

Aggregation of Basic Dyes Induced by Anionic Polyelectrolytes

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Synopsis

The aggregation of basic dyes in aqueous solution in the presence of polyelectrolytes has been studied to determine the nature of the interaction between the dye molecules and the polymer sites. The aggregation induced by carboxymethylcellulose with isolated anionic sites cannot be explained assuming interaction between dye molecules bound to neighbouring sites. Maximum aggregation for various dyes on that polyelectrolyte has been found around $P/D = 0.3$ whereas for PSS that value is found around 1. Dimerization constants calculated for these systems are in good agreement with the values expected from dimerization in aqueous solutions. A new model is proposed to explain these results.

INTRODUCTION

It is well known that a large number of dyes, when in concentrated solution, show deviations from the Beer's law. This is attributed to the formation of aggregates of those species.¹ These aggregates are held together by two different kind of forces, dispersion forces due to the interaction between the π -systems of the dyes,² as well as hydrophobic effects.³ The sum of these forces must be larger than the electrostatic repulsion between the positive charges on the dye molecules.

Similar effects are observed in dilute solutions of the dyes in the presence of polymers, especially those with anionic sites,⁴ and biopolymers like DNA, RNA,⁵ hyaluronates,⁶ and others.⁷

For polyanions the increased aggregation tendency can be ascribed to a partial neutralization of the electrostatic repulsion between the dyes, associated with an ordering effect which tends to put the dye molecules in a configuration more appropriate for interaction. On the other hand, it is assumed that with biopolymers an important factor will be the possibility of intercalation of the dye molecules within the tertiary structure of the macromolecule,⁸ thus providing a geometry favorable to the π -interaction. In both cases, these structures will lead to configurations thermodynamically more stable, mainly due to the hydrophobic character of the dye molecules.

Vitagliano^{9,10} and other authors did propose a spectrophotometric method of determining anionic sites on polymers by titration with basic dyes (Pyronine G or Acridine Orange), assuming that the maximum aggregation (corre-

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sponding to minimum absorbance of the dye monomer) will occur at a 1:1 ratio between anionic sites and dye molecules [$P/D_{\max} = 1$]. On the other hand, several other authors^{7,11} found that the maximum aggregation does occur at $(P/D)_{\max}$ well over 1.

Studies with polymers having variable amounts of anionic sites should provide an insight on the geometry of the aggregates as well as on a definition of $(P/D)_{\max}$. Shirai et al.¹² studied the aggregation of Methylene Blue on polyvinylsulfate with different degrees of sulfation, finding a dependence of $(P/D)_{\max}$ with the percentage of anionic sites on the polymer. Although they found a dependence of $(P/D)_{\max}$ with the substitution, the results cannot be considered conclusive as sulfation was achieved by bulk reaction of polyvinyl-alcohol with chlorosulfonic acid, so that the distribution of anionic sites cannot be assured to be homogeneous.

We want to present here some results obtained comparing the behavior of aggregation of several basic dyes induced by polyelectrolytes of a different kind: a totally sulfonated polystyrenesulfonate with neighboring anionic sites and a carboxymethylcellulose with anionic sites separated by 60 cellulose units.

EXPERIMENTAL

The dyes Methylene Blue (C. Erba), Acridine Orange (Merck), and Safranin (Aldrich) were recrystallized from alcohol before use. Their spectra and extinction coefficients were practically identical with those reported for the pure products. Sulfonated polystyrenesulfonate (PSS) was prepared¹³ from polystyrene ($M_w = 250,000$) by reaction with H_2SO_4 in the presence of Ag^+ . The degree of sulfonation was determined by potentiometric titration and found to be 95%, corresponding to practically all phenyl groups substituted. Hoechst's carboxymethylcellulose (CMC) was also titrated potentiometrically, and the amount of anionic sites correspond to 1.6%, that is, one anionic site every 62 cellulose units. All experiments using CMC were performed at pH 7–8 to assure complete dissociation of the carboxylic groups.

Spectra were measured on a Beckman DU-7 spectrophotometer using cells with path lengths between 1.0 and 0.1 cm.

Calculation of the dimerization constants was performed by an iterative method based on that developed by Arbeloa¹⁴ for aggregation in homogeneous solutions.

RESULTS AND DISCUSSION

Figure 1 shows the absorption spectra of solutions of Methylene Blue $1 \times 10^{-5}M$ in the presence of increasing amounts of CMC. These spectra can be divided in two groups: the first, in which the monomer peak at ~ 660 nm decreases steadily, and the peaks due to dimers at 607 nm and higher aggregates at shorter wavelengths, increase [shown in Fig. 1(left)]; and a second group of spectra in which the monomer peak rises again to an intensity similar, or somewhat higher than at the beginning [Fig. 1(right)]. Similar behavior has been found for the spectra of Methylene Blue and Acridine Orange on CMC and Safranin and Acridine Orange on PSS. When recording the spectra of pure dye solutions at increasing concentrations, the same type

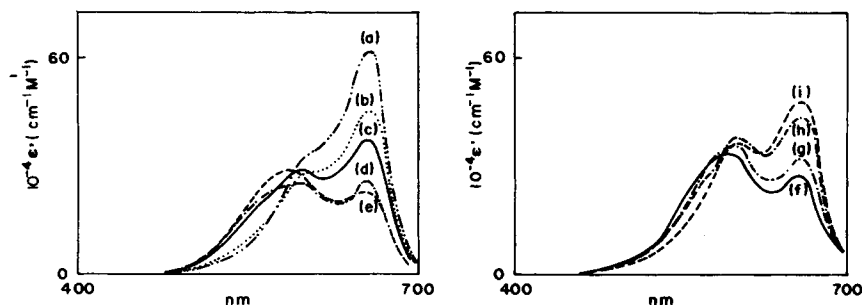


Fig. 1. Spectra of Methylene Blue $1 \times 10^{-5}M$ at $25^{\circ}C$ in aqueous solution with increasing amounts of carboxymethylcellulose: (left) increasing branch: (a) $P/D = 0.00$; (b) $P/D = 0.04$; (c) $P/D = 0.11$; (d) $P/D = 0.18$; (e) $P/D = 0.36$; (right) decreasing branch: (f) $P/D = 1.1$; (g) $P/D = 3.6$; (h) $P/D = 14.2$; (i) $P/D = 35.6$.

of behavior is observed as recorded for the ascending branch, although much higher dye concentrations have to be used to attain the same dimer peak intensities. Whereas practically no dimer absorption is observed in a $1 \times 10^{-5}M$ solution of Methylene Blue in water, the addition of relatively small amounts of polyelectrolyte ($1 \times 10^{-3}meq/L$) to a $10^{-6}M$ solution of Methylene Blue reduced the monomer peak by 50%, increasing proportionally the aggregate peaks.

Figures 2 and 3 show the behavior of the absorbance at the monomer peak maximum as a function of the ratio of polymer anionic sites to dye molecules present in the solution. As can be seen, in all cases the ratio initially decreases due to the formation of n -meric aggregates induced by the anionic sites.

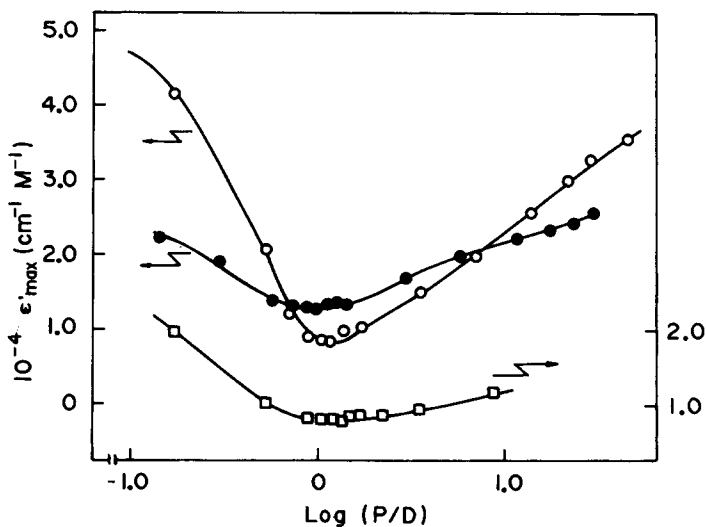
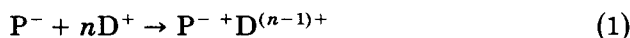


Fig. 2. Behavior of the monomer maximum absorption peaks of basic dyes in aqueous solutions with polystyrenesulfonate at $25^{\circ}C$: (O) Methylene Blue ($\lambda_{max} = 663.5$ nm); (□) Acridine Orange ($\lambda_{max} = 492$ nm); (●) Safranin ($\lambda_{max} = 520$ nm).

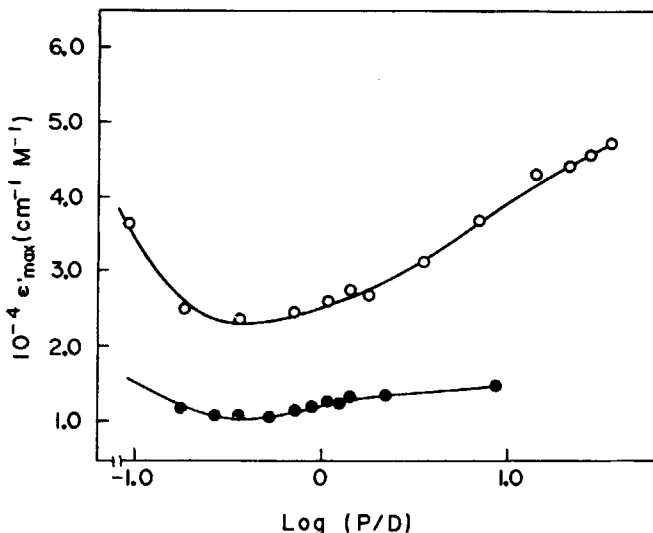
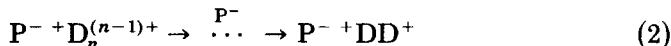
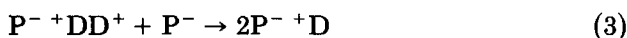


Fig. 3. Behavior of the monomer maximum absorption peaks of basic dyes in aqueous solutions with carboxymethylcellulose at 25°C: (○) Methylene Blue ($\lambda_{\max} = 663.5$ nm); (●) Acridine Orange ($\lambda_{\max} = 492$ nm).

Successive addition of polyelectrolyte will dilute the aggregates over a larger amount of sites, eventually reaching a stage where most of the aggregates are dimers



From this point on, the addition of further anionic sites will redistribute the dye molecules, destroying the aggregates, up to a point when each dye molecule will be electrostatically interacting with only one anionic site



At large dilution, that is, high P/D ratios, the spectra look very much like those of the pure diluted dyes, except for a small shift of the maximum towards the red and an increase in the extinction coefficient. This effect has been attributed to the interaction between the aromatic rings of PSS with the dye π -system.^{3,9} A similar effect is observed when using CMC, so that this possibility has to be ruled out. An electrostatic interaction between the negative sites and the positive dye molecules can be assumed to be responsible for these changes in the spectra, as ion pairs generally have spectra only slightly different from those of the separated ions.¹⁵

As can be seen from Figures 2 and 3 for both dyes on CMC the minimum of the monomer absorption band is found at P/D of about 0.3, whereas on PSS that minimum is found near 1.2. Similar results have been found by Vitagliano et al. for the aggregation of Acridine Orange⁹ and Pyronine G¹⁰ on PSS. Results obtained by Shirai et al.¹¹ for the aggregation of Methylene Blue on PSS and other polyelectrolytes point to higher P/D values at the minimum (2

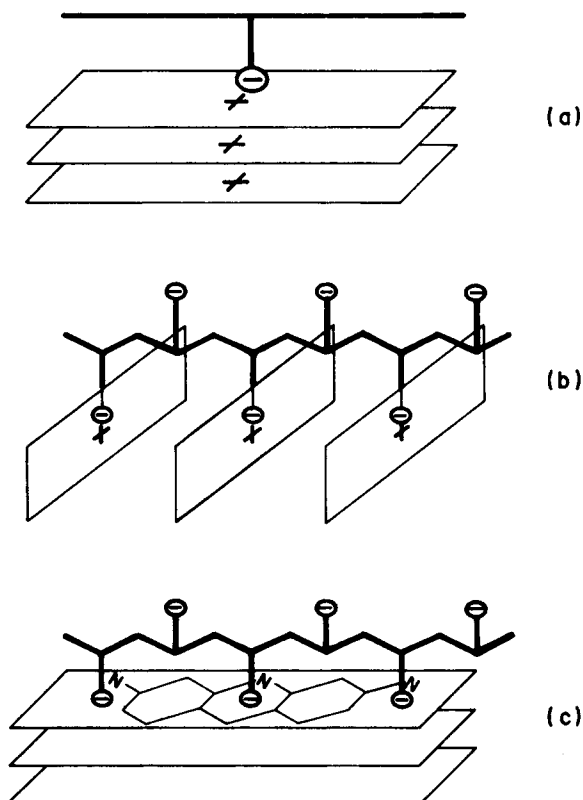


Fig. 4. (a) Aggregation of three dye molecules on an isolated anionic site; (b) conventional model for polyelectrolyte induced aggregation^{5,17}; (c) model for aggregation induced by a syndiotactic-type polyelectrolyte.

on PSS and up to 10 on polyvinylsulfate), in disagreement with those obtained in this work.

Our results suggest that the maximum aggregation of the dyes occurs in a different way on both polyelectrolytes. On CMC the maximum aggregation corresponds to the stacking of three dye molecules on each anionic site. On the other hand, on PSS only one dye molecule is bound at each site at maximum aggregation. This difference is a result of the structural characteristics of both polyelectrolytes used. Whereas in PSS all the anionic sites occupy neighboring positions, in CMC the sites are well separated, not allowing any kind of vicinal interaction. Thus, one must assume that aggregation induced by CMC occurs through the electrostatic binding of a first molecule of the cationic dye, followed by the aggregation (by π -interaction) of other dye molecules. This geometry is shown in Figure 4(a). A similar geometry has been proposed for the adsorption of pseudisocyanine aggregates on silver halide particles.¹⁶ Even if the maximum aggregation of the dyes on PSS at P/D \sim 1 seems to be compatible with the models involving metachromic interactions between dye molecules bound to individual neighboring sites^{5,17} [Fig. 4(b)], it would imply in very high order aggregates in that region, which is not observed experimentally. Using a similar approach as used for CMC, one could assume that the

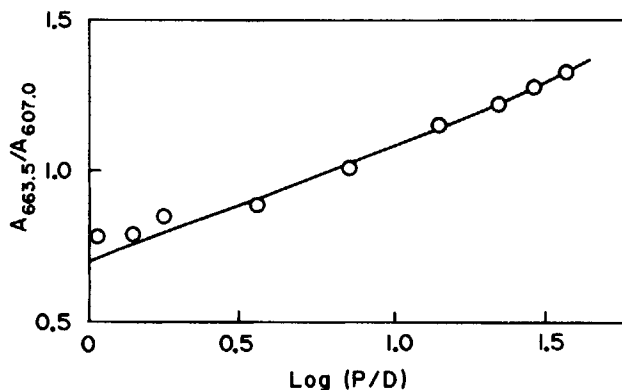
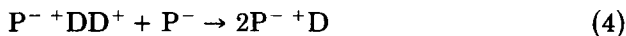


Fig. 5. Calculated (—) and measured (O) monomer to dimer absorption ratios for Methylene Blue in the presence of carboxymethylcellulose.

first dye molecule will be interacting electrostatically with the polyelectrolyte sites with further molecules stacking on the first one, as shown in Figure 4(c).[‡] Vitagliano¹⁸ reports a larger binding strength for Acridine Orange on isotactic-PSS than on the atactic polymer, confirming a smaller dye-dye repulsion when all anionic sites are in positions favorable to electrostatic interaction.

The behavior of the system Methylene Blue-CMC in the dimer deaggregation region can be calculated using the model described above. This calculation corresponds to the ascending branch [Fig. 1(b)] and is shown in Figure 5. As can be seen, a good agreement is found between the calculated and experimental values. These calculations have been made assuming that the addition of anionic sites leads to a deaggregation of the dye molecules:



The absorbance at any wavelength has contributions from the monomer and the dimer so that the ratio between the absorbances at the peak maxima of the monomer and dimer is

$$\frac{A_m}{A_d} = \frac{\epsilon_m^{PD}[PD] + \epsilon_m^{PD_2}[PD_2]}{\epsilon_d^{PD}[PD] + \epsilon_d^{PD_2}[PD_2]}$$

where A_m and A_d are the absorbances of the solution at the maxima of the monomer and dimer peaks, respectively; ϵ_m^{PD} ($= 64,000 \text{ cm}^{-1} M^{-1}$) and ϵ_d^{PD} ($= 34,000 \text{ cm}^{-1} M^{-1}$) are the extinction coefficients of the monomer at those wavelengths and $\epsilon_m^{PD_2}$ and $\epsilon_d^{PD_2}$ the extinction coefficients of the dimer. To proceed with the calculations, two "mass balance" equations have to be used

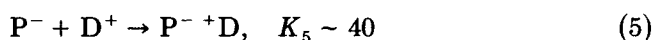
$$\text{for dye molecules } 2(P - D_2) + (P - D) = (D)_0$$

$$\text{for anionic sites } (P_f) + (P - D) + (P + D_2) = (P)_0$$

[‡] Assuming a syndiotactic-type structure for a completely sulfonated PSS, the anionic sites on each other phenyl ring will be separated by about 4.8 Å. This is the same order of the distance between the nitrogen atoms in the dye, which carry most of the positive charge.

where $P - D_2$ are dimer aggregates on an anionic site, P_f are unoccupied sites, $(D)_0$, the total concentration of the dye assumed to be in the monomeric state, and $(P)_0$, the total concentration of anionic sites.

The best fit of the calculated curve in Figure 5 is obtained by using $K_4 = 6 \times 10^{-3}$ and $\epsilon_m^{PD_2} = 11,200 \text{ cm}^{-1} M^{-1}$ and $\epsilon_d^{PD_2} = 19,500 \text{ cm}^{-1} M^{-1}$. This last value is in good agreement with that reported by Ruprecht and Baumgartel for the dimer in aqueous solutions, $18,100 \text{ cm}^{-1} M^{-1}$.¹⁹ The value for K_4 seems to be quite reasonable and can also be evaluated from the equilibria involving the electrostatic interaction of a cationic dye with an anionic site



and the deaggregation of a dye molecule from a dimer bound to an anionic site



The value for $K_4 = 4 \times 10^{-3}$ obtained by multiplying K_5 times K_6 compares quite well with the value used in the calculations.

On the other hand, it was not possible to reproduce the experimental curve for the deaggregation of Methylene Blue on PSS using reasonable values for the dimer extinction coefficients and K_4 . This may be due to the fact that the interaction of each dye molecule with more than one site is not compatible with the mechanism of deaggregation assumed for the calculations.

The equilibrium constants and free energies for dimerization of Methylene Blue in water and induced by polyelectrolytes are shown in Table I. The large differences in free energies between the pure aqueous solutions and those with polyelectrolyte indicate that the aggregation is favored by the presence of anionic species.⁸ The higher value for PSS indicates that the aggregation mechanism will also be different for both polyelectrolytes. This effect can be attributed to the larger effective negative charge of the site-first dye entity that will be more effective in attracting a second cationic dye when using PSS than when using CMC.

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TABLE I
Equilibrium Constants and Dimerization Free Energies for Methylene Blue at 25°C

Polyelectrolyte	K_{dim}	ΔG (kcal/mol)
—	3.7×10^3	4.9
CMC	8.3×10^4	6.8
PSS	1.4×10^6	8.5

⁸Dye aggregation induced by monoanionic species has been observed when using solutions of sodium toluenesulfonate or benzenesulfonate.

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