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Studies on the Interaction of *Klebsiella* K34 Capsular Polysaccharide with Oppositely Charged Dyes and Surfactants

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Abstract —Spectral studies on the interaction of capsular polysaccharide (SPS) isolated from *Klebsiella* serotype K34, with oppositely charged dyes and surfactants have been reported. The SPS was acidic in nature and induced strong metachromacy (~ 110 nm blue shift) in the cationic dye pinacyanol chloride (PCYN) which was due to "card-pack stacking" of individual dye monomers on the surface of the polyanions. Reversal of metachromacy offered a qualitative measurement of stability and nature of binding associated with PCYN-SPS complex. Thermodynamic parameters of dye-polymer complex were evaluated. SPS – cationic dye acridine orange (AO) interaction in aqueous solution have been investigated fluorimetrically. Dye incorporation technique was employed to study cationic surfactant–polymer interactions. Interactions between the polyelectrolyte and oppositely charged surfactants lead to the formation of induced premicelles at surfactant concentrations lower than the CMC of the surfactants and the corresponding binding constant was evaluated. Such studies revealed that the surfactant is not exclusively bound electrostatically, but also through hydrophobic interactions.

Keywords : *Dye, surfactant, SPS, Klebsiella, binding constant.*

INTRODUCTION

The formation of capsular polysaccharides by most of the gram-negative bacteria,

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Klebsiella, which belongs to *Enterobacteriacea* [1,2] family, is a characteristic phenomenon. There are 82 sereologically distinct K-serotype [3, 4] in the *Klebsiella* producing capsular acidic hetero polysaccharides of wide structural variations. The bacterial polysaccharides differ from usual naturally occurring polysaccharides by the fact that they have antigenic properties and definite repeating units, ranging from tri- to hepta-saccharides containing glucouronic acid and galactouronic acid along other neutral sugars. The presence of uronic acid (in some cases, pyruvic acid) in every repeating unit offers potential anionic site in the biopolymers that behave like polyelectrolytes. Primary structures of most of the capsular polysaccharides of *Klebsiella* are now known. [5] Due to the potential use of these bacterial polysaccharides in immunological studies and vaccine preparation, primary structural studies, conformational analysis and studies on various physico-chemical properties of these biopolymers are more significant [6–8].

The capsular polysaccharides from *Klebsiella* K34 consists of D-galactouronic acid, L-rhamnose and D-glucose residues [9]. Evidently, the presence of one galactouronic acid in each repeating unit would provide anionic sites for interaction with cationic dyes as well as cationic surfactants [10, 11]. The present authors also performed a lot of cationic dye-SPS interaction to characterize different SPS isolated from different serotype of *Klebsiella* [12]. Detailed structural aspects of the polysaccharides can be known from the study of dye-polymer interaction.

Studies on dye-polymer interaction inducing metachromasy in different cationic dyes by different synthetic polyanions, DNA, naturally occurring plant polyelectrolytes, etc. are available in literature [13-17]. Different techniques for the isolation and stability determination of metachromatic compounds have been reported [10]. The phenomenon of reversal of metachromasy by addition of urea, alcohol, neutral electrolytes [11] and also by increasing the temperature of the system, may be used to determine the stability of the metachromatic compounds.

The nature of dye-polymer interaction in the metachromatic complex formation and also the suitable conditions for the interaction between the cationic dye and the anionic site of the macromolecules have been studied by determining the thermodynamic parameters of the interaction [18]. Fluorescence of the dye, Acridine orange (AO) molecule and its quenching in polymer matrices has been studied extensively [10, 12, 19].

The interaction between polyelectrolytes and oppositely charged surfactants [20-22] has been extensively investigated due to its importance in both fundamental and applied fields [23, 24] using various experimental techniques. Comprehensive studies of a variety of cationic surfactants with anionic polyelectrolytes, synthetic and biopolymers have been reported [25-33].

The present investigation deals with studies on interaction between anionic biopolymer isolated from *Klebsiella* K34 and the cationic dye, pinacyanol chloride in aqueous medium and the same also associated with surfactant systems. The detailed studies on dye-polymer interaction were carried out by spectrophotometric and spectrofluorimetric techniques. It also includes the evaluation of thermodynamic parameters of interaction and effects of different co-solvents. Interaction of polysaccharides from *Klebsiella* K34 with different cationic surfactant, viz., benzyl dimethyl-(-n-) hexadecyl ammonium chloride (BDHAC), N, N, N-cetyltrimethyl ammonium bromide (CTAB), cetyl pyridinium chloride (CPC) and dodecylpyridinium chloride (DPC) has also been studied by spectrophotometric and spectrofluorimetric measurements using dye incorporation techniques. The binding constants of biopolymer-surfactant complexes were also determined.

EXPERIMENTAL

Materials : The serological test strain of *Klebsiella* K34 was kindly supplied by Dr. S. Schlecht of Max Plank Institute for Immunobiology, Freiburg, Germany. The bacterial cells were grown in nutrient agar medium, harvested and dried. The capsular polysaccharide was isolated and purified by phenol-water-cetavlon method [34]. General experimental details for isolation and purification of SPS are also available in literature [35, 36].

The cationic dyes, pinacyanol chloride (PCYN) and acridine orange (AO), both 99% pure, were purchased from Sigma chemicals, USA and used as received. The experimental surfactants, BDHAC, CTAB, CPC and DPC were purchased from E. Merck, Germany and were recrystallised from ethanol-water mixture prior to use. Other reagents, such as different solvents, were purchased from E. Merck and during the experiments doubly distilled water was used.

Methods : In absorbance study, pinacyanol chloride was used. Absorbance of the solutions was measured at wavelength ranging from 400-700 nm with a "Milton Roy Spectronic 21D" spectrophotometer and Perkin Elmer Lamda 25. Concentrations of dye, surfactants and the polymer used were in the range 10^{-5} to 10^{-3} mol dm^{-3}. One mole of polymer referred to the average mass of one repeating unit of the polymer containing one anionic charge site. The absorption spectra of the aqueous solution of PCYN (1×10^{-5} mol dm^{-3}) upon addition of the capsular polysaccharides at different polymer/dye (P/D) ratios were taken.

Stoichiometry of the dye-polymer compound was determined by isolating the dye-polymer complex according to McIntosh method [11, 12], which was modified by Kornors [37]. From the point of intersection of two linear curves obtained by

plotting the values of complexed dye concentration against polymer concentration, stoichiometry was determined.Stoichiometry was also determined by the centrifugation method [10].

Metachromatic titration was also done spectrophotometrically at 600 nm by usual procedure [12], from which the volume of the polymer solution required for the equivalent consumption of the dye was estimated.

The reversal of metachromasy was studied by measuring absorbance of metachromatic (P/D = 5) as well as pure dye solution at 600 nm (J-band for the pure dye) and also at 490 nm (H-band) upon addition of different co-solvents like methanol, ethanol, n-propanol and urea in increasing amounts. The reversal phenomenon was also confirmed by measuring absorbance of the pure dye solution and the metachromatic solution (P/D = 5) at 450-650 nm with and without addition of co-solvents like ethanol.

The interaction constant (K) of the dye and the polymer was obtained using the Rose and Drago equation [38] in the following form

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

where, C_D = initial molar concentration of PCYN, C_S = molar conc. of SPS, ε_D = molar absorption coefficient of PCYN, ε_{DS} = molar absorption coefficient of the PCYN-polymer complex, K = interaction constant of dye-polymer complex, A_O = absorbance of the pure dye PCYN at 490 nm, A = absorbance of the dye-polymer solution at 490 nm at a particular polymer conc., L = length of light path.

In practice, the values of $(C_D.C_S)/(A-A_O)$ were plotted against C_s [10,19]. A linear relationship was obtained at each temperature. From the slope and intercept of the straight lines as obtained above, the interaction constant (K) values were calculated. From the interaction constant value the standard changes in free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0) of complex formation was obtained by usual procedure.

In fluorescence experiments the cationic dye, acridine orange was used as the probe at a strength of 10^{-5} mol dm^{-3}. The fluorescence intensity of the dye solution as well as of the dye-polymer solutions (at different P/D) was measured using Shimadzu RF5000 spectrofluorimeter. The solutions were excited at 480 nm, the emission spectra were taken within the range 450-650 nm and the fluorescence intensity was measured at 525 nm. The results of fluorescence quenching in acridine orange dye by K34 polymer were treated with Stern Volmer equation [39].

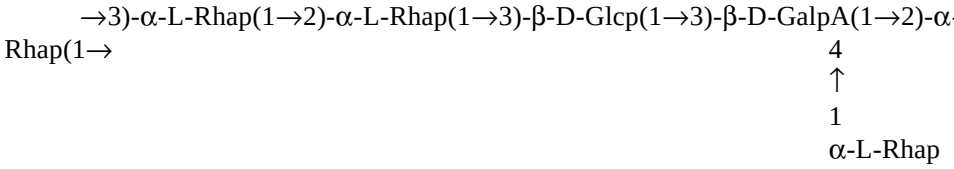
$$F_o/F = 1 + K_{SV}[Q] \quad (2)$$

where, F_o is the fluorescence intensity of the dye solution and F is that of dye-polymer mixture and [Q] is the concentration of the quencher (that is the molar concentration of the polymer) and K_{SV} is the Stern-Volmer constant. The Stern-Volmer plot was drawn by plotting F_o/F against [Q] in the case of acridine orange dye in presence of K34 polymer. Then from the slope of the linear line, the Stern-Volmer constant (K_{SV}) was calculated.

The studies on the effects of different cationic surfactants on the dye-polymer complex were carried out spectrophotometrically [11,40]. To obtain a clear picture about the effects of different cationic surfactants on SPS, absorbance of *Klebsiella* K34-PCYN complex at 298 K were also measured at 450-650 nm. Finally, the binding constant between the anionic polymer and the cationic surfactants were evaluated by Rose and Drago equation [38] from the absorbance results in an approximate manner [40].

RESULTS AND DISCUSSION

The present investigations primarily deal with the interaction of the anionic capsular polysaccharide isolated from *Klebsiella* K34 with cationic dye molecules in aqueous solutions in the absence and presence of different cationic surfactants by absorbance and fluorescence studies. The primary structure of the experimental SPS has already been established by Joseleau et al. [9]. The SPS consists a hexasaccharide repeating unit of L-rhamnose, D-glucose and D-galacturonic acid in the molar proportions of 4 : 1 : 1. The structure for the repeating unit as assigned by Joseleau et al. [9] is represented below :



Primary structure of *Klebsiella* K34

The equivalent weight of the polymer (referred to as the mass of the repeating unit) was determined from the spectrophoto-/fluorimetric titration and was found to be 922 Da, which was very close to the calculated value from its structure. The presence of galactouronic acid in every repeating unit makes the polymer a unique polyelectrolyte (charge density = 0.00108 C g^{-1}) and hence offers potential sites for interaction with oppositely charged dyes and surfactants.

The cationic dye PCYN belongs to cyanine group of dyes and evidently [19] it is more hydrophobic and more aggregating in nature due to its larger size.

The aqueous solution of the dye showed two sharp bands at 600 nm (J-band) and at 550 nm (D-band) corresponding to the absorption peaks of the monomeric and dimeric form due to the vibrationless electronic transition and vibrational electronic transition respectively [40]. With the addition of the K34 capsular polysaccharide, the intensities of the J and D-bands decreased and a new band at 490 nm (H-band) appeared. At higher P/D values a strong H-band appeared with repression of J- and D-bands. Thus a blue shift of ~110 nm was observed indicating induction of strong metachromasy in the PCYN by *Klebsiella* K34 SPS. Initial broadening at the J- and D-bands in association with the formation of H-band indicated multiple band spectrum of the dye [40]. The absorption spectra of the pinacyanol chloride dye (1×10^{-5} mol dm^{-3}) with the addition of the SPS at different P/D ratios (P/D = 0.0 to 50) are shown in Fig. 1.

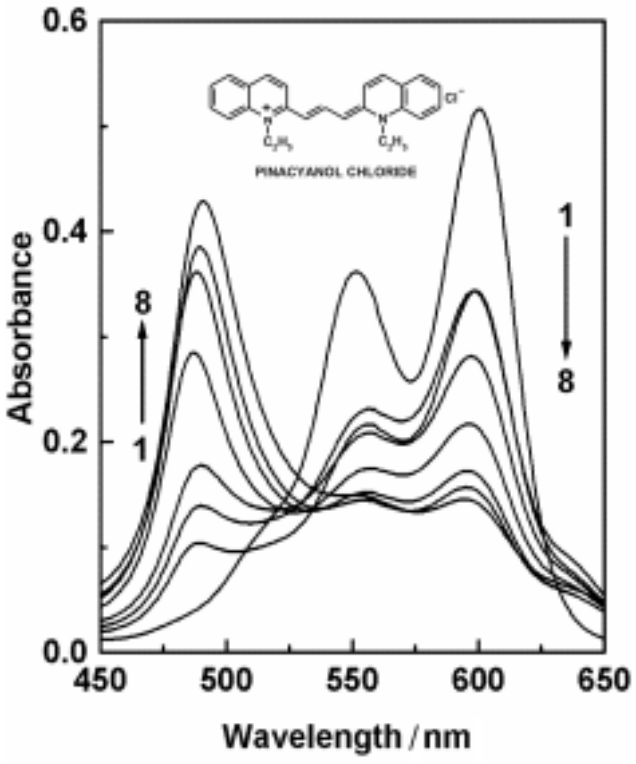


Fig. 1. Absorption spectra of 10^{-5} mol dm^{-3} PCYN in presence of varying amounts of *Kleb siella* K34 SPS at 303 K. 1, No SPS. SPS/PCYN (P/D) ratio : 2, 1; 3, 2; 4, 5; 5, 10; 6, 20; 7, 30 and 8, 50.

The shape of the metachromatic spectra depends on the conformation of the polyanions as well as dye ion in solution. Reports on such conformational influence on the spectrum of PCYN dye by other synthetic polyanions [41,42] and also by biopolymers [19,40] are available. On the basis of our earlier work the metachromatic absorption of the PCYN, induced by *Klebsiella* K34 SPS can be interpreted. Appearance of multiple-banded broad spectra proposed that the polymer might have random coil structure in the solution whereas at higher concentration of the polymer almost a single banded spectrum was observed due to a possible change from random coil to helical form. In this regard the accepted mechanism for polyanion induced metachromasy suggests that the dye cations bind to the adjacent sites of the polymer forming a single individual compound. It is considered to arise from electrostatic interaction among the neighboring dye molecules and fixed sites on polymer as a result of which they suffer effective aggregation resulting in hypochromic and hypsochromic spectral shifts in the absorption band of the dye [41]. It was also reported that the distance between two identical centres of two adjacent dye molecules in their aggregate will depend on the geometry of aggregation [43] and obviously will be larger when the dye molecules are arranged like a stair case instead of being piled one above another like a stack of books. So when the aggregation in a dilute solution of the dye cation is indicated by polyanions through electrostatic binding, a small distance between the adjacent anionic sites on the polyanions will be more favorable for the binding of rigid and planar dye cations (acridine orange, methylene blue) and expected to form stacking arrangement. However, a somewhat larger distance between the anionic sites facilitates the binding of dye cations like pseudoisocyanine (PIC) where molecules aggregate in a suggested way due to the non-planarity.

The metachromatic titration was carried out spectrophotometrically at lower P/D values (0 ~ 2) and yielded identical stoichiometry. In spectrophotometric titration the absorbance values at 600 nm were plotted against volume of polymer solution added as shown in Fig. 2. Stoichiometry of the dye polymer compound was determined from the point of intersection of the linear curves and hence the molecular weight of the polymer per repeating unit was also calculated. Spectrofluorimetric titration also revealed same results.

The stoichiometry of the dye-polymer complex was determined according to McIntosh and centrifugation methods. It was found that the stoichiometry of the dye-polymer complex was 1 : 1 in respect of repeating unit of the polymer : dye (Fig. 3). The molar stoichiometric results indicated that galctouronic acid was the only potential anionic sites for interaction with the cationic dye PCYN and therefore suggested a "card pack stacking" arrangement of the dye molecules to polymer matrices.

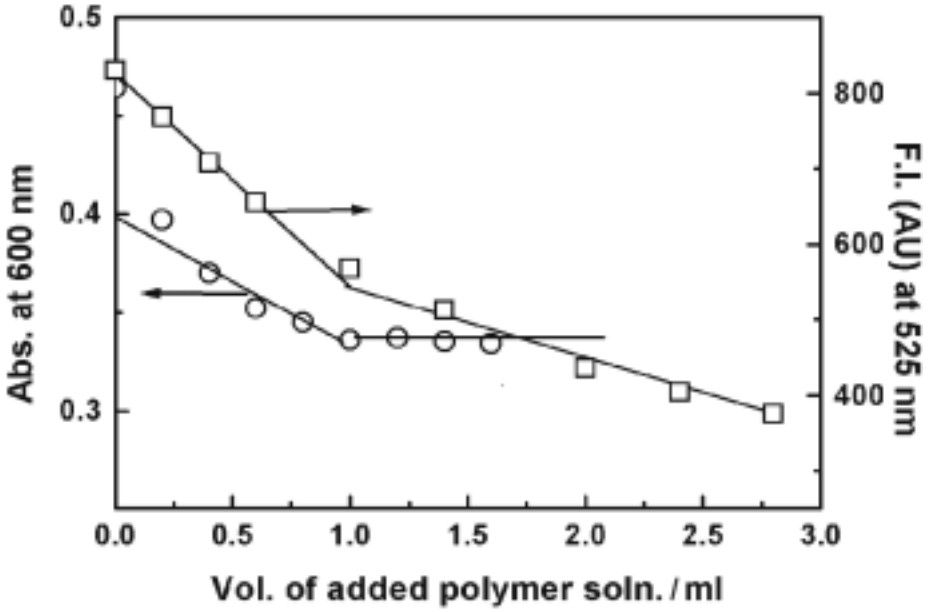


Fig. 2. Spectrophoto-/fluorimetric titration of PCYN by *Klebsiella* K34 SPS at 303 K. [Dye] = 10^{-5} mol dm^{-3}, λ_{ex}AO = 480 nm.

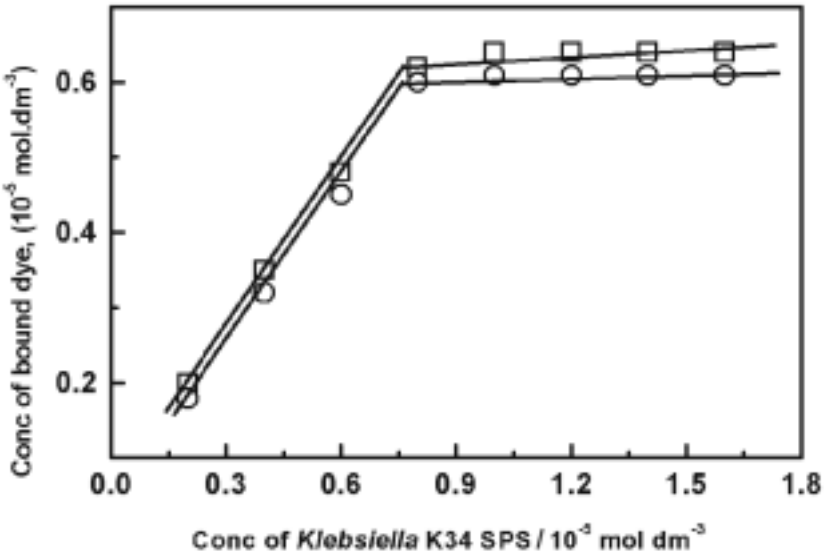


Fig. 3. Determination of stoichiometry of *Klebsiella* K34 SPS-PCYN complex at 303 K. □, McIntosh method and ○, centrifugation method.

Metachromasy destroyed by different means and processes, is termed as reversal of metachromasy. The reversal of metachromasy, induced in PCYN SPS complex, was studied by measuring absorbances both at J- and H-band upon addition of different co-solvents like methanol, ethanol, n-propanol and n-butanol. The results of such study indicated progressive destruction of the metachromatic compound with increasing concentration of alcohols. This may be attributed to reduced hydrophobic effect in presence of alcohols as a result of water structure disruption by the added alcohols [12]. The extent of destruction of metachromatic compounds by alcohols was followed by the increasing absorbances of solution of dye and polymer at the J-band or the decreasing absorbances at the H-band as a function of alcohol content. It was also observed that efficiency of alcohols in disrupting metachromasy was in the order methanol < ethanol < n-propanol < n-butanol, indicating that reversal become quicker with increasing hydrophobic character of the alcohols. This agreed with the results of other dye-polyelectrolyte systems [14,15]. The complete reversal of metachromasy was further confirmed by measuring the absorbances of the pure dye solution and the metachromatic solution at P/D = 5 at wave length range of 450 – 650 nm upon addition of ethanol; a representative case shown in Fig. 4. At the concentration of the ethanol, where the complete reversal of metachromacy was observed, the pure dye solution gave only a single banded spectrum with an intense peak at 600 nm. It also appeared that the metachromatic band (H-band) at 490 nm of the dye-polymer complex solution at P/D = 5 disappeared and the spectrum of the dye-polymer complex became the same as that of the pure dye solution. Thus the concept of reversal of metachromasy might be used to determine the stability of the metachromatic compound as well as the nature of binding involved in the interaction of anionic polymer and cationic dye.

The interaction constant (K) and the thermodynamic parameters of the interaction between the PYCN and SPS are presented in the Table 1. From the thermodynamic results, it was found that the interaction constant (K) values gradually decrease with increasing temperature. The values of ΔG^0 also decrease with increase in temperature and the values were negative. Moreover, the ΔH^0 and ΔS^0 values were also found to be negative. The decreasing trend of the values of the interaction constant (K) with rise in temperature and negative value of ΔH^0 suggested the exothermic nature of the interaction process. Again the negative ΔG^0 value indicated spontaneity of the reaction and its low value suggested a non-chemical type of interaction. The negative entropy change indicated more ordered state of the ions due to aggregation which also supported the aggregation theory of the dye-polymer complex formation during metachromasy.

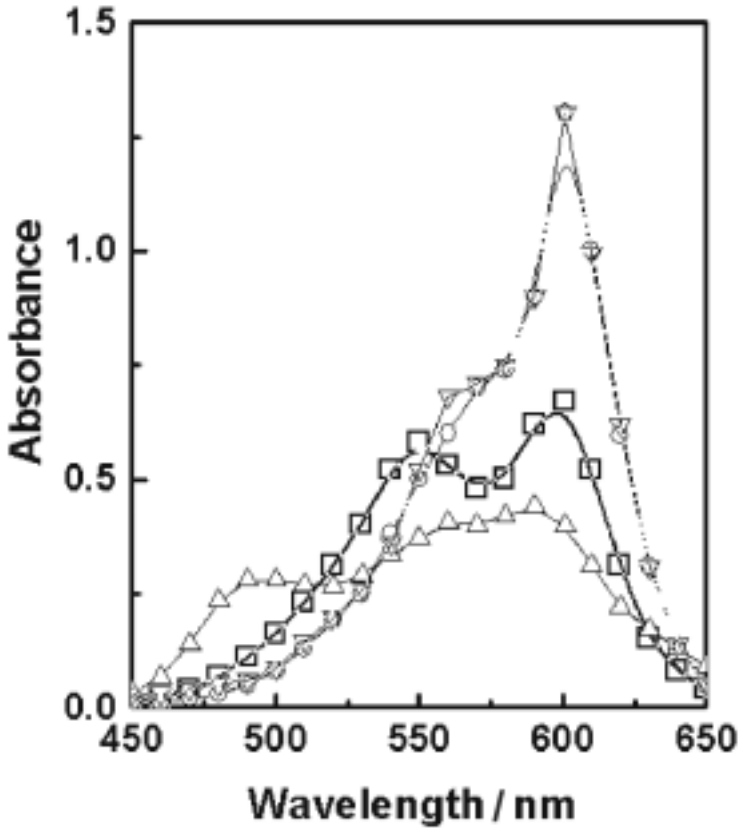


Fig 4. Reversal of metachromasy, induced by ethanol in *Klebsiella* K34 SPS-PCYN complex: □, pure dye; Δ, dye-polymer complex; ○, dye in 40% ethanol; ∇, dye-polymer complex in 40% ethanol. Temperature : 303 K. [Dye] = 10^{-5} mol dm^{-3}, [polymer] = 10^{-5} mol dm^{-3}.

TABLE 1.

Thermodynamic parameters of *Klebsiella* K34 SPS-PCYN complex in aqueous media.

Temp./K	K × 10^{-3}	ΔG^0/kJ mol^{-1}	ΔH^0/kJ mol^{-1}	ΔS^0/J mol^{-1} K^{-1}
303	2.18	–19.37		
308	1.56	–18.82		
313	1.35	–18.75	–42.74	–76.62
318	0.92	–18.04		
323	0.79	–17.91		

Fig. 5 shows the plot of $C_D.C_S/A–A_0$ vs. C_S acording to eq. (1); the interaction constant (K) of the dye-polymer complex was calculated form the slope and intercept of the linear plot.

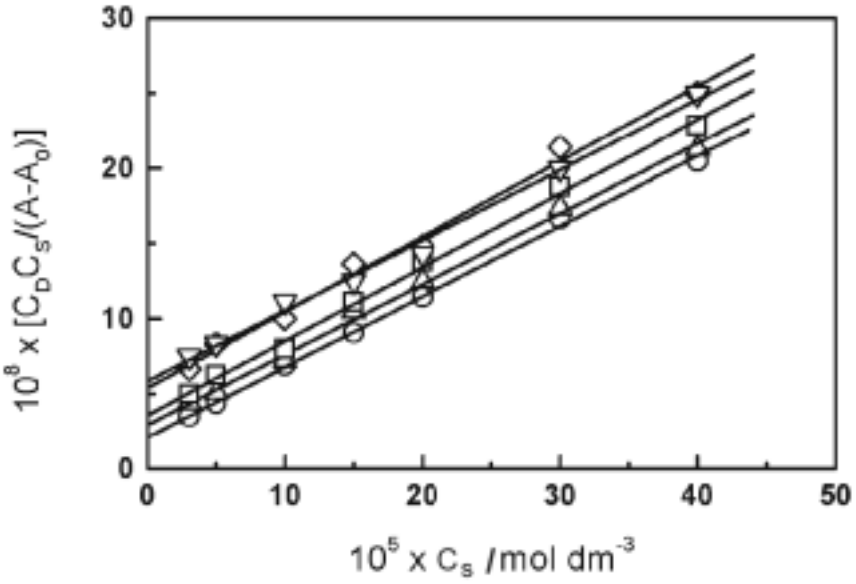


Fig. 5. Plot of $C_DC_S/(A-A_0)$ versus C_S to determine the PCYN-*Klebsiella* K34 SPS binding constant at different temperatures. [PCYN] = 10^{-5} mol dm^{-3}. Temperature (K): ○, 303; Δ, 308; □, 313; ◇, 318 and ∇, 323.

Fluorescence studies were performed with the cationic dye, acridine orange because of its highly fluorescent nature. Fluorescence quenching, an important phenomenon in fluorimetric studies, was applied to study the interaction of fluorescent dye with polymer. The emission spectra of the dye and the dye-polymer mixture at different P/D were recorded at 450–650 nm range using λ_{ex} = 480 nm as shown in Fig. 6. It was found that fluorescence intensity of the dye was progressively quenched with increasing concentration of the polymer as observed in our earlier work [10]. This indicated that the planar fluorescent dye AO preferred to form an aggregation of dye molecules on binding to *Klebsiella* K34 SPS.

The results of fluorescence measurements were also treated by Stern-Volmer equation to study the interaction phenomenon between the dye and the polymer molecules in solution. The Stern-Volmer plot of (F_0 /F – 1) vs [SPS] was found to

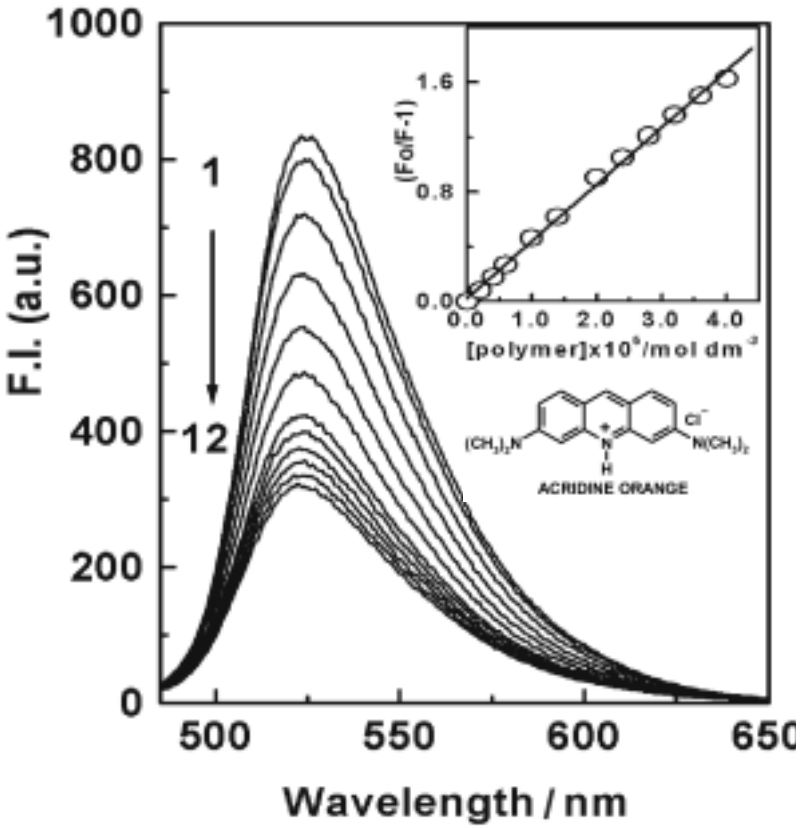


Fig. 6. Emission spectra of 10^{-5} mol dm^{-3} AO at 303 K in presence of varying amounts of *Klebsiella* K34 SPS. [SPS] (10^{-5} mol dm^{-3}) = 1, 0; 2, 1; 3, 2; 4, 5; 5, 10; 6, 20; 7, 30; 8, 50. λ_{ex} = 480 nm. Inset: Stern-Volmer plot for determining K_{sv} *for Klebsiella* K34 SPS – AO complex.

be linear, passing through the origin as shown in the inset of Fig. 6. From the slope of the linear plot the value of Stern-Volmer constant (K_{SV}) that is the binding constant for the dye-polymer complex was calculated and it was found

$$K_{sv} = 4.24 \times 10^4 \text{ dm}^3 \text{ mol}^{-1}$$

with SPS (Quencher) concentration in the range, 0–4 × 10^{-4} mol dm^{-3}. The graph suggested an occurrence of quenching mechanism in the dye-polymer interaction process and the linearity of the plot indicated that the quenching in this case is static in nature [19].

The strength and the nature of interaction between water soluble polyelectrolyte and oppositely charged surfactants depend on the characteristic features of both the

polyelectrolyte and the surfactant. In this regard charge density, flexibility of the polyelectrolyte and the hydrophobicity of the nonpolar part and the bulkiness of the polar part play a vital role in the case of polyelectrolyte-surfactant interaction [26]. Accordingly, the *Klebsiella* K34 polysaccharide being an anionic polymer was expected to interact with cationic surfactant. The cationic dye PCYN was used as a probe for absorbance experiments and the cationic surfactants used were BDHAC, CTAB, CPC and DPC having variable hydrophobicity.

The effects of all the cationic surfactants under investigation on the absorbance of PCYN-*Klebsiella* K34 polymer complex (P/D = 30) at 600 nm are shown in the Fig. 7. From the figure it was found that with addition of cationic surfactants the absorbance values of the dye-polymer complex increased to a considerable extent.

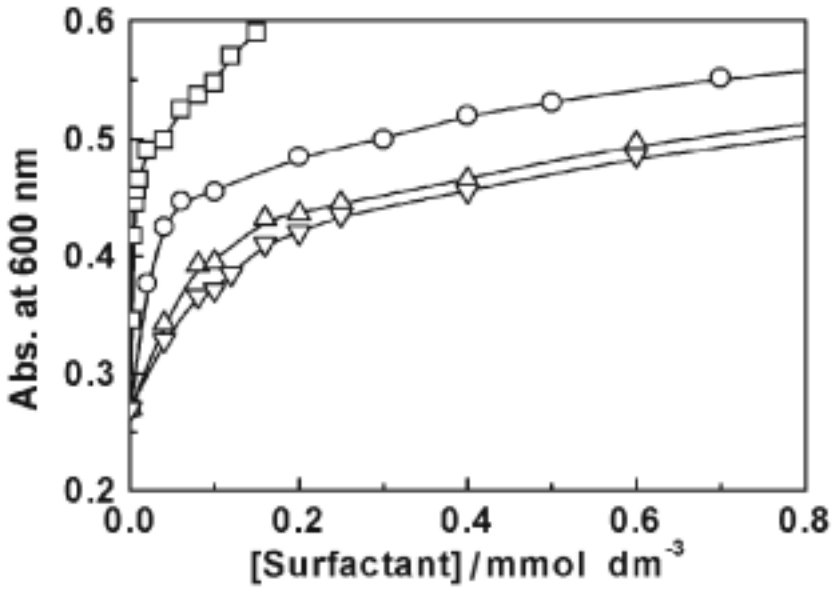


Fig. 7. Effect of cationic surfactants on *Klebsiella* K34 SPS-PCYN complex at 303 K. [SPS]/PCYN = 10, [PCYN] = 10^{-5} mol dm^{-3}. Surfactants : ◇, BDHAC; ○, CTAB; Δ, CPC and ∇, DPC.

This indicated that the surfactant molecules interacted with the capsular polysaccharide, by replacing the cationic dye molecules. The extent of increase in absorbance was considered to be equivalent to the amount of surfactant bound to the polysaccharide. Again the complete absorbance profile indicated that the metachromatic band (H-band) at 490 nm demolished completely with the tendency to return to the original absorbance spectra of the dye molecule with absorbance maximum at 600 nm.

The ability of cationic surfactant to free the dye molecule from the dye polymer complex, was revealed from the difference in the maximum absorbance value and also from the difference in the concentration of surfactants for attaining these values. This followed the order BDHAC > CTAB > CPC > DPC, which is consistent with their hydrophobic chain length. Again the freeing of the dye molecules from the dye polymer complex in presence of cationic surfactants revealed that surfactants interacted electrostatically with the anionic site of the polymer and thus the dye becomes free.

Literature studies [26] showed that the surfactant molecules interacted with polymers above a critical aggregation concentration (CAC) forming polymer supported micelles along the polymer chain. The CAC is usually lower than the cmc of the surfactant in the presence of the polymer. CAC and cmc values of the surfactants follow identical order, i.e., BDHAC < CTAB < CPC < DPC. Thus the binding capability of the surfactants discussed earlier, followed the reverse order as that of both CAC and cmc.

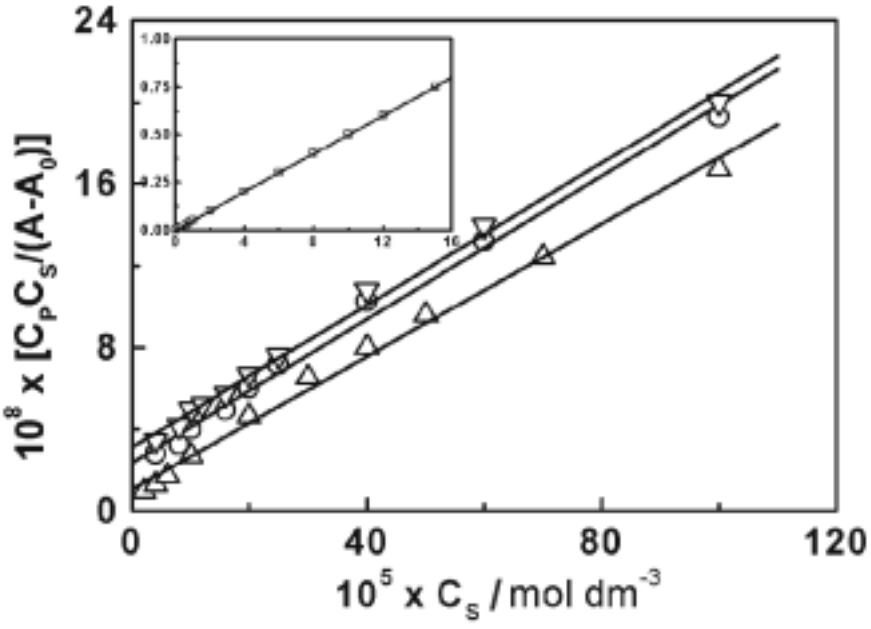


Fig. 8. Plot of $C_P C_S$ / (A-A_0) versus C_S to determine the binding constant of *Klebsiella* K34 SPS-surfactants at 303 K. [SPS]/PCYN = 5, [PCYN] = 10^{-5} mol dm^{-3}. Surfactants: Δ, CTAB; ○, CPC; ∇, DPC. Inset: BDHAC.

The Rose and Drago equation (in a modified form) was also applied to the absorbance values at 600 nm, to determine the binding constant between anionic polymer and cationic surfactants. Using this equation, the values of $(C_P . C_S) / (A-A_0)$ for each surfactant were plotted against Cs, which are shown in Fig. 8. From the slope and the intercept of the straight lines, the binding constant values between the K34 polymers with the surfactants were calculated. The binding constant values are shown in the Table 2 which followed the order BDHAC > CTAB > CPC > DPC. The correlation between the affinity of the surfactant from the K34 polymer and the length of its hydrophobic chain suggests hydrophobic interaction between surfactants and polymer. Thus the binding between oppositely changed polymer surfactant is primarily electrostatic forces which are reinforced by hydrophobic forces. The current observation also supported our earlier observation [40].

TABLE 2.

Binding constants of cationic surfactant-*Klebsiella* K34 SPS at 303 K.

Surfactant	cmc / mmol dm^{-3}	10^{-5} Binding Constant / mol^{-1} dm^3
BDHAC	0.042	1.186
CTAB	0.80	0.686
CPC	0.90	0.616
DPC	14.70	0.572

CONCLUSION

Spectral and flurometric measurements were carried out to investigate the nature and the extent of interaction between the anionic capsular polysaccharide (SPS, isolated from the gram negative bacteria, Klebsiella serotype K34) and cationic dyes and surfactants. Following conclusions are drawn from the study :

1. SPS induced strong metacromasia in pinacyanol chloride, a cataionic dye. 1 : 1 stoichiometry of the polymer dye complex indicated that the primary binding is electrostatic in nature with subsequent "card pack stacking" of the individual dye monomers showing metachromasia as a result of hydrophobic effect. The ease of reversal of metachromasy with increasing concentration of alcohol due to the weakening of the hydrophobic effect, is a measure of the stability of the metachromatic compound.

2. The thermodynamic parameters for the primary binding of the cataionic dye to the anionic sites of SPS points to electrostactic nature of the process.
3. The progressive quenching of the fluorescence intensity of the dye, Acridine orange (AO) with increasing concentration of SPS (Quencher) is due to the fact that the planar AO molecules (cataonic) agreegate because of hydrophobic effect on the primarily bound (by electrostatic forces) dye molecules on the anionic sites of SPS.
4. The degree of binding of the cationic surfactants with SPS decreases with the length of their hydrophobic tails.

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