24.7	18.8	0.88	58.5	—	33.4	—
	CE ₂ AB	18.2	—	—	26.0	23.4	0.71	57.2	—	37.6	—
	CE ₃ AB	21.9	—	—	26.9	22.1	0.75	57.5	—	37.1	—
5.4	CM ₃ AB	0.72	0.73	0.72	32.7	22.9	0.72	65.3	0.72	48.7	8.84
	CEM ₂ AB	0.54	0.67	0.63	32.8	20.7	0.80	65.7	0.61	46.8	11.2
	CE ₂ MAB	0.46	0.55	0.45	32.5	19.4	0.86	65.8	0.54	45.4	14.0
	CAB	26.9	—	—	24.8	29.1	0.57	52.8	—	36.6	—
	CEAB	79.4	—	—	24.0	22.2	0.75	55.5	—	33.9	—
	CE ₂ AB	14.1	—	—	24.5	25.3	0.66	57.0	—	38.2	—
	CE ₃ AB	20.4	—	—	25.4	23.8	0.70	56.9	—	37.3	—

^acmc, γ_{cmc} , Γ_{max} and A_{min} are in mM (for Group A surfactants) and μM (for Group B surfactants), mN m^{-1} , mol m^{-2} and $\text{nm}^2 \text{ molecule}^{-1}$ respectively; $\Delta G_{\text{ads}}^{\circ}$, $\Delta G_{\text{m}}^{\circ}$, ΔH_{m} and are all in kJ mol^{-1} . Standard deviations in cmc, γ_{cmc} , Γ_{max} , A_{min} , f , $\Delta G_{\text{m}}^{\circ}$ and ΔH_{m} are ± 2 , ± 5 , ± 3 , ± 5 , ± 3 , ± 7 and $\pm 6\%$, respectively.

other), depend on the substitution or the modification of the head groups of the two classes of surfactants studied here. They are evaluated using the equations,^{53,54,62,63}

$$\Gamma_{\text{max}} = -1/(2.303nRT) \text{ Lt } d\gamma/d\log[S] \quad (1)$$

and

$$A_{\text{min}} = 10^{18}/N_A\Gamma_{\text{max}} \text{ in nm}^2 \text{ molecule}^{-1}, \quad (2)$$

where n , R , T , $[S]$ and N_A are the number of species formed per surfactant monomer in solution (it was 2 for the surfactants by ionization), the universal gas constant, the absolute temperature, the molar concentration of the surfactant in solution and the Avogadro number, respectively. Here concentration is used in place of activity, as the solutions in use are fairly dilute. The substitution of an ethanolic group for either one $-\text{CH}_3$ in the head group of CM₃AB (Group A) or one-H in the head group of CAB (Group B) produces large changes in the interfacial parameters. The second and third substitutions produce moderate changes. The substitution decreases the head groups' affinity for the counterions in the Group A species at pH 9.0 and 5.4, as found

from the declining magnitudes of the extent of counterion binding (β), evaluated following the relation, $\beta = 1 - (S_2/S_1)$.^{64,65} The conductance and the calorimetric methods are insensitive to elucidate β and the standard enthalpy change for micellization, $\Delta H_{\text{m}}^{\circ}$, respectively for the Group B compounds. The trends in the cmc were reflected on the standard Gibbs free energy of micellization, $\Delta G_{\text{m}}^{\circ}$ (estimated following earlier reported protocols^{53,54,63} $\Delta G_{\text{m}}^{\circ} = (1 + \beta)RT\ln X_{\text{cmc}}$, where X_{cmc} is the cmc of the pure surfactant in the mole fraction scale at temperature T). The $\Delta H_{\text{m}}^{\circ}$ becomes more exothermic with increased substitutions in the Group A surfactants. The phenomenon of micellization is generally entropy controlled at lower temperature, whereas at higher temperature, it is enthalpy controlled.⁶⁶ The first substitution produces comparatively large enthalpy change. The trends in the properties discussed are also dependent on pH; a slight decrease in the $\Delta H_{\text{m}}^{\circ}$ results with decrease in pH (figure 1b). The free energy of adsorption of the amphiphiles have been evaluated using the equation,⁶

$$\Delta G_{\text{ad}}^{\circ} = \Delta G_{\text{m}}^{\circ} - (\pi_{\text{cmc}}/\Gamma_{\text{max}}) \quad (3)$$

where $\pi_{\text{cmc}} = \gamma_{\text{water}} - \gamma_{\text{cmc}}$. It is also presented in table 1. The $\Delta G_{\text{ad}}^{\circ}$ is greater than that of $\Delta G_{\text{m}}^{\circ}$ for all the

systems at all pH. Their difference is a measure of the spontaneity difference of the two processes; the adsorption process is fairly stronger than the bulk process of micellization. Thus, the head group architecture of the surfactants play a decisive role in the pH dependent surface chemical behaviours of the surfactants.

The factors responsible for the self-aggregation of the surfactants in solution are solubility, steric repulsion and shielding of the head group charge. The Group B compounds have lower solubilities than the Group A compounds; the former produces cmc's of magnitudes one order lower than the latter. It is seen that the weakly soluble *N*-octyl-*N,N,N*-trimethylammonium bromide (OM₃AB) produces cmc values comparable with the Group B surfactants.⁶⁷ The addition of ethanolic group on the amphiphile head has two opposing effects on the cmc. It decreases the cmc due to the shielding of the charged head group to interact with its neighbours. Also it enhances the cmc by the steric repulsions among the ethanolic groups of the neighbouring amphiphiles. Thus, the numbers of the ethanolic groups present in the head plays a positive role on the amphiphile self-aggregation. Methyl group imparts more steric restriction than hydrogen. Thus, the cmc of the Group A surfactants are greater than Group B. A decrease in the cmc among the Group A members with increasing number of ethanolic substitution is thus a result of shielding of the positively charged N centre. Increase in the steric repulsion (due to additional methyl or ethanolic groups) in the matter of cmc increase with increased substitution is considered minor. The charge shielding effectivity of the ethanolic group(s) in both is considered equivalent. The factors mentioned above play a competing role towards their interfacial and bulk behaviours. The $A_{\min}^{\text{CAB}} < A_{\min}^{\text{CM}_3\text{AB}}$ whereas substitution of one ethanolic group in CAB or CM₃AB causes $A_{\min}^{\text{CEAB}} \gg A_{\min}^{\text{CAB}}$ and $A_{\min}^{\text{CEM}_1\text{AB}} \gg A_{\min}^{\text{CM}_3\text{AB}}$, but further substitution yielding CE₂MAB and CE₃AB enhances the head group area for the Group A surfactants mildly: thus, $A_{\min}^{\text{CM}_3\text{AB}} < A_{\min}^{\text{CEM}_2\text{AB}} < A_{\min}^{\text{CE}_2\text{MAB}}$ with no such significant variation for the Group B surfactants where $A_{\min}^{\text{CEAB}} > A_{\min}^{\text{CE}_2\text{AB}} \approx A_{\min}^{\text{CE}_3\text{AB}}$. Such a variation in the latter series is due to the reduction in the area of exclusion when substituted with more than one ethanolic group. Intramolecular hydrogen bonding among the multiple ethanolic substitutions is also effective in both CE₂AB and CE₃AB with a resultant decrease in the area. In the concentration region

fairly less than cmc, the surface activity of CE₂MAB at pH 9 is greater than that at pH 5.4 (figure 2) which is the trend for the other analogues in the group (table 1). The head group charge density is less at pH 5.4 favouring a close association among the charged head groups. The β values have a decreasing trend with increasing ethanolic substitution at pH 9 and 5.4; which is only marginal at pH 6.5. The micellization is more exothermic down the series at all pH.

3.3 Interfacial behaviour of gelatin

Gelatin is a random coil polyampholyte with a persistence length of ~ 20 nm,^{68,69} and the composite representation is (Gly-X-Pro)_n, in which 21% of the chain constitutes glycine, another 24% either proline or hydroxyproline (both non-polar residues), and X represents other amino acid residues. X mainly involves 13% basic groups such as lysine, histidine and arginine, 16% acidic groups such as glutamic and aspartic acid, other non-polar side chains constitute the rest 26%. Thus, a significant portion of the chain is neutral leaving sparsely charged portion.⁷⁰ The isoelectric pH of gelatin is 4.8. At pH 9.0, nearly 16% of the chain is negatively charged. It declines significantly at pH 5.4.

Gelatin is a denatured protein and thus prefers to reside at the air-solution interface. It has been reported that surface activity of gelatin enhances with temperature and its concentration.⁴⁹ At pH 5.4, the non-polar fraction in the gelatin chain is greater than

Figure 2. Tensiometric profiles of CE₂MAB at pH 9.0 and 5.4 in presence and absence of gelatin at 303 K.

the polar counterparts. This makes the chain appreciably hydrophobic and it prefers the interface yielding a decreased surface tension of 53.0 mN m^{-1} . At pH 9.0, the biopolymer is obviously less hydrophobic to produce increased surface tension (58.3 mN m^{-1}) of its aqueous solution.

3.4 Gelatin-amphiphile interaction: A schematic illustration with reference to CM_3AB as a representative

Since at higher pH, Group B amphiphiles have poor solubility than Group A amphiphiles, measurements of their interaction are taken only at the lower pH 5.4. Subsequent discussion results of Group A surfactants at the pH 9.0 and 5.4 and for Group B surfactants at pH 5.4 only are considered.

3.4a At pH 9.0: A synopsis of the said interaction taking gelatin- CM_3AB combination as a case is presented below with reference to results illustrated in figure 3 for the CM_3AB . Cationic surfactants like CM_3AB and OM_3AB , etc. interact with gelatin (G) forming composition dependent products at the interface and in the bulk.^{49,50} Initially, monomeric CM_3AB binds to the negative sites on the gelatin at a basic pH 9.0 at the interface (G^{I}) to form an interfacially adsorbed species GS_n^{I} , resulting an initial decrease in the surface tension till T_1 following the process A,

Further addition of CTAB causes the CTA^+ species to favorably combine with gelatin in the bulk (G^{B}),

thereby arresting the decline in $\gamma(T_1 - T_2 \text{ regime})$. This feature is only observable at gelatin concentration greater than 0.05%.

At a critical concentration (termed critical aggregation concentration c_{ac} or T_2), specific for each variety of surfactant, the local concentration of the monomers bound to the polymeric chain reaches its saturation. Then amphiphile monomers start to associate to form small induced micelles that bind with GS_n^{I} forming gelatin-micelle complex or GS_m^{B}

The tiny micelles adhere to the biopolymer chain and the complex becomes soluble in the bulk to get transformed into GS_m^{B} . Replenishment of the relieved interface (I) by the pure surfactant monomer also takes place simultaneously following the process,

The two processes B and C take place in concurrence. If they compensate each other γ remains non-variant with [amphiphile]. If the process B exceeds the process C, free surface is created with a net increase in γ . If otherwise, γ decreases. In the present system, there is more or less equivalent compensation with a slightly greater contribution of the process B from T_2 showing a shallow rise in γ till T_3 . A broad plateau is observed thereafter, when the two processes more or less compensate each other. The bulk concentration of GS_m^{B} becomes large with an onset of precipitation, termed as the coacervation at C_T with the formation of GS_m^{C} following the equilibrium,

Phase separation of GS_m^{C} induces formation of a narrow 'hump' at the higher concentration end of the plateau. The turbidity attains maximum at the hump (C_M). On further surfactant addition, the surfactant

Figure 3. Tensiometric profiles of Group A and B surfactants in 0.05% gelatin. CM_3AB and CE_2MAB at pH 9.0. Profile of CE_2MAB on 0.05% gelatin at pH 5.4 is shown for comparison. The break points are identified with symbols defined in the text.

monomers are adsorbed at the interface to decrease γ . After interfacial saturation at T_4 , free larger micelle formation occurs (with extended cmc) in solution. Thus, γ attains a constant value, i.e. γ_{cmc} at the second plateau. The out of phase GS_m^C (agglomerated complex) disintegrates beyond T_4 , and gradually gets a hydrophobic environment within the micellar core to become solubilized with decrease in turbidity to produce clarity in solution following the process E.

3.4b At pH 5.4: At pH 5.4, the negative binding sites for the biopolymer are smaller in number compared to that at pH 9.0. The formation of GS_n^I at pH 5.4 is less than that at pH 9.0. The γ vs $\log[\text{surfactant}]$ plots for the former are, therefore, of higher altitude than the latter. In the presence of gelatin at pH 9.0, two maxima (the second in the form of a hump) are observed (figure 3) both for CM_3AB and CE_2MAB . At pH 5.4, there appears only one maximum, and the initial rise is fairly flat. The formation of the coacervates and hence the associated turbidity is less. The formation of the hump (at pH 9.0 for amphiphile coprecipitation with the

coacervate) is thus absent. To differentiate between the two properties, the break points in the curve in the main plot of figure 4 at pH 5.4 are designated by 'P' instead of 'T'. As the concentration of GS_n^I is less at this pH, the process B and C have comparable rates to produce a broad plateau between P_1 and P_2 . P_1 corresponds to the cac. The profile is very similar to that of weakly interacting systems.⁷¹ Only a slight coacervation occurs at intermediate concentration between P_1 and P_2 . Dissolution starts soon on the addition of excess of the surfactant yielding a second plateau beyond P_3 similar to that arises at pH 9.0.

3.5 Influence of surfactant head group on gelatin-surfactant complexation

It has been recently reported that formation of DNA-cationic surfactant complexes is hardly affected by the surfactant head group architecture.¹⁷ The surfactants used there were to some extent common with that used here. But the observations on their interaction with gelatin do not fully comply with DNA. There is a report that calf thymus-DNA interaction with Group A surfactants is largely affected by the head group type.¹⁸ Primarily, the variation in the interaction profile arises from its influence on the local environment of the binding site in which the altered state of hydration by the presence of ethanolic groups may have a fare share. It corroborates fairly well with our results. The findings on the gelatin-surfactant systems with regard to the head group variation at pH 9.0 and 5.4 are discussed below.

3.5a Group A at pH 9.0: Substitution of the methyl group by the ethanolic group increases the head group area of the Group A surfactants: the cationic centre becomes sterically hindered to approach the ionic sites of gelatin. In spite of easier amphiphile self-aggregation due to the ethanolic substitution, steric effect defers the formation of the cac. A delayed T_2 (cac) for the Group A species thus arises (table 2a). Such a delayed DNA-induced aggregation of the Group A surfactants were also observed before.¹⁸ As noted before, T_2 appears in the tensiometric illustrations only, other methods fail to register its manifestation as also reported for other systems.^{49,54} At any particular concentration, the differences in the surface activities among the Group A surfactants are only moderate (figure 1a), whereas

Figure 4. Tensiometric interaction profile of Group A and B surfactants in 0.05% gelatin at pH 5.4. Main plot: For CM_3AB and CE_2MAB at 303 K. Inset: For CAB and CE_3AB . Broken and solid line represents CAB and CE_3AB , respectively in pure buffer medium (pH 5.4). The break points in the main plot are identified with symbols defined in the text.

Table 2. The T_2 (cac), T_3 , C_T , C_M and T_4 (cmc₂) (all in mM) for the interaction of gelatin with Group A and B surfactants at (a) pH 9.0 and (b) 5.4 at 303 K.^a

(a)														
pH 9.0	Surface tension			Cond		Mcal		UV		FL				
	T ₂ (cac)	T ₃ [C _T , C _M]	T ₄ [C _B]	T ₃ [C _T]	T ₄	T ₃ [C _T]	T ₄ [C _B]	T ₃ [C _T]	T ₄	T ₃ [C _T]	T ₄ [C _B]			
CM ₃ AB	0.03	0.14 [0.32, 0.38]	1.04 [0.45]	0.14 [0.35]	1.22	0.15 [0.33]	1.20 [0.61]	0.14 [0.32]	1.14	0.12 [0.35]	1.02 [0.49]			
CEM ₂ AB	0.038	0.09 [0.28, 0.36]	0.79 [0.44]	0.08 [—]	0.81	0.09 [—]	0.98 [0.60]	0.08 [0.34]	0.87	0.08 [0.27]	0.85 [0.43]			
CE ₂ MAB	0.045	0.08 [0.19, 0.30]	0.71 [0.38]	0.06 [—]	0.70	0.07 [—]	0.80 [0.50]	0.05 [0.23]	0.78	0.06 [0.23]	0.75 [0.39]			
(b)														
pH 5.4	ST			Cond		Mcal			UV			FL		
	P ₁	P ₂	P ₃	P ₁	P ₃	P ₁	P ₂	P ₃	P ₁	P ₂	P ₃	P ₁	P ₂	P ₃
CM ₃ AB	0.046	0.44	0.82	0.14	0.90	0.14	0.56	0.86	0.12	0.43	0.83	0.11	0.43	0.95
CEM ₂ AB	0.078	0.35	0.72	0.18	0.79	0.18	0.46	0.68	0.17	0.36	0.70	0.19	0.35	0.69
CE ₂ MAB	0.130	0.31	0.62	0.21	0.68	0.22	0.38	0.60	0.19	0.30	0.63	0.25	0.31	0.60
CAB	0.012	0.20	0.34	—	—	—	—	—	0.01	0.21	0.30	—	—	—
CEAB	0.019	0.17	0.58	—	—	—	—	—	0.02	0.14	0.50	—	—	—
CE ₂ AB	0.04	—	—	—	—	—	—	—	0.03	0.09	0.60	—	—	—
CE ₃ AB	0.05	—	—	—	—	—	—	—	0.05	0.20	0.69	—	—	—

^aStandard deviations of the break points are in the range of 3–10%.

appreciable divergences are observed for their interactions with the gelatin (CM₃AB and CE₂MAB in figure 3). It is observed that substitution enhances the surface activity at any particular concentration. This may be explained as follows. Inter-association of CH₂CH₂OH groups enhances hydrophobicity of GS_n¹, the extent of substitution concomitantly enhances the process, and subsequently the surface activity. A similar interaction at the air–liquid interface between NaPSS and DTAB has been reported.⁷² The point T_3 represents the stage at which the two processes B and C proceed at equal rates to yield a constant γ (figure 3). The appearance of T_3 depends on the hydrophobicity of the amphiphile and the nature of its head group. On increasing the number of substitutions at the ammonium head group, an early formation of T_3 results: the slope of the graph in the lower concentration end of the T_2 is greater for the CM₃AB compared with that of the CE₂MAB (figure 3). Formation of relatively lower concentration of GS_n¹, and consequently a lower concentration of GS_m^B results an early occurrence of T_3 for CE₂MAB than CM₃AB. Since the kinetics of a process depends on the concentration of the reactants, the rate of the process B for the substituted

varieties is marginally greater than that of the process C, and the two rates balance to result an early formation of T_3 .

Fluorimetric (I_3/I_1) profiles (figure 5) are indicative of the extent of the hydrophobic microenvironment around the fluorophore, pyrene. The index increases upon the addition of CM₃AB in gelatin solution up to T_3 before forming a crest or plateau similar to that reported earlier.⁷³ At any concentration, the polarity indexes of the ethoxylated compounds are more than that of CM₃AB which further supports the enhancement of the hydrophobicity with substitution. The index forms a flat top plateau for the CM₃AB at C_T , which gradually transforms to a crest in the case of CE₂MAB. A lower GS_m^B concentration results lowering of the GS_m^C concentration. This causes reduced maximum in the turbidity index (100-%T) (figure 6A) for CE₂MAB. C_T marks the appearance of phase separation (point of maximum solubility of the aggregate) in solution, depending on the types of the constituents to form the complex. As reported earlier, the ethanolic groups have an influence on the local environment of the binding sites in which the altered state of the hydration has a fare share.¹⁸ They are less susceptible to

enhance the hydrophilicity of the formed gelatin-surfactant complex, and are more prone to enhance their hydrophobicity due to the substituted ethyl groups. Consequently, all the turbidity related points occur at an early stage for CE₂MAB in the Group A series. Similar conclusion can be drawn from the conductance results. C_T is not visible in the conductance behaviour in the complex formation (figure 7).

Figure 5. Fluorimetric (I_3/I_1) dependence of Group A surfactants in presence of 0.05% gelatin at pH 9.0 and 5.4, at 303 K. CM₃AB, CEM₂AB and CE₂MAB at pH 9.0 in gelatin. CEM₂AB and CM₃AB at pH 5.4 in gelatin. The break points are identified with symbols defined in the text.

Figure 6. Turbidity profiles of Group A and B surfactants in presence of 0.05% gelatin at pH 9.0 and 5.4 at 303 K. (A) Profile of CEM₂AB and CE₂MAB at pH 9.0 and CM₃AB in pH 5.4. Broken line: profile of CE₂MAB in pH 5.4 (shown for a comparison). The break points are identified with symbols defined in the text; (B) CAB, CEAB, CE₂AB and CE₃AB at pH 5.4.

The charged colloidal polyelectrolyte-type complexes contribute to the conductance of the solution with their dissociated ions. The presence of the ethanolic groups reduces the counterion binding of the surfactant micelles that manifests into a reduced β_{T_1} with substitution (table 3). Lower charge density in the head group lowers the specific conductance of the substituted varieties (figure 8). Calorimetry profile (figure 9) for CM₃AB forms two peaks (lower

Figure 7. Conductometric profiles of Group A surfactants at pH 9.0 in 0.05% gelatin at 303 K. Profiles of CEM₂AB and CE₂MAB in buffer without and with gelatin.

Figure 8. Conductometric interaction profiles of Group A and B surfactants at pH 5.4 in 0.05% gelatin at 303 K. Profiles of CM₃AB at pH 9.0 and CE₂MAB at pH 5.4 are shown. Profile of CEM₂AB in buffer at pH 5.4 is illustrated for a comparison of the effects observed at the two pH.

Table 3. Thermodynamic bulk parameters in the interaction of 0.05% gelatin with Group A surfactants in pH 9.0 and 5.4 buffer solution at 303 K.^a

pH	System	β_{T_4}	$-\Delta G_m^\circ/\text{kJ mol}^{-1}$	$\Delta H/\text{kJ mol}^{-1}$		
				ΔH_1	ΔH_3	$-\Delta H_4$
9.0	CM ₃ AB	0.62	56.6	3.33	6.66	7.74
	CEM ₂ AB	0.57	44.0	2.90	5.83	6.44
	CE ₂ MAB	0.44	40.9	2.13	5.12	4.85
5.4	CM ₃ AB	0.51	41.9	2.08	3.56	3.14
	CEM ₂ AB	0.52	42.7	1.50	3.74	2.37
	CE ₂ MAB	0.57	44.7	1.33	4.15	2.54

^aStandard deviations in f_{T_4} , ΔG_m° , ΔH_1 , ΔH_3 and ΔH_4 are ± 5 , ± 3 , ± 5 , ± 4 and $\pm 4\%$, respectively.

Figure 9. Enthalpograms of Group A surfactants in presence of 0.05% gelatin at pH 9.0 and 5.4 at 303 K. Main plot: (a) CM₃AB; (b) CEM₂AB; and (c) CE₂MAB at pH 9.0; (d) CM₃AB; and (e) CE₂MAB at pH 5.4. Inset: Representative sections of the thermograms at pH 9.0.

and higher). The lower peak flattens with the ethanolic substitution to form a broad plateau (Inset, figure 9), the two ends correspond to T_3 and C_B , respectively. The peak heights are prominent at pH 9.0. The four distinct regions observed in the enthalpogram (Inset, figure 9) with their enthalpies are (I) the gelatin-small micelle complex formation (ΔH_1); (II) the preliminary association of the complex (ΔH_2 minor); (III) the advanced aggregation of the complex to form the coacervates (ΔH_3), and (IV) the formation of bigger micelles in solution (ΔH_4). The processes I, III and IV are endothermic, endothermic and exothermic, respectively (table 3). At pH 9.0, the enthalpy change follows the order CM₃AB > CEM₂AB > CE₂MAB. The order of the $\Gamma_{\max}^{T_4}$ and

$A_{\min}$ of the Group A surfactants at pH 9.0 and 5.4 without gelatin (table 1) is reverse than their order with gelatin (table 4). The magnitudes of the interfacial parameters in the presence of gelatin are much different from that in the absence. The complex GS_n^I at the interface plays a distinct role in this matter.

3.5b Group A at pH 5.4: Micellar aggregation on the gelatin chain to produce GS_m^B arises at a later stage at the lower pH 5.4 with less surface activity at each stage for the Group B surfactants (Inset, figure 4 and discussed subsequently). It is observed in the figure 4 (main plot) that the size of the plateau between P₁ and P₂ diminishes with the ethanolic substitution in the amphiphile head group of Group A surfactants. The plateau constitutes the adjustments among the processes B, C and D to maintain a constant surface pressure. The size decrease is due to reduction in the degree of interaction with substitution. Reduced coacervation thereby diminishes the process D. Thus, the formation of the two hump observed in the tensiometric profile at pH 9.0 (figure 3) is absent at pH 5.4. The surface excess ($\Gamma_{\max}^{T_4}$) drops both for CEM₂AB and CE₂MAB, with a concomitant increase in the $A_{\min}$ compared with CM₃AB (table 4). At higher pH, gelatin is a stronger polyelectrolyte and the complexed biopolymer is interacted with more induced micelles and moderately resides at the interface than in the bulk. At pH 5.4, the macromolecule is sparingly complexed, its residence is favorably at the interface. As opposite to pH 9.0, the surface charge density of the gelatin-surfactant complex at any stage increases with the ethanolic substitution at pH 5.4, registering increase in β_{T_4} (table 3). The interaction process decreases the conductance of free surfactant ions in the

Table 4. Interfacial adsorption parameters for the interaction of gelatin with Group A and B surfactants at pH 9.0 and 5.4.^a

pH	System	γ_{T_4} /mN m ⁻¹	$10^7 \tilde{A}_{\max}^{T_4}$ /mol m ⁻²	$A_{\min}$ /nm ² molecule ⁻¹	$-\Delta G_{\text{ads}}^{\circ}$ /kJ mol ⁻¹
9.0	CM ₃ AB	34.2	5.52	3.01	87.4
	CEM ₂ AB	33.7	6.55	2.53	101.1
	CE ₂ MAB	33.2	6.79	2.44	96.7
5.4	CM ₃ AB	34.6	6.15	2.70	101.2
	CEM ₂ AB	34.8	4.40	3.77	125.1
	CE ₂ MAB	35.1	2.93	5.67	167.6
	CAB	25.9	20.6	0.80	52.1
	CEAB	24.7	15.9	1.04	58.0
	CE ₂ AB	25.0	11.7	1.42	75.0
	CE ₃ AB	26.1	11.1	1.49	75.5

^aStandard deviations in γ_{T_4} , $\tilde{A}_{\max}^{T_4}$, $A_{\min}$ and $\Delta G_{\text{ads}}^{\circ}$ are ± 3 , ± 5 , ± 8 and $\pm 5\%$, respectively.

presence of gelatin (figure 8). The head group orientation with pH has a significant say on the surface charge density of the complexes. In the microcalorimetric enthalpogram, the peak heights are significantly lower at pH 5.4, with a decreased enthalpy in all the cases (figure 9 and table 3). The enthalpy of the coacervation (ΔH_3) follows a reverse trend of CE₂MAB > CEM₂AB > CM₃AB at pH 5.4 than that at pH 9.0. An explanation for the observed trends of ΔH_3 is difficult since various processes may participate in the process.^{74,75} Reduced coacervation leads to a decrease in the maximum turbidity index at the lower pH to nearly half of that at the higher pH (figure 6A, CE₂MAB taken as a case). Fluorimetric profile results an initial plateau at the start (figure 5) with a subsequent increase from P₁. Such a profile is also found in literature.⁷⁶ There is an initial steep rise for the Group A surfactants at pH 9.0 essentially due to the formation of the hydrophobic GS_n¹ complex. At pH 5.4, the initial surfactant addition causes negligible enhancement in the polarity index or the hydrophobicity as the interaction is reduced significantly at the lower pH to yield a low concentration of the said complex (GS_n¹).

3.5c Group B at pH 5.4: Only the tensiometric results are presented for pure Group B surfactants at pH 6.5 and 5.4; at pH 9.0, the compound tends to become poorly soluble. The results discussed are with reference to pH 5.4. The CAB and CEAB follow similar profiles (not illustrated) of interfacial and bulk behaviours as discussed in the previous section. The CE₂AB and CE₃AB (Inset, figure 4), on the other hand, produce features similar to that of DTAB and TTAB reported earlier.⁴⁹ The characteristic feature of the broad plateau were also found in

other systems like SDS/poly(vinylpyridinium chloride)/NaCl (0.1 M) system,⁷⁷ sodium tetradecylsulfate/polydimethyldiallylammonium chloride/0.1 M NaCl⁷⁸ and the 7 wt% gelatin-SDS system.⁷⁹ Likewise, for CE₂AB and CE₃AB, the plateaus appear from the point beyond their aqueous cmcs. Substantial precipitation from the starting point for the CE₃AB affects the tensiometric measurements. Turbidimetric maximum decreases with increased substitution (figure 6B). This is also true for the Group A surfactants. The CE₃AB behaves quite odd in this respect. Unlike others, precipitation of its gelatin-surfactant complex does not dissolve at the end. Other methods fail to register the interaction features of the Group B surfactants: this restricts confirmation of the information on the transition points (table 2b). Thus only the cac points can be compared which suggests an order of CAB < CEAB < CE₂AB < CE₃AB by both tensiometry and turbidimetry. Of course, the extended cmc points obtained from the turbidimetry also follows the order as cac between CAB and CEAB found from the tensiometry corroborates with the turbidimetry. In the post interaction stage with gelatin, the $\Gamma_{\max}^{T_4}$, $A_{\min}$ and $\Delta G_{\text{ads}}^{\circ}$ parameters of the Group B surfactants at pH 5.4 follow the orders concomitant with the ethanolic substitution in the head group of the CAB (table 4).

4. Conclusions

Substitution of methyl and/or ethanolic groups in the ammonium head moiety of the surfactant hexadecylammonium bromide plays a crucial role in their surface chemical and bulk behavioural patterns in buffer solution in the absence and presence of gela-

tin. Such head group architectural feature alters the steric behaviour as well as changes in the surface charge density of the amphiphilic head groups with changing pH. The self-aggregation of these amphiphile molecules in solution thus gets affected. The compromising balance between the two effects (steric and surface charge density) thus play a determinant role in the critical micellar concentration (cmc) in solution. Lower pH favours early formation of cmc. Interaction of the surfactants with gelatin at different pH is expected to show differences; two pHs 5.4 and 9.0 are considered to cover the basic region from the vicinity of isoelectric state (pH 4.8) Ethanolic substitution plays an interesting role in the interactional process. It defers cac at both the pH 5.4 and 9.0, enhances hydrophobicity of the protein-surfactant complex through inter-association between its chains and shows less efficiency in removing turbidity. Changes in pH responds in a typical manner: lower pH reduces the total number of binding sites on gelatin, defers cac, and reduces concentration and surface activity of the protein-surfactant complex at various stages in the interaction profile. This causes less presence of the complex at the interface in the far end of the interaction process. Surface charge density lessens with substitution at pH 9.0, whereas it enhances with substitution at pH 5.4. The Group B surfactants are less susceptible to respond to various methods of probing the bulk and the interfacial features; thus a generalized comment on their surface chemical behaviour would be less conclusive than Group A compounds.

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