Spectroscopic Studies on the Interaction of Toludine Blue Dye with Sodium Alginate Polyelectrolyte

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Abstract: *The interaction of Toluidine blue with Sodium Alginate has been investigated by spectrophotometric method. The polymer induces metachromasy in the dye as evidenced from the considerable blue shift in the absorption maxima of the dye. The interaction constant and thermodynamic parameters of polymer-dye interaction have been determined. The effect of alcohols on the reversal of metachromasy has been studied. The thermodynamic parameters of interaction revealed that binding between dye and polymer involved electrostatic forces. The interaction of dye with polymer was studied fluorimetrically which shows the quenching of the intensity of dye with increasing concentration of polymer.*

Keywords: Toluidine blue; Sodium Alginate; Metachromasy; Interaction; Quenching

1. Introduction

Sodium Alginate (NaAlg) is a linear co-polymer of β-D-mannuronic acid and α-L-guluronic acid. It is a natural anionic polymer. Toluidine blue, a cationic fluorescent dye[3-Amino-7(dimethyl amino)-2-methyl phenothiazin-5-ium] which belongs to a thiazine class shows a pronounced ability to permeate the cellular membrane and to perform a photo-bacterial activity [1-2]. More traditionally, it is widely employed in the quantitative determination of an important anticoagulant molecule such as heparin [3-4]. Such a dye molecule is indeed one of the most widely employed in the staining applications.

The studies on the interaction of this polymer with cationic dye Toluidine blue (TB) by spectral analysis will reveal very significant results and valuable information in the field of dye-polymer interaction. Study of dye-polymer complex formation has wide application in histochemistry and the study may be used to determine the free anionic sites of the polymer in aqueous solution and also in tissue reaction. Equivalent weight of a polymer or the concentration of the polymer in the solution can be determined from dye-polymer interaction study. Moreover, chemical identity of different polymers as well as their preferred conformation in solution can be established from the concept of metachromasy[5].

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. The metachromatic change is most pronounced in the visible region and is frequently characterized by the appearance of a new absorption band. Metachromasy is viewed in sodium alginate-toluidine blue interaction in the form of blue shifted spectral band in visible range. Several physicochemical parameters can be evaluated from the study of dye-polymer interaction. These include the molecular weight of each repeating unit of polymer, stoichiometry of the dye-polymer complex, association constant and other related thermodynamic parameters. Such studies are beneficial in understanding the physiology, biochemistry and physical chemistry of macromolecules [6].

Studies on dye-polymer interaction inducing metachromasy in different cationic dyes by different synthetic poly anions, DNA, naturally occurring plants, poly- electrolytes are found in literature [7-10]. The stability of the metachromatic compound may be determined by the phenomenon of reversal of metachromasy which is achieved by addition of alcohol neutral electrolytes [11] and also by increasing the temperature of the system. The interaction of acridine orange with various synthetic polyelectrolytes and acidic polysaccharides [12-14] are available in the literature. Spectrophotometric studies on the interaction of sodium carrageenate with cationic dyes have been studied by Nandinietal [15].

The objective of the present studies is to evaluate the stoichiometry of the dye-polymer complex, binding constant, and other related thermodynamic parameters like free energy, enthalpy, and entropy changes using polymer-dye interactions and also the fluorescence studies between dye and polymer complex. An attempt has been taken to study the extent of reversal by using alcohols which is an indirect evidence for the stability of the metachromatic complex formation. The chemical structure of Toluidine blue and Sodium Alginate are shown in Scheme 1.

2. Experimental

Toluidine blue (TB) was obtained from Sigma –Aldrich (USA) and Sodium Alginate (NaAlg) from Lobachemie and was used as received. Methanol, ethanol, propanol were HPLC grade products from E. Merck, Germany. Absorption spectra were recorded using a spectrophotometer Lambda 25 (Perkin-Elmer, USA). Experimental temperature was controlled by TB-85 circulating water bath (Shimadzu, Japan).Allsolutions were prepared with doubly distilled water. Concentration of aqueous solution of the dye was 10^{-5} M. To a fixed amount concentration of dye, varying amount of polymer is added in different test tubes and an absorbance measurement of each solution has been taken.

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3. Results and Discussion

3.1. Absorption Spectra of Toluidine blue in presence of Sodium alginate

Toluidine blue possesses metachromatic properties.Absorption spectra of the aqueous solution of the dye(10^{-5}M) and those of dye-polymer mixture at different molar ratios(P/D=1to 90) are shown in (**Fig .1**). TB has a sharp intense band (α) at 630nm as well as around 590nm(β) upon addition of the sodium alginate polymer to the aqueous solution of dye, intensity of both the original bands decrease and a new metachromatic band (μ- band) appears at a shorter wavelength (550nm).This blue shift indicated strong metachromatic interaction between the dye and polymer. Dye molecules could form a charge transfer complex with the polymer; hence due to the formation of a charge transfer complex, a blue shift in the original band of the dye molecule occurred. This phenomenon of shifting of the band is metachromasy.

3.2. Determination of stoichiometry

Stoichiometry of the metachromatic complex was determined by isolation technique of MacIntosh [16]. It was found that the metachromatic compound was formed with dye-polymer stoichiometry of $\approx$1:1 as shown in (**Fig.2**) and indicate that the binding is at adjacent anionic sites. This stoichiometry 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 [17].

3.3. Reversal of metachromasy using alcohols

The metachromatic effect is presumably due to the association of the dye molecules on binding with the polyelectrolyte which may involve both electrostatic and hydrophobic forces. The destruction of metachromatic effect may occur on addition of alcohols. The destruction of metachromasy by alcohol is attributed to the involvement of hydrophobic bonding which has already been established [18]. The effectiveness of alcohols in disrupting metachromasy was found to be in the order methanol<ethanol< 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 the original monomeric band of dye species is gradually restored. The effectiveness of the alcohols, namely methanol, ethanol and propanol, on destruction of metachromasy were studied.From the plot of A_{550}/A_{630} (**Fig.3**) against the percentage of alcohols, the amount of alcohols required for complete reversal has been determined. 40% methanol, 30% ethanol and 20% propanol were sufficient to reverse metachromasy.

3.4. Determination of interaction parameters

The interaction constant K_C for the complex formation between TB and NaAlg was determined by absorbance measurement at the metachromatic band at different temperatures taking different sets of solutions containing varying amounts of polymer (C_S) in a fixed volume of the dye solution. The absorbance results were treated using Rose-Drago equation [19].

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

Where, C_D = Initial concentration of the dye, C_S = Initial concentration of the polymer, A_0= Absorbance of the pure dye solution at λ_{max}, A = Absorbance of the dye-surfactant solution at λ_{max}, K_C = Binding constant between the dye and polymer, ε_D = molar absorption coefficient of the dye, ε_{DS} = molar absorption coefficient of the dye-polymer complex, L = Length of the light path.

Plot of $(C_D.C_S)/ (A-A_O)$ versus C_S at different temperature shows a linear relationship as shown in (**Fig 4**). From the slope and intercept of the straight lines, the interaction constant (K_C) values were calculated. Free energy changes were calculated from the relation ΔG^O= -RT $\ln K_C$. The negative sign of ΔG^O indicates the spontaneity of the binding of TB with sodium alginate. $\Delta S^O$was calculated from the slope of plot of ΔG^Ovs temperature. $\Delta H^O$was calculated from the slope of plot of $\ln K_C$vs 1/T. Similar methods for determinations of thermodynamic parameters are available in literature [14,20]. All thermodynamic properties calculated from this method are summarized in Table 1.

3.5. Fluorescence studies

Toluidine blue, being a fluorescent dye, exhibit emission band at 343 nm. Fluorescent studies were performed on TB-NaAlg system and it was found that the fluorescence intensity of TB decreases on the additionof increasing amount of polymer solution as evidenced from (**Fig 5**). 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-Volmer equation[21],

$$\frac{F_0}{F}=1+K_{SV}[Q]$$

whereFo 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, K_{sv} is the Stern-Volmer constant. The Stern-Volmer plot obtained for the present system is shown in (**Fig 6**). From the slope of the plotvalue of the Stern-Volmer constant was found to be 3.0×10^3 lit^{-1}mol^{-1}.

4. Conclusions

Based on the above discussion the work done can be concluded in the following manner:

1) Sodium alginate induced strong metachromasy in cationic dye Toluidine blue.
2) A 1:1 stoichiometry complex was formed between the dye and the polymer.It suggested that every potential anionic site of the repeating unit of the polymer was associated with the dye.
3) Dye-polymer aggregation gets disrupted upon addition of alcohol as revealed by reversal metachromasy. Energetic of dye-polymer complex formation indicated the exothermic nature of binding as well as formation of ordered structure in the process of interaction.
4) From spectral and thermodynamic data the chromotropic character of the polymer was established.
5) The fluorescence spectra between dye and polymer show that with increase in the polymer concentration the intensity of the band of dye decreases. The polymer being anionic in nature, can donate its electron to the cationic dye through the formation of charge transfer complex. Thus the transition probability of electrons of TB bonding molecular orbital to anti/non bonding molecular orbital gets reduced.

Future scope of this work includes characterization of polymer with dye using dynamic light scattering techniques and also layer by layer technique may be used for studying morphology of the molecule using AFM.

5. Acknowledgement

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Table 1: Thermodynamic parameters for interaction of Toluidine blue-Sodium alginate

Temperature (K)	ΔG^0 cal mol^{-1}	ΔH^0 Kcal mol^{-1}	ΔS^0 cal mol^{-1} K^{-1}
303	-5.976	-14.47 x 10^3	-0.0282x10^3
308	-5.704		
313	-5.68		
318	-5.52		

Figures Legends:

Scheme 1: Chemical structure of Toluidine blue (A) and Sodium alginate (B)
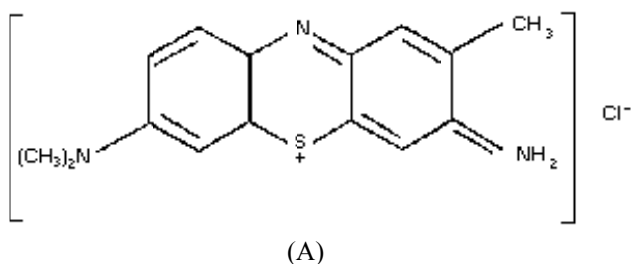Fig 1: Absorption spectra of Toluidine blue- Sodium alginate system at various P/D ratios
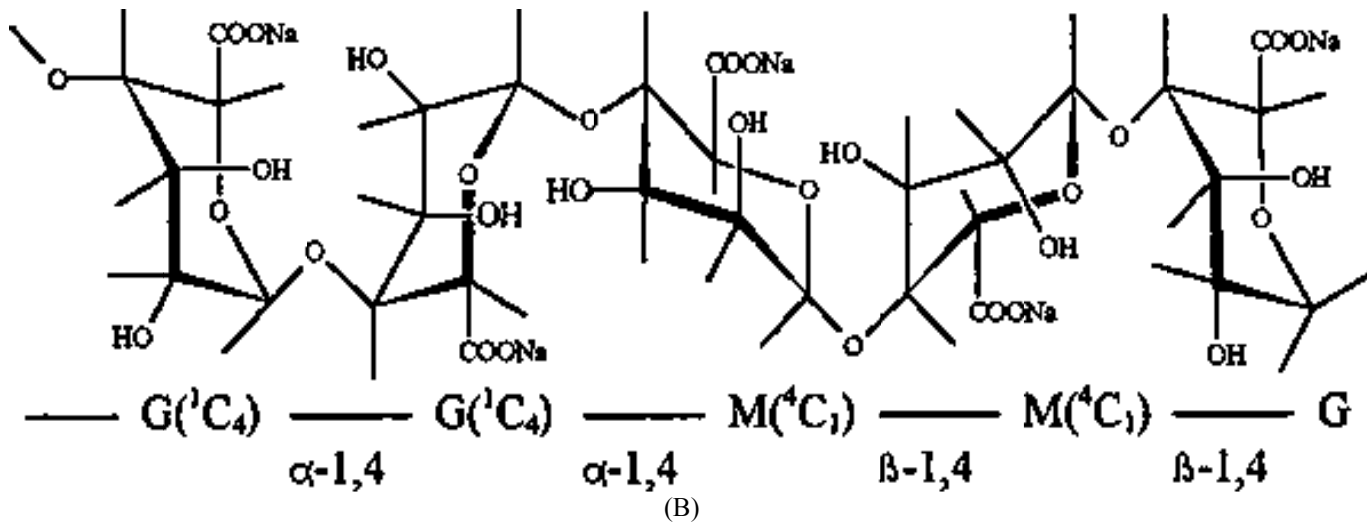Fig 2: Stoichiometry of TB-NaAlg complex
Fig 3: Reversal of metachromasy on addition of alcohols in TB-NaAlg complex
Fig 4: Plots of $C_D C_S$/ $(A-A_0)$ against C_S for TB-NaAlg system at different temperatures
Fig 5: Fluorescence spectra of Toluidine blue- Sodium alginate system at various P/D ratios
Fig 6: Stern-Volmer plot for TB-NaAlg system

(A)

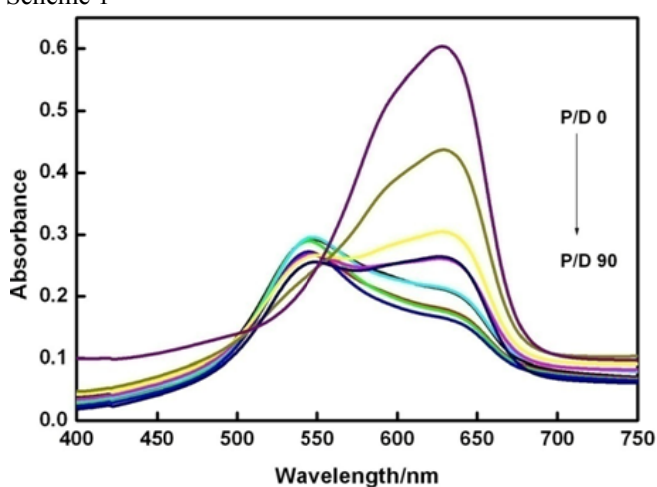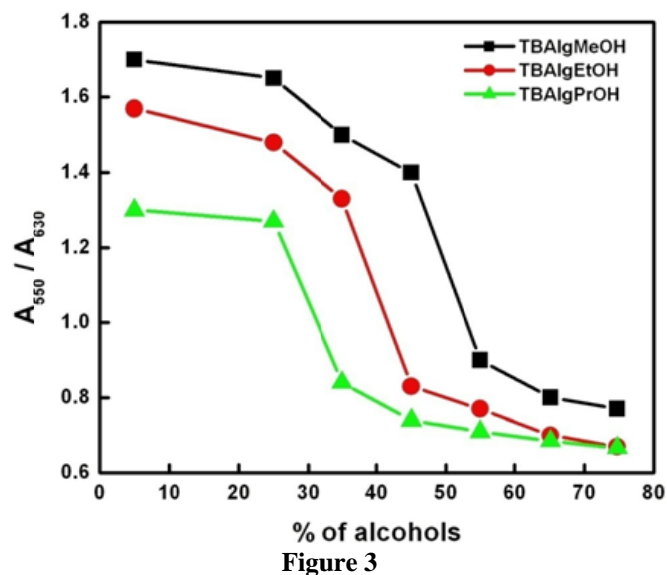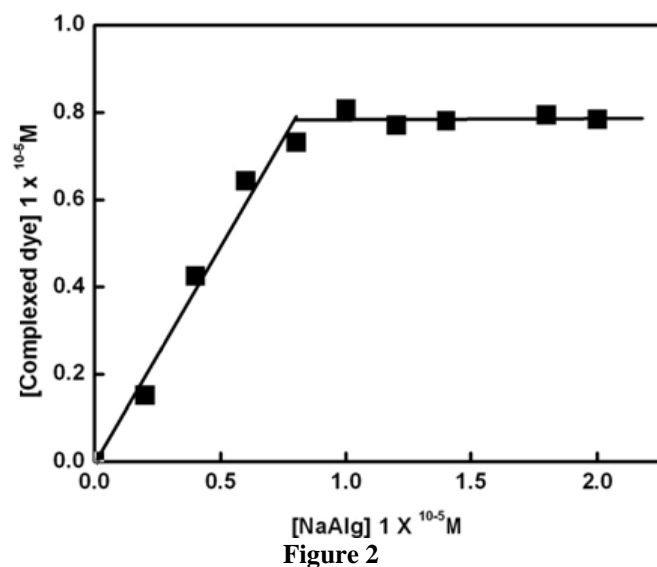$$G(^1C_4) \quad\quad G(^1C_4) \quad\quad M(^4C_1) \quad\quad M(^4C_1) \quad\quad G$$
$$\alpha\text{-}1,4 \quad\quad \alpha\text{-}1,4 \quad\quad \beta\text{-}1,4 \quad\quad \beta\text{-}1,4$$

(B)

Scheme 1

Figure1

Figure 2

Figure 3
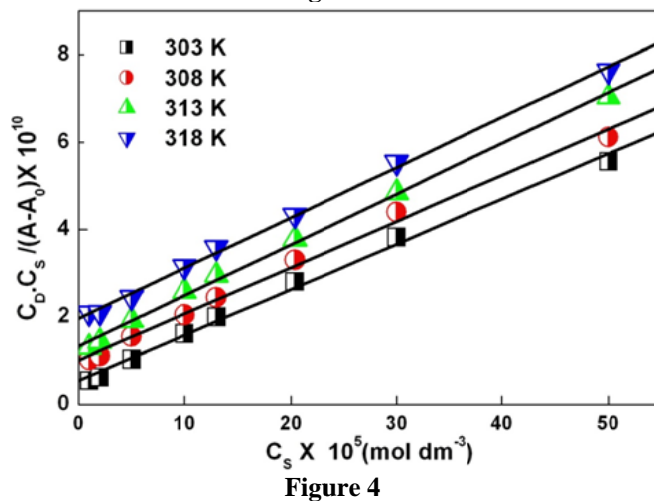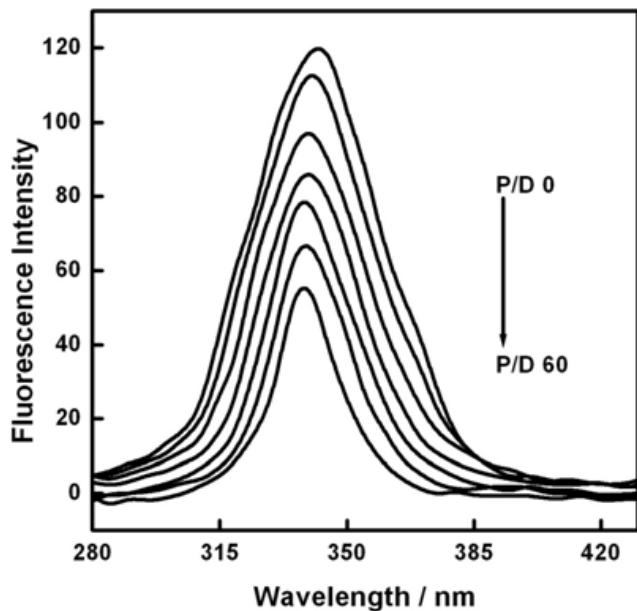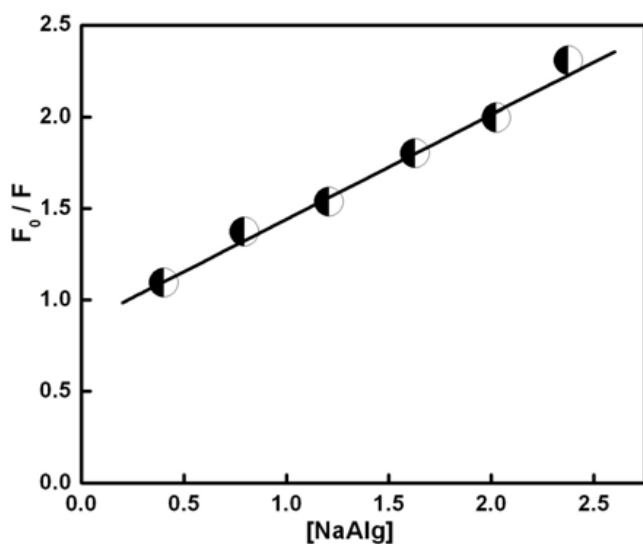

Figure 4

Figure 5

Figure 6

Authors Profile

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