



## Behavior of papain in mixed micelles of anionic–cationic surfactants having similar tails and dissimilar head groups

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### ABSTRACT

The mixed micellar behavior of anionic surfactant, sodium dodecylsulfate (SDS) and cationic surfactant, dodecylethyl-dimethylammonium bromide (DDAB) at varying mole fractions of DDAB, i.e.  $\alpha_{\text{DDAB}}$  in aqueous solution of papain has been investigated with the aid of spectroscopy and physicochemical measurements. Thermodynamic parameters have been computed over the entire mole fraction range of DDAB. The Clint equation and the regular approximation method have been used to investigate the interactions between mixed surfactants in the presence of protein. The two surfactants have similar tails, however, the charges on respective polar head groups are expected to have significant effect on their colloidal behavior. The cmc values of mixed surfactants have been estimated from fluorescence, conductivity, surface tension, ultrasonic speed and density measurements. The results show that lower and higher mole fractions of DDAB give negative departure from ideality whereas intermediate mole fractions have positive deviation. The aggregation number,  $N_{\text{agg}}$  of mixed micelles has also been calculated. The turbidity or cloudiness at intermediate mole fractions demonstrates a reduction in the background free monomer concentration due to neutralization of the monomers of opposite charge. The effect of concentration of papain on mixed micellar behavior indicates that with increase in the concentration of protein, the cac and cmc values increase. The unfolding of polypeptide chain in the presence of mixed surfactant has been observed.

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### 1. Introduction

The interaction of surfactant molecules with water-soluble proteins and enzymes is a matter of interest, due to their importance in many biological, pharmaceutical and industrial systems [1–7]. An understanding of the mechanisms involved in protein–surfactant interactions provides a basis for evaluation of protein stability and rational strategy to optimize the applications of surfactants. Recently, much attention has been given to mixed surfactants since they perform better than single systems [8–11]. The use of mixed micelles has emerged as an alternative to lipids for quantitative study of membrane protein behavior [12]. It is well known that electrostatic interactions play a prominent role during mixing of oppositely charged surfactants [13,14]. Among all the mixed anionic–cationic surfactants, mixtures of alkyl sulfate and tetra ammonium salts are the most commonly studied [15]. However, at low concentrations, the mixtures of anionic and cationic surfactants precipitate at the equimolar mixing. When one of the surfactants is in excess, the precipitate redissolves forming micelles in systems [16]. The precipitation or turbidity is a clear sign of surface activity loss that restricts the practical use of such mixtures. The addition of proteins to mixed surfactant solutions may result in

the modification of both intramicellar and intermicellar interactions and thereby affecting their colloidal behavior.

In the present report, the colloidal behavior of mixed anionic–cationic surfactants, sodium dodecylsulfate (SDS)–dodecylethyl-dimethylammonium bromide (DDAB) has been investigated in the presence of protein, papain. Interesting results have been obtained using several techniques *viz* fluorescence, FT-IR, conductivity, viscosity, turbidity, ultrasonic speed and density over whole mole fraction range of DDAB at 298.15 K. The behavior of various parameters provides insight into the micro-polarity, bulk properties and thermodynamics of the self-organized assemblies in the mixed anionic–cationic surfactant solutions in the presence of protein. The anionic–cationic surfactant mixtures have a significantly higher surface activity than their individual components and mixtures of other types. Thus, the investigation of the properties of the mixed anionic–cationic surfactant systems is promising in the search for highly surface-active systems.

### 2. Materials and methods

#### 2.1. Materials

Sodium dodecylsulfate (SDS, purity >99%) and dodecylethyl-dimethylammonium bromide (DDAB, purity >99%) were purchased from Fluka. Papain (purity >99%) was from Himedia. Papain was

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dissolved in double distilled water and the pH was unadjusted. Double distilled water of specific conductance  $2\text{--}3\ \mu\text{S cm}^{-1}$  was used for the preparation of solutions.

## 2.2. Methods

All the samples were prepared by mole fraction method for the measurements. The estimated uncertainty in the weight was  $\pm 0.1\%$ .

### 2.2.1. Fluorescence

The fluorescence of the mixtures of SDS and DDAB surfactants in aqueous solution of papain was measured using a Varian fluorescence spectrophotometer at  $298.15\ \text{K}$ . Pyrene was used as a probe. It is well known that the ratio of first to third vibronic peaks ( $I_1/I_3$ ) of the pyrene emission spectrum indicates the polarity of the medium in which pyrene is dissolved. The concentration of pyrene was kept constant, i.e.  $10^{-6}\ \text{mol dm}^{-3}$ . Stock solutions of two unlike components of various binary mixtures were prepared by dissolving appropriate amounts of each component. Fluorescence titrations were performed by successive addition of concentrated stock solutions to reference water plus pyrene solution in order to keep the pyrene concentration constant.

### 2.2.2. FT-IR

The FT-IR spectra were recorded on a Perkin Elmer RX spectrometer in the frequency range  $4000\text{--}400\ \text{cm}^{-1}$ . The solutions were prepared by mixing SDS–DDAB and papain in  $\text{D}_2\text{O}$ . The samples were placed in an AgCl cell. The water vapor and solvent absorptions were subtracted from the experimental spectra with subsequent nine-point smoothing by the Savitski–Goley algorithm [17].

### 2.2.3. Surface tension

The surface tension experiments were performed by platinum ring detachment method using a K20 Krüss tensiometer at a controlled room temperature ( $298.15 \pm 2\ \text{K}$ ). The accuracy of the measurement was within  $\pm 0.1\ \text{mN m}^{-1}$ . Before the measurement, the platinum ring was cleaned with highly dilute solution of chromic acid mixture and washed thoroughly with double distilled water and then burned red using the oxidizing portion of the flame of a Bunsen burner. The various concentrations of SDS–DDAB in papain solutions were prepared by addition of small aliquots of concentrated SDS–DDAB solution to 25 mL of sample. The ring was hung from the load cell and lowered about 5 mm below the surface of the liquid. The ring was pulled from the surface and the apparent surface tension was recorded.

### 2.2.4. Conductometry

The conductivity measurements of mixed surfactants in the absence as well as in the presence of papain and at varying mole fractions of DDAB were carried out with the help of digital conductivity meter from LABINDIA. The detailed procedure of conductance measurement has been reported earlier [18].

### 2.2.5. Turbidity measurement

The turbidity was measured using a Digital-Turbidity meter from Decibel Instruments India, after allowing sufficient time (about half an hour) for equilibration. This instrument works on the basis of scattering of light by the colloidal particles. The light coming from the light source was focused on the colloidal solution by passing it through a pair of lenses. The scattered light at a right angle to the incident light was detected by a photo multiplier tube.

### 2.2.6. Viscometry

The viscosity measurements were carried out using Ubbelohde type suspended level capillary viscometer. The viscometer was thermostated at  $298.15 \pm 0.01\ \text{K}$ . All solutions were prepared in

double distilled water. The detailed procedure of viscosity measurement has been reported earlier [18].

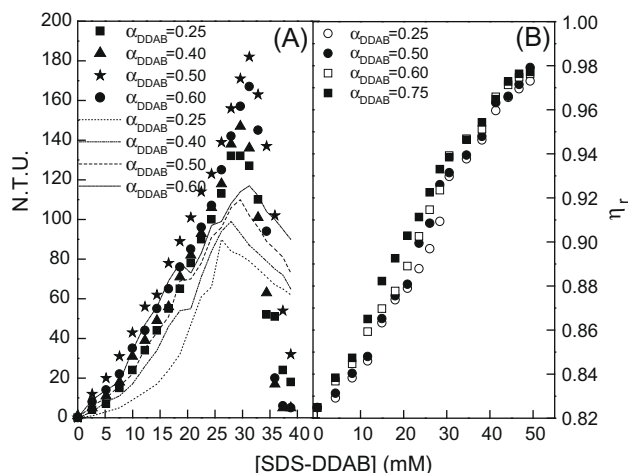
### 2.2.7. Ultrasonic speed and density

Densities and ultrasonic speeds of SDS–DDAB mixtures at varying mole fractions of DDAB in papain were simultaneously and automatically measured using a commercial apparatus (Anton Paar DSA 5000 densimeter and a sound velocity analyser) at  $298.15 \pm 0.001\ \text{K}$ . The reproducibility of the density and ultrasonic speed measurements were  $\pm 10^{-6}\ \text{g cm}^{-3}$  and  $\pm 10^{-2}\ \text{m s}^{-1}$ , respectively. The measurements of density and ultrasonic speed of aqueous solutions of SDS–DDAB solutions in papain were taken as a function of concentration.

## 3. Results and discussion

### 3.1. Turbidity or cloudiness of the systems

It has been reported [19,20] that anionic–cationic surfactant mixtures show turbidity at intermediate compositions. The investigated SDS–DDAB mixture with the same carbon chain length form homogeneous system at higher mole fraction of either SDS or DDAB whereas all other mole fractions lie in between are significantly turbid. Fig. 1A represents the variation of turbidity with increase in mixed surfactant concentration of [SDS–DDAB] at varying mole fractions of DDAB, i.e.  $\alpha_{\text{DDAB}}$ . The turbidity first increases with increase in concentration of SDS–DDAB, reaches maximum and thereafter starts decreasing. The initial increase in turbidity points towards the unfolding of polypeptide chain [7]. The unfolding has been elaborated in later sections. When one of the surfactants is in excess, the precipitate redissolves and low turbidity is observed [21]. Further, increase in concentration of SDS–DDAB, neutralization of oppositely charged electrical site available both on the surfactant head group take place. The strong electrostatic interactions between the oppositely charged surfactants and protein might be responsible for the formation of insoluble dimers. The possibility that turbidity is due to precipitation or phase separation is ruled out since no phase separation was observed in the samples even after keeping these for several days under observation. In the absence of protein, the lower turbidity values (Fig. 1A) demonstrates the weaker interactions between the surfactant mixtures. The formation of dimer is



**Fig. 1.** Variation of (A) turbidity of mixed surfactant in water (represented by lines) and in 0.001%, w/v papain (represented by solid symbols); (B) relative viscosity,  $\eta_r$ , with increase in mixed surfactant concentration of [SDS–DDAB] at various mole fractions of DDAB, i.e.  $\alpha_{\text{DDAB}}$ .

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