International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Metachromasia Induced in Cationic Dyes by Neem (*Azadirachta indica*) Polysaccharide

Amitabha Saha

Department of Chemistry, Women's College, Agartala-799001, India

Abstract: ***Aqueous extract of Neem (Azadirachta indica) tree exudate gum furnishes, a polysaccharide material (NP) having high equivalent weight (1085±5). NP induces slight spectral shift in toluidine blue (TB, $1x10^{-5}$M) and distinct multiple banded metachromasia in more aggregating thiazine dye 1,9-dimethylmethylene blue (DMMB, $1x10^{-5}$M).Weak chromotropic ability of NP is reflected in its failure to induce metachromasia in methylene blue (MB, $1x10^{-5}$M). NP induces strong metachromasy (blue shift ~100nm) in dye pinacyanol chloride (PCYN, $1x10^{-5}$M). Half plateau values signifying destruction of metachromatic compounds to the extent of 50% correspond to ~25%, ~18% and ~ 28% ethanol for DMMB-NP, TB-NP and PCYN-NP systems respectively. Negative ΔS values for the DMMB-NP and PCYN-NP systems indicate the formation of ordered structures during the formation of dye-polysaccharide complexes.***

Keywords: Metachromasia, Cationic dyes, Neem Polysaccharide, Chromotrope, μ-band

1. Introduction

Spectrophotometrically metachromasia can be defined as the blue shift of the main absorption band of a dye observed in dilute aqueous solution induced by the presence of suitable chromotropes[1]. Metachromatic spectral shift is thus always hypsochromic in nature and in most cases hypochromic also. All the cationic dyes are not metachromatic and all the metachromatic dyes are not potentially equal. A metachromatic dye consists of large hydrophobic aromatic portion and small hydrophilic cationic charge centre. Aggregating tendency of a dye increases with the increase of hydrophobic portion of the dye. The grater metachromatic potentiality of dimethylmethylene blue (DMMB) over other members like methylene blue (MB) and toluidine (TB) of the same thiazine group has been reported[2]. Of the dyes belonging to cyanine group, pinacyanol chloride (PCYN) is a good metachromatic dye but its lower homologue N, N′-diethylpseudoisocyanine chloride(PIC) is not[3].Pinacyanol chloride has a strong aggregating tendency in aqueous solution. It is known that the dye pseudoisocyanine(PIC) does not give blue-shifted metachromasia but gives a sharp red-shifted band called Jelley-Scheib[4] band, popularly known as J –band.

A chromotrope is usually polyanionic in nature and its ability depends on anionic charge density and nature of anionic group. Chromotropic abilities of biopolymers like chondroitin sulphate, heparin etc.; plant products like aliginic acid,pectic acid, carrageenan etc.;synthetic polyanions like poly acrylate, polyvinyl sulphate, polystyrene sulphonate etc.; inorganic salts like ammonium molybdate, mercuric chloride, potassium ferrocyanide, ferricyanide, potassium persulphate, ammonium metavanadate etc. have been reported.

We report here spectrometric, conductometric and thermodynamic interactions of neem polysaccharide with different cationic dyes.

2. Experimental

1,9-Dimethylmethylene blue (Sigma-Aldrich), toluidine blue (E. Merck), methylene blue (E.Merck), pinacyanol chloride (Serva) and pseudoisocyanine Chloride (Serva) were used. Toluidine blue and methylene blue were purified through recrystallization. Stock solutions of the dyes DMMB, TB, MB, PCYN and PIC were made in double–distilled water. The dye solutions and other experimental solutions were stored in dark when they were not in use.

Isolation of neem polysaccharide (NP): The exudate gum obtained from injured trunk of neem tree (4.0 gm) was dissolved in distilled water (100ml) by thoroughly shaking with a magnetic stirrer. The solution was filtered under suction. To this filtrate potassium acetate was added with stirring. The polysaccharide was precipitated on gradual addition of absolute ethanol (1 : 3) and was allowed to settle overnight. The precipitate (NP ~ 1.5 gm) was collected by centrifugation, washed with ethanol and dried by acetone

Equivalent weight of NP : Equivalent weight of NP was determined by direct conductometric titration of its aqueous solution with standard HCl solution.

Absorbance was measured with a Toshinwal CL 10A4 spectrophotometer and conductance with a Systronic 303 conductometer. Stability of metachromatic compounds were studied by recording the absorbances of the metachromatic compound of different systems and the dye concerned at the monomer band (α-band) on addition of ethanol in increasing amounts.

Thermodynamic parameters of the dye-polysaccharide interactions were determined by measuring spectral changes of the DMMB-NP and PCYN-NP mixtures at the metachromatic band peak at 570 nm and 500 nm, respectively under different temperatures (35-50 ^{0}C). The interaction constant

(Kc) of the dye-polysaccharide system, D+P ⇌ DP was determined according to the modified equation of Rose and Drago[5]

$$\frac{C_D \times C_S}{(A-A_0)} = \frac{1}{K_C\, L(E_{DS} - E_D)} + \frac{C_S}{(E_{DS} - E_D)L}$$

Where C_D is the initial concentration of dye; C_S is the initial molar concentration of polysaccharide; A_O is the absorbance of the dye solution; E_{DS} is the molar absorption co-efficient of the dye; L is the length of the light path and A is the absorbance of the dye-polysaccharide mixture. The value of K_C was calculated from the intercept and slope of the linear plot of $C_D C_S / (A - A_O)$ Vs C_S. Free energy change (ΔG) and entropy change (ΔS) was calculated from the thermodynamic equations relating K_c, ΔG & ΔS.

Equations :ΔG = - RTln Kc

and ΔG = ΔH-TΔS

3. Results and discussion

The neem polysaccharide has been reported [6] to contain L-arabinose, L-fucose, D-galactose, D- glucuronic acid as the monosaccharide units and equivalent weight of neem polysaccharide is 1080. Nayak *et al*[7] reported that the monosaccharide units galactose, arabinose, glucuronic acid, fucose and glucosamine were present in the molar ratio of 86:70:30:10:1

The polysaccharide materials (NP**)** present in the neem gum was isolated by precipitation with ethanol in presence of KOAc. The product NP was isolated as its potassium salt. Conductometric titration result (**Fig.1**) gives equivalent weight 1085±5 for NP,which is close to the reported value of Srivatsava *et al*[6] . The equivalent weight is considered to be the molar mass of one repeating unit.

Fig. 2A shows absorption spectra of $1.0\times10^{-5}M$, DMMB solution in water exhibits of prominent dimer band (β-band) around 600 nm in addition to its monomer band (α-band) around 650 nm, reflects strong aggregation tendency of DMMB. DMMB shows distinct but multiple banded metachromasia in presence of NP (**Fig. 2B, C, D**).TB is a dye commonly used for the study of metachromasia and has a λ_{max} at 630 nm in dilute aqueous solution($1.0\times10^{-5}M$) (**Fig. 3A)**.On adding NP to the dye in a polysaccharide/dye of mole ratio 5 to 25, α band of TB undergoes hypsochromic shift and a new band (μ-band) appears around 600 nm(**Fig. 3B, C, D**) with appreciable absorbance. NP fails to induce metachromasia in MB (Figure not shown) .Dextran sulphate, heparin and chondroitin sulphate are polyanions of high charge density and are known to induce sharp metachromasia in thiazine dyes DMMB, MB and TB. 'Bael' (*Aegle marmelos*) fruit gum polysaccharide and Bael tree exudate gum polysaccharide[8], 'Tal' (*Borassus flabellifer*) fruit gum polysaccharide[9] and 'Chalta' (*Dillenia indica*) fruit mucilage polysaccharide[10] are polyanions of relatively lower charge density and are known to induce metachromasia in the stronger metachromatic dye DMMB but not in MB; TB undergoes only modest metachromatic shift in presence of these polyanions

Like DMMB, cyanine dye PCYN is also a very strong aggregating dye, having its α-band around 600 nm and a prominent β-band around 550 nm (**Fig. 4A**) even in very dilute solution ($1.0 \times 10^{-5}M$). In presence NP, μ-band appear around 500 nm (**Fig. 4B, C, D**) and another prominent band appears around 600nm, λ_{max} of the dye. Shapes of the metachromatic curves of the PCYN-NP system, resemble those of PCYN-Tal fruit (*Borassus flabellifer*) polysaccharide[9] and also PCYN-Chalta (*Dillenia indica*) polysaccharide[10]. NP fails to induce any spectral shift in PIC (figure not shown).

Role of disruptive factor like ethanol has been studied and the half-plateau values signifying destruction of metachromatic compound to the extent of 50% and hence their stabilities, correspond to 25% ,18% and 28% ethanol, respectively for DMMB-NP(**Fig. 5D**), TB-NP(**Fig. 5E**) and PCYN-NP system (**Fig. 5F**). Stabilities of metachromatic compounds evaluated on the basis of disruptive role of ethanol are found in the order: PCYN-NP> DMMB-NP> TB-NP

The binding constant [11], K_C is calculated using the modified equation of Rose and Drago at different temperature (**Fig. 6 & 7**). The ΔS[12] value is calculated from the thermodynamic equations relating K_C, ΔG and ΔS for the system (**Fig.8 &9**).In case of toluidine blue dye the absorbance of the dye solution (A_O) is greater than the absorbance of the dye-polysaccharide mixture (A) therefore Rose and Drago equation is not used to calculate the binding constant(K_C).

From Table-1& 2, the values of binding constant K_C decrease with the rise in temperature in both DMMB-NP and PCYN-NP suggest that binding process is exothermic in nature [13]. Negative ΔS values for the DMMB-NP and PCYN-NP system indicate the formation of ordered structures during the formation of dye-polysaccharide complexes [14].

4. Conclusion

Equivalent weight of an acid polysaccharide indicates the frequency of the acid groups in its repeating units; the higher the equivalent weight, lower is the frequency of the acid groups; lower is the charge density and weaker is the chromotrope. Neem polysaccharide has high equivalent weight so it is a weak chromotrope. . Weak chrotropic behavior of NP is reflected in its exhibition of metachromasia in strong metachromatic dyes like DMMB, PCYN and its failure to cause blue shift in MB or its inducing little shift in TB. This is also corroborated on the basis of half-plateau values and thermodynamic parameters of different systems.

5. Acknowledgement

The author thanks U.G.C, NERO, Guwahati, for financial assistance.

References

[1] M.Schubert,D.Hamerman:J.Histochem.Cytochem.,195, **4**,159
[2] M.K. Pal, A. Ray: Indian J. Biochem. Biophys, 1988, **25**, 378
[3] M.K. Pal, B.K. Ghosh: Makromol. Chem., 1979,**180**, 959
[4] E. E. Jellley: Nature, 1936, **138**, 1009
[5] N.J.Rose, R.S.Drago : J.Am.Chem.soc.,1959,**81**,6138
[6] S.Mukherjee,H.C.Srivatsava: J. Am. Chem. soc., 1955, **77**, 422
[7] B.R. Nayak, R. Rao , T.N. Pattabiraman :Proc.Indian Acad.Sci., 1978,**87B** (Experimental Biology 4), No.10,261
[8] A.K.Bhattacharyya , N.C.Chakraborty: Indian J. Chem., 1990, **29A**, 32.
[9] A. K. Bhattacharyya, M. K Pal , A. Das: *J. Indian Chem. Soc*., 1986, **63**, 138.
[10] A.K. Bhattacharyya , G. Bhattacharya: J. Indian Chem. Soc., 1996, **73**, 463
[11] A.Saha, S Bhaumik : Res. J. Chem. Environ , 2014, **18(4)**, 72.
[12] S.Dasgupta, R. K. Nath, S.Biswas, A. Mitra, A. K.Panda: Indian J. Biochem, Biophys, 2009,**46**, 192
[13] A.K. Bhattacharyya , A. Saha: J. Indian Chem. Soc., 1998, **75**, 451
[14] 14. A. Mitra, R.K.Nath, A.K.Chakraborty: Indian J. Biochem. Biophys,1992,**29**,411

Table 1: Thermodynamic parameters for the interaction between DMMB and NP

Temp. (K)	K_C	*ΔG K Cal mol^{-1}*	*ΔS Cal mol^{-1}deg^{-1}*
308	15476	-5.942	
311	13125	-5.897	
314	11886	-5.892	-7.37
317	10506	-5.870	

Table 2: Thermodynamic parameters for the interaction between PCYN and NP

Temp. (K)	K_C	*ΔG K Cal mol^{-1}*	*ΔS Cal mol^{-1}deg^{-1}*
308	22368	-6.169	
311	20238	-6.167	
314	17000	-6.117	-8.27
317	15178	-6.103	

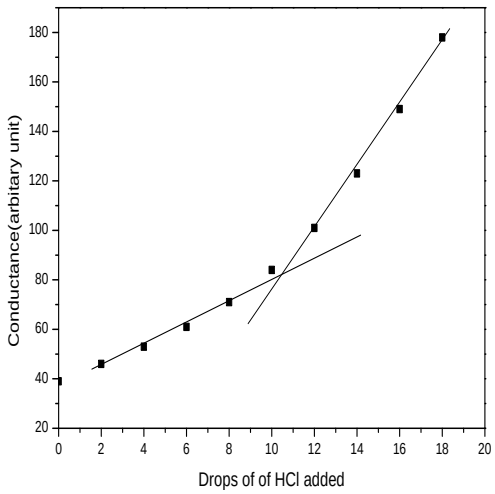

Figure 1: Conductometric titration of 25 ml NP (0.103 gm in 100ml) Vs HCl (.045N)

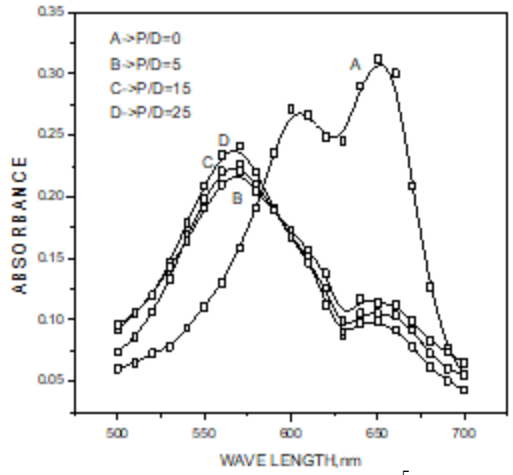

Figure 2: Absorbance spectra of 1.0×10^{-5}M DMMB in presence of NP at different polysaccharide/dye molar ratio(P/D= 0 to 25)

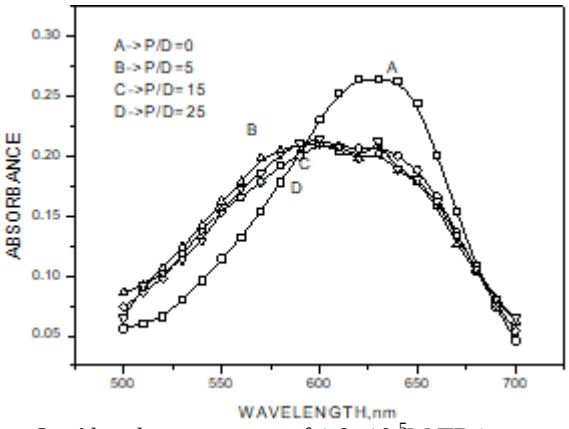

Figure 3: Absorbance spectra of 1.0×10^{-5}M TB in presence of NP at different polysaccharide/dye molar ratio (P/D = 0 to 25)

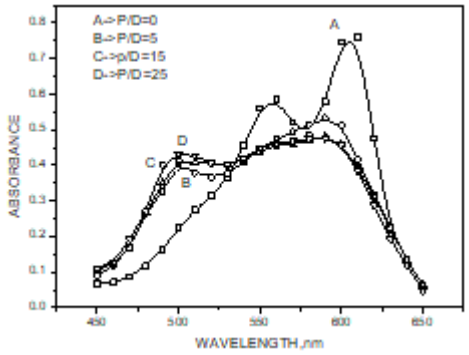

Figure 4: Absorbance spectra of 1.0×10^{-5}M PCYN in presence of NP at different polysaccharide/ dye molar ratio(P/D =0 to 25)

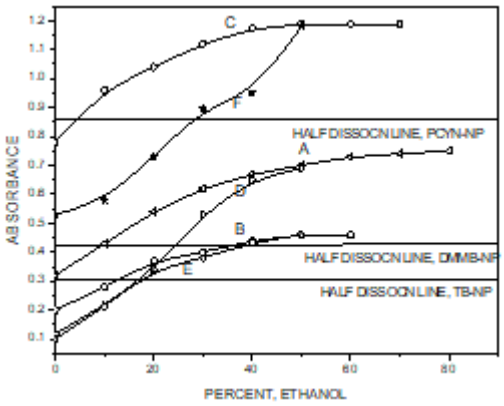

Figure 5: Plots of absorbance at α – band of $1.0X10^{-5}$M DMMB,TB, and PCYN Vs percent ethanol in water(A,B,C) and in presence of NP (D,E,F; P/D = 20)

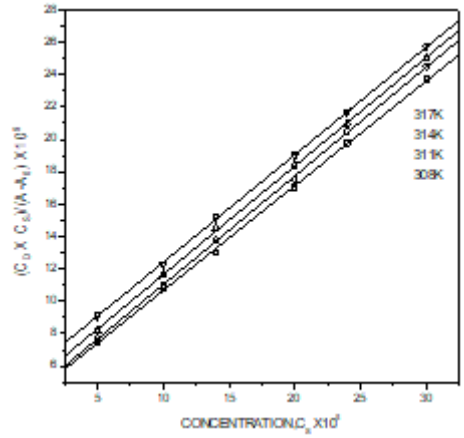

Figure 6: Plots of $(C_D\times C_S)/(A-A_0)$ against C_S in NP-DMMB interaction at different temperatures

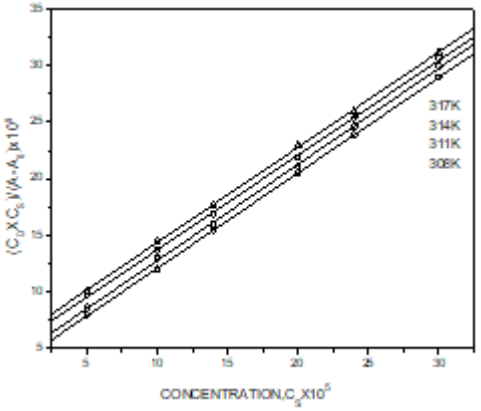

Figure 7: Plots of $(C_D\times C_S)/(A-A_0)$ against C_S in NP-PCYN interaction at different temperatures

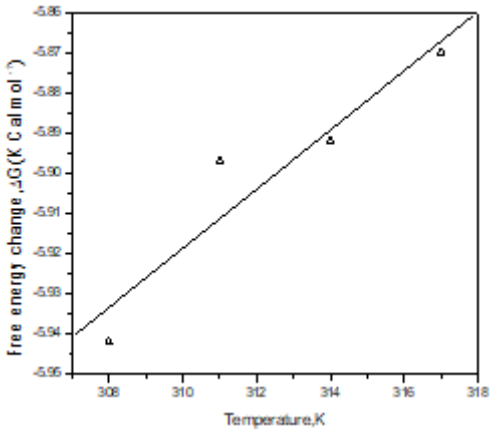

Figure 8: Variation of free energy change(Δ G) with temperature during interaction between NP and DMMB

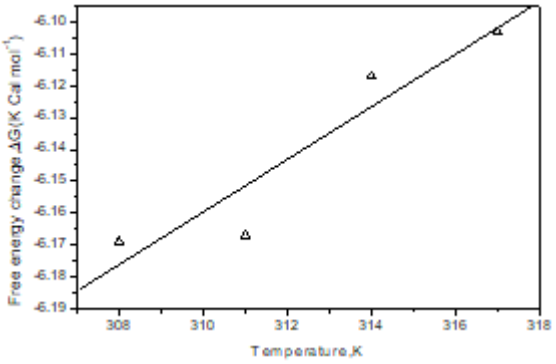

Figure 9: Variation of free energy change(Δ G) with temperature during interaction between NP and PCYN