



# A Comparative Study of Metachromasy Induced by Anionic Polelectroytes in Toluidine Blue

Nandini R., Vishalakshi B.\*

Dept. of Chemistry, MITE, Moodbidri, India

## Email address:

[hodche@mite.ac.in](mailto:hodche@mite.ac.in) (Vishalakshi B.)

\*Corresponding author

## To cite this article:

Nandini R., Vishalakshi B. A Comparative Study of Metachromasy Induced by Anionic Polelectroytes in Toluidine Blue. *Science Journal of Chemistry*. Special Issue: Polyelectrolyte-Dye Interaction. Vol. 5, No. 4-1, 2017, pp. 1-7. doi: 10.11648/j.sjc.s.2017050401.11

**Received:** February 13, 2017; **Accepted:** February 15, 2017; **Published:** March 6, 2017

---

**Abstract:** The interaction of two anionic polyelectrolytes, namely, sodium alginate (NaAlg) & sodium heparin ate (NaHep) with a cationic dye Toluidine Blue (TB) has been investigated by spectrophotometric method. The polymers 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 TB and sodiualginate was found to be lesser than that formed between TB and sodiumheparinate. This fact was further confirmed by reversal studies using alcohols, urea and electrolytes. The interaction parameters revealed that binding between TB and anionic polyelectrolytes was mainly due to electrostatic interaction andhydrophobicforces. The effect of the temperature on to metachromasy has beendiscussed.

**Keywords:** Cationic Dye, Metachromasy, Polyelectrolyte's, Aggregation, Temperature

---

## 1. Introduction

A blue shift in the spectrum of the dye takes place when a dye is bound to the polymer. This phenomenon is known as metachromasy [1-4]. Metachromasy is related to the interaction of cationic dyes with polyanions where a single individual compound is formed by the interaction of the dye cation and the chromo trope polyanionic polymer. Several physiochemical parameters such as molecular weight of each repeating unit, stoichiometry of the dye-polymer complex, binding constant, and other related thermodynamic parameters like free energy, enthalpy, and entropy changes can be evaluated using polymer-dye interactions. Biological activity of macromolecules depends on its tertiary conformation. Conformation of the polyanions controls the induction of metachromasy of aqueous dye solution. Although there are several reports on metachromasy of various classes of acidic polysaccharides and different synthetic polyanions [5-10]. Studies on polymer-surfactant interactions in aqueous solution have been attracting widespread attention due to multiple practical uses in biology [11-14]. 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 [15]. Several physicochemical properties of macromolecule - surfactants are quite relevant in this context. Formulation procedures based on suitable mixture may have appealing applications [16-18] has been noted that oppositely charged surfactant binds to polymer surfaces through both electrostatic and hydrophobic interaction [19]. The interaction of toluidine blue and Heparin was studied [20-21] spectrophotometrically. The interaction of TB with Heparin in the presence of sodium chloride and albumin has been reported [22]. The interaction of toluidine blue with acid mucopolysaccharides and nucleic acids were recorded over the temperature range 10-70°C [23]. Metachromasy of Toluidine blue-stained materials was examined at 5-95°C microspectrometrically [24, 25]. The interactions of Toluidine blue with poly ( $\alpha$ -L-glutamic acid) of helical and coiled conformation were determined by spectrophotometric method [26]. The objective of the present study the interaction of the cationic dye Toluidine Blue with two anionic polyelectrolytes namely, sodiualginate & sodiumheparinate and to evaluate the thermodynamic parameters of interaction and 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.

## 2. Materials & Methods

### 2.1. Apparatus

The spectral measurements were carried out using Shimadzu UV-2550 Spectrophotometer using 1cm quartz cuvette.

### 2.2. Reagents

Toluidine Blue (TB) was obtained from (Hi Media, India) and used as received. sodium heparinate (NaHep) & sodium alginate (NaAlg) (Hi Media, India) were used as received; Methanol, (MeOH) ethanol (EtOH) and 2-Propanol (PrOH) (Merck, India) were distilled before use. Urea, sodium chloride and potassium chloride were obtained from (Merck, India) and were used as received. Absorption Spectra were recorded using a Shimadzu UV-2550 Spectrophotometer.

## 3. Methods

### 3.1. Determination of Stoichiometry of Polymer-Dye Complex

Increasing amounts of polymer solution ( $0.0$ - to  $4 \times 10^{-3}M$ ) were added to a fixed volume of dye solution Toluidine Blue ( $0.5ml$ ,  $1 \times 10^{-3}M$ ) in different sets of experiments and the total volume was made up to  $10ml$  by adding distilled water in each case. The absorbances were measured at  $606nm$  for pure dye and at  $516nm$  in case of TB-NaAlg complex and at  $504 nm$  in case of TB-NaHep complex.

### 3.2. Study of Reversal of Metachromasy Using Alcohols and Urea

For measurements of the reversal of metachromasy, solutions containing polymer and dye in the ratio  $2.0:1.0$  were made containing different amount of alcohol. The total volume was maintained at  $10ml$  in each case. The absorbances were measured at  $606 nm$  and  $516 nm$  in case of TB- NaAlg and at  $606 nm$  and  $504 nm$  in case of TB-NaHep.

### 3.3. Study of Reversal of Metachromasy Using Electrolytes

For measurements of the reversal of metachromasy, solutions containing polymer and dye in the ratio  $2.0:1.0$  were made containing different amount of electrolytes or ( $1 \times 10^{-7}M - 1 \times 10^{-2}M$ ) were made in case of TB- NaAlg and TB-NaHep complexes. The total volume was maintained at  $10ml$  in each case. The absorbances were measured at  $606 nm$  and  $516 nm$  in case of TB-NaAlg and at  $606 nm$  and at  $504 nm$  in case of TB- NaHep. A plot of absorbance at  $606 nm$  Vs molar concentration of electrolytes were made to determine the concentration of surfactants and electrolytes needed for complete reversal of metachromasy.

### 3.4. 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 ( $36^{\circ}C-54^{\circ}C$ ). The above experiments were repeated in presence of the polymers at various polymer-dye ratios ( $2, 5, 8$  and  $10$ ).

## 4. Results & Discussion

The absorption spectra of Toluidine Blue shows an absorption maximum at  $606 nm$  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  $516 nm$  in case of TB- NaAlg and at  $504 nm$  in case TB-NaHep complex. The blue shifted band is attributed to the stacking of the dye molecules on the polymer backbone and this reflects high degree of cooperativity in binding [27]. Appearance of multiple banded spectra proposed that the polymer might have a random coil structure in solution. Whereas at higher concentration of the polymer almost a single banded spectrum was observed due to possible change from random coil to helical form [28]. The absorption spectra at various P/D ratios are shown in (Fig. 1 and Fig. 2) respectively.

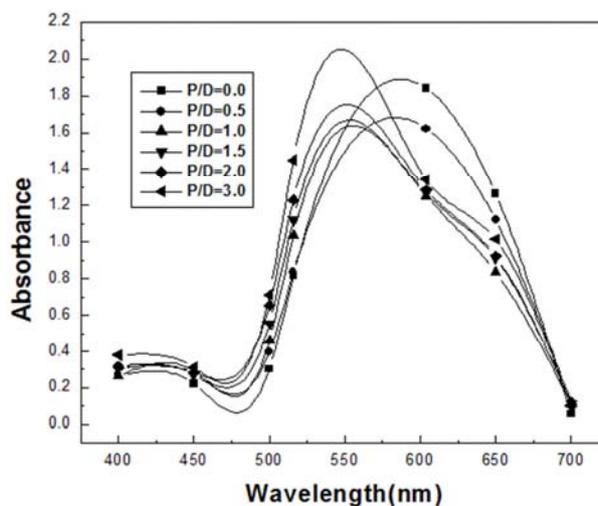


Figure 1. Absorption spectrum of TB-NaAlg at various P/D ratios.

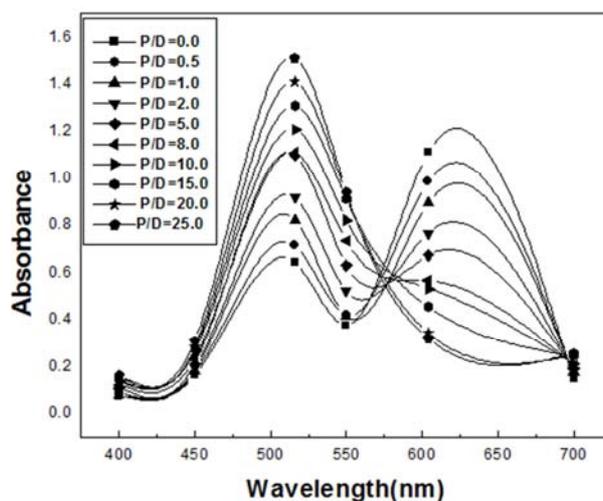


Figure 2. Absorption spectrum of TB-NaHep at various P/D ratios.

#### 4.1. Determination of Stoichiometry

To determine the stoichiometry of the polymer-dye complex, a plot of  $A_{516}/A_{606}$  versus the polymer/dye ratio was made for system TB- NaAlg. A similar procedure was repeated complex TB-NaHep also. The stoichiometry of both TB- NaAlg & complex was found 1: 1 which indicates that the binding is at alternate anionic sites. This indicates that every potential anionic site of the polyanion was associated with the dye cation and aggregation of such dye molecules was expected to lead to the formation of a card pack stacking

of the individual monomers on the surface of the polyanion so that the allowed transition produces a blue-shifted metachromasy. The results were in good agreement with the reported values for interaction of similar dyes with polyanions. Similar results were reported in case of binding of Pinacyanol chloride on poly (methacrylicacid) & poly (styrenesulfonate) systems [29-31]. The stoichiometry results are obtained by plotting  $A_{606}/A_{516}$  or  $A_{606}/A_{504}$  Vs P/D ratio in each case. The results are shown in (Fig. 3).

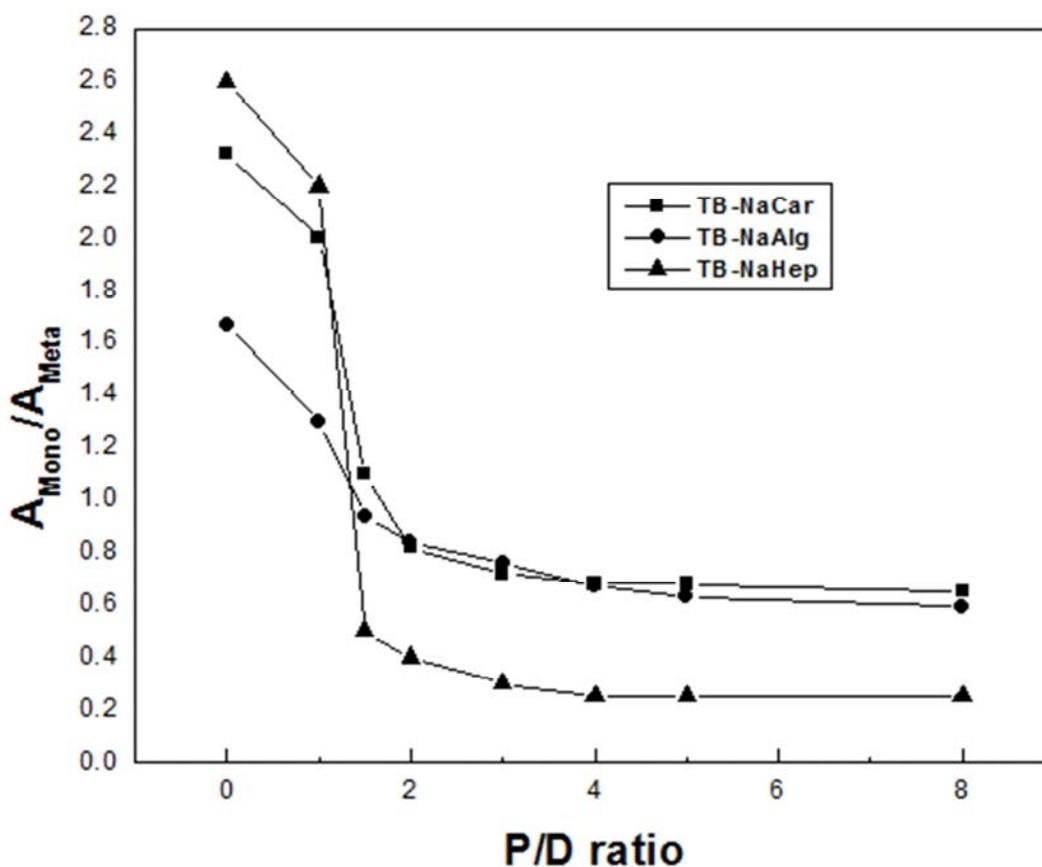


Figure 3. Stoichiometry of TB-polymer complexes.

#### 4.2. 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 interactions. 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 has already been established [30-34]. The efficiency 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 at P/D=2.0, the original

monomeric band of dye species is gradually restored. The efficiency of the alcohols, namely methanol, ethanol and 2-propanol, on destruction of metachromasy were studied. In case of TB- NaAlg system 40% methanol, 30% ethanol, 20% 2-Propanol were sufficient to reverse metachromasy 60% methanol, 50% ethanol, 40% 2-Propanol were required to reverse metachromasy in TB-NaHep system. From the plot of  $A_{516}/A_{606}$  or  $A_{504}/A_{606}$  (Fig. 4 and Fig. 5) against the percentage of alcohols or molar concentration of urea, the percentage of alcohols or molar concentration of urea, needed for complete reversal has been determined. The concentration of urea to reverse metachromasy is found to be as high as 5.5M in TB-NaHep system and 3.5 M in case of TB- NaAlg system (Fig. 6). Similar reports are available in literature for reversal of metachromasy in anionic polyelectrolyte/cationic systems by addition of alcohols or urea [35].

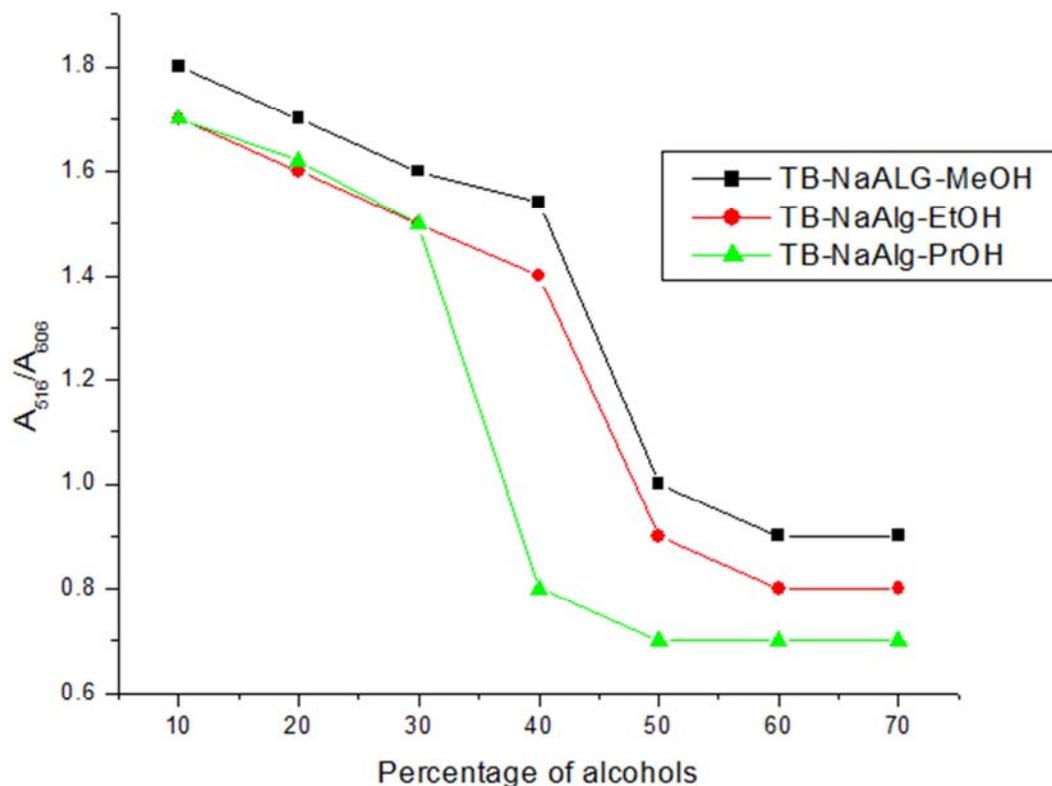


Figure 4. Reversal of metachromasy (TB-NaAlg).

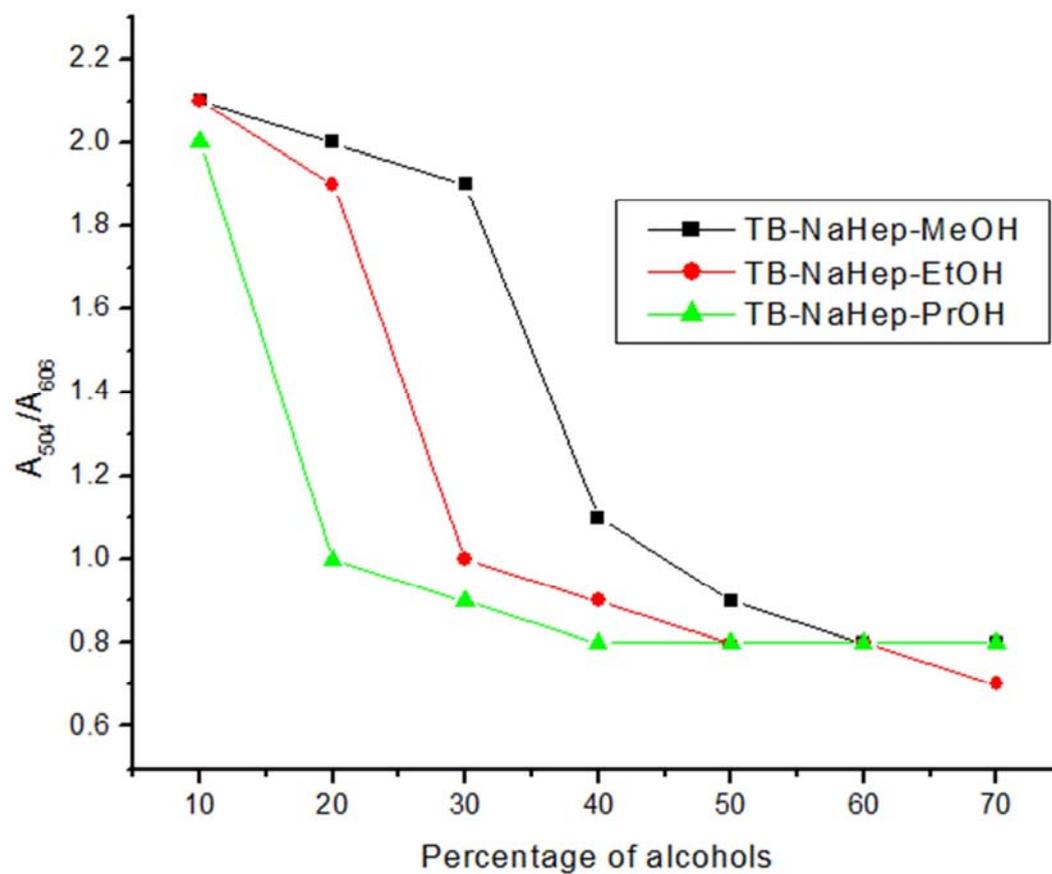


Figure 5. Reversal of metachromasy (TB-NaHep).

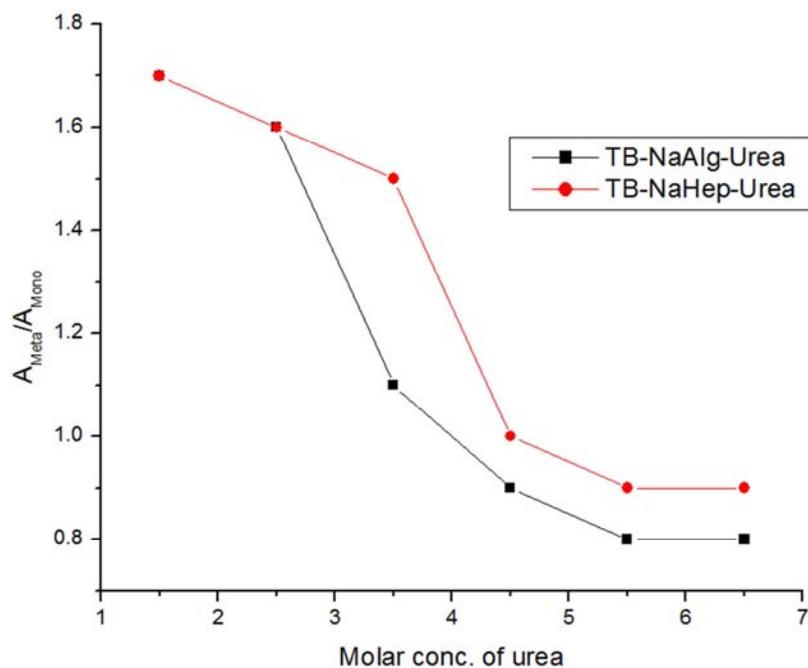


Figure 6. Reversal of metachromasy using urea.

#### 4.3. Reversal of Metachromasy Using Electrolytes

Tan & et al [36] have reported the disruption of metachromatic band with the variation of ionic strength. When sodium chloride of varied ionic strengths were added to TB- NaAlg & TB-NaHep complexes and the absorbances were measured in the range 350-700 nm. In case of TB-NaHep complex the monomeric band reappears at higher ionic strength ( $1 \times 10^{-3} M$ ) than compared to that of TB-NaAlg complex ( $1 \times 10^{-4} M$ ). In aqueous solutions the charged polymer molecule will be in the extended conformation due to the repulsion between the charged groups. On adding the

dye the conformation of the polycation changes to a compact coil owing to dye binding resulting from electrostatic interaction thus giving rise to metachromatic band. The concentration of sodium chloride required to reverse metachromasy was greater in case of complex than in the case of complex. From the plot of absorbance at 606 nm against molar concentration of electrolytes, the molar concentration of electrolytes needed for complete reversal of metachromasy has been determined. The results are shown in (Fig. 7).

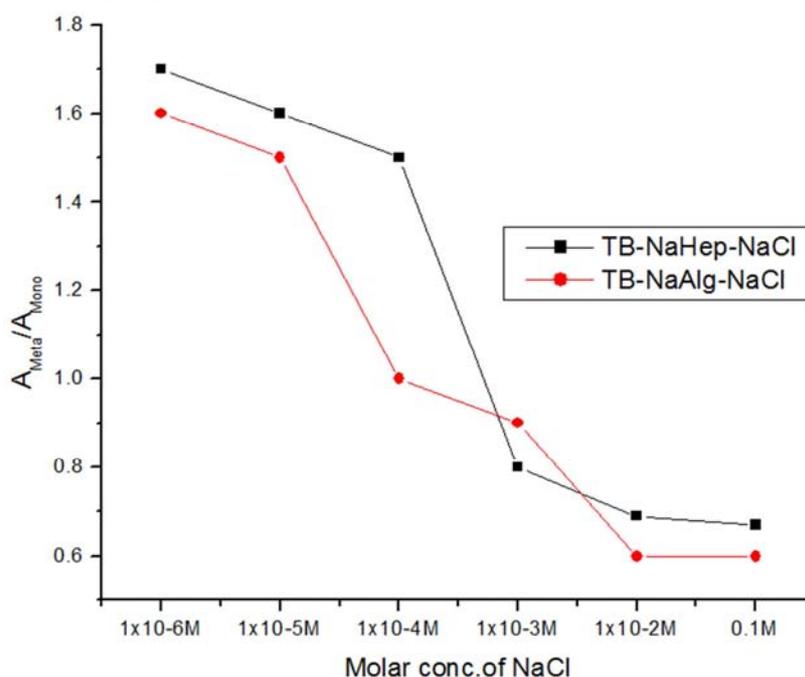


Figure 7. Reversal of metachromasy using NaCl.

#### 4.4. Determination of Interaction Parameters

The interaction constant  $K_c$  for the complex formation between TB- NaAlg and TB-NaHep complexes was determined by absorbance measurements at the metachromatic band at four different temperatures taking different sets of solutions containing varying amounts of

polymer ( $C_s$ ) to affixed volume of the dye solution ( $C_D$ ). The value of  $K_c$  [37] was obtained from the slope and intercept of the plot of  $C_D C_s / (A - A_0)$  against  $C_s$ . In each case the thermodynamic parameters of interaction, namely  $\Delta H$ ,  $\Delta G$  and  $\Delta S$  were also calculated [38]. The results are tabulated below.

Table 1. Thermodynamic Parameters of interaction for TB- NaAlg & TB- NaHep systems.

System	Temp (K)	$K_c(\text{dm}^3\text{mol}^{-1})$	$\Delta G(\text{kJmol}^{-1})$	$\Delta H(\text{kJmol}^{-1})$	$\Delta S(\text{Jmol}^{-1}\text{K}^{-1})$
TB-NaAlg	309	4954	-21.8		
	315	3780	-21.5	-47.1	-25.0
	321	2586	-20.9		
	327	1987	-20.6		
TB-NaHep	309	5430	-22.1		
	315	4735	-22.0	-33.3	-18.0
	321	3820	-21.8		
	327	2915	-21.6		

- a Calculated according to Rose-Drage equation.  
 b Calculated from the thermodynamic equation  $\Delta G = -RT \ln K_c$   
 c Calculated graphically by plotting  $\ln K_c$  against  $1/T$  according to Van't Hoff equation  $\ln K_c = -\Delta H/RT + C$   
 d Calculated from the thermodynamic expression  $\Delta G = \Delta H - T\Delta S$ .

## 5. Conclusion

From the above results it is evident that both sodium alginate and sodium heparinate were effective in inducing metachromasy in the thiazine dye, toluidine blue. The extent of metachromasy induced by sodium heparinate was found to be higher than that induced by sodium alginate which is evident from

The interaction constant and other the modynamic parameters, electrostatic forces of attraction play a major role in formation of polymer-dye complex as is evident from reversal studies using electrolytes.

Hydrophobic forces also contribute to a small extent.

## References

- [1] A Mitra, R. K Nath, SBiswas., A. K., Chakraborty, A. K., Panda Mitra A., J. Photochem. Photobiol, A: Chem, vol. 111, 1997, pp 157.
- [2] A. Mitra, A. K. Chakraborty, J. Photochem. Photobiol. A: Chem., vol. 178, 2006, pp198.
- [3] R. W Horbin., Biochemie. Biochem., vol.77, 2002, pp 3.
- [4] Bergeron J. A., M. J Singer, Biophys. Biochem. Cytol. vol. 4, 1958, pp. 433.
- [5] M. K. Pal, B. K. Ghosh, Makromol. Chem. vol. 181, 1980, pp1459.
- [6] Norden B., Kubista M., in: B. Samori, E. W. Thulstrup (Eds.), Polarized Spectroscopy of ordered systems, vol. 242, Kulwer Academic Publisher, Dordrecht, Holland, 1988.
- [7] Sabate R., Esterlich J. J. Phys. Chem B, vol. 107, 2003, pp. 4137.
- [8] Meszaos R., Varga, I., Gilanyi T., J. Phys. Chem. B vol.117, 2005, pp.13538.
- [9] Monteux C., Williams C. E., Meunier J., Anthony O., Bergeron V., Langmuir Vol. 20, 2004, pp. 57.
- [10] Honda C., Kamizono H., Matsumoto K., Endo K., J. Colloid Interface Sci vol. 278. 2004, pp. 310.
- [11] Moulik S. P., Gupta S., Das A. R., Makromol. Chem, vol. 181, 1980, pp. 1459.
- [12] Lee J., Moroi Y, J. Colloid Interface Sci. 2 vol. 273, 2004, pp. 645.
- [13] Mesa C. L., J. Colloid Interface Sci. vol. 286, 2005, pp. 148.
- [14] Konradi R., Ruhe J., Macromolecules vol. 38, 2005, pp. 6140.
- [15] Villeti M., Borsali A., Crespo R., Soldi V., Fukada K., Macromol. Chem. Phys. vol. 205, 2004, pp. 907.
- [16] Fundin J, Hansson P., Brown W., Lidegran I., Macromolecules vol.30, 1997, pp. 1118.
- [17] Ohkuma S., Furuata T, M. J. A. Proc. Japan Acad., vol 46, (1974), 857.
- [18] Stone A. L., Bradely D. F., Biochim. Biophys. Acta; vol. 148, (1967), 172.
- [19] Ohkuma S., Fukawa K., Furihata T., Proc. Japan Acad; vol. 46, (1970), 713.
- [20] Dorfman A., Am. Acad. Pediat Proc; vol. 22, (1958), 573.
- [21] Ohkuma S., Furuata T., Proc. Japan. Acad. vol. 46, (1970), 884.
- [22] Kelley J. W., Chang L., Rothstein F., The journal of Histochemistry and Cytochemistry, vol. 17, (1969), 651.
- [23] Ushio H., Uchimura H., Sano T., Yasunaga T., Polymer Journal, vol. 10, (1978), 153.
- [24] Gupta A. K., and Rohatgi-Mukherjee K. K, J. Indian Chem. Soc.1 vol. 59, 982, pp.578.

- [25] Pal M. K., Schubert M. J. *Histochem Cytoche.*, vol. 9, 1961, pp. 673.
- [26] Pal M. K. and Ghosh B. K., *Macromol. Chem*, vol. 181, 1980, pp. 1459.
- [27] Pal M. K and Ghosh B. K., *Macromol. Chem*. vol. 180, 1979, pp. 959.
- [28] Pal M. K and Schubert M., *J. Phys. Chem.*, vol. 17, 1963, pp. 182.
- [29] Frank. H. S., and. Evans M. W., *J. Chem. Phys.* vol. 17, 1945, pp. 507.
- [30] Kauzmann W., *Advan. Protein. Chem.* vol. 14, 1959, pp1.
- [31] Bruning and Holtzer A., *J. Am. Chem Soc.*, vol. 83, 1961, pp. 4865.
- [32] Whitney P. L., and Tanford C., *J. Biol. Chem* vol. 237, 1962, pp. 1735.
- [33] Mukerjee P., and Ray A., *J. Phys. Chem.* vol. 67, 1963, pp. 190.
- [34] Tan J. S, Schneider R. L, *J. Phys. Chem.* vol. 79, 1975, pp.1380.
- [35] RoseNJ; DragoRS; *Journal of American Chemical Society*, 1 vol. 81, 959, pp. 6138.
- [36] Nandini R, Vishalakshi B, *J. Polym Eng*, vol 31., 2011, pp. 191.
- [37] Nandini R, Vishalakshi B, *spectrochimica acta Part A*, vol 75., 2010 pp. 14.
- [38] Rose N. J., and Drago R. S., *J. Am. Chem. Soc.*, 81, 1959, 6138.
- [39] Gummow B. D., and Roberts G. A. F., *Makromol. Chem.*, 187, 1985, 995.