Spectral studies on the interaction of cationic dye/surfactants with

*Klebsiella* K28 capsular polysaccharide

**Abstract**

Spectral studies on the interaction of acidic capsular polysaccharide (SPS), isolated from *Klebsiella* K28, with cationic dye and surfactants have been reported. The polymer induced strong metachromasy (~100 nm blue shift) in the cyanine dye pinacyanol bromide (PCYB). The SPS consists of D-glucose, D-galactose, D-mannose and D-glucuronic acid (2:1:2:1 molar ratio) in hexasaccharide repeating units. Glucuronic acid acted as potential binding sites. 1:1 stoichiometry of the SPS-dye complex indicated the association of every potential anionic binding site of the polymer and its stacking conformation. Addition of different cosolvents resulted reversal of metachromasy. Oppositely charged surfactant-SPS binding was evaluated by dye incorporation technique. Cationic surfactants replaced bound dye molecules, thus the original band intensity of dye got increased. From the spectral data, binding constants of polymer-surfactant aggregates were calculated.

1. Introduction

The Gram-negative bacteria *Klebsiella* belongs to *Enterobacteriaceae* family, and are the causative factors for several human diseases which result significant morbidity and mortality [1-3]. The severe diseases include liver abscesses [4] septic endophthalmitis [5, 6], infections in urinary tract, pneumonia, bacteremia [1], meningities [3,7]. Less virulent diseases include wound infection. *Klebsiella* born diseases are transfected nosocomically and as community-acquired infections [7,8]. *Klebsiella* is considered as an extra cellular pathogen whose virulence is associated with the production of an encapsulated polysaccharide. The polysaccharide capsule provides protection against host defense mechanism [9]. There are about 82 serological stains of *Klebsiella* and certain serotypes are causative factors of certain type of infections / diseases [10]. Thus, a detailed study on the structure and conformation of the capsular polysaccharides are assumed to be highly important. Such studies would be beneficial to develop prototype vaccines against the bacterium [11]. Moreover, it has also been found that *Klebsiella* capsular antigens are safe in human and these antigenic polysaccharides are now used as human vaccines [3,12-14]. Primary structures of different *Klebsiella* capsular polysaccharides (SPS) are now known and it has been found that most of the polysaccharides are composed of definite repeating units, which may vary from 3 to 7. Primary structure of *Klebsiella* K28 reveals the presence of hexasaccharide repeating units where one glucouronic acid in every repeating unit makes it a unique anionic polyelectrolyte [15]. *Klebsiella* K28 is one of the virulent serotypes which specially infect blood, urinary tract and respiratory tract [3]. This further encouraged the authors to study the solution behavior of SPS isolated from this particular serotype. During last two decades, the present authors have been engaged in the characterization of SPS isolated from different serotypes of *Klebsiella* [16]. Still the results could be considered to be only fragmentary in the context of all the
different serotypes of *Klebsiella* family. Thus, such studies were assumed to be significant scientific activities.

Studies on the interaction of biomacromolecules with dyes are considered to be significant as such studies are beneficial in understanding the physiology, biochemistry and physical chemistry of macromolecules. Such studies are usually done by spectroscopic techniques, as very dilute solutions of dyes are needed. Usually, the spectra of dye-polymer complex are different from the spectra of the pure aqueous dye solution in case of effective interaction. Complexation of the dye may be mediated by electrical charges or through hydrophobic interactions. As already mentioned that bacterial polysaccharides are usually polyanionic in nature, these polysaccharides can act as chromotrope to cationic cyanine dyes. A blue shift in the spectrum of the dye takes place, the phenomenon, which is known as metachromasy [16-19]. Metachromasy is related to interaction of cationic dyes with polyanions where a single individual complex compound is formed by interaction of dye cation and the chromotrope polyanionic polymer. Several physiochemical parameters can be evaluated/assessed from dye polymer interaction. These include the molecular weight of each repeating unit, stoichiometry of dye-polymer complex, binding constant and other related thermodynamic parameters like free energy, enthalpy and entropy changes. Biological activity of a macromolecule depends on its tertiary conformation. Conformation of the polyanion controls the induction of metachromasy of aqueous dye solution. Although there are several reports on metachromasy of various classes of acidic polysaccharides [16-19] and different synthetic polyanions [20,21] with different cationic dyes, but similar studies using bacterial polysaccharides are not plenty. Such studies could help in understanding the conformation of the bacterial polysaccharides in aqueous solution, and hence can be used as models for drug-biopolymer interaction [16].
Cyanine dyes, which are cationic in nature, have widely been used to probe biological systems such as the helical structure of DNA [17], tertiary conformation of bacterial polysaccharides [16-18] and other polymers [19-21]. As these dyes have high light absorptivity, they can be used as optical probes in studying membranes, micelles and other host systems [22,23-25]. The cationic dye pinacyanol bromide (1, 1′-diethyl - 2, 2′-carbocyanine bromide, PCYB) belongs to the class of conjugated cyanine dye, which is amphipathic in nature and has a tendency to aggregate in aqueous media. Local environment surrounding PCYB strongly controls its absorption spectra. When it interacts with negatively charged species, a blue shift in the spectra takes place [16-21]. PCYB, like other cyanine dyes, shows multibanded spectra. In water, the maxima appear at 600 and 550nm with a broad hump at ~520nm [23-25]. The band at 600 nm corresponds to the J-aggregates according to the name of the inventor [24]. Existence of multiple bands (at 600 and 550nm) corresponds to the monomer and dimmer forms of the dye [25]. Upon addition of anionic polyelectrolytes [16-21], or when the dye gets adsorbed onto a colloidal surface [26,27], a new band appears at~ 500nm (known as H-band) at the cost of the 600 and 550 nm bands.. This unique feature of PCYB has made the dye a potential marker for studying aqueous biomacromolecular solution. Absorption spectra help in quantitative determination of the binding constant of dye-polymer complex, biomacromolecular conformation in aqueous media and other physicochemical parameters [16-21], as already mentioned earlier.

Studies on polymer-surfactant interaction in aqueous media have been attracting widespread attention due to multifarious practical uses in biology [28-30] and their associated properties [31]. Such studies are also assumed to be important as the mixed systems/aggregates can give rise to advanced functions that are unobtainable from a single component [32]. Several physicochemical properties of macromolecule-surfactant (or lipid) are quite relevant in this context. Formulation procedures based on a suitable mixture may
have many appealing applications [29,33-35]. Surfactant-polymer interaction studies include synthetic polymers like polydiallyldimethylammonium chloride [32,36], copolymers of maleic acid [37], polyvinyl pyrrolidone [38], polyethylene oxide [29], etc. The more biologically relevant systems include triptophan dipeptides [39], serum albumin [30], polypeptides [40], plasmid DNA [41], and carbohydrate based polymers [42-45]. But literature studies reveal the non-abundance of plenty reports on bacterial polysaccharide-surfactant interaction. Such studies, besides the aforementioned objectives, would shed light in understanding the complex lipid-protein and/or lipid-polysaccharides interaction prevalent in cellular membranes [46-48]. It has been noted that oppositely charged surfactant bind to the polymer matrices through both electrostatic and hydrophobic interaction [49].

There are various ways by which polymer-surfactant interactions can be studied. These include turbidimetry, viscometry, light scattering, small angle neutron/X-ray scattering, potentiometry, conductometry [42,50] and dye incorporation technique [50,51], etc. Among different techniques, dye incorporation has been found to be convenient due to its simplicity and time consumption. Spectral pattern of a dye-polymer-surfactant system are different from the spectra of pure dye or even in presence of polymer. When a cationic surfactant is added to an anionic polymer-cationic dye aggregate, the bound dye gets released /dislodged from the polymer matrix. Thus, the intensity of the dye at its original band increases. This principle has been employed to study the polymer-surfactant interaction by the authors previously [50]. But no quantitative explanation could be provided.

The present investigation deals with visible spectral studies on interaction of Klebsiella K28 bacterial polysaccharide with a cationic dye pinacyanol bromide (PCYB) and four different cationic surfactants, benzyl dimethyl-(n)-hexadecylammonium chloride (BDHAC), N,N,N-cetyltrimethylammonium bromide (CTAB), cetylpyridinium chloride
(CPC) and dodecylpyridinium chloride (DPC). Initially, the dye polymer interaction was studied to characterize the SPS and some physicochemical parameters for the dye-SPS interaction. Then the said cationic surfactants were added to dye-SPS complex, thereby increasing the original band intensity of pure dye. The binding constant of SPS-surfactant complexes was determined from the data of these reverse phenomena of dye polymer interaction.

2. Materials and methods

2.1. Materials

General experimental details regarding the isolation and purification of bacterial polysaccharides can be found elsewhere [16-18, 52]. Briefly describing, the serological test strain for *Klebsiella K28* capsular antigens was kindly supplied by Dr. Schlecht of Max-Planck Institute for Immunobiology, Freiburg, Germany. The stain was checked for agglutination in Difco type-specific antisera. The bacterial cells were grown in nutrient agar medium, harvested, dried and capsular polysaccharides were isolated and purified by phenol-water-cetavlon method.

The dye PCYB was purchased from Sigma Chemicals, USA. It was 99% pure and was used as received. The surfactants BDHAC, CTAB, CPC and DPC and solvents were products from E. Merck, Germany. They were recrystallized from ethanol water mixture and purities were checked with thin layer chromatography. Other reagents were purchased from SRL, India. Double distilled water was used throughout the experiments. Spectral measurements were done at 400-700nm using a Milton Roy Spectronic 21D spectrophotometer. Concentration of dye, surfactants and the polymers used were in the range $10^{-3}$ to $10^{-5}$ M.
2.2. Methods

Stoichiometry of the metachromatic compounds was determined using McIntosh (isolation) [16-18] as well as Centrifugation method. This method has recently been modified, as method of continuous variation, by Konors [53]. In McIntosh method, increasing amounts of the K28 polymer solution were added to a fixed quantity of PCYB dye solution (10^{-5} M) taken in different test tubes. It was thoroughly homogenized using a vortex mixer. Five milliliter petroleum ether was then added to each test tube and shaken for another 15 min. The metachromatic polymer-dye complex was thus transferred into the organic layer. The uncomplexed aqueous dye solution was separated out using a separating funnel and the concentration was measured colorimetrically at 600nm (the original \(J\)-band of PCYB). Stoichiometry was obtained from the intersection point of the plots of complexed dye concentration versus polymer concentration. In centrifugation method the metachromatic solutions were centrifuged at 11,000 rpm (23,000 g) for 15 min, where the metachromatic compounds were sedimented. From the supernatant the concentration of the un-complexed dye was estimated and hence stoichiometry was determined graphically as in earlier case.

By metachromatic titration the volume of the polymer solution required for the equivalence of the anionic groups of the polymer and the dye cation was measured. Increasing amount of the polymer solution (0.1-10 mL, 10^{-4}M) was added to a fixed quantity of dye solution (10^{-5}M) in different test tubes. Absorbance of the solutions was measured at 600nm. These absorbance values were plotted against volumes of the polymer solutions added which gave two intersecting straight lines. From the point of intersection, the volume of the polymer solution required for the equivalent consumption of the dye was calculated.

The reversal of metachromasy was investigated by measuring the absorbance of the pure dye and dye-polymer solution at 600nm (\(J\)-band for the pure dye) as well as at 500nm
(H-band) upon addition of different solvent like ethanol, methanol, 1-propanol, DMF, DMSO and urea.

For studying the micellar effect on the bacterial polysaccharide, to a fixed concentration of dye-polymer mixture ([Dye] = 10^{-5} M, P/D = 30), increasing amounts of single cationic surfactants were added and spectral measurements of each solution was measured at 450-650 nm.

3. Results and discussion

*Klebsiella* K28 capsular polysaccharide consists of D-glucose, D-galactose, D-mannose and D-glucuronic acid in the approximate molar ratios of (2:1:2:1). The equivalent weight was found to be 980. The primary structure of the polysaccharide has been published by Curval et al. [15]. The polysaccharide is composed of hexasaccharide repeating unit having the following structure:

![Hexasaccharide Structure](image)

The presence of glucuronic acid in every repeating unit makes the polymer a unique polyelectrolyte.

As already mentioned, that PCYB belongs to the class of conjugated cyanine dye. Being amphipathic in nature, the molecule has a tendency to aggregate. The dye shows two
absorption maxima: one at 600nm and another at 550nm, respectively (Figure 1).

![Absorption Spectra](image)

**Figure 1.** Effect of *Klebsiella* K28 capsular polysaccharide on the absorption spectra of aqueous $10^{-5}$ M PCYB at 298 K. Curves 1 → 7: P/D 0, 1, 3, 5, 10, 20 and 30.

Although reported by others [23-25], in the present study no significant band was observed at ~520nm. The band corresponding to 600nm was due to the vibrationless electronic transition ($S_0 \rightarrow S_1$) whereas the 550nm band was due to a vibrational electronic transition. The $J$-band (at 600nm) intensity decreased with the rise in 550nm ($D$-band) band intensity when concentration of PCYB was increased, as also reported by others [23,25]. These two multibanded spectra were due to dimerization of the dye molecule [26,28,54]. The present observation was also in conformity with the earlier reports. The dye dimer was always in equilibrium with its monomer. Pure dye in aqueous medium showed an overlapping of the two spectra. When SPS from *Klebsiella* K28 was added gradually (from $10^{-4}$ to $3.0 \times 10^{-4}$M), to the aqueous dye solution, a blue shifted band appeared at 500nm ($H$-aggregate). This metachromatic band was due to the formation of charge transfer complex between the capsular polysaccharide and PCYB. At lower polymer/dye molar ratio [P]/[D], the sharp bands at 600 and 550nm got broadened with the appearance of a small hump at 500nm. At
P/D = 3 the polymer induced a distinct metachromatic band, when the blue shifted peak appeared at 500nm. On further increase in P/D values, there was no spectral shift of the $H$-band. At higher P/D molar ratios $J$- and $D$-band almost got depressed and the intensity of $H$-band increased sharply. The blue shift value of about 100nm exhibited strong induction of metachromasy in the dye pinacyanol bromide dye by *Klebsiella* K28 polymer. Initial broadening at the $J$- and $D$- bands, along with the formation of $H$-band resulted multiple band structure of the dye. Appearance of multiple banded structures at lower P/D values suggested random coil conformation of the polymer. On the other hand, at higher polymer concentration, the polymer changed its conformation to helical form as evidenced from the appearance of a single banded spectrum on and after P/D = 10 [16-18, 21]. Stoichiometry of polymer/dye in the metachromatic compounds was determined by McIntosh method and Centrifugation method [16-18,55]. It was observed that the metachromatic compound was formed with the dye/polymer stoichiometry of 1: 1 (shown in Figure 2).

![Figure 2. Determination of stoichiometry of *Klebsiella* K28 polysaccharide- PCYB complex by centrifugation (O) and McIntosh (Δ) method at 298 K.](image)

This result was in good agreement with the reported values [16-18,55]. Reaction between PCYB and SPS belongs to so-called double displacement reaction, where the cation of the SPS (herein Na$^+$ ion, as they are isolated as Na$^+$ salt) gets replaced by the dye cation.
Now when varying amounts of SPS was added, intensity of the $J$-band should decrease and should correspond to the amount of free dye. Thus, by plotting complexed dye concentration versus polymer concentration one should get a break point as after the break point only the conformation changes in the polymer and/or stacking of dye to polymer matrices could take place. Appearance of an isosbestic point at $\sim$535nm also suggests the formation of 1:1 stoichiometric complex between the dye molecule and SPS [21]. Metachromatic titrations (using the theoretically calculated values) also supported 1:1 stoichiometric complex formation.

The effect of non-aqueous solvent (such as ethanol) on the reversal of metachromasy is shown in Figure 3 as representative one.

*Figure 3. Effect of ethanol (40%, v/v) on the absorption spectra of Klebsiella K28 polysaccharide-PCYB complex. at 298 K. [PCYB]= 10^{-5} M. Curves: ($\triangle$) P/D=0; (O) P/D=3; ($\Delta$) P/D=O in presence of 40% (v/v) ethanol; (V) P/D = 3 in presence of 40% (v/v) ethanol.*

Addition of ethanol increased the intensity of the $J$-band with the corresponding decrease in $D$-band and in presence of 40% ethanol at P/D= 3 the metachromatic band ($H$-band) disappeared and the spectrum became identical with that of the pure dye solution (in 40% ethanol). Short chain alkanols like methanol and ethanol are often used to dissolve poorly soluble dyes. Thus, these alcohols can have a strong influence on the dye aggregation
behavior [24]. Upon addition of alcohol, overall polarity of the medium decreases, thus electrostatic interaction between dye and polymer becomes less favourable. Moreover, ethanol enhances the solubility of the dye monomer, which resulted an increase in the intensity of the J-band of PCYB. Medium polarity decreases with the increase in alkanol chain length. Thus, it was found the efficiency in disrupting the metachromatic band to follow the order n-butanol > n-propanol > ethanol > methanol. Experimental results suggested that the dye only interacts with SPS through predominant electrostatic binding in the anionic charge centers of the polymer. Urea is a common denaturant and can destroy the secondary interaction forces in biomacromolecules, or even small biomolecular aggregates. Therefore, reversal of metachromasy should take place upon addition of urea to a dye-SPS complex. So reversal of metachromasia, upon addition of urea, DMF and DMSO could act as marker to the extent of SPS denaturation [55]. Effects of the addition of latter category agents on the stability of PCYB-SPS complexes were not shown to save space.

Interaction constant ($K_c$) between SPS and PCYB was determined at different temperatures using Rose and Drago equation [16-18]. Results are summarized in Table 1. Binding constant was found to be comparable with earlier findings [16-18]. $K_c$ values were found to decrease with the rise in temperature, revealing the binding process to be exothermic in nature [18]. Free energy changes were calculated from the relation $\Delta G^0 = -RT\ln K_c$ while the enthalpy and entropy changes were calculated from the graphical plots of $\Delta G$ versus $T$ according to the relation $\Delta G^0 = \Delta H^0 - T\Delta S^0$. Higher values of free energy change indicated the dye-polymer aggregation to be controlled both by electrostatic and hydrophobic interactions [18]. Values of the enthalpy and entropy changes were within the range of a reversible biological process.
Table 1. Thermodynamic parameters for the interaction of *Klebsiella* K28 capsular polysaccharide-pinacyanol bromide in aqueous media at 298 K

<table>
<thead>
<tr>
<th>Temp.(K)</th>
<th>$10^{-3} \times K_c(M^{-1})$</th>
<th>$\Delta G^\circ$ (kcal mol$^{-1}$)</th>
<th>$\Delta H^\circ$ (kcal mol$^{-1}$)</th>
<th>$\Delta S^\circ$ (cal mol$^{-1}$ K$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>303</td>
<td>2.40</td>
<td>-4.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>308</td>
<td>2.06</td>
<td>-4.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>318</td>
<td>1.56</td>
<td>-4.68</td>
<td>-5.73</td>
<td>-2.54</td>
</tr>
<tr>
<td>323</td>
<td>1.37</td>
<td>-4.67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interaction between the capsular polysaccharide and surfactants were studied by the dye incorporation technique [50]. The absorbance of the dye pinacyanol bromide decreased considerably at 600nm, when it interacted with the capsular polysaccharide K28. At a definite $P/D$ ratio of 30, cationic surfactant of increasing concentration was added separately. It was found that upon addition of cationic surfactants the absorbance values of the dye-polymer complex at its $J$-band increased considerably. It indicated that the surfactant molecules interacted with the capsular polysaccharide by replacing the cationic dye molecules. In other words, the cationic dye molecules got free from the polymer matrix with the addition of cationic surfactants. The increase in absorbance was considered to be equivalent to the extent of surfactant bound to the polysaccharide. The increase in absorbance can also be correlated with the ability of the surfactant in freeing the dye molecules from the dye-polymer complex.

The effect of different cationic surfactants on the absorbance of pinacyanol bromide and *Klebsiella* K28 polymer at 600nm are shown in Figure 4. In all the cases it was observed that the metachromatic band at 500nm of the dye-K28 capsular polysaccharide was depleted with the addition of surfactants. From the Figures, it was clear that the increase in the absorbance value at the $J$-band upon addition of different surfactants followed the order BDHAC $>$ CTAB $>$ CPC $>$ DPC. Also it was found that the concentration of surfactants required to reach to the corresponding increased absorbance value followed the descending
order BDHAC < CTAB < CPC < DPC. Thus, it could be said that the ability in freeing the dye from the dye-polymer complex followed the order BDHAC > CTAB > CPC > DPC.

![Figure 4](image)

*Figure 4.* Effect of cationic surfactants on the absorbance of *Klebsiella* K28-PCYB complex at 298K. [PCYB] = 10^−5 M; P/D = 30. Surfactants (1.0 m moldm⁻³): (O) none; (Δ) DPC; (¶) CPC; (x) CTAB and (□) BDHAC.

Surfactant-polyelectrolyte interactions are electrostatically prevalent when they are oppositely charged. In synthetic polymer-surfactant systems polyelectrolytes and oppositely charged surfactants produce the strongest association [36,46-48,50,51]. Usually, such kind of interactions is initiated by the salt, like electrostatic interactions among oppositely charged polyelectrolytes and surfactant and then gets stabilized by the hydrophobic interactions of the bound surfactant tails [46]. The binding constant between the anionic polymer and the cationic surfactants were calculated by Rose and Drago equation from the absorbance results. The equation is given below:

\[
\frac{C_D}{A - A_0} = \frac{1}{K_C L (\varepsilon_{DS} - \varepsilon_D)} + \frac{C_S}{L (\varepsilon_{DS} - \varepsilon_D)}
\]  

(1)

where, \(C_D\) is the concentration of the polymer added, \(C_S\) the concentration of the surfactant added, \(A_0\) the absorbance of pure dye solution at 600nm and \(A\) is the absorbance of the dye-
polymer solution at 600nm when \( C_S \) concentration of surfactant added. \( K \) is the binding constant between the polymer and surfactant; \( \varepsilon \) is the molar absorption coefficient.

The values of \( (C_D C_S)/(A-A_0) \) was calculated for surfactant and then the values of \( (C_D C_S)/(A-A_0) \) were plotted against \( C_S \) (shown in Figure 5).

\[ Figure 5. \text{Plots of } (C_D C_S)/(A-A_0) \text{ vs. [surfactant]} \text{ for the determination of binding constant at 298K.} \]

Surfactants: (•)CTAB (O)CPC (▼) DPC and inset: BDHAC.

From the slope and the intercept of the straight line, the binding constant values between the K28 polymer and the surfactant were calculated. Results are summarized in Table 2.

\[ Table 2 \text{ Values of binding constants of different cationic surfactants with Klebsiella K28 capsular polysaccharide and their CMC values} \]

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>CMC (mM)</th>
<th>( 10^3 \times \text{Binding constant} (M^3) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDHAC</td>
<td>0.042</td>
<td>1.67</td>
</tr>
<tr>
<td>CTAB</td>
<td>0.80</td>
<td>0.55</td>
</tr>
<tr>
<td>CPC</td>
<td>0.90</td>
<td>0.245</td>
</tr>
<tr>
<td>DPC</td>
<td>14.70</td>
<td>0.146</td>
</tr>
</tbody>
</table>
The binding constant values in case of BDHAC, CTAB, CPCI and DPCI were \(1.67 \times 10^{-5}\), \(0.55 \times 10^{-5}\), \(0.245 \times 10^{-5}\) and \(0.146 \times 10^{-5}\) M\(^{-1}\) respectively, which followed the order BDHAC > CTAB > CPC > DPC and the values also were in accordance with CAC (or CMC) values of the surfactants. It was also clear from the results that the extent of surfactant binding to the polymer was sharply regulated by the hydrophobic chain length. As the surfactants have their chain lengths following the order BDHAC > CTAB > CPC > DPC, hence the binding constants also followed the same order. Again, between CTAB and CPC the former has higher head group charge density that the later, hence CTAB showed a higher binding affinity than CPC with the SPS. Longer hydrophobic chain in surfactant could contribute higher hydrophobic interaction; hence, such an observation was noted. CTAB has higher chain length than DPC and hence have lower CMC value in water. In succession it should have a higher binding affinity with an anionic polyelectrolyte. Thus, from the present study one could conclude that the extent of freeing of dye by a surfactant molecule from a polymer-dye aggregate depended on the head group charge and chain length of the surfactant molecules. Therefore, a decrease in chain length resulted the decrease in binding constant. The present observation supported the earlier observations [50]. In Figure 6, variation of

![Figure 6](image.png)

*Figure 6. Variation of binding constant between cationic surfactant and *Klebsiella* K28 capsular polysaccharide with the critical micelle concentration of cationic surfactants. Temp. 298K.*
binding constant as a function of CMC has been graphically presented. The variation was found to exponentially decay with the increase in CMC of the cationic surfactants. It may also be mentioned that the CMC values of surfactants could be influenced by the presence of SPS and/or dye molecules. But in the present case the effects were negligible as very low concentration of the polymer and dye were used. Thus, one could assume that the CMC values of the surfactants would hardly change. Even if there are any changes, they will be same, and in the present comparative study, these changes would be insignificant.

4. Conclusions

(1) Anionic bacterial polysaccharide, isolated from *Klebsiella* K28, induced strong metachromasy in cationic dye pinacyanol bromide. A 1:1 stoichiometric complex was formed between the dye and the SPS.

(2) Dye-polymer aggregation got disrupted upon addition of alcohols, DMF, DMSO, as revealed by the reversal of metachromasy.

(3) Energetics of dye polymer complex formation were determined and found to be comparable with reversible biological processes.

(4) Cationic surfactants, by way of displacing the dye molecules, from the polymer matrix, got bound to the polymer. Hence, the original band intensity of the dye molecules increased upon cationic surfactant addition.

(5) Binding affinity of surfactant with polymer followed the order BDHAC >CTAB >CPC >DPC which was in accordance with their hydrophobic chain length and/or head group charge density.
References


