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A comparative study on the physico-chemical properties of bacterial capsular polysaccharides from different serotypes of *Klebsiella*

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Abstract

Comparative studies on various physico-chemical properties of bacterial capsular polysaccharides from different serotypes of *Klebsiella* have been investigated. A correlation of the primary structures with the solution properties of different polymers has been established based on spectrophotometric, spectrofluorometric and viscometric measurements. Absorption studies on four experimental biopolymers (K7, K14, K17 and K26) with four cationic dyes, pinacyanol choloride (PCYN), acridine orange (AO), toluidine blue (TB) and phenosafranin (PSF) indicated that chromotropic capability of the polymers with respect to induction of metachromasy followed the order: K7 > K14 > K26 > K17. Formation of (1:1) stoichiometric polymer–dye complexes suggested stacking conformation in all the four polymers. Fluorescence quenching of AO and PSF dye induced by the polymers also revealed the quenching efficiency of polymers to follow the same order as revealed in the absorption studies. The Stern–Volmer constant (K_{SV}) also supported similar trend. The order of chromotropic character and fluorescence quenching of the polymer were in conformity with their charge densities. Viscosity measurements of all the four capsular polysaccharide solutions showed the characteristic behavior of polyelectrolytes indicating sharp increase in reduced viscosity of the aqueous polysaccharide solution at lower dilution. Molecular weight of the bacterial polysaccharides were also determined from viscometric measurements.

Keywords: Capsular polysaccharides; Dye–polymer interaction; Metachromasia; Viscosity; Fluorescence quenching

1. Introduction

At present about 82 serologically classified strains of *Klebsiella* K-serotypes have been recognized [1,2], which produce polysaccharides of different chemical structures. Primary structures of most of the capsular polysaccharides of *Klebsiella* are now known [3]. Almost all of them are acidic in nature having antigenic properties and are composed of definite repeating units. The *Klebsiella* capsular antigens have been found to be safe in human and these antigenic polysaccharides are now used as human vaccines [4,5], which are non-pyrogenic, non-toxic and immunogenic. Due to the potential use of the bacterial polysaccharides in immunological and vaccine preparation; in addition to primary structural studies, conformational analyses, as well as studies on various physico-chemical properties of these biopolymers in a comparative manner is of great importance. But such studies are very rare and the present studies aim at it. Studies on the interaction of small dye molecules with such biopolymers can produce useful informations regarding the structure and conformation [6–8] of the polymers. Specificity in the interaction of different dyes with polysaccharides has also been well studied [9,10]; such systems have potentiality in terms of drug–biopolymer interaction.

Chemical identity of different polymers as well as their conformation in solution can be established from the concept of metachromasy. The term metachromasia is a long studied phenomenon and it still have viability in quantitative and qualitative characterization of biopolymers and/or tis-

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sues. Metachromasia is basically a staining property by virtue of which a small molecule (may it be a drug or a dye) binds to a tissue or polymeric substrate whereby the color of the dye or drug molecule changes [11]. The shift in color band, usually in shorter wave length region, also occurs with increasing dye concentration. The long and well studied phenomenon was elaborately explained by Schibe and Zanker [12] and Szira-mai and Balazs [13]. Yamabe also reported about the analysis and application of metachromasia [14]. Theoretical prediction of induction of metachromasia was reported in detail by Czikkely et al. [15]. Elaborate discussion on metachromasia was also reported by McKusick [16]. Metachromasia is best viewed usually when a basic molecule binds with highly acidic tissue components like bacterial capsular polysaccharides [17]. Recent studies on metachromasia also supported earlier observed facts, although earlier methods needed to be improvised. Recent review on metachromasia includes Horbin [18]. Most of all the reported studies include cellulose [19], glycosaminoglycans from connective tissues [20], heparin and/or its derivatives [21], etc. But studies on the interaction of dye molecules with bacterial polysaccharides are not very common. During last three decades our laboratory has been actively involved in dye–polymer interaction studies, even though a few comparative studies on the capsular polysaccharides have been done. Such studies are believed to enlighten the structural variation among the different serotypes of a Gram-negative bacterium like *Klebsiella*. The serological identity of two capsular polysaccharides from *Klebsiella* K20 and *Escherichia coli* K30 was established [22] by measuring metachromatic spectral changes with the cationic dye neutral red. It has been reported that the conformation of the polyanionic chromotrope reflected on the shape of the metachromatic spectra [23] and stoichiometry of the dye–polymer complex depends on the conformation of the polymer [24]. Different techniques for the isolation and stability determination of the metachromatic compound have been reported [25]. The concept of reversal of metachromasy may be used to determine the stability of the metachromatic compound.

Fluorescence spectroscopic measurements are assumed to yield useful information about dye–polymer complex formation. Number of binding sites on the polymer molecule can be evaluated from fluorescence quenching technique [26]. Electronic excitation transport processes in polymeric systems allow fluorescence experiments to provide informations about macromolecular structure. Fluorescence techniques were also used [27,28] to study the conformational mobility of polymers in dilute solutions, the interpenetration and association of chains and the cooperative transitions of polymers containing carboxylic groups from a compact to an expanded state.

Viscosity of polymeric solution in absence and presence of additives is one of the important parameters capable of providing useful information on polymer solution [29]. Different molecular properties of macromolecules like shape, non-electrolytic or polyelectrolytic nature, molecular weight, etc. influence the viscosity of polymer solution.

In order to understand the correlations of chemical structure and immunological specificity and to account for the serological cross reactions amongst the different K-antigens of *Klebsiella* and between the *Klebsiella* K-antigens and the surface antigens of other bacteria, it is very much important to investigate physico-chemical properties of the polysaccharides of all the different serotypes of *Klebsiella*. The present investigation has been carried out to achieve a comparative behavior in connection with different physico-chemical properties of the capsular polysaccharides isolated from four different K-serotype K7, K14, K17 and K26 belonging to the same bacterial genus *Klebsiella*.

2. Experimental

The serological test strains for *Klebsiella* K7, K14, K17 and K26 capsular antigens were kindly supplied by Dr. Schlecht of Max-Planck Institute for Immunobiology, Freiburg, Germany. The stains were 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 [30]. The four experimental dyes pinacyanol chloride (PCYN), acridine orange (AO), phenosafranin (PSF), toluidine blue (TB) were purchased from Sigma Chemical Co. USA. The first one was used as received other dyes were used after purification by recrystallization techniques.

All the absorption spectra were recorded on a Milton Roy Spectronic 21D spectrophotometer and the fluorescence measurements were done in Shimadzu RF-5000 spectrofluorimeter. Concentrations of the aqueous solutions of dyes and polymers were in the range of 10^{-4} to 10^{-6} M; 1 mol of polymer referred to the average mass of one repeating unit of the polymer containing one anionic charge site. The general experimental details for the measurement of absorption and fluorescence, determination of stoichiometry, spectrophotometric and spectrofluorometric titration have been described earlier [31–33].

Viscosity of the polymer solution was measured in a Ubelhode Viscometer and the intrinsic viscosity [η] was calculated according to the equation of Fuoss and Strauss [34] as follows:

$$\frac{\eta_{sp}}{C} = \frac{A}{1 + B\sqrt{C}} \tag{1a}$$

$$\frac{C}{\eta_{sp}} = \frac{1 + B\sqrt{C}}{A} \tag{1b}$$

$$\frac{C}{\eta_{sp}} = \left(\frac{1}{A}\right) + \left(\frac{B}{A}\right) \times \sqrt{C} \tag{1c}$$

The values of C/η_{sp} were plotted against $\sqrt{C}$ and then extrapolated to $\sqrt{C} \rightarrow 0$.The intercept at $\sqrt{C} \rightarrow 0$ gave the value of $1/[\eta]$ and thus the value of $[\eta]$ was calculated. From the viscosity data, molecular weights of all the polysaccharides were calculated by using Mark–Howink equation [35], $[\eta] = kM^{\alpha}$.

All the experiments were performed at 298 K in aqueous media.

3. Results

The primary structures of all the polymers under investigation have been published earlier [36–39] and their repeating units are shown in Fig. 1. All the four polysaccharides were polyelectrolytes having different anionic charge densities. Charge density of the polyelectrolyte was calculated as the mass of potential anionic charge group (–COOH) per unit mass of the repeating unit. The calculated values for K7, K14, K17 and K26 polymers were 0.0928, 0.0865, 0.0566 and 0.0570, respectively. Chromotropic character of all the four capsular polysaccharides were investigated by studying spectral properties of the cationic dyes viz. PCYN, AO, TB and PSF in the UV–vis range. These four dyes were chosen after screening a good number of cationic dyes for their spectral behavior on interaction with the polymers.

The absorption spectra of the aqueous solution of AO dye in presence of K7, K14, K17 and K26 polymers in the UV–vis region indicated significant behavior of the polymers in inducing metachromasy in the dye. There was a considerable blue shift of the monomeric dye band (from 490 to 470 nm) in the dye–polymer mixtures upon addition of K7, K14 and K17 polymers, but K17 polymer did not show any appreciable change. The spectra at the UV region, however, did not show any significant change in the peak at 270 nm.

The aqueous solution of TB dye showed a broad band with the peak at 630 nm in the visible region and a sharp peak at 290 nm in the UV region. The K7, K14 and K26 polymers induced metachromasy in the dye quite appreciably in the visible range by shifting the peak from 630 to 575 nm. The UV peak at 290 nm was blue-shifted to a certain extent by the interaction of the dye with K7, K14 and K26 polymers with the change in the intensity. However, the K17 polymer did not show any considerable effect on the dye solution.

The UV–vis spectra of PSF dye with the four polymers were also investigated. The absorption band of the aqueous dye solution in the visible range was blue-shifted upon addition of K7, K14 and K26 polymers almost to the same extent. The peak at 520 nm shifted to 500 nm indicating weak induction of metachromasy. In the case of K17, intensity of the peak at 520 nm decreased, but no blue shift was recorded. The effects of polymers on the UV peak at 275 nm were not considerable. The spectral behavior of PCYN dye was significantly different from all the other three dyes and was studied extensively in presence of four polymers in our previous publications [8,32,33,40]. The aqueous solution of the dye showed two bands in the visible region with peaks at 600 and 550 nm corresponding to monomeric band (α) and dimeric band (β). Upon addition of four different polymers, the dye–polymer mixture exhibited blue-shifted metachromatic band (μ) with a peak (near 500 nm) of considerable

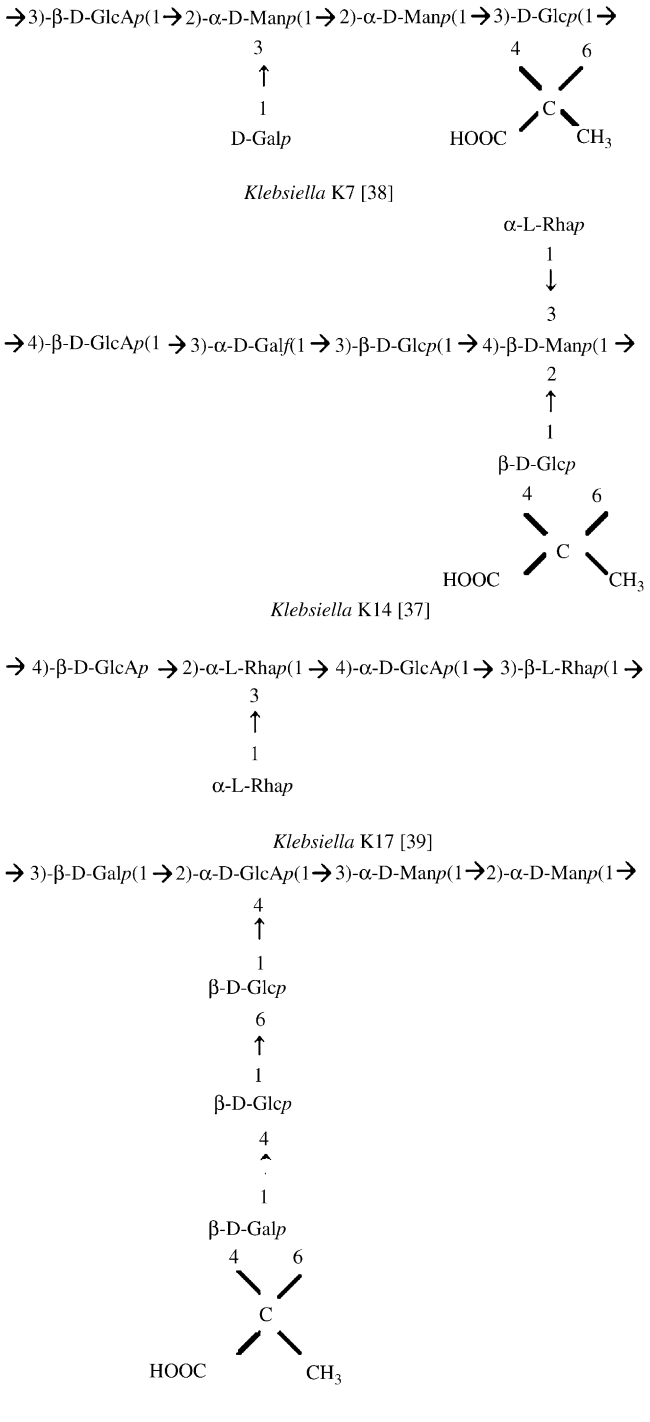

Fig. 1. Primary structures (repeating unit) of *Klebsiella* capsular polysaccharides under investigation. Anomeric configurations for D-*Glcp* and terminal D-*Galp* were not known for *Klebsiella* K7.

Table 1
Stoichiometry and equivalent weight of different *Klebsiella* capsular polysaccharides by spectrophoto/fluorometric titrations

Polymer	Spectrophotometric titration using PCYN			Fluorometric titration using		Eqivalent weight	
	MacIntosh method	Centrifugation method	Metachromatic titration method	AO	PSF	Theoretical[a]	Observed[b]
K7	0.65 (708)	0.66 (722)	0.68 (742)	0.66 (722)	0.64 (699)	727	719
K14	0.54 (1122)	0.53 (1081)	0.52 (1102)	0.53 (1102)	0.53 (1102)	1040	1102
K17	1.15 (877)	1.06 (810)	1.02 (778)	1.06 (810)	1.05 (802)	794	815
K26	0.69 (1223)	0.65 (1152)	0.67 (1190)	0.67 (1190)	0.66 (1167)	1183	1184

[a] Mass of the repeating unit was the theoretical equivalent weight (with respect to glucuronic acid). Parentheted values are experimentally observed equivalent weight.
[b] Average of all the experimentally observed values, using different methods.

difference that depends upon the nature of polymers. The spectral studies with the four polymers and the four dyes indicated that chromotropic character of the polymers with respect to induction of metachromasy was of the order: K7 > K14 > K26 > K17 and the dye pinacyanol chloride was found to be the most suitable one for further detailed studies on metachromasy, particularly, in the visible absorption range.

The determination of stoichiometry of the dye–polyanion was very much important to study the observed difference in the metachromasy of the dye. It was found that the stoichiometry of the dye–polyanion complex has been reflected in the metachromatic spectrum. The results of stoichiometry of pinacyanol chloride and four polymers in the dye–polymer compounds are shown in Table 1. A suggested model for dye–polymer interaction of different polymers has been depicted in Fig. 2.

Fig. 3 shows comparative titration curves of PCYN, AO and PSF by the four polymers. Results of metachromatic titrations yielded identical stoichiometry ratios as obtained by isolation method and centrifugation method, shown in Fig. 3A. Equivalent weights of the polymers, calculated from the results of metachromatic titrations, were in good agreement with the values as expected from the respective chemical composition (Table 1). Fluorescence studies were performed with cationic dyes AO (λ_{ex} = 491 nm and λ_{em} = 522 nm) and PSF (λ_{ex} = 531 nm and λ_{em} = 572 nm) because these two dyes were found to be strongly fluorescent in nature. Other two dyes pinacyanol chloride and TB were not found suitable for the fluorescence studies. The emission spectra of AO in presence of K7, K14, K17 and K26 polymers were studied in our earlier publications [15,31–33]. From the studies it was observed that with the addition of polymers to the dye solution, fluorescence intensity of the dye was progressively quenched with increasing concentration of the polymers. Progressive quenching of fluorescence by gradual additions of polymer to the dilute solution of the dye was also utilized for spectrofluorometric titration and stoichiometric ratio of dye–polymer compounds were calculated (Table 1). Equivalent weights of the polymers were calculated from the end points of the titration curves (Fig. 3B and C). The results agreed well with those obtained by metachromatic titrations, as shown in Table 1. Thus spectrofluorometric titration results also gave the identical stoichiometry values as obtained by spectrophotometric method.

Reversal of metachromasy induced in PCYN dye by all the four polymers were studied by measuring absorbances of the metachromatic solution upon addition of different alcohols and urea. The results are given in Table 2. The results

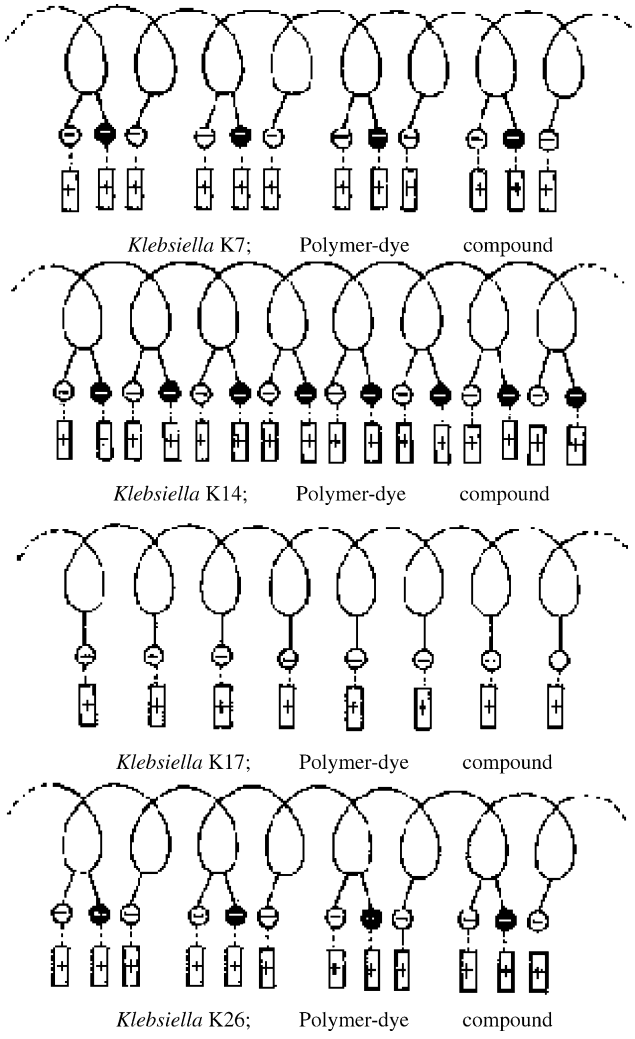

Fig. 2. Proposed model for showing the dye–polymer interaction process in case of different polymers. (Θ), glucouronic acid; (●), pyruvic acid and (+), dye cation.

Table 2
Effects of additives on reversal of metachromasy in different *Klebsiella* polymer–dye complexes at 298 K

Klebsiella serotype	Amount of additive required for complete disruption of metachromatic band			
	Methanol% (v/v)	Ethanol% (v/v)	1-Propanol% (v/v)	Urea (mol dm^{-3})
K7	50	40	30	8
K14	45	35	25	8
K17	40	30	20	7
K26	40	35	20	8

Dye used: 1.0×10^{-5} mol dm^{-3} PCYN; [polymer]/[dye] = 5.0.

indicated that the minimum concentration of the co-solvents required for complete reversal of metachromasy were different for different co-solvents and also the amount of a specific solvent was dependent on the type of metachromatic compound formed by different polymers.

The results of fluorescence quenching in AO and also PSF by the four polymers were treated with Stern–Volmer equation to study the interaction phenomenon. In the case of AO, Stern–Volmer plots for all the four polymers were linear (Fig. 4A). But in the case of PSF (Fig. 4B) Stern–Volmer plots were linear up to a certain P/D only. At higher P/D, there was deviation in each case. From the slopes of the Stern–Volmer plots, K_{SV} values were calculated (Table 3). The K_{SV} results obtained from the plots of both the dyes indicated that the polymers had quenching efficiency in the following order: K7 > K14 > K26 > K17. The rate of quenching of fluorescence of AO and PSF dyes by the polymers

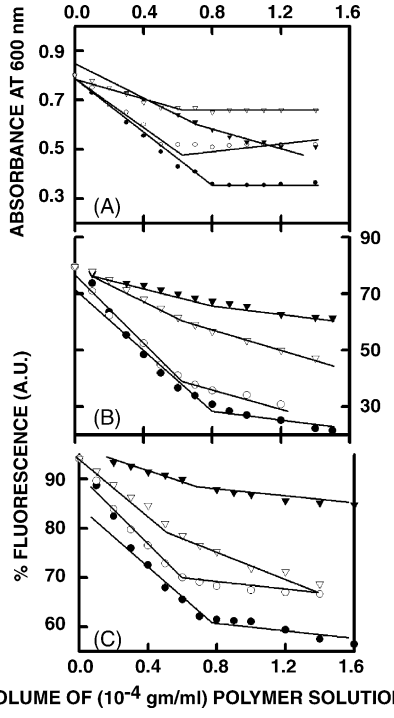

Fig. 3. Comparative (A) spectrophotometric (PCYN) and spectrofluorimetric ((B), AO; (C), PSF) titration curves of dyes with different *Klebsiella* polymers at 298 K. [Dye] = 1.0×10^{-5} mol dm^{-3}. Polymers: (●), K7; (◯), K14; (▼), K17 and (▽), K26.

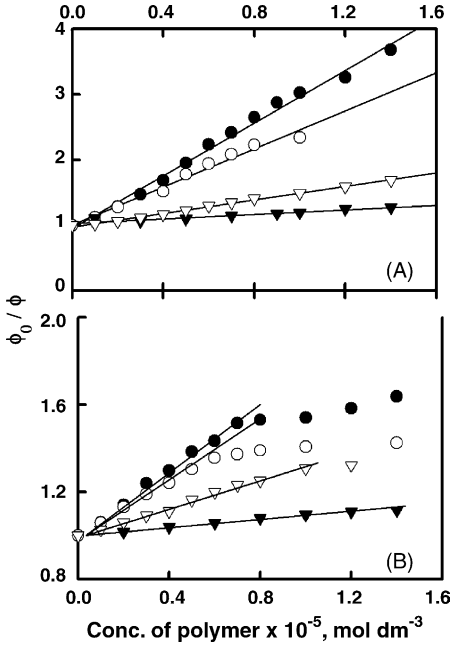

Fig. 4. Stern–Volmer plots for the interaction of (A), AO and (B), PSF with different *Klebsiella* polymers at 298 K. [Dye] = 1.0×10^{-5} mol dm^{-3}. Polymers: (●), K7; (◯), K14; (▼), K17 and (▽), K26.

also indicated the identical order of efficiency as found in metachromasy.

The results of viscometric properties of all the four polysaccharides are shown in Fig. 5 and it was found that reduced viscosity (η_{sp}/C of the aqueous solution of the polymer increased very sharply at low concentration as in the case of other polyelectrolytes [30]. Whereas in the case of non-electrolytes [41,42], reduced viscosity (η_{sp}/C usually rises with increasing polymer concentration. The limiting value of

Table 3
Stern–Volmer constant (K_{SV}) values for the fluorescence quenching of AO and PSF induced by different *Klebsiella* polymers in water at 298 K

Klebsiella serotype	$K_{SV} \times 10^{-4}$ dm^3 mol^{-1}	
	AO	PSF
K7	20.00	7.50
K14	15.00	6.00
K17	2.00	1.00
K26	5.00	3.25

[Dye] = 1.0×10^{-5} mol dm^{-3}.

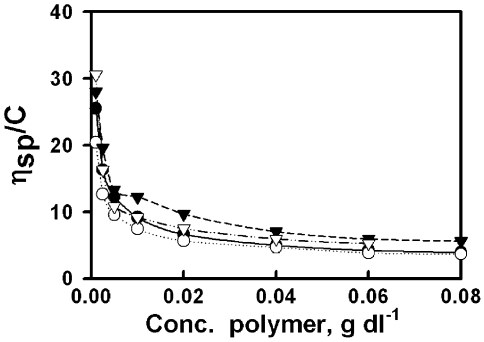

Fig. 5. Variation of reduced viscosity with concentration for capsular polysaccharides isolated from different *Klebsiella* serotypes at 298 K. Polymers: (●), K7; (◯), K14; (▼), K17 and (▽), K26.

η_{sp}/C, obtained by extrapolation of the η_{sp}/C versus C plot to zero concentration, is the intrinsic viscosity, $[\eta]$. Such an extrapolation is not always possible for determining the $[\eta]$ of the polyelectrolyte. In the present case, intrinsic viscosity of the aqueous solution of the polysaccharide was, however, obtained by plotting the values of C/η_{sp} versus $\sqrt{C}$ (Fig. 6) according to the equation of Fuoss and Strauss [34]. The linear plots were extrapolated to $\sqrt{C} \rightarrow 0$ and the intrinsic viscosity was calculated from the intercept on C/η_{sp} axis. The values of $[\eta]$ for *Klebsiella* K7, K14, K17 and K26 were found to be 34.50, 29.40, 40.00 and 38.46 dl g^{-1}, respectively. From the viscosity data, molecular weights of *Klebsiella* polymers were calculated by using Mark–Howink equation [35] for polymeric substances. In the equation $[\eta] = kM^{\alpha}$, both k and α are essentially empirical parameters whose values depend on the molecular shape in solution and on the type of solvent used. The values of k and α for different polymers in different solvents are available in the literature [43,44]. Molecular weights of a number of *Klebsiella* capsular polysaccharides by gel-permeation chromatography and their intrinsic viscosities have been determined earlier [45,46] by a number of workers. The value of k (1.20×10^{-2}) was calculated from the literature value taking the value of $\alpha = 0.8$ (assuming random coil model) [33]. The results of the calculated molecular weights of the four polysaccharides are summarized in Table 4. The results of the molecular weights were found to

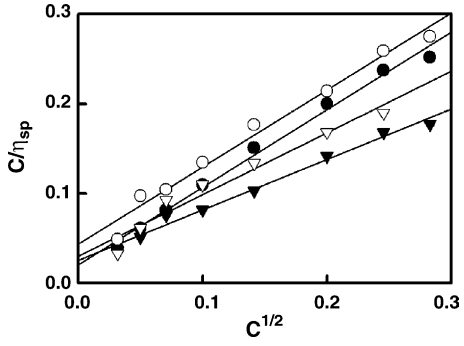

Fig. 6. C/η_{sp} vs. $C^{1/2}$ profile for capsular polysaccharides isolated from different *Klebsiella* serotypes at 298 K. Polymers: (●), K7; (◯), K14; (▼), K17 and (▽), K26.

Table 4
Molecular weight of different *Klebsiella* capsular polysaccharides from viscosity measurements in water at 298 K

Klebsiella serotype	Values of $[\eta]$ in ml g^{-1}	Molecular weight $\times 10^{-6}$
K7	3450	6.6
K14	2940	5.4
K17	4000	8.0
K26	3846	7.6

$\alpha = 0.8$ and $k = 1.20 \times 10^{-2}$ for all the polymers.

be comparable with the published values for similar other polymers.

4. Discussion

From the primary structural information [35–39], it was observed that there were remarkable variations in the structures of the four polysaccharides under investigation viz. regarding size of the repeating unit, sugar constituents, pattern of linkages, branch points, etc. In spite of such structural variations these capsular polysaccharides of *Klebsiella* K-serotype have some intrinsic similarities. Glucuronic acid was present as a potential anionic site in the repeating units of all the four polysaccharides, whereas pyruvic acid was also present in all the polysaccharides of K7, K14 and K26, except K17 polymer. Consequently, the polysaccharides possessed different anionic charge densities and played important role in the subsequent studies.

The absorption spectra in the UV–vis region of the aqueous solutions of AO, TB and PCYN dyes in presence of K7, K14, K17 and K26 were studied thoroughly and revealed the weak metachromatic behavior of the dyes. In this regard TB was superior to the other two dyes and in case of all the dyes the peaks in the corresponding UV region remain insignificant. Besides, in the visible region K7, K14 and K26 polymers exhibited considerable blue shift of the corresponding monomeric band of the dye, but K17 did not show any appreciable change. On the contrary, spectral behavior of PCYN dye was significantly different from other three dyes. The spectral studies with the four polymers and the four dyes indicated that chromotropic character of the polymers with respect to induction of metachromasy followed the order: K7 ≥ K14 > K26 > K17 and the dye pinacyanol chloride was found to be the most suitable one for detailed studies on metachromasy, particularly, in the visible absorption range. Usually, the extent of metachromatic shift increases with the increase in the hydrophobic character of the dye compared to other dyes. Pinacyanol chloride being larger in size is expected to be more hydrophobic and more aggregating in nature.

From the absorption spectra [31–33] of the metachromatic solutions it was found that the conformations of the polyanionic chromotropes were reflected on the shape of the metachromatic spectra. Appearance of multiple-banded

broad spectra indicated that all the four polymers might have random coil structures in the solution and an overcrowding of the dye on the chromotrope resulted. At higher concentration (P/D = 20–30), the conformations of the polyanions K7, K14 and K26), possibly, changed from random coil to helical form, exhibiting single-banded spectra [12]. At the intermediate P/D values, the spectra demonstrated a transition in conformation.

The results of molar stoichiometry indicated that both pyruvic and glucuronic acid offered potential anionic sites for interaction with the cationic dye. All these are reflected in the proposed model (Fig. 2). From the results of reversal of metachromasy it was clear that the minimum concentrations of the co-solvents required for complete reversal of metachromasy were different for different co-solvents and the values were also dependent on the stability of the metachromatic compounds formed by the different polymers. Progressive destruction [33,47] of the metachromatic compounds by different co-solvents suggested an involvement of hydrophobic bonds in aggregation of dyes leading to dimidiation as well as metachromatic compound formation. Such results can also be regarded as an important parameter to determine the stability and nature of binding of the metachromatic compounds. It was revealed that the metachromatic compound formed by K7 polymer was most stable and that of K17 was the least stable.

Results of metachromatic titrations yielded identical stoichiometric ratios as obtained by isolation method and centrifugation method. The relative order of the chromotropic characters of the four polymers was also evident from the increasing residual absorbance at the respective equivalent points. In the fluorescence studies [48], progressive quenching of the fluorescence intensity of the dye with increasing concentration of the polymers indicated that the experimental fluorescent dyes preferred to form a staggered aggregation on binding to *Klebsiella*. The Stern–Volmer type of quenching phenomenon was observed in both the cationic fluorescent dyes AO and PSF due to the interaction between the dyes and different polymers. In the case of AO, Stern–Volmer plots for all the polymers were linear, suggesting static quenching [47]. But in the case of PSF, linearity of the plot was obtained only up to a certain concentrations of the polymer, beyond which there was deviation in each case. This was possibly [31] due to either association of the quencher with the fluorescer or the self-association of the quencher molecule. The Stern–Volmer constant values obtained from the plots of both the dyes indicated that the polymers had quenching efficiency in the order: K7 > K14 > K26 > K17. The relative orders of the quenching characters of the four polymers were evident from the increasing residual fluorescence at the respective equivalent points. Spectrofluorometric titration results also yielded the identical stoichiometry values as obtained by spectrophotometric method.

The results of viscometric studies of all the four polysaccharides indicated that the reduced viscosity (η_{sp}/C) of the aqueous solution increases very sharply at low concentration (Fig. 5), indicating usual behavior of polyelectrolytes [41]. This anomalous shape of the curves, observed by the polymers were explained by the fact that the degree of ionization of the polyelectrolyte increased with decreasing concentration. In the case of polysalts (sodium salt of the polysaccharides) an ion atmosphere was formed by the gegenions (Na^+ ions) around the chains of the polyelectrolyte macro ions. In the absence of any added salt, in very dilute solutions of the polyelectrolyte, the diameter of the polyion atmosphere was greater than the diameter of the coiled molecule. The viscosity of the polysaccharides increased in dilute solution due to expansion of the polymer coil in order to increase the chain rigidity as the carboxylate ions (COO^-) of the polysaccharide repelled each other. Another contributing factor might be that because of the expansion of the macro ions, they could interfere with each other, causing an influence of concentration on configuration of the polymer molecule. Thus viscometric studies yielded useful results on solution properties like polyelectrolytic nature of the polysaccharides. Besides, molecular weight of the four polysaccharides were determined by viscometric method and shape of the polymers in the solution was ascertained.

Therefore, the results on dye–polymer interaction as discussed above established the chromotropic character and quencher behavior of all the four *Klebsiella* polymers. It also revealed that in all cases dye–polymer interaction was incorporated with electrostatic as well as hydrophobic bond to a certain extent [48]. From the results of the relative absorption spectra at different P/D values, stability of the metachromatic compound and the residual absorption values at the respective end points of the metachromatic titrations, etc. it was evident that the polymers possessed chromotropic character in the order: K7 > K14 > K26 > K17. Fluorescence studies also revealed identical order of efficiency of the polymer as fluorescer. The charge density on the polyanions also supported the above trend. Generally, a polyelectrolyte with high charge density was found to be more efficient in inducing metachromasy. Thus from the results of the different measurements carried out in the current investigation a clear comparative picture of the properties of the four different *Klebsiella* capsular polysaccharides was revealed and a correlation of the primary structures with the solution properties was established. These studies will lead a valuable account to understand the correlations of chemical structure and immunological specificity of various *Klebsiella*. The results can be utilized as models for studying competitive binding characteristics of the biopolymers with different types of drugs.

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