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ORIGINAL CONTRIBUTION

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Studies on the interaction of bacterial capsular polysaccharide- *Klebsiella K16* with cationic and mixed surfactants

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Abstract The spectral studies of cationic dyes, pinacyanol chloride (PCYN) and acridine orange (AO) with capsular polysaccharide *Klebsiella K16* (PK16) biopolymer in micellar media reveal many interesting phenomena. Intensity of the metachromatic band (μ) at 490 nm decreases gradually on addition of cationic single surfactant to the biopolymer PK16–dye system of P/D = 30, whereas the intensity of α and β bands reach to the value of original pure dye. As a result, the cationic surfactant destroys the metachromatic compound and forms a new complex with biopolymer PK16 by freeing the dye molecule. Enhancement of fluorescence intensity of AO-PK16 system with cationic surfactant is another evidence for the binding between the biopolymer and the surfactant. Interaction between the biopolymer and mixed surfactant has also been studied. Finally, the binding ability of cationic surfactants with or without non ionic surfactant, the idea of the critical aggregation concentration (cac) of the surfactant, mole fraction and the charge density of mixed surfactant for binding with PK16 and also the site of interaction have been pointed out.

Keywords Mixed micelle · Charge density · Cac · Binding constant · Metachromatic compound

Introduction

Surface active species i.e., surfactants are amphiphilic in nature capable of forming organized structure like micelle or reverse micelle in an aqueous and a nonaqueous solution, respectively. Surfactants play a major role in the adherence and food emulsification in micro-organisms and surface tension in the mammalian lung [1]. One of the outstanding properties of surfactants is their ability to solubilize molecules which otherwise would not be soluble in water.

Polymer–surfactant interaction in aqueous medium is a developing field of research. Interaction between water soluble non ionic polymer and ionic surfactants has been extensively studied and a lot of information gained about the polymer–surfactant interaction under varying conditions [2]. Aqueous polymer–surfactant systems are of great importance in industrial applications such as enhanced oil recovery, detergency and pharmaceutics due to their amphiphilic aggregation behaviour and delicate rheological characteristics [3, 4].

In most of the cases, such studies are involved between polymer and single surfactant. But there is very little work in the literature devoted to the study of the binding of mixed surfactants to oppositely charged biopolymer. Systems with mixed surfactants are of scientific interest as well as technological value [5]. Dubin et al. [6, 7] studied the effect of added non ionic surfactant on the interaction of the mixed surfactant and oppositely charged polymer. Li et al. [8] used the spectroscopic method to study the properties of various polymer / sodium dodecyl sulphate (SDS) / cosurfactant

solutions. Panda et al. [9] also studied the micellar effect of pure cationic and cationic-non ionic surfactants on *Klebsiella 03* lipopolysaccharide and found the sequence of binding capability of the surfactants with polymer.

To know the behaviour of the capsular polysaccharide, *Klebsiella K16* (PK16), biopolymer towards surfactants, we studied the interaction between PK16 and single cationic surfactant and also mixed cationic/non ionic surfactant systems by dye-incorporation technique. For this purpose, the thermodynamic and spectrophotometric properties of these systems are reported in this paper. Furthermore, we have compared the binding capability of cationic surfactants to *Klebsiella K16*.

Experimental details

The serological test strain of *Klebsiella K16* was obtained from Dr. S. Schlecht of Max Plank Institute for Immunobiology, Freiburg, Germany. Cationic surfactants: BDHAC (Benzyldimethyl-n-hexadecyl ammonium chloride), CTAB (Cetyltrimethyl ammonium bromide), CPCl (Cetylpyridinium chloride), DPCl (Dodecylpyridinium chloride) and non ionic surfactants: Triton X-100 (Octylphenyl-polyoxyethylene glycol ether), Tween-20 (Polyoxyethylene sorbitan monolaurate), Tween-40 (Polyoxyethylene sorbitan monopalmitate), Tween-60 (Polyoxyethylene sorbitan monostearate) and Brij-35 (Polyoxyethylene lauryl ether) were obtained from E. Merck. Cationic dyes: Pinacyanol chloride (PCYN) and Acridine orange (AO) of Sigma were used after recrystallization. Cationic and non ionic surfactants were purified by recrystallization from appropriate water/ethanol and water/chloroform mixture, respectively and then dried under vacuum.

Absorption spectra were recorded on spectrophotometer (Milton Roy Spectronic-21D) with a matched pair of stoppered quartz cell of 1 cm path. The fluorescence spectra were recorded on a Shimadzu spectrofluorometer (model RF 5000) by exciting at the absorption maximum 480 nm of the AO dye in aqueous solution, using quartz cell of 1 cm path.

All measurements were done with freshly prepared solutions using double distilled water.

Results and discussion

Interaction between biopolymer and single surfactant

Interaction between the capsular polysaccharide *Klebsiella K16* and the cationic surfactants were studied by dye - incorporation technique. Aqueous solution of the PCYN chloride dye (1×10^{-5} mol dm^{-3}) showed sharp peaks at 600 nm and 550 nm corresponding to monomeric band (α) and dimeric band (β), respectively

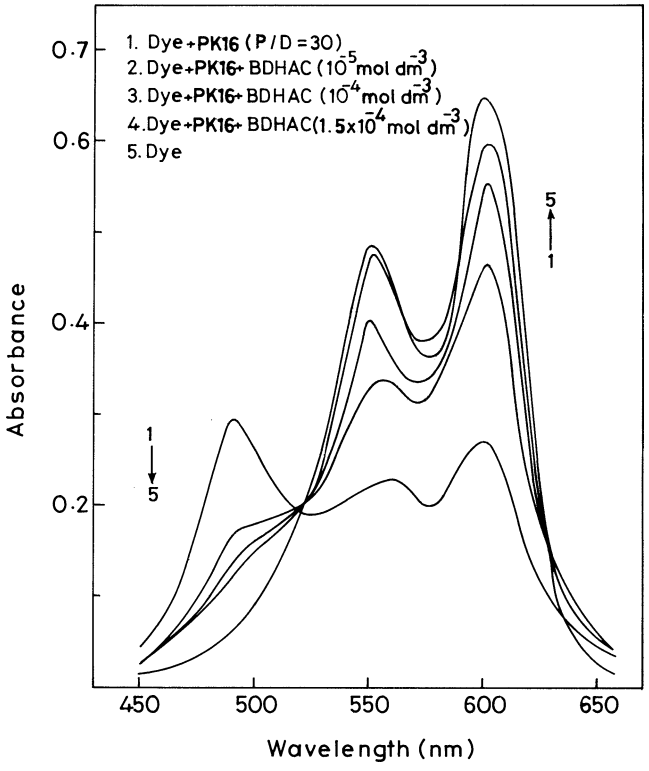

Fig. 1 Absorption spectra of PCYN–*Klebsiella K16* biopolymer complex (P/D = 30) with the addition of different concentration of BDHAC at 298 K. Concentrationof dye PCYN: 1.0×10^{-5} mol dm^{-3}

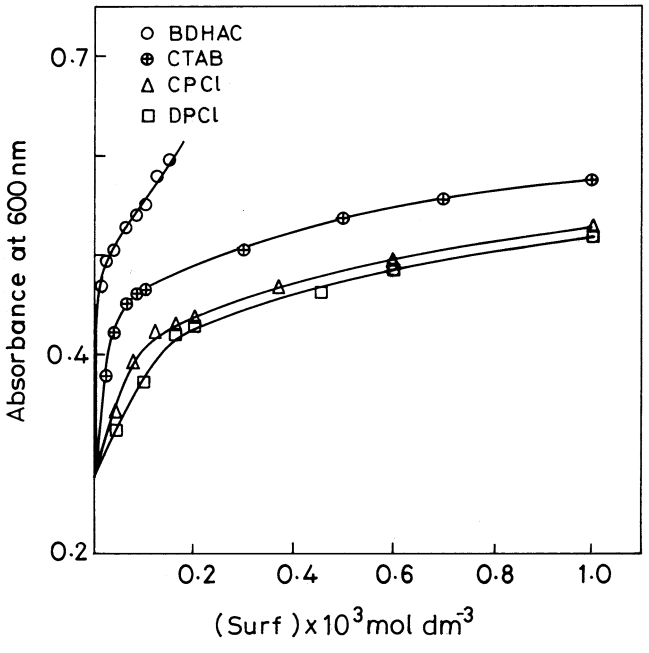

Fig. 2 Effect of cationic surfactants on the absorbance of PCYN–*Klebsiella K16* biopolymer complex (P/D = 30) at 600 nm

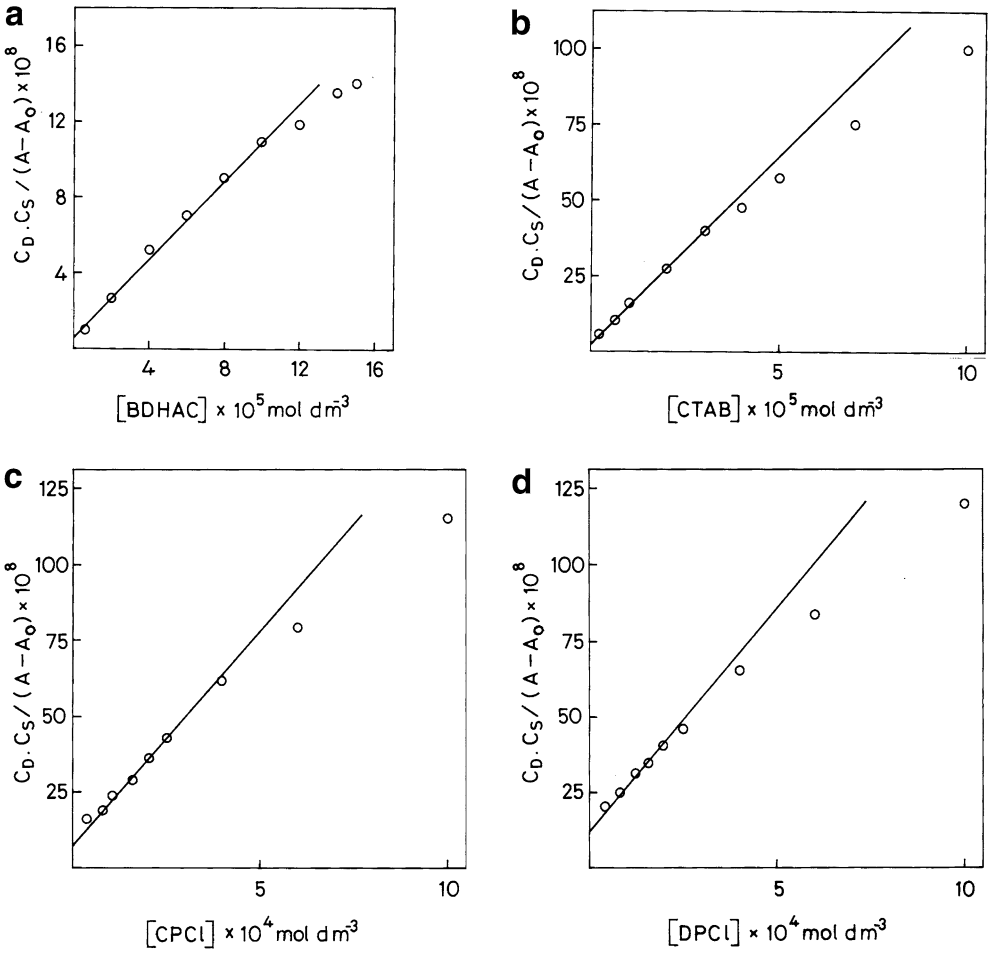

Fig. 3 Plots of $C_D.C_S/A\text{-}A_O$ versus C_S i.e., concentration of surfactant in PCYN–*Kebsiella K16* biopolymer complex (P/D = 30) with the addition of different cationic surfactant: a) BDHAC, b) CTAB, c) CPCl and d) DPCl

Table 1 Binding constant between biopolymer *Klebsiella K16* polysaccharide and cationic surfactants at 298 K

Surfactant	CMC mol dm^{-3}	K_C (Absorbance study) dm^3 mol^{-1}	K_T (Fluorescence study) dm^3 mol^{-1}
BDHAC	0.042×10^{-3}	1.73×10^{-5}	1.66×10^{-5}
CTAB	0.80×10^{-3}	0.50×10^{-5}	0.60×10^{-5}
CPCl	0.90×10^{-3}	0.229×10^{-5}	0.256×10^{-5}
DPCl	14.70×10^{-3}	0.132×10^{-5}	0.159×10^{-5}

as shown in Fig.1. It was found that upon addition of a biopolymer, intensities of both α and β bands decreased and a new band (μ) appeared at 490 nm due to formation of metachromatic compound by biopolymer PK16 and dye with their ratio (P/D) above 10. This work has been published [10]. At a definite P/D ratio of 30, with addition of different concentration of cationic surfactant BDHAC i.e., 1×10^{-5}–1.5×10^{-4} mol dm^{-3}, absorbance of the metachromatic compound at 600 nm (α band) and 550 nm (β band) increase and attain pure dye absorbance whereas the metachromatic band at 490 nm is destroyed.

Like BDHAC, other cationic surfactants e.g. CTAB, CPCl and DPCl also destroy the metachromatic compound formed by PCYN and PK16, and simultaneously increase absorbance at α and β bands. In Fig. 2, the effect of these cationic surfactants on absorbance of the PCYN–PK16 complex at 600 nm have been exhibited. From this, it is clear that increase in absorbance value at 600 nm for different surfactants followed the order: BDHAC > CTAB > CPCl > DPCl, which was found to be in accordance with their CMC values.

From Fig.1, it is observed that surfactant BDHAC molecules interacted with PK16 by replacing cationic

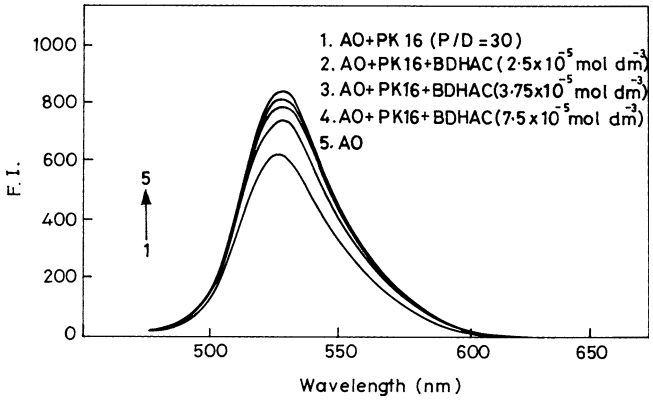

Fig. 4 Fluorescence spectra of AO–*Klebsiella K16* biopolymer complex (P/D = 30) with the addition of different concentration of BDHAC at 298 K. Concentration of dye AO: 1.0×10^{-5} mol dm^{-3}

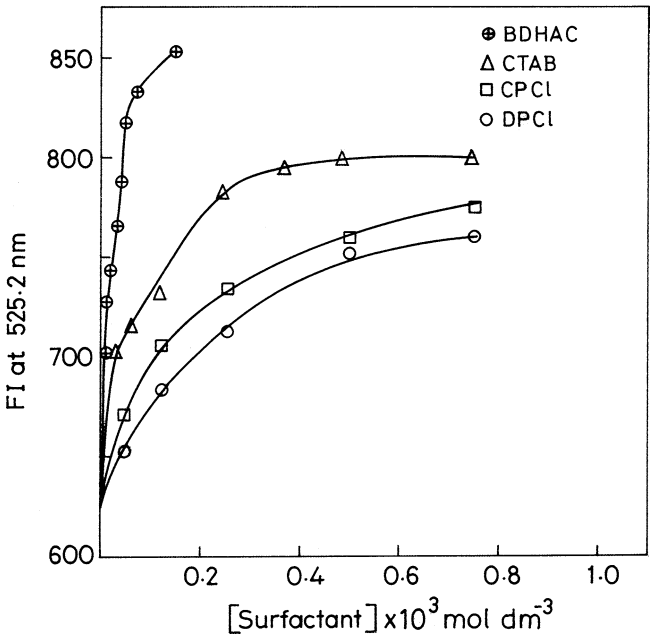

Fig. 5 Effect of cationic surfactants on the fluorescence of AO–*Klebsiella K16* biopolymer complex (P/D = 30) at 525.2 nm

dye molecules i.e., freeing dye molecules. The extent of increase in absorbance was considered to be equivalent to the amount of BDHAC bound to the polysaccharide. Spectrophotometric data were employed to calculate the binding constant of biopolymer-cationic surfactant interaction. For a 1:1 complex, the binding constant K_C between cationic surfactants and biopolymer PK16 can be determined using the Rose and Drago Eq. [11] in the following form:

$$C_D.C_S/A\text{-}A_O = 1/K_C L(\varepsilon_{DS} - \varepsilon_D) + C_S/L(\varepsilon_{DS} - \varepsilon_D) \quad (1)$$

where C_D and C_S are the initial concentration of *Klebsiella K16* and cationic surfactant, respectively; L is the optical path of the solution; A_O and A are the absorbance of the dye-polymer solution at 600 nm without and with surfactant, respectively and ε_{DS} and ε_D are the respective molar extinction coefficient of the polymer–surfactant complex and the polymer at the maxima of the complex, and K_C is the binding constant between the polymer and surfactant. In Fig. 3, $C_D.C_S/A–A_O$ versus C_S was plotted for the PCYN–PK16 polymer complex by addition of different cationic surfactants which were found to be linear, confirming 1:1 complex formation between PK16 and cationic surfactant. From the slope of each plot, K_C of the polymer–surfactant complexes was calculated and presented in Table 1.

Like absorbance, fluorescence study with cationic surfactants in the dye–polymer system exhibits a similar phenomenon. When the fluorescent dye AO (λ_{em}, 525.2 nm) was used, the fluorescence intensity was quenched in the presence of the PK16 biopolymer as shown in Fig.4. But with addition of cationic surfactant to the AO–biopolymer system, fluorescence intensity was enhanced indicating interaction between PK16 and surfactant, and removal of dye molecules. The fluorescence intensity of the AO-PK16 complex at 525.2 nm with P/D = 30 increased gradually with increase in concentration of surfactant, BDHAC. The effect of other cationic surfactant e.g. CTAB, CPCl and DPCl on the fluorescence intensity of the dye–polymer complex is similar to BDHAC. In Fig. 5, it is shown that the fluorescence intensity at 525.5 nm of the AO-PK16 system with P/D of 30 reached optimum values with specific concentrations of different cationic surfactants. Here also, it could be said that the binding capability of the surfactants to the biopolymer followed the order: BDHAC > CTAB > CPCl > DPCl as seen from the absorbance study.

From the spectrofluorometric data of Fig.5, the binding constant between the biopolymer and surfactants was determined using Chlang and Lukton's Eq. [12] in the following form:

$$1/F.I = 1/\alpha_T[D] + n/\alpha_T K_T[M][D] \quad (2)$$

where [M] and [D] are the surfactant and total dye concentrations, respectively; α_T and K_T are the temperature coefficient and binding constant between polymer and surfactant; n is the aggregation number of surfactant and F.I is the fluorescence intensity of the dye–polymer complex in the presence of the surfactant. In Fig. 6, the straight lines were obtained by plotting 1/F.I versus 1/[M], and K_T values were determined from the slope and intercept of these straight lines which are inserted in Table1.

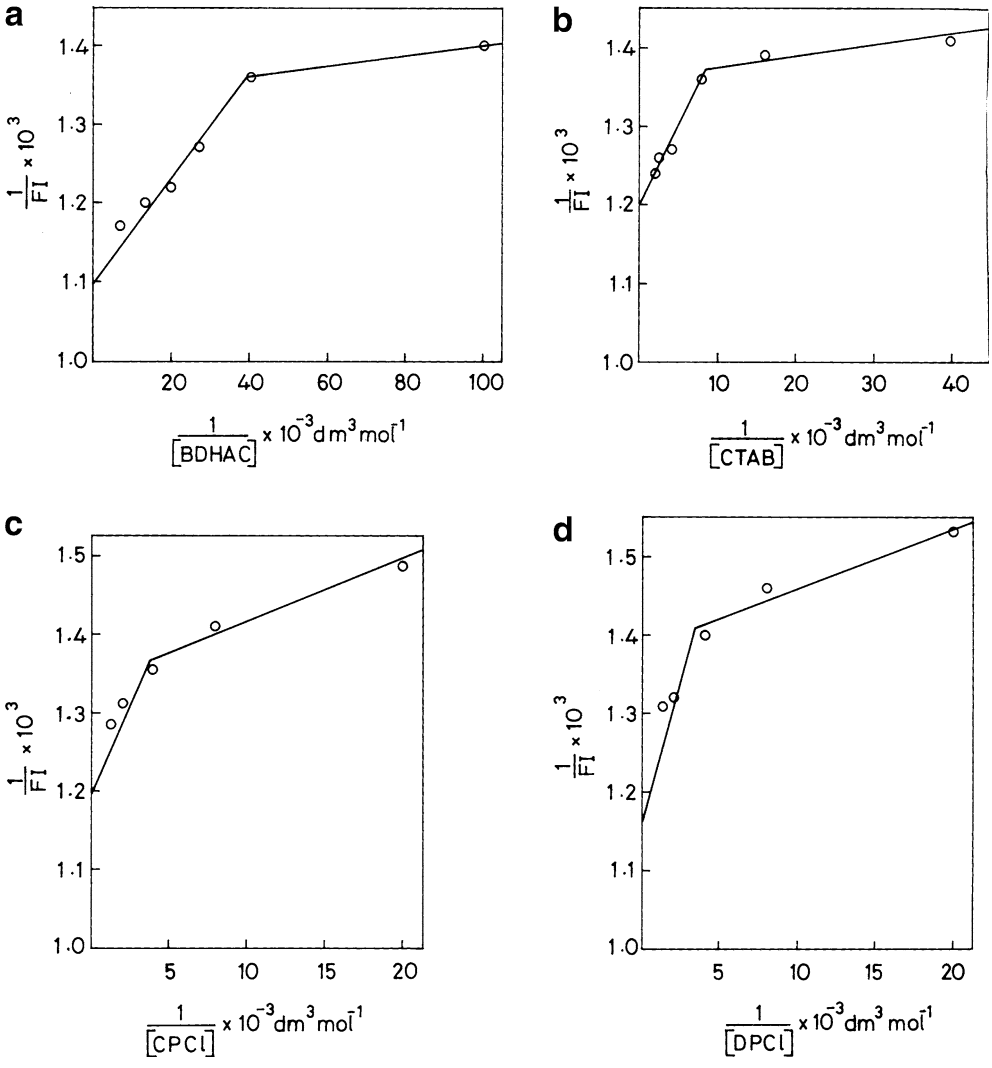

Fig. 6 Plots of 1/F.I versus 1/[M] i.e., 1/[surf.] in AO–*Klebsiella K16* biopolymer complex (P/D = 30) with the addition of different cationic surfactant: a) BDHAC, b) CTAB, c) CPCl and d) DPCl

Interaction between biopolymer and mixed surfactant

Instead of a single cationic surfactant, ionic-non ionic mixed surfactants with different mole fraction of non ionic surfactant (X_2) i.e., 0.2 to 0.8 were added in the dye-polymer system at P/D = 30 and spectral studies were carried out. The variation of absorbance of the dye-polymer complex with different concentration of BDHAC/nonionic mixed surfactant is shown in Fig.7. The different non ionic surfactants used were Tween-20 (T 20), Tween- 40 (T 40), Tween- 60 (T60), Triton X–100 (TX 100) and Brij –35 (B 35). The absorbance of dye-polymer complex at 600 nm with BDHAC–non ionic surfactants of different composition were less than with BDHAC alone. The absorbance of the dye-polymer complex with other cationic and non ionic surfactants were greater than specific cationic surfactant at definite mole fraction. For CPCl / non ionic-dye-polymer and DPCl/non ionic-dye-polymer systems, the absorbance was found to be greater than with the single cationic surfactant at 0.5 mole fraction whereas it is at $X_2 = 0.2$ for CTAB/non ionic-dye-polymer system than with CTAB alone.

In Fig. 8, it is shown that fluorescence intensity values of dye-polymer at 525.2 nm with BDHAC-non ionic surfactants of different composition were less than with BDHAC alone, like absorbance profile. The fluorescence intensity of CPCl/non ionic-dye-polymer and DPCl/non ionic-dye-polymer systems were found to be greater at 0.5 mole fraction of non ionic than cationic surfactant whereas it is at $X_2 = 0.2$ for CTAB/non ionic-dye-polymer system rather than with CTAB alone.

From the Figs. 2 and 5, it was observed that with the addition of individual cationic surfactant in the dye-polymer system, the first absorbance at 600 nm and the florescence intensity at 525.2 nm increased sharply and thereafter the extent of increase became less prominent. This indicates that the dye gets free with addition of

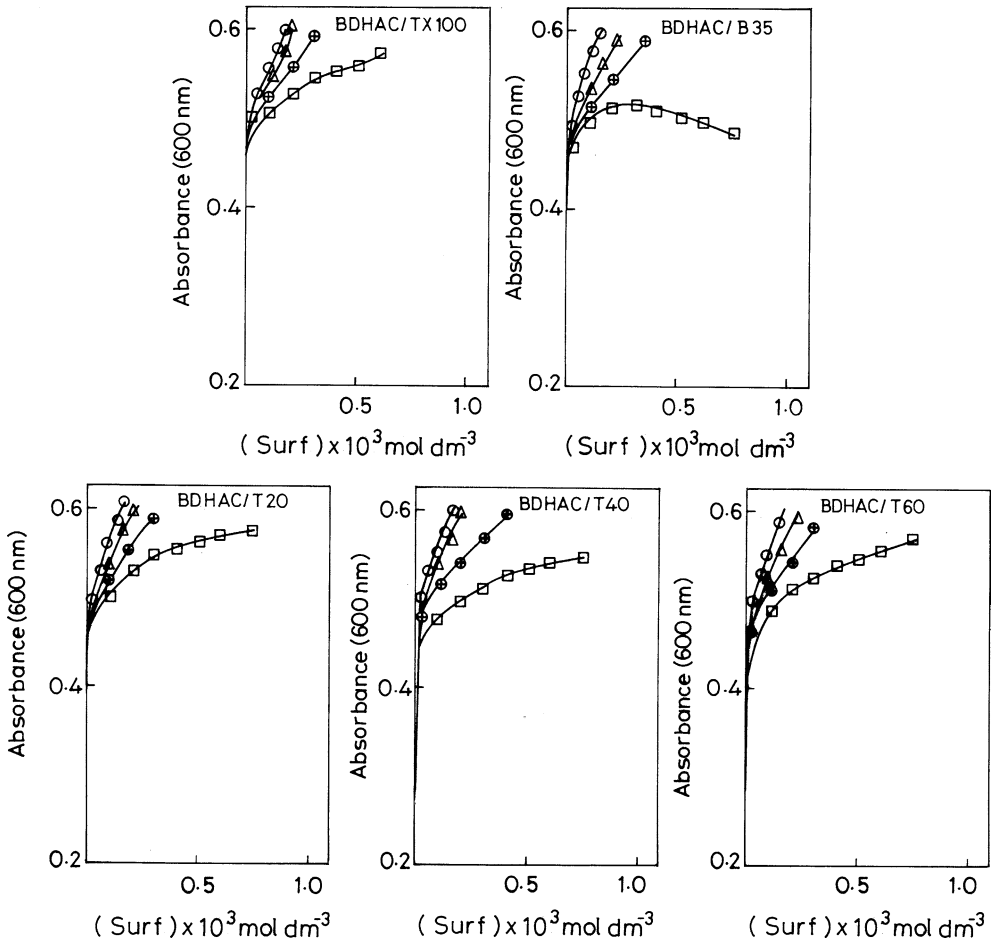

Fig. 7 Effect of BDHAC/non ionic mixed surfactant on the absorbance of PCYN–*Klebsiella K16* biopolymer complex (P/D = 30) at 298 K. Concentration of dye PCYN: 1.0×10^{-5} mol dm^{-3} and mole fraction of non ionic surfactant (X_2): 0.0 (○), 0.2 (Δ), 0.5 (⊕) and 0.8 (□)

surfactant and the enhancement in absorbance and fluorescence values can be correlated to the ability of the surfactant in freeing dye molecules from the dye-polymer complex. In the absorbance profile (Fig.1), it is observed that the metachromatic band at 490 nm demolished completely with the tendency to return of the original absorbance spectra of the dye molecules with sufficient absorbance maxima value at 600 nm in the presence of cationic surfactants. In the fluorescence profile (Fig.4) also, it is seen that the quenching effect of polymer on the dye molecule gets reversed and the original fluorescence spectra of pure dye tends to return with the addition of cationic surfactants.

Conclusions

Binding ability of cationic surfactant to biopolymer

The ability of cationic surfactant to free the dye molecule from the dye-polymer complex is revealed from the difference in the maximum absorbance value or the fluorescence intensity value for different cationic surfactants and also from the difference in the concentration of surfactants for attaining those values which follow the order: BDHAC > CTAB > CPCl > DPCl. The freeing of dye molecules from the dye-polymer complex in the presence of cationic surfactant shows that surfactants interact electrostatically with the anionic site of the polymer and thus the dye becomes free.

Gjerde et al. [13] and others [14, 15] gave the idea of a new term, critical aggregation concentration (cac), which is much lower than the CMC of any surfactant during interaction of the surfactant with an oppositely charged polymer. It is also observed that cationic surfactants are able to free a dye from the dye-polymer complex at lower concentration than their CMC. These lower concentrations of surfactants might be regarded as their cac though the exact value was not determined. The binding capability of surfactants with biopolymer follows the reverse order of cac or CMC i.e., the sequence of order given earlier.

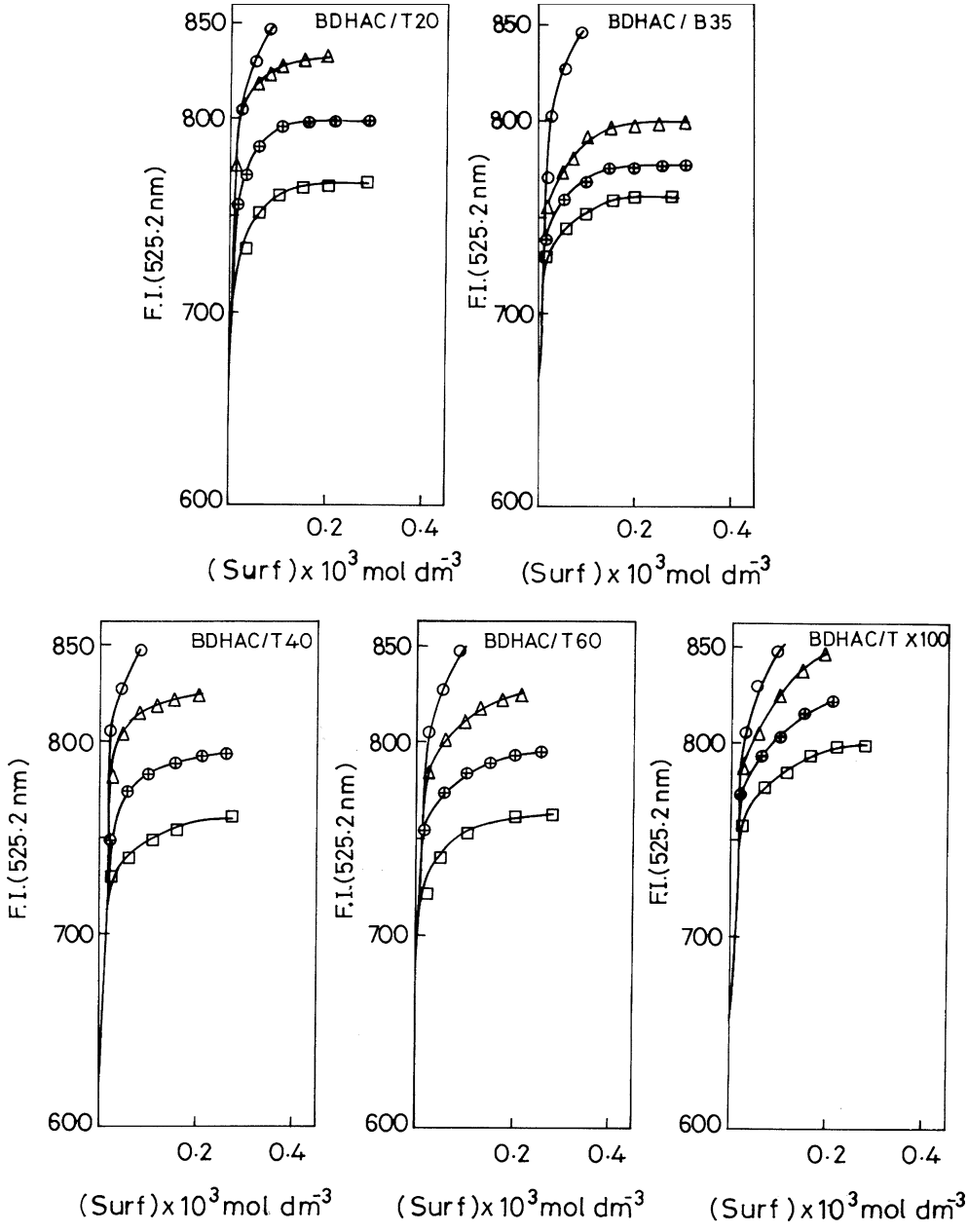

Fig. 8 Effect of BDHAC/non ionic mixed surfactant on the fluorescence of AO–*Klebsiella K16* biopolymer complex (P/D = 30) at 298 K. Concentration of dye AO: 1.0×10^{-5} mol dm^{-3} and mole fraction of non ionic surfactant (X_2): 0.0 (○), 0.2 (Δ), 0.5 (⊕) and 0.8 (□)

Effect of mole fraction and charge density of mixed surfactant

For the mixed surfactant systems, the CMC (or cac) value mainly depends on the amount of non ionic surfactant. Since non ionic surfactants have much lower CMC values than ionic ones, the values of CMC of mixed surfactants decreased with increasing mole fraction of the non ionic surfactant (X_2). The decrease was very sharp at the beginning, then decreased gradually up to X_2 = 0.5, beyond which the value remained almost constant until it attained the value of pure non ionic surfactant [16]. Here, it can be noted that the overall decrease of CMC value of the mixed surfactant system may in turn decrease the overall cac value also, in the presence of polymer. Thus, at X_2 of 0.2 or 0.5 of non ionic surfactant, the overall decrease in CMC (cac) value requires a relatively lower concentration of surfactants to bind with the polymer.

However, it should be noted that in a mixed micellar system consisting of cationic and non ionic surfactants, the overall charge density decreases and the aggregation number increases compared to the individual cationic surfactant. At X_2 of 0.8, the overall decrease in CMC

(cac) cannot supersede the decrease in the charge density and increase in the aggregation number, for which again a higher concentration of the surfactant is required to bind with the polymer. Thus, it was found that in CTAB, CPCl, DPCl and different non ionic mixed surfactant systems, a mole fraction of 0.2 or 0.5 was most effective for binding with biopolymer, *Klebsiella K16*.

BDHAC has very low CMC value. Thus, the addition of non ionic surfactants has little effect in further lowering the CMC value of the BDHAC - non ionic system. On the other hand, addition of non ionic surfactants decreases charge density and increases the aggregation number of the micellar system, which in turn lowers the binding capability of the system to the polymer. Thus, the binding capability decreases as the mole fraction of non ionic surfactant increases.

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