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Dynamic Light Scattering and Viscometric Studies on the Interaction of Bacterial Capsular Polysaccharide, *Klebsiella* K40, with Pure and Mixed Surfactant Systems in Aqueous Media

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Abstract — Capsular polysaccharide (SPS) is an integral component of gram negative bacteria, *Klebsiella*. The interaction of SPS isolated from *Klebsiella* K40 serotype with oppositely charged pure surfactants and cationic-nonionic mixed surfactants were investigated by dynamic light scattering and viscometric technique. Variation in the hydrodynamic radius and zeta potential were studied by dynamic light scattering method. Nature of the interaction is governed by concentration of the surfactants, charge density, surfactant head group and the CMC of the surfactants. Micellar effects of the pure and mixed surfactants on binding with the SPS are presented in this paper.

Keywords : *Capsular polysaccharide, dynamic light scattering, viscometry, CMC.*

INTRODUCTION

Klebsiella belongs to gram negative bacteria of the *Enterobacteriaceae* family [1]. Bacterial capsular polysaccharides are acidic in nature. These polysaccharides are also known as “sauy” polysaccharides (SPS), sau means ‘sour’ in German. The primary structure of *Klebsiella* K40 capsular polysaccharide (SPS) was analysed in our laboratory [2]. The K40 SPS consists of pentasaccharide repeating units, having one residue of glucuronic acid in each repeating unit which leads to its anionic

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polyelectrolytic nature [2]. *Klebsiella* are causative factors for several human diseases [3–6]. However, these polysaccharides also have antigenic properties and possess potential uses in immunological and vaccine preparation [7–8]. Interaction between polymers and surfactants in aqueous solutions have attracted significant interest because of their widespread commercial applications and academic viewpoints [9–12]. Comprehensive studies on a variety of cationic surfactants with anionic polyelectrolytes, synthetic and natural, are available in literature [13–21]. Studies on various physico-chemical parameters on the above mentioned aspects in the presence of SPS are however, limited.

In the present paper, efforts have been made to characterize the nature of interaction of anionic SPS with cationic surfactants, *viz.*, benzylhexadecyltrimethylammonium chloride (BDHAC), didodecyldimethylammonium bromide (DDAB), hexadecyltrimethylammonium bromide (CTAB), tetradecyltrimethylammonium bromide (TTAB), dodecyltrimethylammonium bromide (DTAB), hexadecylpyridinium chloride (CPC) and dodecylpyridinium chloride (DPC). Also the cationic-nonionic mixed surfactants (DPC-Tween-20) were used a different combinations in order to understand the effect of charge density and critical micelle concentration on the nature and extent of interaction. For dynamic light scattering studies only BDHAC, CTAB, CPC and DPC have been used as pure cationic surfactants. Further, we tried to correlate the binding of the surfactants to the SPS through rheology (viscosity) and size measurements by dynamic light scattering technique. The zeta potential of the various systems were also determined in order to explain the SPS-surfactant interactions on the basis of surface charge variation. These studies are expected to shed light on the binding nature of SPS with various surfactants and may be helpful in industrial preparation and pharmaceutical formulations.

EXPERIMENTAL

Materials :

The serological test strain of *Klebsiella* K40 was kindly supplied by Prof. S. Strim of the University of Giessen, Germany. The bacterial strain was grown on nutrient agar plates and the capsular polysaccharide was isolated from the dry bacterial cells by phenol-water-cetavlon method [22–24]. The experimental surfactants benzyltrimethyl-n-hexadecylammonium chloride (BDHAC), didodecyldimethylammonium bromide (DDAB) N,N,N-hexadecyltrimethylammonium bromide (CTAB), hexadecylpyridinium chloride (CPC), dodecylpyridinium chloride (DPC), dodecyltrimethyl ammonium bromide (DTAB), tetradecyltrimethylammonium bromide (TTAB), nonionic surfactants polyoxyethyleneglycol-*tert*-octylphenyl ether (TritonX-

100) and polyoxyethylenesorbiton monolaurate (Tween-20) were purchased from E. Merck, Germany. Purity of the surfactants were checked by measuring their conductance and/or surface tension values [25]. Double distilled water with a specific resistivity of $18\text{m}\Omega\text{cm}^{-1}$ was used during the experiments and all experiments were performed at 298 K.

Viscosity measurements :

Viscosity measurements were carried out using a small Ubbelohde viscometer (5.0 mL capacity, $\sim 300\text{s}$ flow time for water). Pure as well as mixed surfactants of different compositions were combined with fixed concentration of SPS sample. Reduced viscosities (η_{sp}/C) at different SPS concentrations (C) were calculated with the help of Huggin's equation : [26]

$$\frac{\eta_{sp}}{C} = \frac{[\eta]}{k'[\eta]-C} \quad (1)$$

where, η_{sp} is the specific viscosity ($\eta_{sp} = (t - t_0)/t_0$), t is the flow time of the polymer solution, t_0 is the flow time of pure solvent, C is the polymer concentration in g dl^{-1} k' is Huggins' constant and $[\eta]$ is the intrinsic viscosity.

The nature of interaction between the SPS and surfactants is deduced from the plot of η_{sp}/C vs. C .

Dynamic light scattering (DLS) studies : measurement of hydrodynamic diameter (d_h) and zeta potential (ZP) :

The dynamic light scattering (DLS) and zeta potential (ZP) measurements were done using Nano-ZS90 (Malvern-USA). He-Ne laser emitting a light of 632.8 nm was used as the light source. The DLS experiments were carried out at a fixed scattering angle of 90° . To a fixed amount of SPS solution in a 4.0 mL cylindrical quartz cuvette, surfactant was gradually added using a microsyringe (Hamilton, USA) and the mixture was homogenized before the data were recorded. Although the SPS - surfactant systems are polydispersed, the mean hydrodynamic diameter is used for the presentation of the changes in the hydrodynamic size distribution caused by the addition of the surfactant.

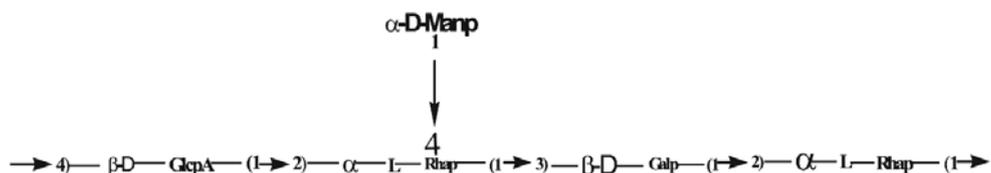
DLS measurements directly give the hydrodynamic diameter of the aggregates according to the Stokes-Einstein equation [27] :

$$\bar{D} = \frac{k_B T}{6\pi\eta R_h} \quad (2)$$

where, k_B is Boltzmann constant, η is viscosity of the medium at the experimental temperature T , R_h is the hydrodynamic radius and \bar{D} is the corresponding diffusion co-efficient.

RESULTS AND DISCUSSION

The primary structure of the bacterial polysaccharide extracted from *Klebsiella* K40 contained galactose, mannose, rhamnose and glucuronic acid in appropriate molar ratios of 1 : 1 : 2 : 1. The pentasaccharide repeating unit of the SPS has the following structure [2] :



The mass of the SPS containing one anionic group on average referred to the equivalent weight of the SPS was found to be 840Da [2].

Viscosity measurements :

Reduced viscosity (η_{sp}/C) of the SPS solution in presence of pure cationic surfactants and cationic-nonionic mixed surfactants of different compositions were measured. The plot of η_{sp}/C vs. surfactant concentration in case of pure cationic surfactant has been shown in Fig. 1. Viscosity decreased initially with increasing surfactant concentration in all the cases and depending on the CMC of the individual surfactants the viscosity-surfactant concentration profile attained constancy at post micellar region. The initial decrease in viscosity of the systems was the outcome of cationic surfactants binding to the anionic polymer by electrostatic attraction leading to the formation of polymer-surfactant complexes [23] and at post micellar region slight increase in viscosity attributed to the coiling up of polymer. The coiling up of polymer is enhanced in case of surfactant with lower CMC value [24]. Capability of the surfactants to reduce the viscosity of the SPS was mainly governed by two factors (i) nature of surfactant head groups (i.e., nature of electrostatic interactions) and (ii) CMC of the surfactants. The phenomenon of contraction of the polymer chains were due to the gradual neutralization of their negative charges by the positive surfactant heads. The capability of the pure cationic surfactants to reduce the viscosity of the SPS is in the order: BDHAC > DDAB > CTAB > TTAB > DTAB > CPC > DPC. In spite of having higher

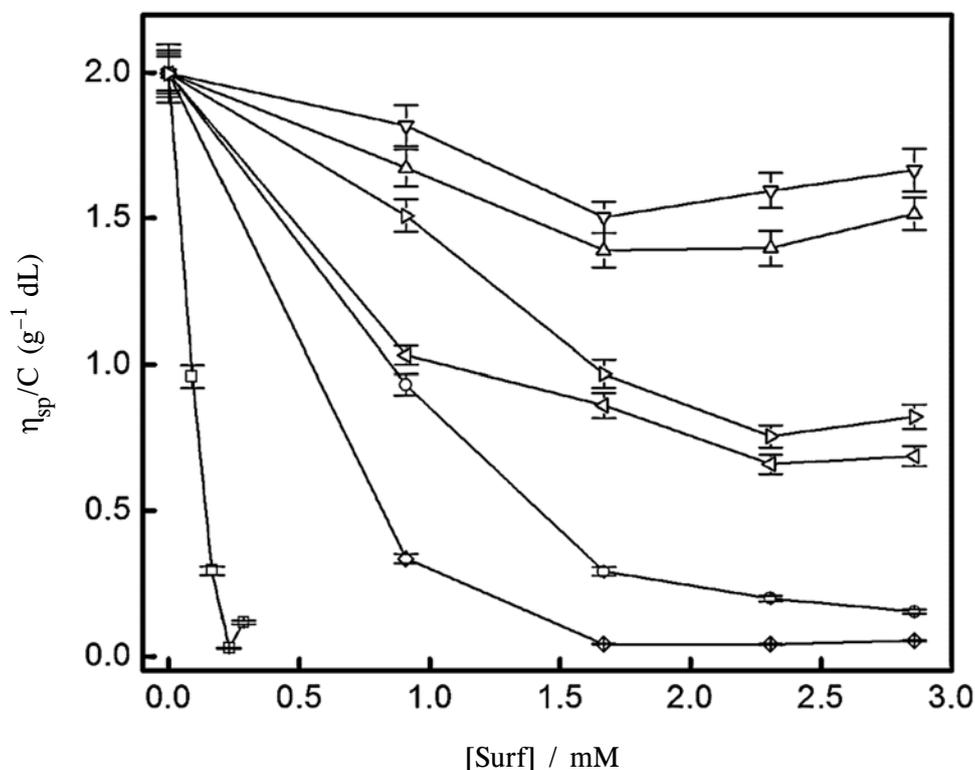


Fig. 1. Variation in reduced viscosity of *Klebsiella* K40 SPS (0.1 mM), in presence of cationic surfactants at 303 K. Cationic Surfactants : □, BDHAC; ○, DDAB; △, CTAB; ▽, TTAB; ◊, DTAB; ◁, CPC and ▷, DPC.

CMC values, TTAB and DTAB were found to exert stronger effect in reducing the viscosity of SPS than CPC and DPC. This suggests that, the electrostatic attraction plays key role in the process of decrease in viscosity. TTAB and DTAB having same head (quaternary ammonium) group possess higher charge density than CPC and DPC which have pyridinium head group and exert stronger electrostatic effect to SPS.

The viscosity of SPS-DPC-Tween-20 systems are presented in Fig. 2. The capacity of DPC to reduce the viscosity of the SPS is very low. Since surfactant-polymer interaction is governed by both electrostatic as well as hydrophobic behaviour [23], this nature of DPC may be attributed to its high CMC value and low alkyl chain length. On addition of nonionic surfactant Tween-20, the capacity to reduce the

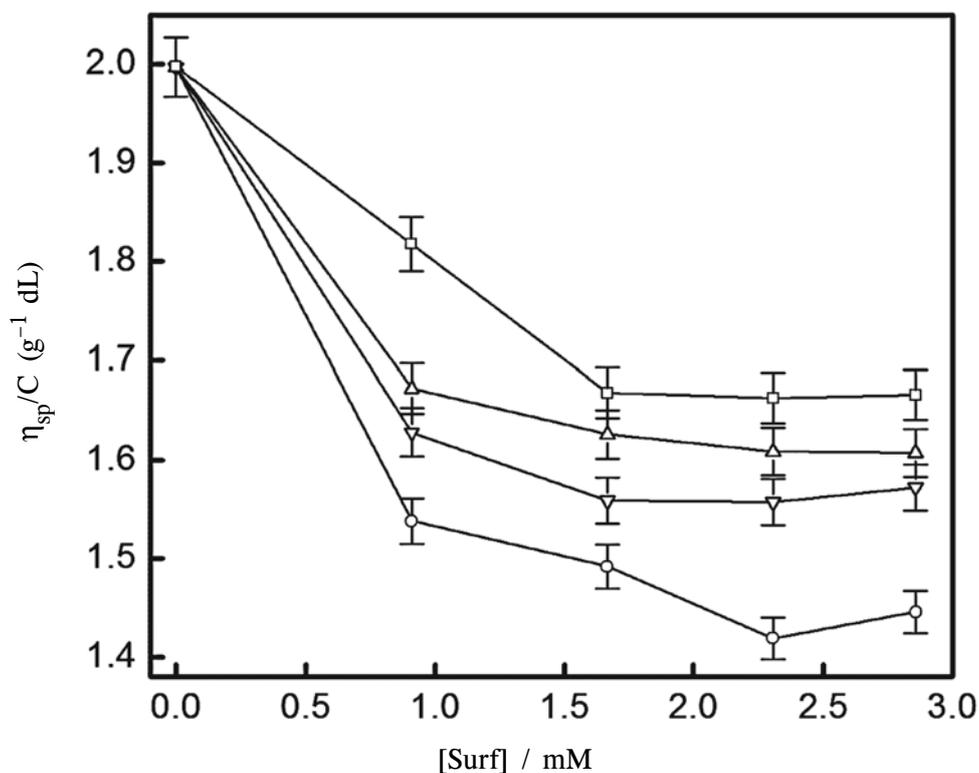


Fig. 2. Variation in reduced viscosity of *Klebsiella* K40 SPS (0.1 mM), in presence of DPC-Tween 20 mixture at 303 K. Mole fraction of Tween 20 : \square , 0; \circ , 0.2; Δ , 0.5 and ∇ , 0.8.

viscosity was enhanced as a result of decrease in CMC of the cationic–nonionic mixed surfactant systems [23]. However, increase in the mole fraction of nonionic surfactant (mole fraction of the nonionic surfactant $x_2 > 0.2$), suppressed the extent of decrease in viscosity. Therefore, it can be assumed that, the decrease in charge density leads to decrease in the electrostatic attraction between SPS and CPC with the increase in mole fraction of non-ionic surfactant which resulted the further increase in viscosity [23].

Dynamic light scattering studies :

The trends in the variation in d_h of pure SPS as well as SPS-surfactant mixed systems were found to follow reverse trend with viscosity and which were similar for different surfactants (Fig. 3). Here also the variation in d_h were dependent on two factors (i)

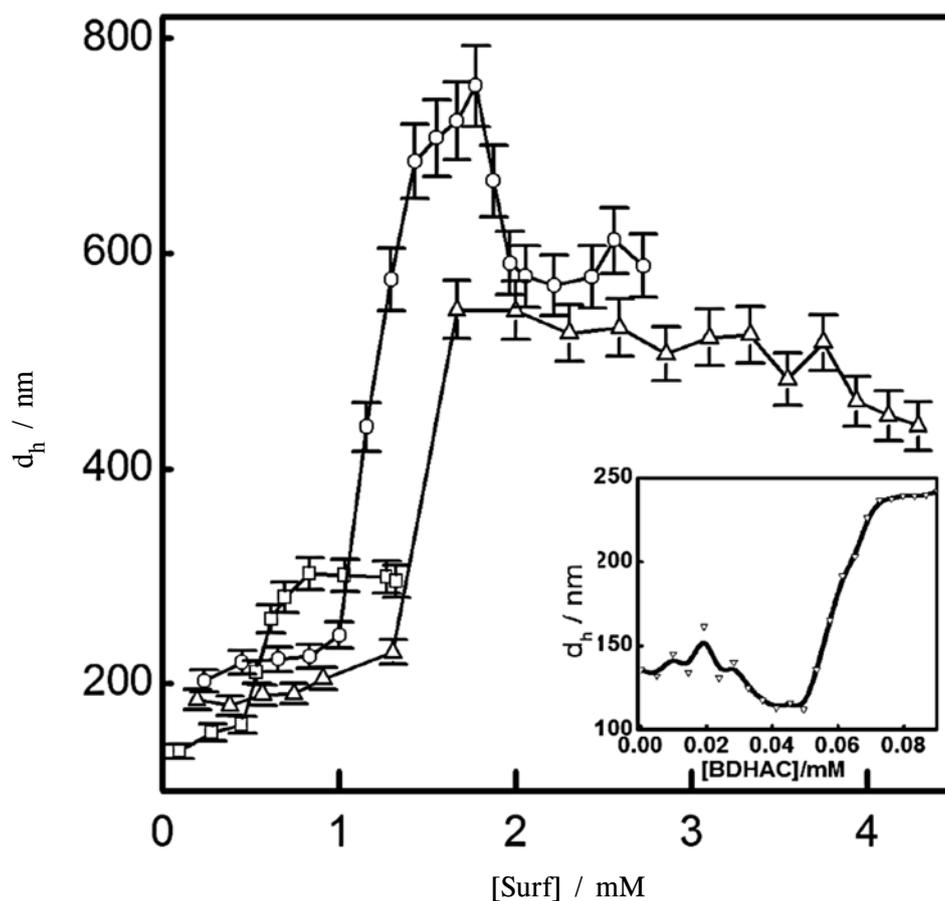


Fig. 3. Variation in size of *Klebsiella* K40 SPS (0.1 mM) in presence of cationic surfactants at 303 K. Cationic Surfactants : \square , CTAB; \circ , CPC and Δ , DPC. [Inset : ∇ , BDHAC].

nature of head groups and (ii) CMC of the surfactants. The d_h -surfactant concentration profile passes through a maximum, initially size increases due to formation of polymer-surfactant complex as a result of electrostatic attraction, but at the onset of micellization of individual surfactants coiling up of polymer could lead to the decrease in d_h . The order of binding capability of surfactants to SPS is as follows : BDHAC > CTAB > CPC > DPC. In case of cationic-nonionic mixed surfactant, the result for CTAB/Tween-20 is shown in Fig. 4 as representative. The onset of size

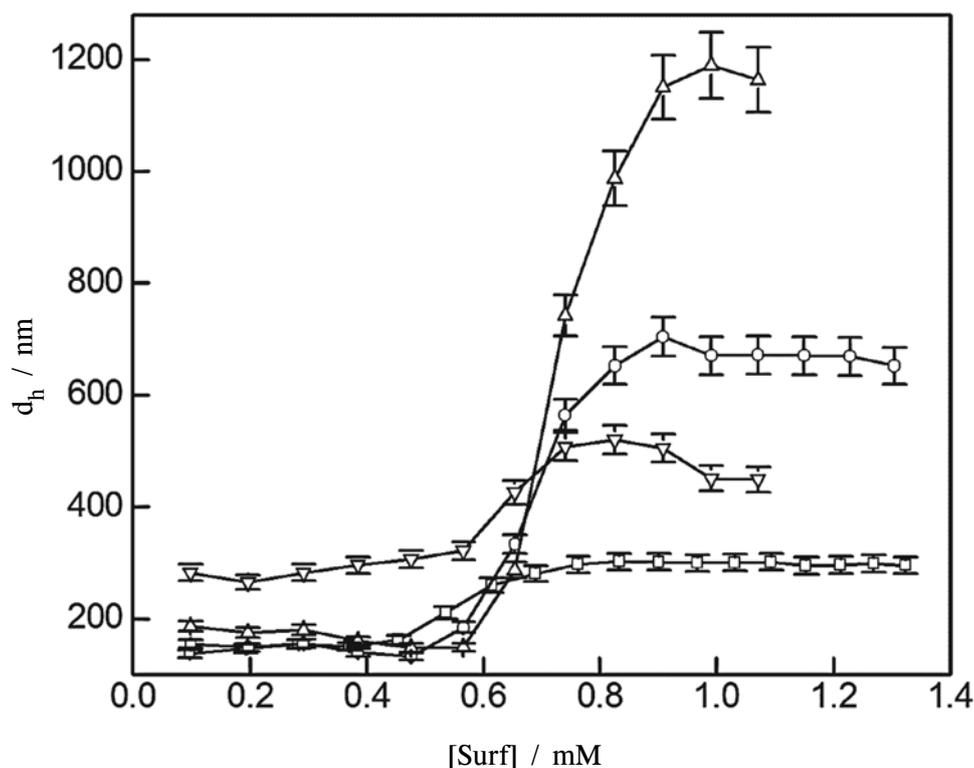


Fig. 4. Variation in size of *Klebsiella* K40 SPS (0.1 mM) in presence of CTAB-Tween 20 mixture at 303 K. Mole fraction of Tween-20 : \square , 0; \circ , 0.2; Δ , 0.5 and ∇ , 0.8.

enhancement started at a mixed surfactant concentration of 0.5 mM. The interaction of SPS is more extensive which leads to more effective size variation. This is due to decrease in CMC value on addition of nonionic surfactant (21–25, 28–30). The suppression of size enhancement after 0.5 mole fraction of non-ionic surfactant attributed to the decrease in charge density of the mixed surfactant systems which led to the less electrostatic interaction.

Zeta potential of SPS-surfactant systems :

The zeta potential of the SPS solution was measured using DLS instrument. The change in zeta potential of SPS in presence of pure cationic surfactants is shown in Fig. 5. The addition of oppositely charged surfactants to the SPS led to the charge neutralization. Reversal of charge occurs when excess surfactant binds with the SPS.

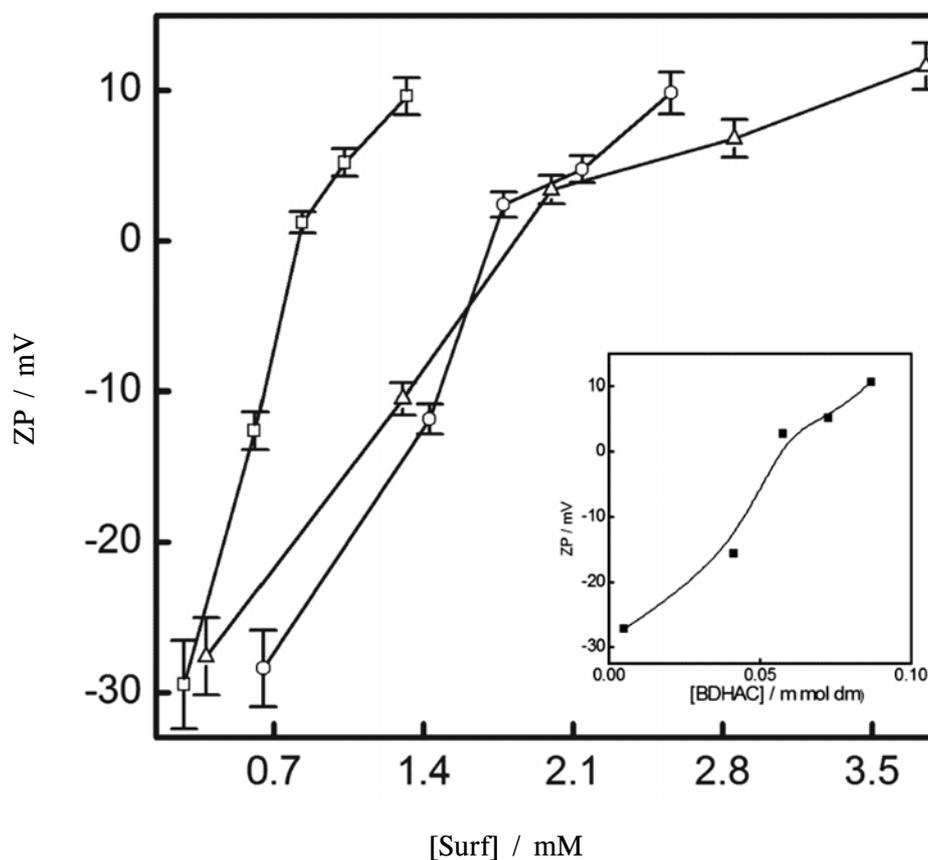


Fig. 5. Change in zeta potential of aqueous solution of *Klebsiella* K40 SPS (0.1 mM) in presence of cationic surfactants at 303 K. Pure Surfactants : □, CTAB; ○, CPC; △, DPC. [Inset : ▽, BDHAC].

Due to presence of some free surfactants which are unable to bind to the SPS, the charge neutralization does not appear at equimolar ratio. The reversal of charge is determined by the extent of binding which is further controlled by the CMC of the surfactant (30). From the figure, it is observed that the reversal of charge follows the order : BDHAC > CTAB > CPC > DPC. The variation of the zeta potential on addition of mixed surfactants CTAB-Tween-20 has also been depicted in Fig. 6. In case of mixed surfactants, due to decrease in CMC, the binding capacity is enhanced and the concentration of surfactant required to neutralise the charges of the SPS is

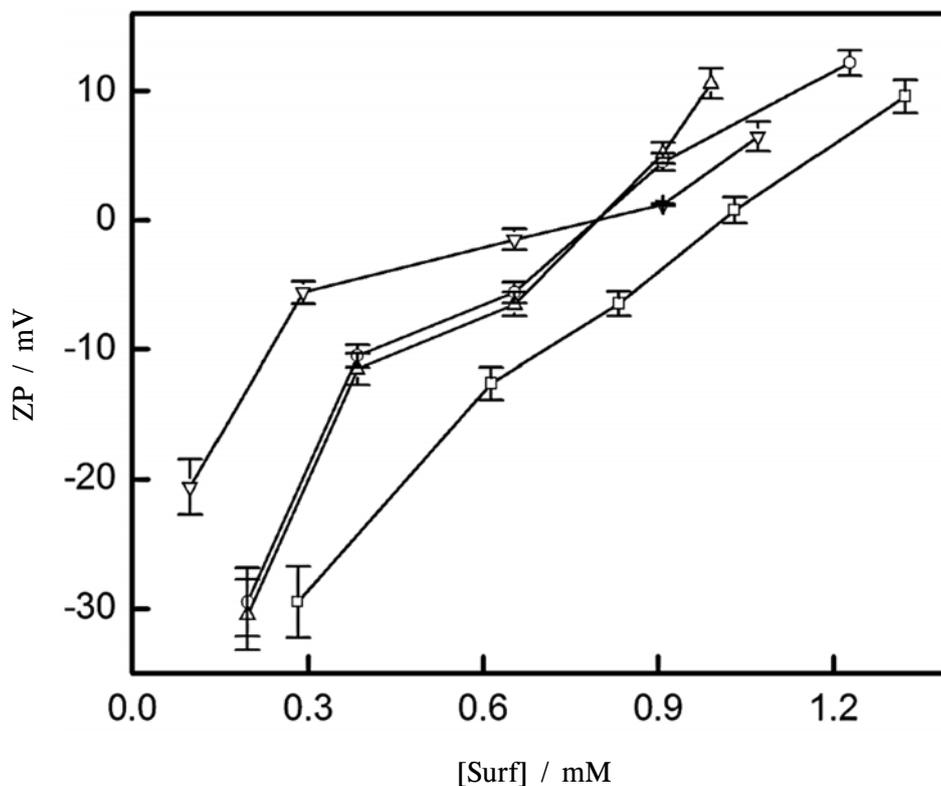


Fig. 6. Change in zeta potential of aqueous solution of *Klebsiella* K40 SPS (0.1 mM) in presence of CTAB-Tween-20 mixture at 303 K. Mole fraction of Tween 20 : \square , 0; \circ , .2; Δ , .5 and ∇ , .8.

less. Thus the extent of reversal of charge is more effective than the pure surfactants (30).

Summary and conclusions :

The interaction of anionic SPS isolated from *Klebsiella* K40 with cationic and cationic-nonionic mixed surfactant systems were studied using various techniques. Based on the experimental results the following conclusions can be drawn :

1. The capacities of reducing the viscosity of SPS by pure surfactants are in accordance to their CMC values. In case of mixed surfactants, addition of nonionic surfactant further decreases the viscosity.

2. The size of mixed aggregates followed reverse trend to the viscosity.
3. Charge neutralization of the anionic SPS takes place on addition of both cationic as well as cationic-nonionic mixed surfactants, resulting in reversal of zeta potential. The onset of neutralization points are also in accordance to the CMC values of the surfactants used.
4. The present set of studies strongly reveal the presence of two forces, viz., electrostatic and hydrophobic forces during the aggregation process of polymer-dye and polymer- surfactant mixed systems.

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