Spectophotometric Study of Interaction between Sodium Carrageenate and Cationic Dyes

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Abstract: The interaction of two cationic dyes, namely, methylene (MB) and acridine orange (AO) with an anionic polyelectrolyte, namely, sodium carrageenate (NaCar) has been investigated by spectrophotometric method and spectrofluorimetric method. The polymer induced metachromasy in the dyes resulting in the shift of the absorption maxima of the dyes towards shorter wavelengths.The stability of the complexes formed between acridine orange and sodium carrageenate was found to be lesser than that formed between methylene blue and sodium carrageenate. This fact was further confirmed by reversal studies using alcohols, urea, surfactants and electrolytes. The interaction parameters revealed that binding between acridine orange and sodium carrageenate was mainly due to electrostatic interaction while that between methylene blue and carrageenate is found to involve both electrostatic and hydrophobic forces. The effect of the structure of the dye and its relation to metachromasy has been discussed.

Keywords: Cationic dyes, Metachromasy, Sodium carrageenate, Binding

Introduction

Metachromasy is a well known phenomenon in dyes and dye-polymer systems[1]. The metachromatic change is most pronounced in the visible region and is frequently characterized by the appearance of a new absorption band. The change has been attributed to the formation of various dye aggregates of the bound dye molecules on the ionic polymer sites[2]. The metachromatic change are frequently specific rather than general and constitutes an experimental basis for histochemical and cytochemical applications. For a particular polymer species it depends on the specific substituent's attached to the dye, even if they belong to the same class[3,4]. The phenomenon of reversal of metachromasy by addition of alcohols, urea and electrolytes and also by increasing the temperature has been used to determine the stability of the metachromatic band[5].

The interaction of acridine orange with various synthetic polyelectrolytes and acidic polysaccharides are available in the literature[6-14]. The fluorescence of dye acridine orange and its quenching in polymer matrices has been extensively studied[15-17].

The interaction of methylene blue with various synthetic polyelectrolyte such as poly (potassium styrenesulfonate) and poly (sodium 4-vinylphenylsulphate) has been reported in the literature[18]. The effect of alkali metal chlorides and 1-substituted 3-carbomylpyridinium bromides on the metachromatic behavior of methylene blue induced by poly (potassium styrenesulfonate) and poly (potassium vinylsulfate) was investigated spectrophotometrically[19]. The structural effect of polyanion on the metachromatic behavior of methylene blue was investigated spectrophotometrically using poly(sodium acrylate), conventional poly(sodium methacrylicacid), isotactic poly(sodium methacrylate) and the copolymer poly(sodium maleate-covinyl alcohol) and the differences between $-OSO_3-$ and $-COO-$ as binding sites were discussed[20]. The interaction of methylene blue with Poly (potassium vinylsulfate) and poly (sodium acrylate) are available in the literature and the thermodynamic parameters of interaction have been evaluated[21]. The interaction of methylene blue with potassium poly(vinyl sulfate) has been studied and the effect of KCl and urea on the binding has been reported[22].

Cyanine dyes, which are cationic in nature, have widely been used to probe biological systems such as helical structure of DNA[14], tertiary conformation of bacterial polysaccharides[23-25] and other polymers. As these dye having high light absorptivity, they can be used as optical probes in studying membranes, micelles and other host systems. Studies on polymer-surfactant interaction in aqueous solution have been attracting widespread attention due to multiple practical uses in biology. Such studies are also assumed to be important as the mixed systems/aggregates can give rise to advanced functions that are unobtainable from single component[26]. Several Physicochemical properties of macromolecule - surfactant are quite relevant in this context. Formulation procedures based on suitable mixture may have appealing applications[27-29]. It has been noted that oppositely charged surfactant binds to polymer surfaces through both electrostatic and hydrophobic interaction[30].

The objective of the present study is to compare the extent of metachromasy induced by sodium carrageenate in the cationic dyes methylene blue and acridine orange and to evaluate the thermodynamic parameters of interaction. It is also attempted to study the extent of reversal by using alcohols, urea, surfactants and electrolytes which is an indirect evidence for the stability of the metachromatic complex formed.

Experimental

Acridine orange (AO) was obtained from (S.D.fine chemicals, Mumbai) and used as received. Methylene blue (MB) was obtained from (Acros Media) and used as received. Sodium carrageenate (NaCar) (S.D.fine chemicals, India) were used as received. Methanol (MeOH), ethanol (EtOH) and 2-Propanol (PrOH) (Merck, India) were distilled before use. Urea, sodium chloride (NaCl) and potassium chloride (KCl) (Merck, India), were used as received. Sodium laurylsulphate (SLS) and sodium dodecylbenzenesulphonate (SBS) (Lobachemie, India) were used as received.

Apparatus

All the absorbance measurements were recorded using a Shimadzu UV-2550 spectrophotometer.

Determination of stoichiometry of polymer-dye complex

Increasing amounts of polymer solution (0.0- to 9 mL, 1×10^{-3} M) were added to a fixed volume of dye solution (0.6 mL, 1×10^{-3} M) in case of acridine orange and (0.5 mL, 1×10^{-3} M) of dye to increasing amount of polymer solution (0.0-1.2 mL, 1×10^{-3} M) in case of methylene blue in different sets of experiments and the total volume was made up to 10 mL by adding distilled water in each case. The absorbances were measured at 492 nm and 454 nm in case of AO-NaCar and at 628 nm and 528 nm in case of MB-NaCar complex.

Study of reversal of metachromasy using alcohols and urea

For measurements of the reversal of metachromasy, solutions containing polymer and dye in the ratio 2:1 were made containing different amount of alcohol (10-80%) or urea (1-8 M). The total volume was maintained at 10 mL in each case. The absorbances were measured at the appropriate wavelengths as mentioned earlier.

Study of reversal of metachromasy using surfactants and electrolytes

For measurements of the reversal of metachromasy, solutions containing polymer and dye in the ratio 2:1 containing different amount of surfactants or (0.1 M - 1×10^{-2} M) were made in case of AO-NaCar and (1×10^{-8} M-1×10^{-2} M) in case of MB-NaCar The total volume was maintained at 10 mL in each case. The absorbances were measured at 492 nm and 454 nm in case of AO-NaCar and at 628 nm and 528 nm in case of MB-NaCar. Similarly, polymer-dye solutions containing different amounts of electrolytes (1×10^{-7} M-1×10^{-2} M) in case of AO-NaCar and (1×10^{-8} M-1×10^{-2} M) in case MB-NaCar were made and the absorbances were measured at the two wavelengths as mentioned earlier.

Determination of thermodynamic parameters

The thermodynamic parameters were determined by measuring the absorbances of the pure dye solution at the respective monomeric band and metachromatic band in the temperature range (36 ^{0}C-54 ^{0}C). The above experiments were repeated in presence of the polymers at various polymer-dye ratios (2, 5, 8 and 10).

Results and Discussion

Effect of polymer concentration on metachromasy

The absorption spectra of acridine orange and methylene blue at various concentrations are shown in Figure 1 and Figure 2 respectively. The absorption maxima were found to be 492 nm in case of acridine orange and 628 nm in case of methylene blue indicating the presence of a monomeric dye species in the concentration range studied. On adding increasing amounts of polymer solution the absorption maxima shifts to 454 nm in case of AO-NaCar and to 528 nm in case of MB-NaCar system. The blue shifted band is attributed to the stacking of the dye molecules on the polymer backbone and this reflects high degree of co-operativity in binding[31,32]. The absorption spectra at various P/D ratios are shown in (Figure 3 and 4) respectively.
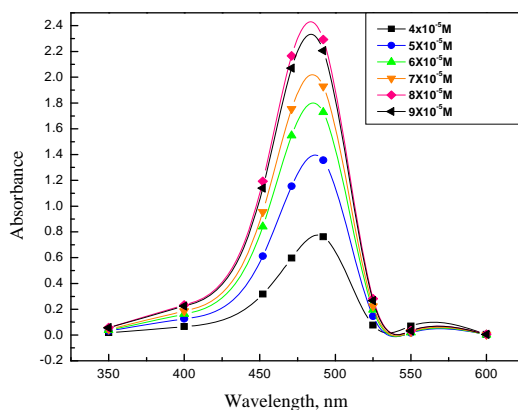

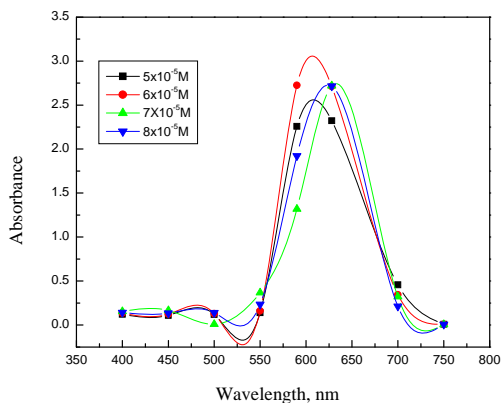**Figure 1.** Absorption spectrum of AO at various concentrations

Figure 2. Absorption spectrum of MB at various concentrations
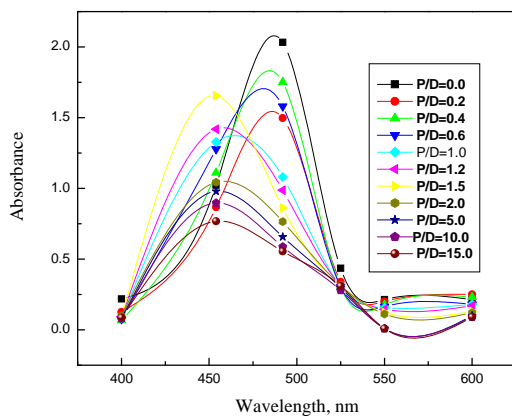

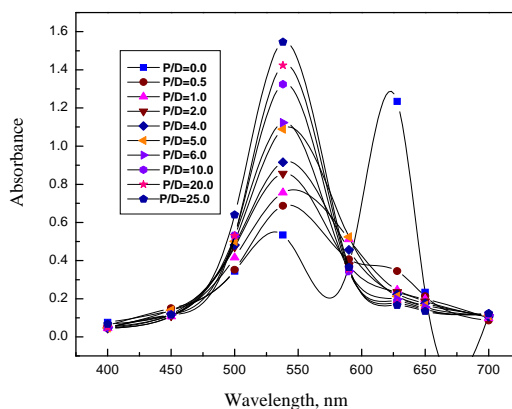**Figure 3.** Absorption spectrum of AO-NaCar system at various P/D ratios

Figure 4. Absorption spectrum of MB-NaCar system at various P/D ratios

Determination of stoichiometry

To determine the stoichiometry of the polymer-dye complex, a plot of A_{492}/A_{454} *versus* the polymer/dye ratio was made for AO-NaCar system and a plot of A_{628}/A_{528} was made in the

case of MB-NaCar as shown in Figure 5 and Figure 6 respectively. The stoichiometry of AO-NaCar complex was found 2: 1 which indicates that the binding is at alternate anionic sites[33]. The results were in good agreement with the reported values for interaction of similar dyes with polyanions[34]. While in case of MB-NaCar complex the stoichiometry is 1.5:1 and the binding is at adjacent anionic sites. This indicates that there is lesser overcrowding and more aggregation of the bound dyes on the polymer chain in the latter case than in the former case. Similar results were reported[35] in case of binding of pinacyanol chloride on poly (methacrylicacid) & poly (styrene sulfonate) systems.
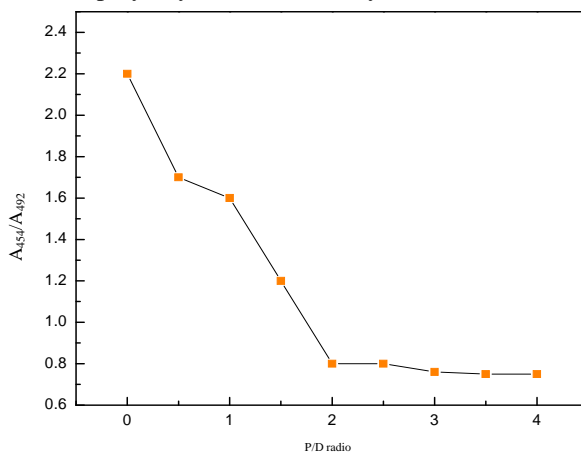
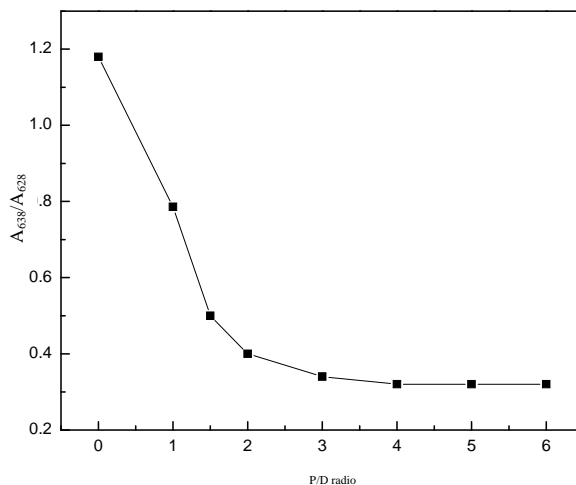**Figure 5.** Stoichiometry of AO-NaCar complex

Figure 6. Stoichiometry of MB-NaCar complex

Reversal of metachromasy using alcohols and urea

The metachromatic effect is presumably due to the association of the dye molecules on binding with the polyanion which may involve both electrostatic and hydrophobic forces. The destruction of metachromatic effect may occur on addition of low molecular weight electrolytes, alcohols or urea. The destruction of metachromasy by alcohol and urea is attributed to the involvement of hydrophobic bonding which has already been established[36-40].

The effectiveness of alcohols in disrupting metachromasy was found to be in the order methanol<ethanol< 2-propanol, indicating that reversal becomes quicker with increasing hydrophobic character of the alcohols. The above facts are further confirmed in the present system. On addition of increasing amount of alcohol to the polymer/dye system with P/D=2.0, the original monomeric band of dye species is gradually restored. The effectiveness of the alcohols, namely methanol, ethanol and 2-propanol, on destruction of metachromasy were studied. From the plot of A_{454}/A_{492} or A_{628}/A_{528} (Figure 7 & 8) against the percentage of alcohols or molar concentration of urea, the amount of alcohols or molar concentration of urea, required for complete reversal has been determined**.** In case of AO-NaCar system 60% methanol, 40% ethanol, 20% 2- propane were sufficient to reverse metachromasy. Whereas in case of in MB-NaCar system, 55% methanol, 45% ethanol, 35% 2- propanol were required to reverse metachromasy. The concentration of urea to reverse metachromasy is found to be as high as 4 M in AO-NaCar system and 4.5 M in case of MB-NaCar system (Figure 9 & 10). Similar reports are available in literature for reversal of metachromasy in anionic polyelectrolyte/cationic systems by addition of alcohols or urea[41,42].
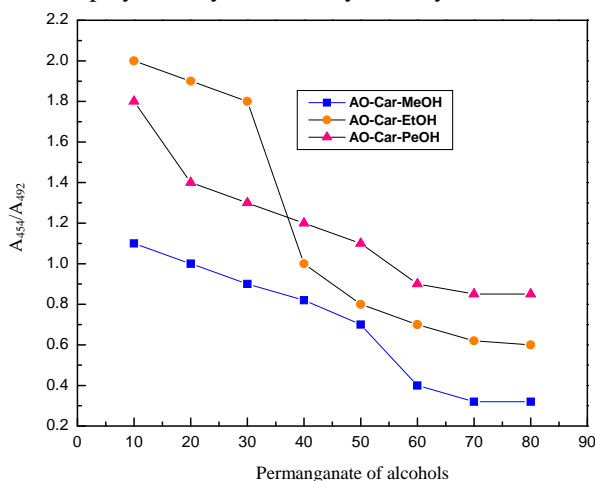

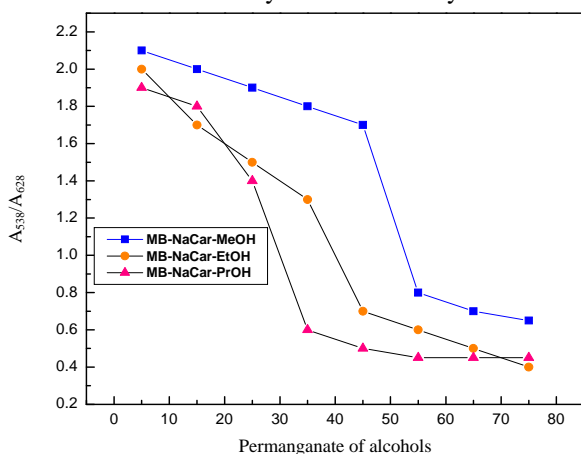**Figure 7.** Reversal of metachromasy in AO-NaCar system on addition of alcohols

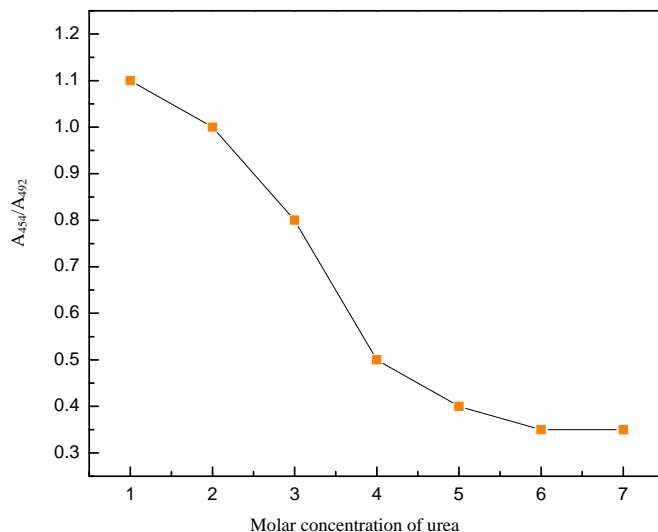**Figure 8.** Reversal of metachromasy in MB-NaCar system on addition of alcohols

Figure 9. Reversal of metachromasy in AO-NaCar system on addition of urea
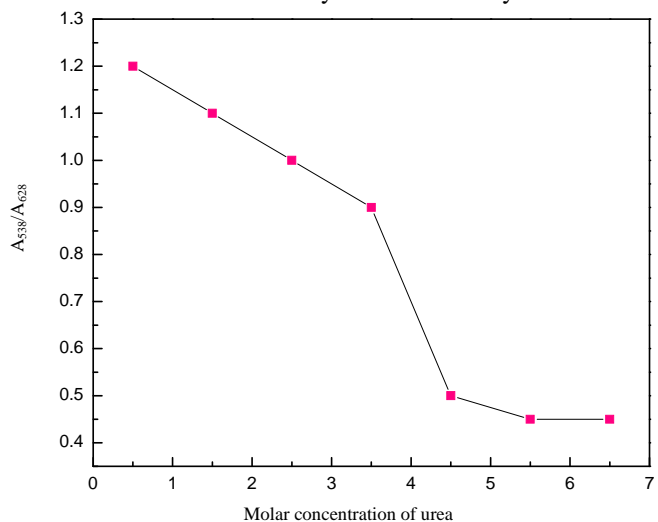

Figure 10. Reversal of metachromasy in MB-NaCar system on addition of urea

Effect of surfactants

It is observed that on adding increasing amounts of surfactants to polymer-dye complex, the metachromsy is gradually reversed. From the plot of A_{454}/A_{492} or A_{628}/A_{528} (Figure 11 and Figure 12) against the molar concentration of surfactants, the molar concentration of surfactants needed for complete reversal of metachromasy has been determined. The molar concentrations of sodium laurylsulphate and sodium dodecylbenzenesulphonate needed to cause reversal was found to be 1×10^{-6} M in case of AO-NaCar and 1×10^{-5} M and 1×10^{-4} M in case of MB-NaCar system. These results agree with those reported earlier in literature[43,44]. Thus the cationic surfactant molecules interacted with the anionic sites on the polymer replacing the cationic dye.
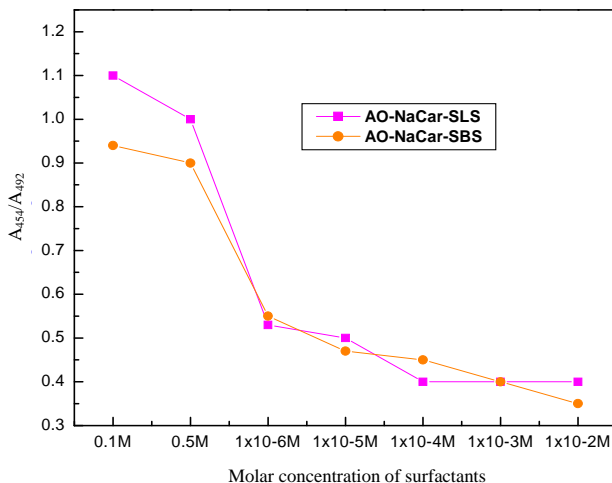
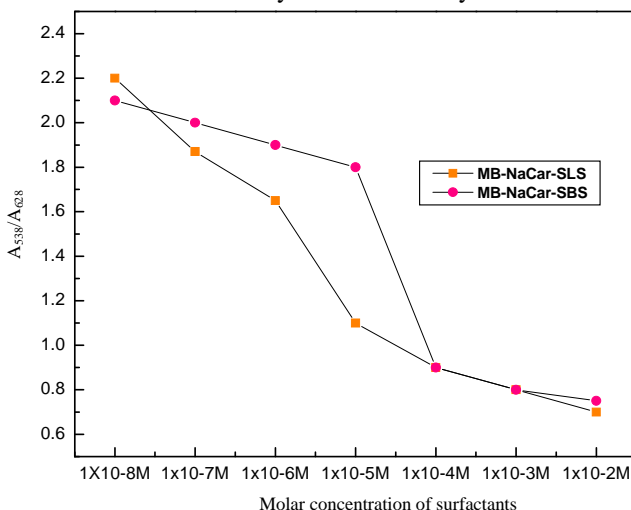**Figure 11.** Reversal of metachromasy in AO-NaCar system on addition of surfactants

Figure 12. Reversal of metachromasy in MB-NaCar system on addition of surfactants

Effect of electrolytes

Tan *et al*[45] have reported the disruption of metachromatic band with the variation of ionic strength. In aqueous solutions the charged polymer molecule will be in the extended conformation due to repulsion between the charged groups. On adding the dye the conformation of the polycation changes to a compact coil owing to reduced repulsion due to dye binding, thus giving rise to metachromatic band. In the present study NaCl and KCl solutions of different concentrations were added to AO-NaCar and MB-NaCar complexes and the absorbances were measured at the two wavelengths. In case of AO -NaCar the monomeric band reappears at higher ionic strength (1×10^{-4} M) when compared to that of MB-NaCar (1×10^{-5} M). From the plot of A_{454}/A_{492} or A_{528}/A_{628} (Figure 13 and Figure 14) against the molar concentration of electrolytes, the molar concentration of electrolytes needed for complete reversal of metachromasy has been determined. The concentration of

sodium chloride required to reverse metachromasy was greater in case of AO-NaCar complex than in case of MB-NaCar complex. The addition of KCl showed similar effect in case of NaCar-Dye complexes in both cases.
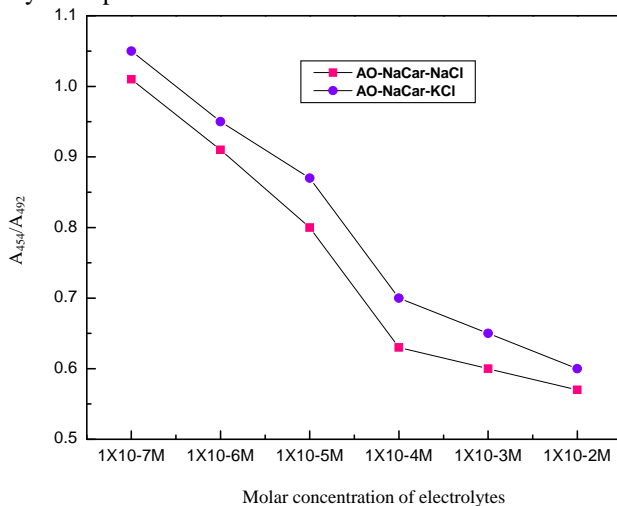

Figure 13. Reversal of metachromasy in AO-NaCar system on addition of electrolytes
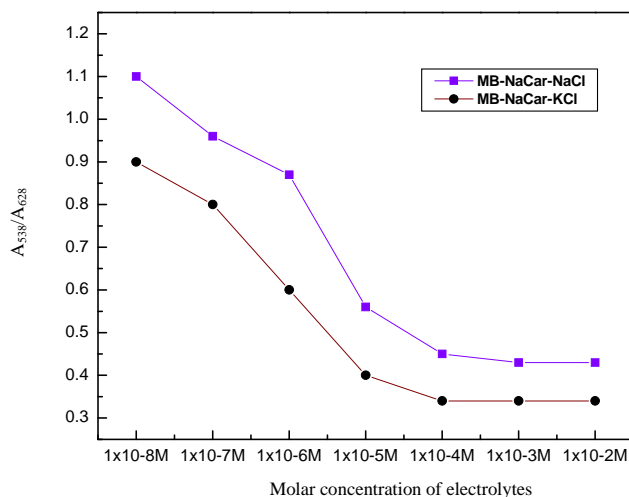

Figure 14. Reversal of metachromasy in MB-NaCar system on addition of electrolytes

Fluorescence studies

Acridine Orange, being a fluorescent dye, exhibits emission band at 529 nm. Fluorescent studies were performed on AO-NaCar system and it was found that the fluorescent intensity of AO decreases on the addition of increasing amount of polymer solution as evidenced from (Figure 15). The binding of dye on to polymers is observed to quench the emission characteristics of dye. Finally, to study the interaction between the polymer and dye, the fluorescence data were fitted to Stern-Volker equation[23], Fo/F=1+Ksv[Q], where Fo is the fluorescence intensity of the dye solution and F is that of the dye-polymer mixture and [Q]

is the molar concentration of the polymer, Ksv is the Stern-Volmer constant. The Stern-Volmer plot obtained for the present system is shown in (Figure 16). From the slope of the plot value of the Stern-Volmer constant was found to be $4.0 \times 10^3 \, \text{lit}^{-1} \, \text{mol}^{-1}$.
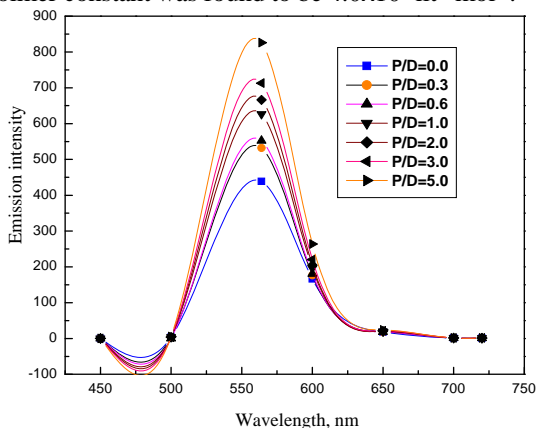

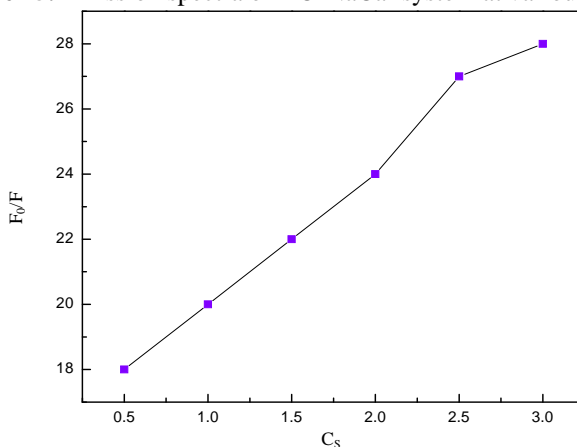**Figure 15.** Emission spectra of AO-NaCar system at various P/D ratios

**Figure 16.** Stern-Volmer plot for AO-NaCar system

Determination of interaction parameters

The interaction constant K_c for the complex formation between AO and NaCar and MB-NaCar was determined by absorbance measurements at the metachromatic bands at four different temperatures taking different sets of solutions containing varying amounts of polymer (C_S) in a fixed amount of the dye solution (C_D). The absorbance results were treated using Rose-Drago eqn. $C_D.Cs/ (A-A_0) = 1/K_cL (\varepsilon_{ds}- \varepsilon_d) + Cs/L (\varepsilon_{ds}- \varepsilon_d)$[46]. C_D refers to the initial molar concentration of dye and C_S refers to the concentration of the polymer sample. ε_{ds} and ε_d refers to the molar extinction coefficients of the complex and that of the dye at the absorption maximum of the complex. The value of K_c was obtained from the slope and intercept of the plot of $C_DC_S/ (A-A_O)$ against Cs for AO-NaCar (Figure 7) and MB-NaCar (Figure 18) systems. In each case the thermodynamic parameters of interaction, namely ΔH, ΔG and ΔS were also calculated. The results are tabulated in the Table 1 for both the systems studied.

**Table 1.** Thermodynamic parameters for AO-NaCar &MB-NaCar systems

Temp, K	K_C [a] $dm^3 mol^{-1}$		ΔG [b] $kcal.mol^{-1}$		ΔH [c] $kcal. mol^{-1}$		ΔS [d] $cal. mol^{-1}K^{-1}$	
	I	II	I	II	I	II	I	II
309	3457	7015	-3.164	-5.436				
315	3178	6154	-3.264	-5.460	-5.63	-4.88	-16.67	-16.30
321	2976	5215	-3.364	-5.459				
327	2151	4528	-3.464	-5.469				

I –AO-NaCar system, II-MB-NaCar system; a: Calculated from Figure 17 and Figure 18 according to Rose-Drago equation, b: Calculated from the thermodynamic equation ΔG = -RTln K_c, c: Calculated graphically by plotting ln K_c against 1/T according to Van't Hoff equation ln K_c = -Δ H/RT +C, d) Calculated from the thermodynamic expression ΔG =Δ H - TΔS.
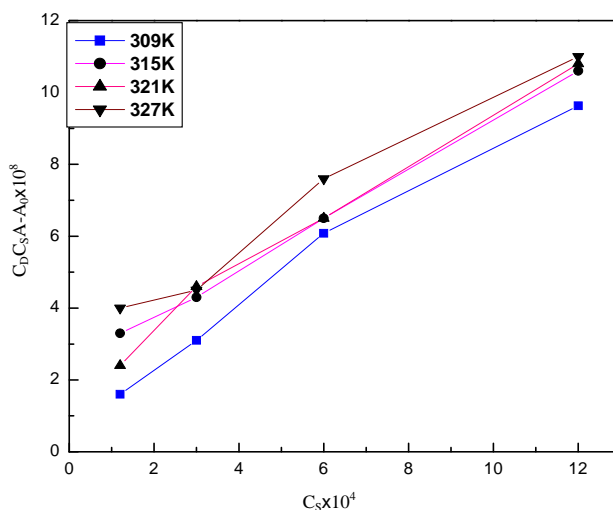

Figure 17. Plots of $C_D.C_S/$ (A-A_O) against Cs for AO-NaCar system at different temperatures
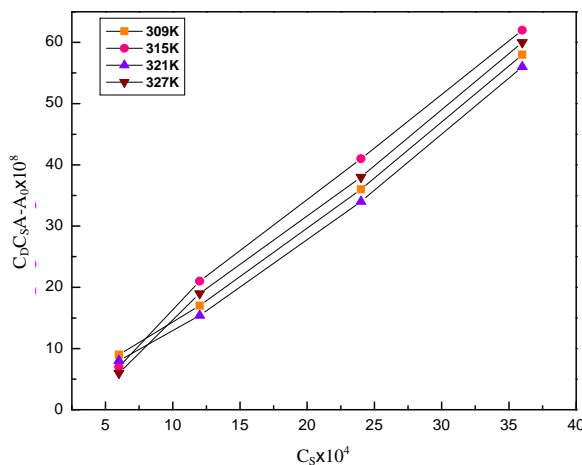
Figure 18. Plots of $C_D.C_S/$ (A-A_O) against Cs for MB-NaCar system at different temperatures

Effect of structure of dye

The structures of two dyes used in the present study are given in Scheme 1 and 2. It is evident that acridine orange, is a rigid planar cationic dye and a shorter distance between the adjacent anionic sites on the polyanion will be more favorable for binding resulting in stacking arrangement. On the other hand methylene being larger in size binds on alternate sites on the polymer. Also it is more hydrophobic and induces greater aggregation[6]. Thus in this case the distance between the two adjacent dye molecules will be greater and the dye molecules are bound on the alternate sites of the polymer and are oriented like a stair case which agrees with the reported literature[7].
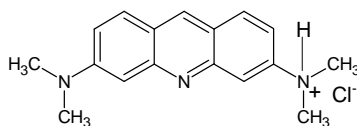
Scheme 1. Structure of acridine Orange
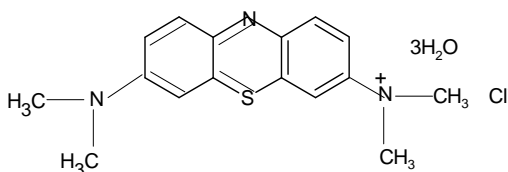
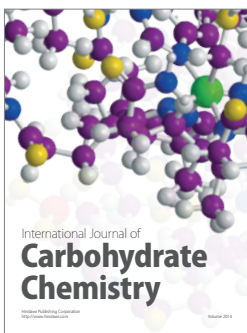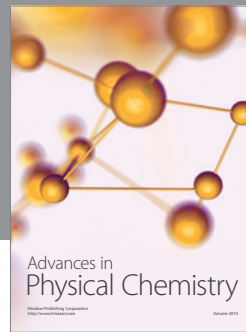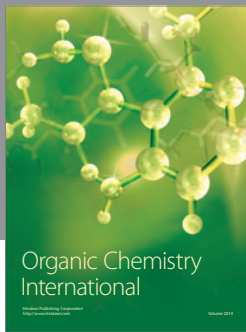**Scheme 2.** Structure of methylene Blue

Conclusion

The polymers sodium carrageenate induced metachromasy in the dye acridine orange and methylene blue. The monomeric band occurs at 492 nm and 628 nm while the metachromatic band occurred at 454 nm in the case of AO-NaCar and at 528 nm in the case of MB-NaCar. The spectral shifts are higher in the case of MB-NaCar (100 nm) than in case of AO-NaCar (38 nm).These results are further confirmed by the reversal studies using alcohols, urea, sodium chloride and surfactants. The thermodynamic parameters were found to be greater for MB-NaCar complex than for AO-NaCar system. It is thus evident from the above studies that both electrostatic and non-ionic forces contribute towards the binding process.Based on the results it can be concluded that the methylene blue is more effective in inducing metachromasy in sodium carrageenate than acridine orange.

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