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Chromotropic Behaviours of Chalta (*Dillenia indica*) Fruit Mucilage Polysaccharide towards some Cationic Dyes[#]

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Chalta (*Dillenia indica*) fruit mucilage polysaccharide (DI) is an acid polysaccharide of low charge density (eq. wt. 996). Weak chromotropic ability of DI is reflected in its failure to induce metachromasia in methylene blue (MB). DI induces slight spectral shift in toluidine blue (TB) and distinct but multiple banded metachromasia in more metachromatic dye 1,9-dimethylmethylene blue (DMMB) at different polymer-to-dye concentrations (P/D). Blue-shift of principal absorption band (~ 110 nm) of pinacyanol chloride (PCYN) in its dilute aqueous solution (1.0×10^{-5} *M*) in presence of DI at different P/D values, reflects stronger metachromatic potentiality of PCYN. Spectrum of aqueous solution of pseudoisocyanine chloride (PIC, 1.0×10^{-5}*M*) has been found to remain unperturbed by DI. Disruption of metachromatic compounds of PCYN-DI and DMMB-DI to the extent of ~50% by ~21% and ~20% ethanol respectively, signifies lower stability of the systems.

A substance inducing metachromasia in a suitable cationic dye in its aqueous solution is called a chromotrope. A chromotrope is polyanionic in nature. Polyanions with high charge densities and containing sulphate groups[1] are known to induce sharp metachromasia in different cationic dyes. Metachromatic abilities of cationic dyes belonging to thiazine group have been reported[2] to be DMMB > TB > MB. Nature of the polyanion sharply influences its interaction with the cyanine dyes PCYN[3] and PIC[4]. A polysaccharide material DI[5] obtained on saponification of aqueous extract of Chalta (*Dillenia indica*) fruit mucilage is an acid polysaccharide (eq. wt. 996) consisting of galactose, arabinose, mannose and galacturonic acid. We report here our observations on chromotropic abilities of DI with respect to different thiazine and cyanine dyes. Stabilites of metachromatic compound[6] of different systems have also been studied.

Results and Discussion

A chromotrope facilitates cooperative aggregation of dye cations resulting in blue-shifted metachromasia[6]. A chromotrope may be a biopolymer, a synthetic polymer or an inorganic salt like ammonium molybdate, mercuric chloride etc. Small molecules capable of causing aggregation of dye cations to trimer or tetramer have been reported[7] to induce metachromasia in a potentially metachromatic dye. Chromotropic ability of a polyanion depends on its charge density as well as on the nature of the anionic groups[1,8]. DI, an acid polysaccharide containing only carboxylic group as the anionic group, was extracted as its potassium salt. The equivalent weight of DI (996) as its potassium salt indicates the presence of one acid monosaccharide unit per six monosaccharide units on an average. Equivalent weight of the acid polysaccharide indicates the frequency of the acid groups in its repeating unit; the lower the equivalent weight, the higher is the frequency of the acid groups and higher is the charge density of the polyanion.

DI being a polyelectrolyte of high equivalent weight and hence of relatively low charge density, is expected not to be a very efficient chromotrope. This is evident from the fact that it fails to induce metachromasia in methylene blue (1.0×10^{-5} *M*). Metachromatic blue-shift of λ_{max} is the result of regular aggregation of the dye cations on the polyanion chain, and hence a high charge density will facilitate closer packing of the dye cations with stronger Coulombic interaction of the π-electrons[7]

Toluidine blue (1.0×10^{-5} *M*) has its α-band around 630 nm (Fig. 1A) and does not have any distinct β-band. α-band and β-band represent monomeric and dimeric state of the dye respectively. In presence of DI, α-band of TB undergoes hypsochromic shift and a new band (μ-band) appears around 590 nm (Fig. 1B) with appreciable absorbance at P/D 3.0. With the increase of P/D ratio (6.0) absorbance around α-band and μ-band are diminished (Fig. 1C). DI, however, causes very modest blue-shift of the principal absorption band of TB (Table 1).

1,9-Dimethylmethylene blue (DMMB), a thiazine dye with enriched hydrophobic element, is more metachromatic than TB and MB. Enhanced aggregating tendency of DMMB is evident from the appearance of sharp β-band around 595 nm in addition to its monomer band around 650 nm (Fig. 2A). DI induces sharp but multiple banded metachromasia in DMMB at different P/D values (Curves 2B, C, D), μ-band appearing around 550 nm with small peaks around α-band and β-band of the dye. Weak chromotropic nature of DI is also evident from the lesser magnitude of blue-shift (Table 1) induced by it in comparison to that of DMMB induced by dextran sulphate (DS), a polyanion with high charge density (eq. wt 167). ATP has been claimed to be one of the smallest

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chromotropes to induce metachromasia in DMMB[9], but not in acridine orange.

TABLE 1–MAGNITUDE OF BLUE-SHIFTS

Systems	α-band nm	μ-band nm	P/D	Blue-shift nm
DMMB-DS	650	500	3.0	150
DMMB-DI	650	550	6.0	100
PCYN-DS	600	485	3.0	115
PCYN-DI	600	490	6.0	110
TB-DS	630	490	3.0	140
TB-DI	630	590	12.0	40

Like DMMB, the cyanine dye pinacyanol chloride (PCYN) is also a very strong aggregating dye, having its α-band around 600 nm and a prominent β-band around 545 nm (Fig. 3A) even in its very dilute solution (1.0×10^{-5} *M*). In presence of DI, μ-band appears around 490 nm (Fig. 3B) and another prominent band appears

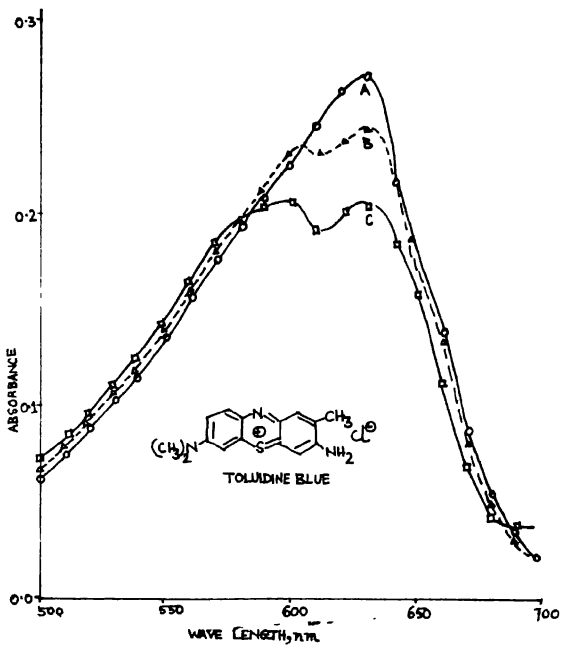


Fig. 1

around 600 nm, the λ_{max} of the dye. The positions of the metachromatic bands and the shapes of the metachromatic curves appear to be insensitive towards changes of P/D values, except that absorbance around λ_{max} of the dye is a bit higher in the case of lower P/D values (Fig. 3). Shapes of the metachromatic curves of the PCYN-DI system, resemble those of PCYN -Tal fruit (*Borassus flabellifer*) polysaccharide[10] and also those of PCYN-polysodium acrylate[3] systems. Bael fruit gum polysaccharide and bael tree exudate gum polysaccharide[11], bacterial cell wall polysaccharide[12], ATP and even ADP[13] have been reported to induce metachromasia in PCYN.

Pseudoisocyanine (PIC), the lower homologue of PCYN, suffers red-shift in presence of suitable polyanion instead of blue-shift. DI fails to induce any spectral shift

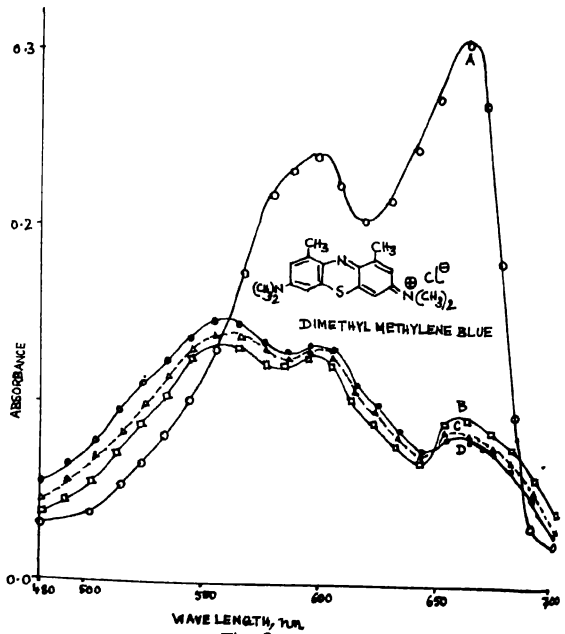


Fig. 2

(either red or blue) in PIC[14]. Induction of metachromasia in PCYN causing blue-shift of about 110 nm by DI, points more to the stronger metachromatic potentiality of the dye rather than to the stronger chromotropic ability of the polyanion. Magnitude of blue-shift in just one dye PCYN does not seem to be a surer test of the chromotropic

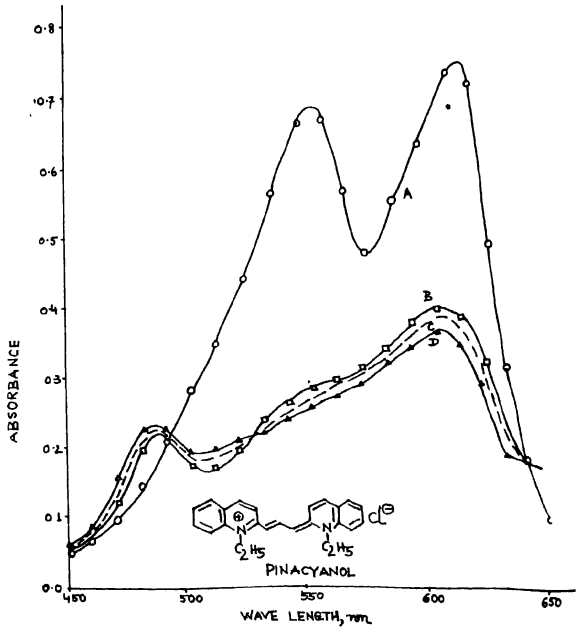


Fig. 3

ability of a polyanion. Chromotropic ability of a polyanion should, therefore, be tested through interaction of the polyanion with several metachromatic dyes as well as through evaluation of half-plateau values[6] signifying disruption of its metachromatic compounds with different dyes to the extent of 50%. Nature of both the cationic dye and the polyanion decides the stability of the metachromatic compound. Relative stabilities of metachromatic compounds are evaluated on the basis of disruptive role of ethanol, urea etc. The extent of disruption of metachromasia by ethanol/urea is obviously a function of ethanol/urea concentration. Decrease in stability of metachromatic compounds is accompanied by the decreasing absorbance of dye-chromotrope solution at the

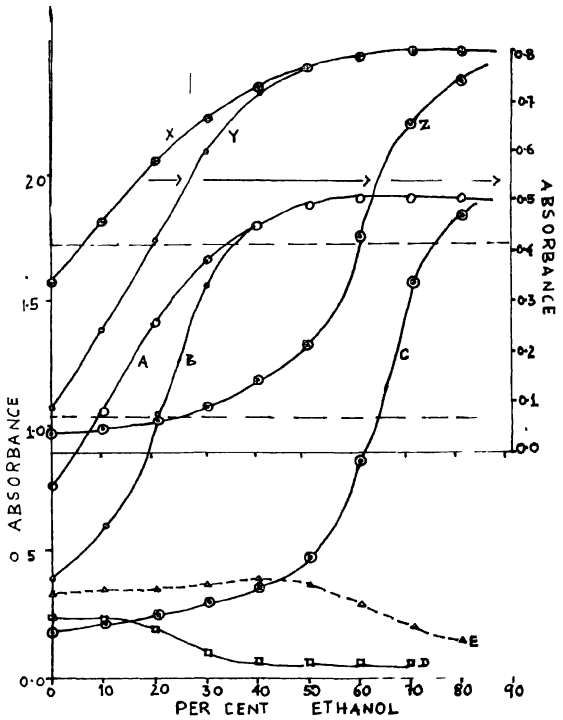


Fig 4

metachromatic band (μ-band) (Fig. 4D,E) and increasing absorbance at the monomer band (α-band) (Fig. 4B,C,Y,Z) of the dye. Evaluation of half-plateau value[6] provides a sharper measure of stability of metachromatic compounds. Fig. 4 represents the role of ethanol over the different systems. Curve 4A is the absorbance at the α-band of the 1.0×10^{-5} *M* PCYN in water and 4X is the corresponding curve for DMMB. Disruption of metachromatic compounds of PCYN-DI and DMMB-DI to the extent of 50% by ~21%(Curve 4B) and ~20% (Curve 4Y) ethanol respectively, compared to ~63% and ~59.9% ethanol for PCYN-DS and DMMB-DS respectively, points to lower stability of metachromatic compounds of DI with different cationic dyes and to poor chromotropic ability of DI.

Experimental

The mucilaginous material from the core of four Chalta (*D. indica*) fruits (half-ripe, ~1 kg each) was stirred with water (200 ml) and the viscous solution (pH 5.5) was filtered, centrifuged and the supernatant filtered. The polysaccharide material in the filtrate was precipitated with ethanol (300 ml) in presence of potassium acetate (7.5 g). The precipitate (1.65 g) was collected by centrifugation and dried by solvent (ethanol, acetone) exchange. The precipitate (1.5 g) was then dissolved in KOH (0.1 *M*), flushed with nitrogen and left overnight under nitrogen atmosphere at room temperature (25°). The solution was centrifuged and the supernatant neutralised (pH ~8) with cold AcOH and dialysed (24 h) against water. The bag content was centrifuged and a small precipitate appearing was rejected. Polysaccharide material in the supernatant was precipitated with ethanol (250 ml) in presence of potassium acetate (7 g). The polysaccharide (DI, 0.5 g) was dried in the usual way.

Methylene blue (MB) and toluidine blue (TB) (both E. Merck) were purified through recrystallisation[6]. Dimethyl methylene blue (DMMB), pinacyanol chloride (PCYN) and dextran sulphate (DS) (Serva, Fein biochemica, Heidelberg) were used as such. Stock solutions of the dyes MB, TB and DMMB were made in double-distilled water. Stock solution of PCYN (~30 mg) was made in methanol (5 ml) and the volume made up to 100 ml with double-distilled water. The dye solutions as well as the experimental solutions were stored in dark.

Equivalent weight of the polysaccharide was determined through conductometric titrations[15] of its aqueous solution (50 mg) with 0.1255 *N* HCl. Conductance was measured with a Systronics 303 conductometer. Absorbance was measured with a Toshniwal CL 10A4 spectrophotometer.

Disruptive factors like urea and ethanol lead to reversal of metachromasia[6] and signify stabilities of the metachromatic compounds. Reversal of metachromasia was studied by measuring the absorbance of the metachromatic compound of different systems at both the monomer band (α-band) and the metachromatic band (μ-band) and also of the dye concerned at the monomer band (α-band) on addition of ethanol/urea in increasing amounts.

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