

MECHANISM OF CHITOSAN-INDUCED METACHROMASY

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Abstract

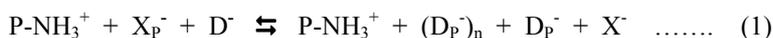
Three mechanisms to explain polyelectrolyte-induced metachromasy are discussed in the light of new experimental evidence obtained from studies involving interaction between chitosan and anionic dyes. The results from experiments including viscosity measurements, determination of the stoichiometry of interaction between chitosan and a divalent anionic dye, and the metachromatic changes produced on casting films from polymer/dye solutions confirm the inadequacy of any mechanism involving ion-pair formation between dye ions and polyelectrolyte chain. The importance of the volume occupied by the random coil of a polyelectrolyte chain in determining its efficiency as a chromotrope is demonstrated by the effect of solution concentration on the levelling-off absorbance value in metachromatic titrations, and the influence of the molecular weight of the chitosan on the ease of reversal of metachromasy by excess polyelectrolyte. These results support the solution aggregation mechanism which does not require ion-pair formation for metachromasy to be induced. Although these results were all obtained using chitosan as the sole polyelectrolyte they may be applied generally to polyelectrolyte-induced metachromasy since chitosan-induced metachromasy has been shown to exhibit all the characteristic features of the more recognised metachromasy induced in cationic dyes by anionic polyelectrolytes.

Introduction

The currently accepted mechanism of polyelectrolyte-induced metachromasy involves the formation of ion-pairs between dye ions and the oppositely charged sites on the polyelectrolyte chain, followed by aggregation of the bound dye ions. Within this overall mechanism there are two theories regarding the mode of dye ion binding to the polyelectrolyte chain prior to their aggregation. In the Michaelis theory [1-4] dye ion binding does not occur at random but rather at a site adjacent to an already occupied site. In the Shirai theory [5-10] dye ion binding does occur at random along the chain and this is followed by conformational changes in the polyelectrolyte chain which bring the bound dye ions close enough together to permit aggregation.

Both these versions of the ion-pair mechanism were developed from observations solely on the induction of metachromasy in anionic polyelectrolyte/cationic dye systems. It is only in the last 20 years that chitosan has been recognised as inducing metachromasy in anionic dyes [11-14]; prior to this it was assumed that it could only be induced by anionic polyelectrolytes [15, 16]. The use of chitosan rather than an anionic polyelectrolyte as the chromotrope has a number of advantages including the ease with which the cationic charge density along the chain may be altered and the fact that the structural variety available in anionic dyes is very much greater than that available in cationic dyes. Based on studies of chitosan-induced metachromasy Gummow and Roberts [13]

proposed an alternative mechanism, the solution aggregation mechanism, that does not involve initial ion-pair formation. It depends on the fact that the counter ions of polyelectrolytes are not uniformly distributed throughout the solution but are concentrated within the electrostatic domains of the individual polyelectrolyte chains, with only a small fraction distributed throughout the bulk of the solution [17]. Thus the counter ions form a relatively concentrated solution within the electrostatic domains of the polyelectrolyte chains. In the solution mechanism the dye ions exchange for the counter ions of the polyelectrolyte chain, thereby forming a concentrated dye solution within the confines of the random coil of the polyelectrolyte chain, thereby giving rise to normal concentration-induced aggregation of the dye ions within the coil domain:



where

P-NH_3^+ = protonated amine groups on the chitosan chain

X_p^- = counter ions within the electrostatic domain of the protonated chitosan chain;

X^- = counter ions without the electrostatic domain of the protonated chitosan chain;

D^- = unaggregated dye ions without the electrostatic domain of the protonated chitosan chain;

$(\text{D}_p^-)_n$ = aggregated dye ions within the electrostatic domain of the protonated chitosan chain;

D_p^- = unaggregated dye ions within the electrostatic domain of the protonated chitosan chain.

Within the coil domain there will be a dynamic equilibrium between the concentration of aggregated and unaggregated dye that will shift towards the unaggregated form as the total concentration of dye within the electrostatic domain decreases.

Aggregation of the dye ions lowers the total energy of the system by reducing the extent of contact between water and the hydrophobic surfaces of the dye molecules, and by increasing the entropy of the system through the release of structured water molecules from the hydrophobic surfaces removed from contact with the water [18]. It is this lowering of the total energy of the system which is the driving force that moves the equilibrium of equation (1) over to the right-hand-side.

Materials and Methods

Materials

Chitin was prepared in the laboratory from squid pen supplied by Dr A Falshaw of Industrial Research Ltd., Lower Hutt, New Zealand, and this was converted to chitosan using 45% (w/w) NaOH at a temperature of 60°C. A portion of the high molecular weight chitosan obtained initially was degraded by nitrous acid treatment to obtain lower molecular weight materials.

The dyes CI Acid Orange 7, CI Acid Red 13 and CI Acid Red 88 were commercial samples which were purified as described previously [11].

Methods

Visible spectroscopic measurements were made using a Perkin Elmer 551S spectrophotometer and metachromatic titrations were carried out as described previously [11].

Viscosity measurements were carried out at $25.0 \pm 0.05^\circ\text{C}$ using a suspended-level viscometer containing an internal sintered glass filter.

Results and Discussion

A series of experiments have been carried out in an attempt to distinguish between the solution aggregation mechanism [13] and the two ion-pair mechanisms [1-10]. The experiments fall into two groups:

1. those that cannot be explained by either of the two ion-pair mechanisms;

- those that demonstrate the importance of the coil domain volume of a polyelectrolyte chain in the metachromasy-induction process.

Before discussing these experiments however it is worth pointing out that both ion-pair mechanisms must be considered improbable on theoretical grounds since an aggregation process involving dye ions bound to the polyelectrolyte chain through ion-pair formation would result in a very large loss of the conformational entropy of the polyelectrolyte chain.

Experimental results that cannot be explained by either ion-pair mechanism

1. Film formation

The metachromasy induced by addition of chitosan to a solution of C.I. Acid Red 88 (AR 88) is reversed on casting a film from the solution; as the film forms the colour reverts from the metachromatic orange, with $\lambda_{\max} = 450$ nm, to the orthochromatic red, with $\lambda_{\max} = 505$ nm (see Table 1). Conversely, the addition of the non-ionic hydroxyethyl cellulose (HEC) to a solution of AR 88 does not induce any metachromasy, both the dye and the polymer/dye solutions having $\lambda_{\max} = 505$. However a film cast from this solution exhibits the orange metachromatic colour with $\lambda_{\max} = 455$ nm (Table 1).

Table 1. Values of λ_{\max} for solutions of C.I. Acid Red 88 in the presence of chitosan and of hydroxyethyl cellulose, and of films cast from the solutions.

Co-solute	Measured λ_{\max} value		
	Solution of dye	Solution of dye + co-solute	Cast film
Chitosan	505 nm	450 nm	505 nm
HEC	505 nm	505 nm	455 nm

These results show that the dye aggregates that are present in the chitosan/AR 88 system due to the induced metachromasy break down as the water evaporates. As ion-pair formation is more likely to occur as the concentration increases, the result for chitosan/AR 88 suggests that metachromasy is destroyed as the conditions become more and more favourable for ion-pair formation. This is supported by the fact that the precipitate that may form in solutions of chitosan/AR 88 on standing, and which may be assumed to be the dye anion salt of the protonated chitosan, exhibits the red colour that is characteristic of the orthochromatic state. Under conditions where ion-pair formation is not possible, such as the HEC/C.I. Acid Red 88 system, dye aggregation leading to metachromasy occurs through increase in dye concentration as the water evaporates.

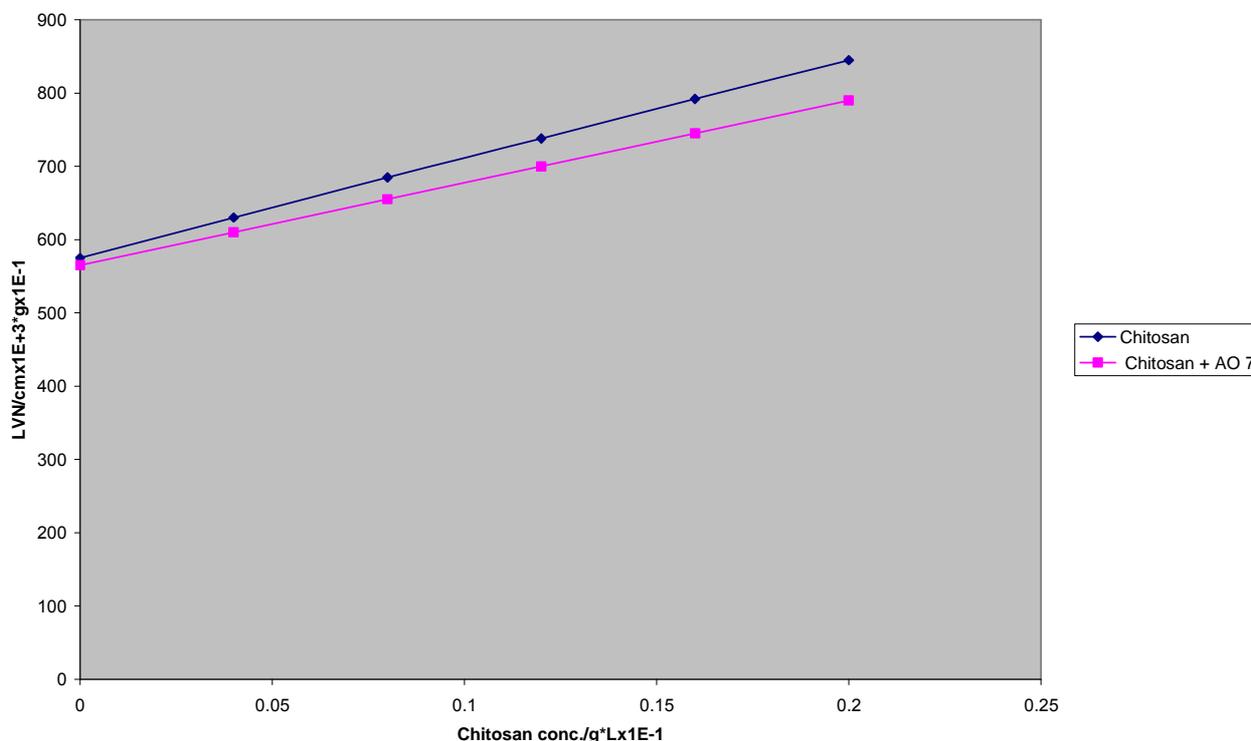
2. Viscosity measurements

If polyelectrolyte-induced metachromasy does arise from ion-pair formation followed by aggregation, then the presence of a metachromatic dye should have a considerable effect on the viscosity of a solution of the polyelectrolyte involved. Aggregation based on the Michaelis model [1-4] would increase the rigidity of the chains considerably due to the reduction in rotation around the backbone bonds, and hence give rise to a pronounced increase in viscosity. Alternatively, aggregation based on the Shirai model [5-10] would tend to convert the polyelectrolyte chains to compact spheres thereby causing a large decrease in viscosity. On the other hand it would be expected that the presence of aggregated dye ions within the coil domains of the polyelectrolyte chains, as proposed in the solution aggregation theory [13], would have little effect on the viscosity.

A 0.4 g l⁻¹ solution of chitosan in 0.1 M acetic acid/0.01 M NaCl was mixed with an equal volume of a solution of C.I. Acid Orange 7 in 0.1 M acetic acid/0.01 M NaCl. The concentration of NaCl present should reduce the induced metachromasy by less than 20% [12] and this was confirmed by u.v/visible spectroscopy. This solution was diluted by addition of aliquots of 0.1 M acetic acid/0.01 M NaCl solution and the flow times recorded. The Limiting Viscosity Number was determined and

compared with that for another solution of chitosan in 0.1 M acetic acid/0.01 M NaCl but without any C.I. Acid Orange 7 present (Figure 1).

Figure 1 : Determination of Limiting Viscosity Number of chitosan in the presence and in the absence of the metachromatic dye C.I. Acid Orange 7.



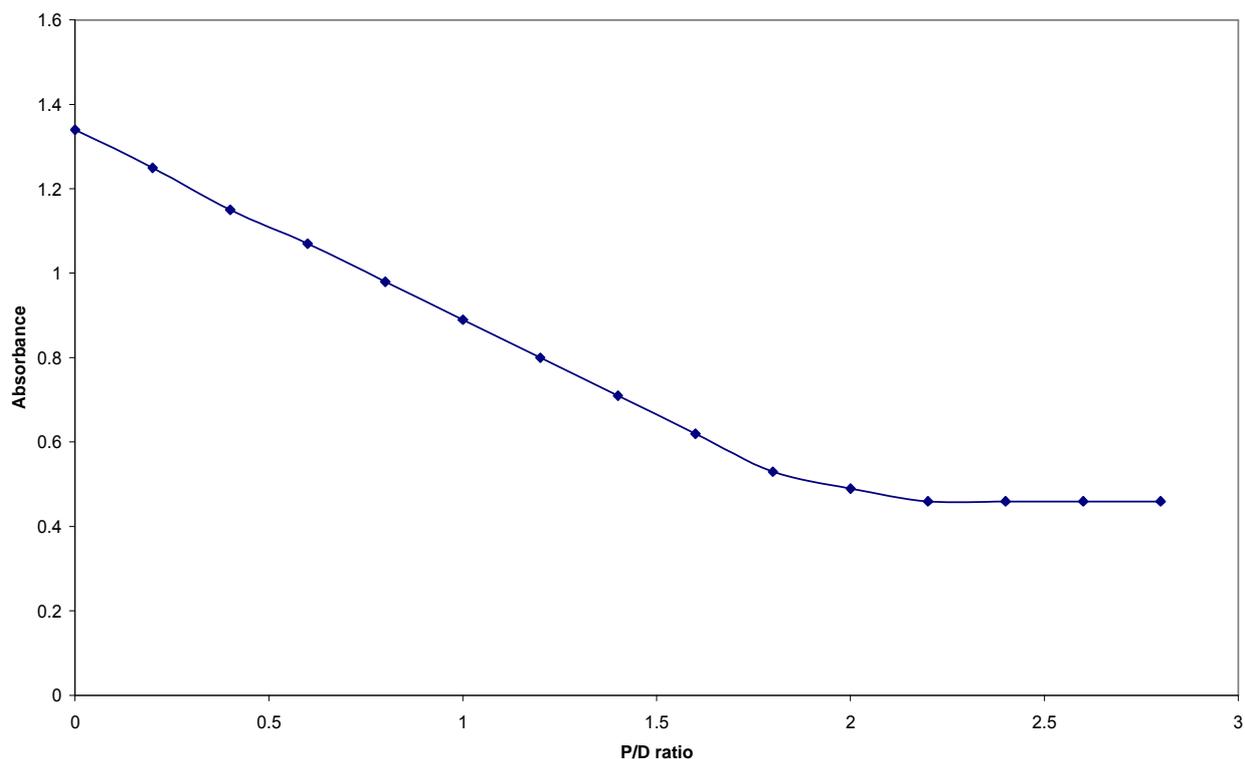
It is obvious from Figure 1 that the induction of metachromasy in a dye solution has very little effect on the viscosity of the solution. In the current example the LVN values were 575 and 565 $\text{cm}^3 \text{g}^{-1}$ in the absence and in the presence respectively of AO 7 in the metachromatic state. A much greater effect on the viscosity would have been expected if the mechanism of chitosan-induced metachromasy involved ion-pair formation followed by any form of aggregation. However, because of the slightly higher electrolyte content due to the dye content, a slight decrease in viscosity would be expected if the solution aggregation mechanism is correct.

3. Stoichiometry of metachromatic titrations of divalent anionic dye with chitosan.

In metachromatic titrations the ratio in the solution between the molar concentrations of charged sites on the polyelectrolyte chains and of dye ions is called the Polymer:Dye ratio (P/D). Typically, since to date all metachromatic titrations have been carried out using monovalent dyes, the end point or equivalence point occurs at a P/D ratio of 1:1. The use of chitosan as a chromotrope enables this to be tested with dyes containing more than one charged group.

A titration was carried out using C.I. Acid Red 13 as the metachromatic dye; this has a similar structure to C.I. Acid Red 88 except that it contains two $-\text{SO}_3\text{Na}$ groups. Figure 2 is the plot of Absorbance *versus* P/D ratio for this titration and clearly shows that the equivalence point at a P/D ratio of approximately 2.0. That it occurs at slightly less than 2.0 is due to the fact that commercial C.I. Acid Red 13 contains a small proportion of C.I. Acid Red 88, due to incomplete sulphonation of 2-naphthol during synthesis of the intermediates, and this component is not normally by recrystallisation [19]. Since the latter dye would have its equivalence point at a P/D ratio of 1.0, the presence of it as an impurity in C.I. Acid Red 13 would cause a small reduction in the P/D ratio at the equivalence point.

Figure 2 : Metachromatic titration of the divalent dye C.I. Acid Red 13 with chitosan



This P/D value means that at the equivalence point is reached when there are two positively charged sites on the polyelectrolyte chains for every dye ion present and it is not possible to construct a realistic model that would enable each dye ion to form two ion-pairs with the polyelectrolyte chain. However if aggregation of dye ions in solution within the electrostatic domain of the polyelectrolyte chains is responsible for metachromasy, and ion-pair formation is not involved, then the stoichiometry at the metachromatic equivalence point should indicate a P/D ratio of 2:1 (a site:sulphonic acid group ratio of 1:1).

Experimental evidence that demonstrates the importance of the random coil volume of a polyelectrolyte chain in the metachromasy-induction process

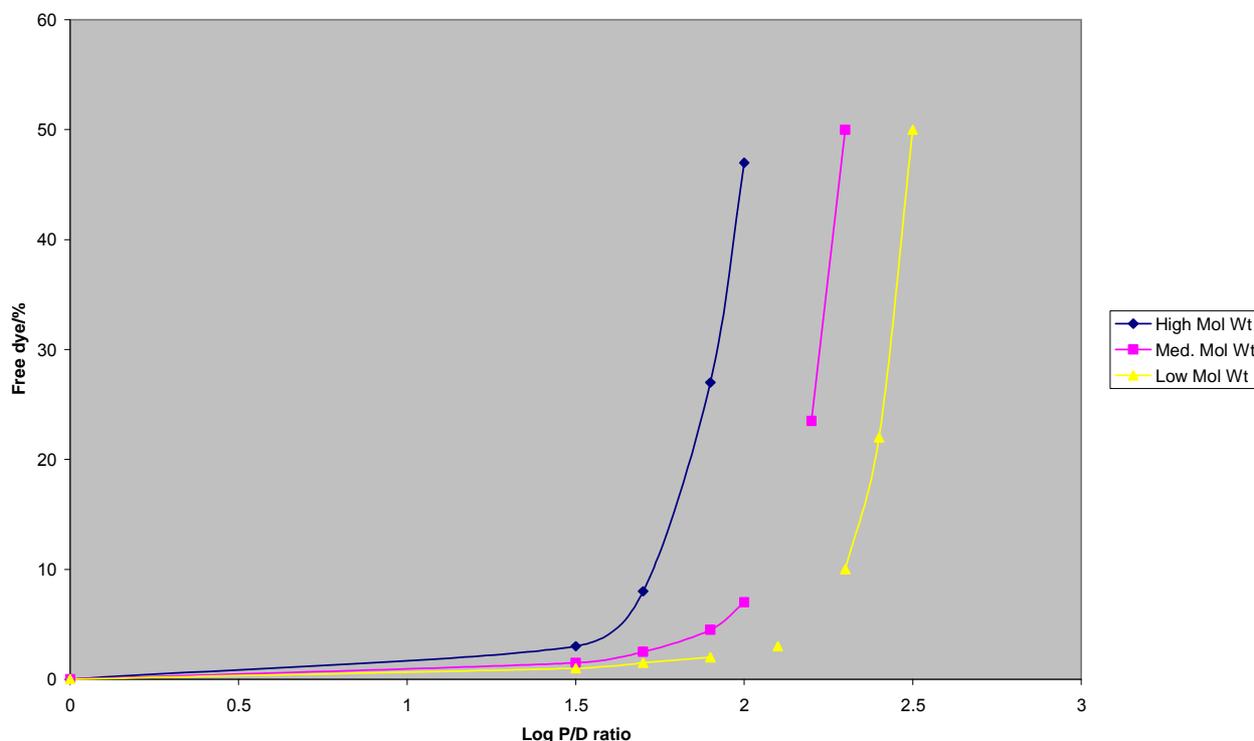
If the solution aggregation mechanism is correct then any factor that increases the volume of the random coil domain of the polyelectrolyte chains, without altering the linear charge density, will reduce the concentration of any ionic species within the domain. This would have the effect of reducing the extent of dye aggregation within the domain, as this will be concentration dependent, and hence affect the observed metachromasy. However no such effect should be observed if the ion-pair mechanism is correct since the local environment for any dye bound to the chain will be the same regardless of the volume occupied by the chain in solution.

1. Effect of chitosan molecular weight on the ease of reversal of metachromasy.

One interesting aspect of chitosan-induced metachromasy, and of polyelectrolyte-induced metachromasy in general, is that the effect can be gradually reduced by adding polymer in considerable excess over that required to reach the equivalence point. Since the effective coil volume of a polymer chain in solution increases more rapidly with increasing molecular weight than does chain length [20], reversal of metachromasy by addition of excess chitosan should be easier – that is, it should require less polyelectrolyte to produce a given percentage of free dye – the greater the molecular weight of the chitosan.

The ease of metachromasy reversal using three chitosan samples of low, medium and high molecular weight but with similar F_A values was investigated and the results are plotted in Figure 3.

Figure 3 : Reversal of metachromasy in the chitosan/C.I. Acid Orange by addition of excess chitosan: effect of chitosan molecular weight



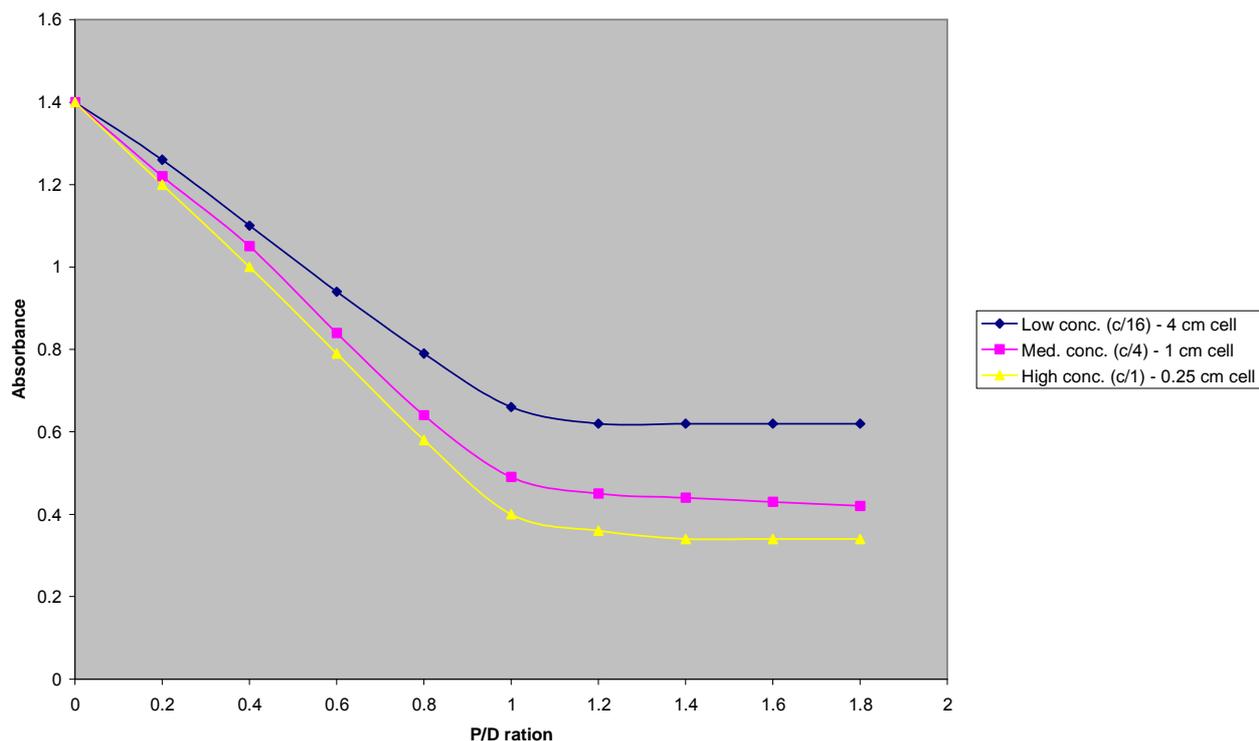
This clearly shows that the effectiveness of excess chitosan in reversing metachromasy increases with increase in molecular weight and hence with increase in the volume of the random coil domain of the chitosan chains.

3. Effect of chitosan concentration on the leveling-off absorbance in metachromatic titrations.

The amount of dye associated with a given polyelectrolyte chain at the equivalent point in metachromatic titrations, that is at a P/D ratio of 1:1 in the case of a dye ion carrying a single charge, will be the same regardless of the volume of the random coil domain of the chain. However polyelectrolyte chains expand their random coil volume on dilution so that the concentration of the polyelectrolyte within this volume will be less the lower the concentration of the polyelectrolyte, although the actual amount of dye will be the same. Hence the equilibrium between unaggregated and aggregated dye ions should be shifted in favour of the unaggregated species as the polyelectrolyte concentration used in the titration is reduced.

A stock solution of 0.4016 g L^{-1} in 0.1 M acetic acid was made up together with an approximately $2 \times 10^{-3} \text{ M}$ solution of C.I. Acid Orange 7 (the 'high concentration' solutions). These were each diluted by a factor of 4 to give the 'medium concentration' solutions which were further diluted by a factor of 4 to give the 'low concentration' solutions. Metachromatic titrations were then carried out using match cells of 0.25 cm , 1.0 cm and 4.0 cm path lengths for the 'high', 'medium' and 'low' pairs of solutions respectively (Figure 4).

Figure 4 : The effect of polyelectrolyte concentration on the absorbance value at the equivalence point in metachromatic titrations.



The results show clearly that although the equivalence point occurs at the same P/D ratio in each case, the absorbance value at this point increases with decrease in the chitosan concentration, indicating the predicted shift towards unaggregated dye as the concentration of polyelectrolyte used in the titration is decreased.

Conclusions

The observed spectral changes on casting films from solutions of chitosan and hydroxyethyl cellulose containing C.I. Acid Red 88, the results from viscosity measurements on solutions of chitosan both in the presence and absence of a metachromatic dye, and the stoichiometry of the metachromatic titration of the divalent anionic dye C.I. Acid Red 13 with chitosan, clearly demonstrate that neither proposed mechanism of polyelectrolyte-induced metachromasy that involve ion-pair formation between dye ions and oppositely charged groups on the polyelectrolyte can be correct for chitosan-induced metachromasy. However the results are readily explained by the solution aggregation mechanism.

The influence of the chitosan molecular weight on the ease of reversal of metachromasy by addition of excess chitosan, and the effect of chitosan concentration on the equivalence point absorbance value demonstrate the importance of the volume of the random coil domain of the chitosan chain in determining its effectiveness as a chromotrope. These effects may be predicted from the solution aggregation mechanism but again highlight the incorrectness of the mechanisms based on ion-pair formation.

Although these results have been obtained solely for the chitosan/anionic dye system, the fact that chitosan-induced metachromasy has been previously shown to be the same in all characteristics as the traditional anionic polyelectrolyte/cationic dye systems strongly suggests that the solution aggregation mechanism applies to all polyelectrolyte-induced metachromasy regardless of the ionic character of the chromotrope.

Acknowledgements

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