CHAPTER - I

Introduction and Review of Previous Work

A. Dye-dye interactions in solution:

For about a century it has been known that the colour and luminescence properties of ionic dyes can vary with their concentration or substrate composition. These colour changes are first recognised and used for histological purposes in connection with the differential staining of tissues (1). It also became apparent, particularly in the field of textile colouring, that certain dyes disobey the Beer's law relation and change their absorption characteristics. The spectra of aqueous solutions of several cationic dyes are dependent on the concentration of the dye (2). In the visible region these spectra are characterised by several maxima, the intensities of which depend on the dye concentration. The absorption band of the dye at longer wave length is characteristic of very dilute solutions and usually referred to as α band. Increasing dye concentrations results in the gradual replacement of α-band by a band with a shorter wave length which is assigned as β band (2-6). Further increase in dye concentration may cause substitution of β band by another diffuse band closer to the blue range of the spectrum. This band is assigned as γ band. Such concentration changes may also cause the appearance of a new maximum at wave lengths longer than the α-band; if this new transition is narrow, intense and exhibits resonance fluorescence, it is called a J-band after Jelley (7) who, together with Scheibe (8), first described its properties.

The α-band is usually referred to the unperturbed transitions obtained in highly dilute aqueous or alcoholic solutions and is attributed to the monomeric form of the dye while the deviations from Beer's law and the appearance of the β, γ and J bands are attributed to dimeric and higher aggregates of the dye respectively (9-11). This spectroscopic phenomenon is called metachromasy and cationic dyes which show this effect are known as metachromatic dyes (12). The phenomenon is exhibited only by dyes in which the characteristic ionic charge is an integral part of the chromophoric system and is distributed throughout the system by resonance (13), that is, most common basic dyes and a few acid dyes and the same arguments apply to dyes, such as fluorescein, with a distributed negative charge.

Many dyes when used to colour certain tissue constituents (chromotropes) absorb light of wave length shorter than that absorbed by the dyes in solution (1). Similar metachromatic behaviour is exhibited in solution when the dyes interact with soluble chromotropes, for example nucleic acids (14), Polyphosphates (15) and gelatine (16). Metachromasy may also be induced in solution in the absence of chromotropic substances in three different ways (17); by increasing the dye concentration (dye-induced), by adding salt (salt-induced) and by lowering the dielectric constant of the solvent (solvent-induced).

The dimerization of many organic acids and similar compounds is usually attributed to hydrogen bonds. But the association of

dye stuffs must be explained in a different way for the following reasons:

(i) Dye stuffs dimerize more strongly in aqueous solution than in organic solvents, whereas carboxylic acid dimers exist in the vapour and in non-polar solvents, but are dissociated in water.

(ii) In contrast to the behaviour of the dimerizing carboxylic acids, the polymerization of dye stuffs does not stop at the dimeric state, but often proceeds to trimers and higher polymers.

(iii) Colourless leuco dyes dimerizing to a smaller extent than the corresponding coloured forms.

These facts agree with the postulate that the polymerization of dyes is due to additive forces of van der Waals type. It may seem that these forces are too weak to account for dimerization energies of the order of 4 to 7 K Cal/mole (18). However, according to London's theory (19), the mutual potential energy of two identical molecules possessing a single long-wave electronic absorption band λ_o (a description which fits most dye stuffs) is to a first approximation proportional to $f\lambda^4$, where f is a measure of the excitation probability (called number of absorption electrons or oscillator strength). Since large values of f and λ are exactly the properties which make a compound strongly coloured, the attractive forces between dye stuff molecules must be considerably larger than those between similar coloured molecules (e.g. the corresponding leuco dyes). These forces are additive and thus account for the formation of dimers as well as polymers.

The (virtual) dipole-dipole attraction of London in the case of dye stuffs should be supplemented by dipole-quadrupole and quadrupole-quadrupole terms. Since the two ions are of the same sign, one must substract the coulombic electrostatic repulsion energy, $\frac{z^2e^2}{Dr}$, from the London-Margenau (20) attraction energy. Finally, the usual repulsion energy between neutral molecules must also be taken into account. So the dimer is probably held together by London dispersion forces and "hydrophobic bonding" (21) which overcome the electrostatic repulsion between two dye cations. The dispersion forces should be greatest when the monomer units are in a sandwich with principal molecular axes parallel. The coulombic repulsion will be minimized when the charged amino groups (e.g. in the case of thiazine dyes like methylene blue) lie along opposite edges of the sandwich.

Deviations from Beer's law with the appearance of β, γ or J bands have been rationalized in terms of dye aggregation, i.e., the formation of dimers, trimers, and n-mers, and the accompanying spectral changes were first interpreted by Förster with a classical oscillator model (22,23). These spectral changes of dye-dye interactions were later explained with the theory of energetically delocalized states, i.e., excitons by a number of researchers (24-28). The essential feature of this treatment (29, 30) is that the excitation achieved in a single molecule of a periodic molecular assembly is transferred by coupled oscillation from molecule to molecule in a period which is shorter than the vibration time of the component molecules in the assembly. The

McRae Kasha exciton model (25) is described here in short restricting our initial considerations to the specific case of dimeric dye molecules. It is assumed that the axis of the composite molecule, the dimer in this case, parallels its transition polarization axis, i.e., the transition dipole is placed along the long axis of the molecule as shown schematically in Fig. 1. Upon

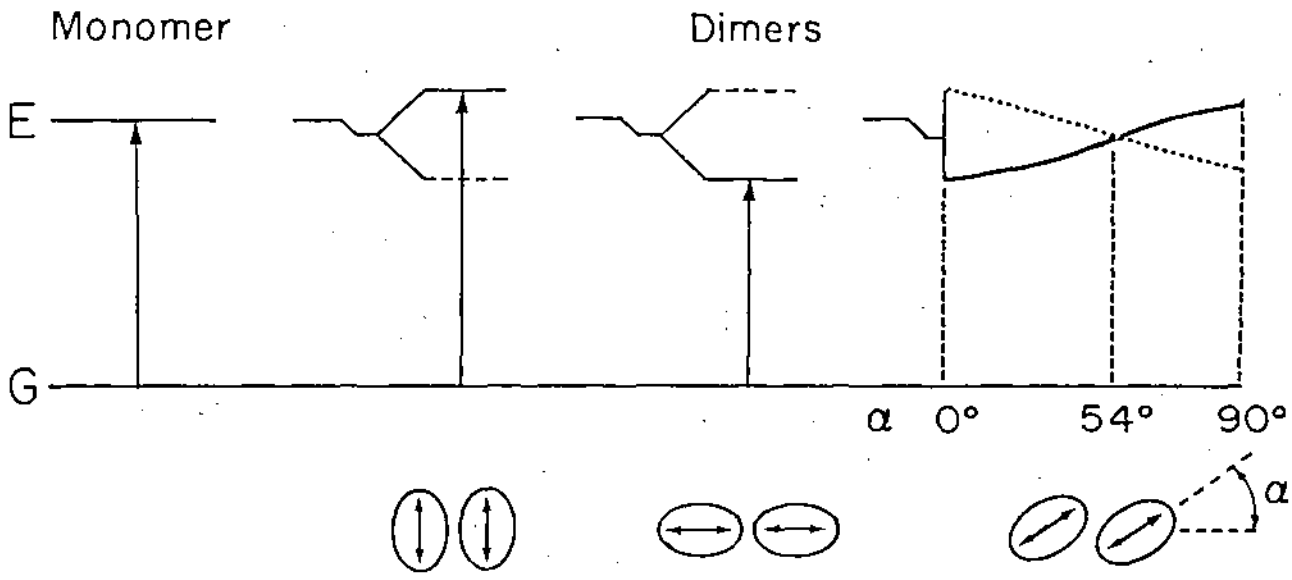

Figure 1.
The energy level diagram for a dye monomer and its dimers shows that the "allowedness" for dimer transitions from the ground state (G) to the split excited states (E) depends on the angle (α) between the transition dipoles and the molecular axis of the aggregate.

excitation of the dimer from its ground state (G), the model provides for splitting of the excited state (E) because of electronic degeneracy. The angle (α) between the transition dipoles and the molecular axis of the aggregate determines whether a transition is allowed to the lower or the higher excited-state levels. The ground states of the monomeric and dimeric molecules are shown in Fig. 1

to be fixed at the same relative position although the point-multipole expansion employed by Kasha provides for a displacement of the dimer ground-state because of van der Waals interaction. A common ground state assumption seems to be an over simplication in view of the effect of dye aggregation on such ground-state properties as infrared transitions (31-33) redox potential and basicity (34). Excitonic interactions, however, can be conveniently discussed in terms of energies normalized to a common ground state. Thus Fig. 1 shows that when the transition dipoles are in line with the molecular axis of the dimer, i.e., when $\alpha = 0$, then the transition to the lower excited level will be allowed and as a result the maximum absorption of the dimer will be red-shifted relative to the absorption of the monomer. Therefore, a red shift, as observed in J-band formation, will occur as long as the angle α is less than about 54° while if the angle is greater, the transition to the higher of the split excited levels will be allowed so that the singlet-singlet transition of the dimer will be blue-shifted relative to the monomeric dye. The latter case is the one most frequently observed and the interaction between two conjugated, (near) planar dye molecules — the van der Waals or London-force dimers - suggests sandwich structures in which the transition dipoles are parallel to each other.

One can, therefore, conclude in general that the dimeric structures of the dyes consists of two monomers held together with their planes, or at least their chromophores essentially parallel to each other, with some deviations from parallelism because of thermal vibrations or structural asymmetry.

One important consequence of the sandwich dimer model and its split excited-state levels is the expectation of an intense blue-shifted transition relative to the $0 \longrightarrow 0$ maximum of the monomer, as well as weak red-shifted band associated with the forbidden transition of low energy. The energy difference between the monomer maximum and that of the blue-shifted dimer should be approximately proportional to the dye's extinction coefficient or, more precisely, to its transition dipole moment.

The spectral and thermodynamic characteristics (i.e. ΔG, ΔH) for dimer formation of various thiazine and cyanine dyes in aqueous solution (35) and related results, like those of Wörz and Scheibe for non aqueous dimers (36) lead to the following generalizations:

(a) Delocalization of π-electrons favours dimerization of cyanines. Accordingly, dimerization is enhanced by the number of HC = CH groups in a vinylogous series (37).

(b) Dimerization is favoured by increased hydrophobicity of the dye such as from lengthened N-alkyl chains (35,36).

(c) The dimerization process is insensitive to the sign of the cyanine's ionic charge although an increase in the charge density per molecule is expected to interfere with aggregation(35).

(d) Non planarity of the dye leads to diminished dimerization. This structural feature can be induced by cis-configurations in cyanines or by highly crowding substituents which also depress

the dye's extinction coefficient (36,38).

(e) The β-band is diminished and the α-band is intensified by increasing the temperature of a dye solution (3,10) and this reversibility suggests that the α and β bands correspond to distinct species of the dye.

Thermodynamic considerations indicate that the standard free energy of aggregation of charged dyes includes positive terms from electrostatic contributions and from mixing, e.g. the conversion of two monomers to a single dimer, as well as negative terms from dispersion and from hydrophobic forces (39-41).

In sharp contrast to the above aggregation theory of metachromasy of dyes in solution, Millson and McKay (17,42-44) during mid sixties in a series of papers attempted to explain metachromasy of cyanine dyes, rhodamine B, methylene blue, and some other dyes in terms of an hypothesis involving the association of the dye with its counter ion, following the suggestion of Feichtmayr and Schleg (45) for some triphenyl methane dyes. They argued that because the dyes in question are all salts, so as their concentration is raised from infinite dilution, interactions between dye ions and counter-ions would be expected, not merely because of Debye-Hückel effects, but because the dye ions are large and polarizable and much less soluble in water than simple salts. The close approach of such a dye ion and a counter-ion involves strong electrostatic interactions which would be expected to affect the distribution of charge over the chromophoric system of the dye ion, and hence its visible spectrum. McKay and Millson

further maintained that their hypothesis was consistent with the same type of metachromasy that occurred with the addition of salts as well as when the dielectric constant of the solvent was lowered, as such lowering greatly enhanced the ion-counter ion interaction. It thus appeared that their hypothesis was in direct conflict with that of Scheibe (9). But now this view seems no longer viable following Paddy's potentiometric evidence of normal counterion activity under conditions where these bands are formed (6,46). Moreover, the influence of salt on the spectra of cationic dyes can be at least partly predicted from activity coefficients calculated by the Debye-Hückel limiting law (39,40,41).

Paddy suggested that, independant of the possible existence of ordered water at the hydrocarbon surface of large organic dye molecules, the attraction between water molecules at the expense of water-dye interactions made significant contributions to the energetics of dye dimerization (6,46,36). Following the early work of Scheibe and Sheppard (8,9,47,48), the importance of the participation of London-van der Waals dispersion forces for the self-association of dyes was recognised. The observation of West and Pearce (37) that increasing the vinylogs in thiacyanines causes both blue-shifting and enhanced dimerisation strongly supports the view that dispersion forces associated with delocalized electrons provide the major contribution to dimerization of dyes. Insensitivity of this process to the ionic charge of the dye also supports this conclusion (35).

Aside from the cited hydrophobic and dispersion forces, electrostatic forces also have to be considered since they may play a dominant role in dye-dye interactions. Although formamide, with a dielectric constant greater than water, does not induce dye aggregation under ambient conditions, the preeminence of water in promoting the self-association of large surfactant or dye ions was often ascribed to the high dielectric constant of this solvent. Since its bulk value is likely to be misleading when applied to the solvation shell of an organic solute, a screening factor was introduced to express the effective dielectric constant experienced by the microscopic solvent/dye system (39-41). In general, additives that increase the effective dielectric constant will reduce repulsion between similarly charged organic ions and thus facilitate their interaction. The opposite effect is found from additives that diminish this constant. As a broad generality it can be stated that increase salt concentration causes an increase in the screening factor or effective dielectric constant and thus promotes aggregation. Different inorganic salts have slightly different effects on the dye aggregation (13,49,50) and the extent of dimerization in the case of alkali metal chlorides at higher concentrations is in the order : CsCl > NaCl > LiCl. This effect may be due to the presence of positive ions which build up solvent regions from which the dye molecules are excluded because of electrostatic repulsion; as a consequence dye concentrates in the remaining free volume.

In this respect the behaviour of tetraalkylammonium salts is peculiar as reported by Berone et al (51); they act like salts at lower concentration promoting the dye association while at higher concentrations the hydrophobic component of the molecule prevails and behave like non-ionic cosolute promoting the dye dissociation.

Independent of the magnitude of their dielectric constants, non aqueous organic media are often good solvents for dye ions, and with few exceptions (52-54), such media require low temperatures or high concentration of the dye to induce formation of dimers or higher aggregates (55,31,56,57). A shift from the α to the β absorption band was found for acridine orange in methyl-alcohol and formamide in the concentration range 0.01 to 0.1 molar. The dimerisation constants in these cases are two to three orders of magnitude lower than in water (58). Protic solvents are not required for dimerisation; in fact, the dimer spectrum of a phthalocyanine dye in benzene or CCl_4 is virtually identical with the dye's crystal spectrum (52). For deaggregation to occur, it would be sufficient if the dye experiences a micro environment of low effective dielectric constant that promotes stability of dye monomers. Regarding mixture of solvents, unsettled questions remain about their effective dielectric constants; nevertheless, the increasing deaggregation activity of methanol, acetone and

dioxane in aqueous dye solutions may have a similar basis and is consistent with a decrease in the bulk dielectric constants of the mixture (59).

Robinson et al (41) had investigated in detail the thermodynamics of association of the positively charged dye, acridine orange spectrophotometrically in water as a function of ionic strength and in the presence of added methanol, urea and dioxan. The extinction coefficients ϵ_M, ϵ_D and $\epsilon_{st.}$ were measured which are characteristic of the spectra of monomer, dimer and long aggregates of the dye molecules respectively. A theoretical model system, based on short-range stacking and long-range electrostatic interactions, had been used to interpret the thermodynamic data. Their results suggested that both factors are of importance in determining the tendency to aggregate. Furthermore, when the solvent is varied they are related by a compensation effect; decreases in stacking interactions are paralleled by decreases in repulsive electrostatic interactions.

The marked difference between the dimerization constants of dyes in water and in non-aqueous polar solvents claims for some other cause which is not solely the trivial high medium dielectric constant but must be connected with the peculiar structure of water. Actually, the data obtained in CH_3OH and $HCONH_2$ for acridine orange seem to show some dielectric constant effect, even if it does not explain the 8500/30 ratio, between the K_{12}'s in water (ϵ=80) and formamide (ϵ= 109) (58,60). Furthermore, from a comparison of thermodynamic data along a homologous series it

results that molecules having groups to interfere directly with the water structure through hydrogen bonding (i.e. the amino hydrogens) have a much lower dimerization constant and a higher negative value of the dimerization entropy (3,61).

In the formation of a dimer from two monomers, the number of molecules decreases. As a result, an ideal entropy of mixing term is associated with ΔS_D, the so called "cratic entropy" : $\Delta S_C = -R\ln X_2 = -33.4$ joules/^oK mol (in water), X_2 being the solute mole fraction in 1 molar solution (40,21). By substracting ΔS_C from ΔS_D , the entropy change ΔS_U is obtained, characteristic of the interactions associated with dimerization (unitary entropy of dimerization). The data reported in literature (58) show that ΔS_U is generally positive. Since the formation of a dimer from two monomers involves a certain loss of rotational and translational entropy, the positive ΔS_U of dimerization is the result of an extra contribution cancelling several negative terms. Such a contribution has to be found in the structural changes of water connected with dye dimerization. It is noteworthy that increasing the number and/or the length of the aliphatic side chains one observes an increase of the unitarian entropy of dimerization (62).

It is a well known fact that hydrocarbons do not dissolve in water. The unfavourable process of dissolution of hydrocarbons ($\Delta G > 0$) is a consequence of negative hydration entropy ($\Delta S < 0$) the enthalpy effects being small. The negative entropy of hydration

is interpreted as resulting from enhancement of structure of neighbouring water molecules surrounding the hydrocarbon. This special type of interaction of non-polar groups with water is known as hydrophobic hydration (63). The association or aggregation of polar groups which is known as hydrophobic interaction should then be regarded as partial reversal of the thermodynamically unfavourable process of solution. The peculiar features exhibited by aqueous solutions of hydrocarbons or solutes having apolar groups with one or two functional groups have been attributed to the peculiarity of water as solvent.

It has been suggested by Frank and Evans (63) that water molecules at the hydrocarbon portion of dissolved large organic molecules form structural regions of low entropy, the so-called icebergs (64). The aggregation of individual dye molecules in aqueous solution thus results in an increase in entropy owing to the breaking up of these ordered regions of water by the individual dye molecules as they bind to one another. This gain in entropy is accordingly considered to be the driving force in the association of dye molecules in aqueous solution by hydrophobic bonding (65,66). As a corollary, compounds that accept hydrogen bond or break those of water, e.g., urea, phenols should eliminate structured water at hydrocarbon interface and thus eliminate the driving force for their self association.

These concepts had been applied initially to the study of proteins and surfactants but were introduced to dye aggregation

by Mukerjee and Ghosh (39), who established that urea caused deaggregation of methylene blue and the dimerization of this dye was indeed accompanied by an entropy gain (39,40). Such a gain was also reported for Rhodamine B by Rohatgi and Singhal (67); on the other hand, acridine orange thionine and a thiacarbocyanine lost entropy on dimerization as shown by Robinson et al (41), Rabinowitch and Epstein (3), and Maddy (6,46) respectively. Hence, these findings do not support the view of Mukerjee and Ghosh (39) that the driving force in dye aggregation is the entropy gain that results when structured water — the so-called iceberg of low entropy — is displaced by dye/dye contacts, i.e. hydrophobic bonding (65,66). So it appears that except for the long fatty-tail dyes (or micelle formation by surfactants (68-70) which exhibit a near-zero enthalpy) a clear distinction between entropy - and enthalpy-controlled dye-aggregation processes may be premature. This conclusion is supported by the observation that the water-water interaction model can be applied to both hydrophobic and to dispersion forces (41,46).

From all the experimental evidence we may conclude with certainty that the appearance of the β and γ bands in the absorption spectrum of the ionic dyes is due to the sandwich-like stacking of molecules independently of the medium that promotes such a stacking. The blue shift from α to β band due to dimerization has been also predicted theoretically (71,72).

On the other hand some doubts still exist about the forces ruling the dye stacking especially in water solution. According to

some authors (41,73) the main factor is the energy release upon π-electron clouds overlap in the association of dyes, while others claim the existence of strong hydrophobic interaction (40,65,74-76). A critical analysis of the previously reported results seems to suggest in favour of the last hypothesis because the dye association process deeply involves structural changes in the solvent.

B. Dye-Polyelectrolyte interactions:

The interaction between cationic dyes and polyelectrolytes has been widely studied in recent years (77) because of the great interest of these dyes in various fields of biology. These dyes are derivatives of a variety of aromatic compounds, such as acridine, thiazine, xantene, triphenyl-methane, etc. They bind strongly to any poly acid substrate, particularly to biological tissues as the later are constituted of proteins, nucleic acids, polysaccharides that bear ionized groups on their chains. A number of these dyes are used for staining living tissues, some of them have pharmacological properties and their molecules bear similarities with antibiotic molecules such as dannomicine and actinomicine. Many of them have a remarkable mutagenicity being able to interfere in the replication of DNA by causing the deletion or insertion of base pairs (78-81). These facts are valuable reasons to address research to the study of the interaction mechanism between dyes and polyionic substrates.

The binding of acridine dyes to DNA and polyribonucleotides has been particularly studied in detail using several techniques which include dialysis equilibrium (82), viscosity (79,83), optical spectrometry (80,82,84-86), induced optical rotation (87-89), circular dichroism (90,91), fluorescence (83,92), X-ray on fibers (79, 80), and low angle X-ray scattering (93). The great interest in the acridine dyes binding to polynucleotides is mainly due to the remarkable mutagenicity of acridines which can interfere in the replication of DNA by causing deletions or insertions of base

pairs (81,94). However, more than fifteen years ago, Bradley and Lifson (95) pointed out that in spite of the great amount of experimental data gathered with a variety of methods on the interaction between dyes (especially of the acridine series) and biological polyelectrolytes, there was no complete picture comprehensive of all available information on the subject and has not yet led to a unified picture of the binding process. Since then information round-up, focused on the biological interest found in compounds having mutagenic properties, has noticeably increased. Nevertheless, it seems Bradley and Lifson's statement is still standing.

The main feature of the binding of the cationic dyes to biopolymers and to synthetic polyelectrolytes is the so-called "metachromasia" that is a change of the absorption and fluorescence spectrum in the visible region (14). This effect has been suggested as due to different binding modes of the dye molecules on the substrate (84,96) which can exhibit different absorption spectra in the visible region. The absorption bands show that the dye molecules bind as monomer, or they interact with each other on the polyelectrolyte molecule to form dimers or aggregates similar to those existing in concentrated aqueous solutions (3,4). Although during the last decades a large amount of work has been published on the interaction of dyes with synthetic polypeptides, similar study on the binding of the dyes to synthetic polyelectrolytes has been neglected. Synthetic polyelectrolyte molecules are simpler systems and the study of their interaction with dyes should give interesting information of relevance to problems connected with

biological polymers.

The interaction of synthetic polyelectrolytes with metachromatic dyes is similar to that of biological polymers (97,98). The tendency of dye molecules to stack each other is much higher (97-100), and generally it is difficult to observe the spectrum of monomer bound dye. The simple Bradley and Wolf model (84) of dilution of the dye molecules along the polymer chain owing to the increased number of available binding sites does not apply satisfactorily in this case but can be applied only to rigid polymers (101). The behaviour of synthetic polyelectrolytes can be attributed to the higher flexibility of their chains (84,97, 99) with respect to those of biopolymers. In this respect the poly (styrene sulphonic acid) has got more resemblance to DNA and to polyribonucleotides than to the synthetic electrolytes with a polyvinyl chain (97,99).

By adding a DNA solution to a dilute dye solution such as acridine orange one can see the gradual substitution of the absorption band of the free dye (α-band) with that of the dye bound to the polyelectrolyte (γ-band), characteristic of the aggregated dye. The spectrophotometric graphs are similar to those obtained by increasing the dye concentration in water. The γ band is much broader and has a maximum shifted 50-60 nm towards the blue region of the spectrum. Here we are in the presence of a dye excess and so, we can assume that the γ band belongs to the dye that saturates the DNA polyanion. With a further addition of DNA (i.e. when the concentration of the absorption sites on the polyelectrolyte

molecules becomes greater than the dye concentration) the α -band reappears with a 5-10 nm red shift of its maximum, and in the same time the γ band is gradually substituted by a new one (β) with a maximum 20 nm red shifted. The β band and the new α band belong to the bound dye and were interpreted by Bradley and Wolf (84) as a dilution of the dye along the polyelectrolyte molecule with the increasing of the available binding sites.

A statistical treatment allowed defining and computing a "stacking coefficient" which accounts for the tendency of the dye molecules to aggregate when bound along the polymer chain. The acridine orange binding sites on DNA were assumed to be the DNA phosphoric groups (85,102) as they are titrated by the dye within a few percent. It should be pointed out that all metachromatic dyes behave in an almost identical way with DNA and all polyacids exhibit similar effects on the same dye.

The γ and β absorption bands of the spectra have been attributed to dye molecules stacked as aggregates or as dimers inside the domain of the macroion (stacked bound dye). The α band is attributed to monomeric bound dye while the transition from γ and β to α band is interpreted as due to the dilution of the dye inside the domain of the polymeric chain.

With most synthetic polyelectrolytes, such as polyacrylates, polymethacrylates, polyphosphates, carboxy methyl cellulose etc., the bound dye dilution is not so evident as in the DNA case because of the strong stacking tendency of bound dye (77) and it is

necessary to reach very high values of the ratio P/D of polymer equivalent concentration to dye concentration in order to see the reappearance of the α absorption band. A peculiar behaviour is, however, shown by polystyrene sulphonic acid (PSS) which shows a spectrophotometric behaviour similar to DNA, exhibiting the α absorption band at P/D ratios not much higher than unity (98, 101,103). The sulphonic groups can be easily titrated stoichiometrically by using a dye such as acridine orange to an accuracy of 1-2% (101,103). The stacking coefficient of the bound dye, on the other hand, is low, indicating specific interactions between the dye and the polymeric chain, that favour the dye monomerization. According to Vitagliano and Costantino (101) this behaviour could be due to the PSS benzene rings favouring a partial intercalation of dye molecules between neighbouring rings, similar to that found in the DNA-dye systems, the stacking coefficient values are also very low in this case (84,104).

It is worth mentioning that the polymer matrix does not increase the stacking tendency of the dye, as compared with the one in aqueous solution. On the contrary the binding to a polymer promotes the monomerization of the dye molecules. The marked association effect apparently promoted by the binding process is actually due to the fact that bound dye molecules are constrained within the polyelectrolyte "phase" so that their actual local concentration is much higher than the stoichiometric one.

Two different equilibria characterize the dye-polyelectrolyte interactions in solution:

(i) The adsorption of dye on the polyelectrolyte :

$$P_n + 2D_{free} \rightleftharpoons P_n 2D_{bound} ,$$

where P is generally chosen as the macroion monomeric unit. The above adsorption reaction implies an exchange with an equivalent amount of polyelectrolyte counterions.

(ii) The association, or stack, of the dye bound to the polyelectrolyte.

However, authors have usually preferred a model for the macroion as a linear sequence of binding sites so that the stacking equilibrium can be considered as a distribution of different species along one single direction (105,106). This model is certainly realistic for polymers with a rigid structure implying open chain conformations in solution; it may fail for flexible chains that can assume compact coiled conformations similar to micelles. The model has been successfully applied to the interaction of dyes with nucleic acids (84,104,107), polystyrene sulphonates (101,103,108,109), polysaccharides (110) and polyglutamates (12,49,111).

The simplest expression to describe the binding equilibrium is the Langmuir isotherm (112), and often experimental data fit well in such an expression. A more accurate expression is derived if one assumes the linear chain model of Schwarz (113) of course, the binding process can not be considered separately from the stacking one and in fact the stacking tendency of the dye favours the binding, promoting a cooperative effect.

It has been suggested by Pal and Schubert (114) that the appearance of metachromasy of cationic dyes by adding to polyanions depends on the interaction of three types : (i) electrostatic interaction between dyes and anionic sites of polyanions, (ii) hydrophobic interaction between bound dye via the effective conformational change of polymer chain; (iii) interaction of π electrons between bound dyes. They suggested that the first interaction was probably the strongest of the three and that the third interaction was essential for the appearance of metachromasy, although it was the weakest of the three. The fact that large flat molecules are bound to the polyanion molecules much more strongly than small ions such as Li^+, Na^+, etc., is a strong evidence that other interactions besides the electrostatic ones play a relevant role on the dye binding process. However, the electrostatic effect must be the main one because unionized polyelectrolyte molecules bind a very little amount of dye as shown by Barone et al (97). We can assign to the electrostatic effect the following experimental facts (a) the evidence that binding sites correspond to ionized groups; (b) the increasing binding strength by increasing charge density, and (c) the competition of metal ions, including alkaline metal ions, for the binding sites.

Experimental evidence shows that the amount of available binding sites on a polyelectrolyte generally corresponds to that of its ionised groups (65,102,103,111); for some polymers such as DNA or PSA, the titration of ionized groups with a dye is stoichiometric with an error lower than 2-3%, so that dyes can be used for titrating the polyelectrolyte in very dilute solution. Experimental data also demonstrate that the binding strength is an increasing

function of the macro ion charge density (77). So, for partially ionized polyions (such as polymers bearing carboxylic groups along the chain), the amount of dye that saturates the polyelectrolyte increases proportionally to the degree of neutralization, while the concentration of free dye in equilibrium with the bound one decreases (97). The wave length of the metachromatic band reflects the strength of the stacking of bound dyes : the shorter the wave length of the metachromatic band, the stronger the stacking of bound dyes (115).

Addition of salts to a dye-polyelectrolyte system promotes the release of the dye from the polymeric matrix to solution (116, 117). There is competition with metal cations, even with those such as alkali metals which are thought to bind to polyelectrolyte molecules mainly or only by electrostatic interactions (118-121). The competing ion charge is the most effective factor controlling the dye-salt ion exchange equilibrium, though different effects were observed for series of ions bearing the same charge (e.g. Li^{+}, Na^{+}, Cs^{+} , ... or Mg^{++}, Ca^{++}, Ba^{++}, ...) (122-123) presumably due to differences in the size of the ions.

Shirai et al (124) had recently investigated the effect of alkali metal chlorides and 1-substituted 3-carbamoylpyridinium bromides on the metachromatic behaviour of methylene blue induced by poly(ptassium styrene sulphonate) (PSS) and poly (ptassium vinyl sulphate) (PVS) spectrophotometrically. The size of hydrated ion and the hydrophobicity of substituent were significant factors for inorganic and organic ions, respectively. They observed that the concentration of salts required to destroy the metachromasy

induced by PSS and PVS decreased in the order : $Li^+ > Na^+ > K^+ > Cs^+$, which is in good agreement with that of the binding affinities of these ions to polystyrene sulphonate (125,126) and polyvinylsulphonate (127). This relationship suggests that the replacement of the bound methylene blue by metal cations resulting in the destruction of metachromasy is induced by non-specific electrostatic forces. Shirai et al (108,128) further studied the structural effect of polyanions on the metachromatic behaviour of methylene blue and concluded that the flexibility and charge density of the polymer are the important factors in the aggregation of the bound dye and that the difference among $-SO_3^-$, $-OSO_3^-$ and COO^- as binding sites is not a significant factor. Their results suggest that polyanions having a higher flexibility and charge density show a higher dye binding affinity. However, it was generally observed that for polyanions of similar macromolecular structure the order of binding strength of dyes is : $-CO_4^- > -CO_2^- > -PO_2^- > -CH_2 - CO_2^-$ (129). Similarly for polymers with a polyvinyl chain, sulphonic groups bind dye more strongly than carboxylic groups (97,98,103). In general it was found that the metachromasy of dyes induced by polyanions is extremely affected by such factors as degree of polymerization of polyanion, number of anionic sites per polymer molecule, flexibility of polymer chain, and polymer-dye ratio in solution.

The effects of aliphatic tail of N-alkylated acridine orange derivatives on the interaction of poly styrene sulphonic acid have been studied with spectrophotometric and electric

dichroism measurements by Yanagishi and Watanabe (109). From the analysis of the experimental results they concluded that the length of the aliphatic tail effects the cooperative interaction among bound dye species to an appreciable extent and that the dynamics of a bound dye molecule is influenced by the length of the alkyl chain more delicately than by the equilibrium of its steric hindrance due to the alkyl chain, for example, may be important toward the attack of a different polymer chain.

c. Dye-clay minerals interactions:

The chromotropic effects in clay minerals were observed by Vedeneva (130) by studying sorption of malachite green and brilliant green by montmorillonite and kaolinite in 1947. She concluded that formation of ionic bonds between clays and dye caused a bathochromic shift, while the effect of intensification of the dipole bond was hypsochromic. In 1963 Bergmann and O'Konski (131) described a spectrophotometric study of methylene blue sorbed on sodium bentonite. Spectral changes were found to follow changes in the amount of methylene blue sorbed on the clay surface. Due to the fact that these changes were similar to the spectral shifts accompanying dimerization and polymerization of methylene blue in aqueous solution, these shifts were also attributed to dye-dye interaction on the surface of montmorillonite. According to these authors the dimer is held together by London dispersion forces and hydrophobic bonding.

Yariv and Lurie (132) in 1971 investigated in detail the metachromatic behaviour of methylene blue adsorbed on montmorillonite and showed that the adsorption takes place by a cation-exchange mechanism and the dye can be sorbed either at the edges of the clay platelets or on the oxygen sheets of the silicate layer. The latter type of sorption leads to metachromasy of the dye with a shift of the absorption band in the visible region to lower wave lengths. Those authors showed through X-ray diffraction studies, that metachromasy of methylene blue in montmorillonite occurs even when there is only a monolayer of the organic compound

lies parallel to the silicate sheets and concluded that π -interactions between the dye cations and the oxygen plans of the aluminosilicate layer of the montmorillonite lead to metachromasy when clay dye interactions take place.

Yamagishi and Soma (133) in 1981 studied the adsorption of several N-alkylated acridine orange by Na-montmorillonite. In their study they showed that adsorption of cationic dyes which belong to the acridine orange family also results in metachromasy. However, they considered the clay mineral as a normal polyelectrolyte and believed that the interaction between the negatively charged mineral and the dye cation is a pure electrostatic attraction and that metachromasy resulted from the electronic interaction between neighbouring adsorbed cations.

More recently in 1984 Cohen and Yariv (134) extended their earlier studies (132) and investigated metachromasy of acridine orange using H^{+}-, Na^{+}-, Mg^{2+}-, Al^{3+} and Cu^{2+} montmorillonite by visible and IR spectroscopy and by X-ray diffraction method. Two types of associations between acridine orange and montmorillonite were noted : (i) a monolayer of the dye located in the interlayer space, with the aromatic rings parallel to the aluminosilicate layer and (ii) a bilayer in the interlayer space or tilting of the cationic dye relative to the alumino-silicate layer. They concluded that in the association of type (i) metachromasy cannot be attributed to dimerisation of the dye but must be caused by π-interactions between the oxygen of the aluminosilicate and the aromatic ring.

In view of the above conflicting opinions on the causes of metachromasy when cationic dyes are sorbed on clay minerals, the present study has been carried out to throw further light in this field by investigating the spectral behaviour of trimethyl propyl thionine (TMPT), trimethyl butyl thionine (TMBT), diethyl methyl propyl thionine (DEMPT) and diethyl methyl butyl thionine (DEMBT) when sorbed by montmorillonite, vermiculite and Laponite suspensions.