March, 1981] © *1981 The Chemical Society of Japan* NOTES *Bull. Chem. Soc. Jpn.*, *54*, *923—924 (1981)* 923

Effect of Ionic Strength on the Metachromatic Behavior of Dye-Polymer Systems[1)]

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(Received August 28, 1980)

Synopsis. The effect of added sodium chloride on the metachromatic interaction between cationic dyes (Crystal Violet, Trypaflavine, and 9-aminoacridinium chloride) and sodium polyphosphate and poly(ethylenesulfonate) was studied in aqueous solutions by measuring the visible absorption spectra and by determining the bound-dye spectra and the equilibrium constants with the principal component analysis.

Previous reports have shown that metachromatic changes of the absorption spectra of Crystal Violet (CV) and Trypaflavine (TF) in the presence of sodium polyphosphate (NaPP) are affected by addition of simple electrolytes.[2–4)] The degree of metachromasy was decreased with the increase of ionic strength, *i.e.*, the absorption spectra of dye–NaPP solutions returned to the original spectra of the NaPP-free dye solutions. On the basis of the ultrafiltration of dye–NaPP solutions, this result was attributed to the dissociation of bound-dye species from NaPP by competition with Na^+ ions.[3,4)]

Recent applications of the extended-principal-component-analysis (EPCA) procedure to a variety of dye–polymer solutions demonstrated that it is possible to determine the number of light-absorbing components, the equilibrium constant, the pure spectra of dye species bound to polymer, and the fraction of the bound-dye species from a family of experimental absorption spectra of a dye–polymer system.[5–8)] By utilizing this EPCA procedure, therefore, the amount of dye species dissociated from the polymer site by an increase of ionic strength can now be estimated on a precise and quantitative basis. In this Note, the effect of added NaCl on the metachromatic behavior of four different dye–polymer systems will be reported. In all dye–polymer combinations, the fractions of bound-dye species indeed decrease with an increase of added NaCl but, surprisingly, the spectra of the bound-dye species remain unchanged.

Experimental

Materials. Samples were sodium polyphosphate (NaPP)[5)] and sodium poly(ethylenesulfonate) (NaPES),[5)] and the chlorides of Crystal Violet (CV),[5)] Trypaflavine (TF),[5)] and 9-aminoacridinium chloride (AA).[8)]

Measurements and the Procedure for Data Analysis. Absorption spectra were measured at 25 °C.[8)] Optical titration of a dye solution, which contains either 1 mM ($=10^{-3}$ mol/dm^3) or 0.1 mM NaCl, was carried out by the dropwise addition of a polymer solution which contains the same amount of NaCl. The observed series of spectra of each dye–polymer system were analyzed by the EPCA procedure, which has been described together with other precautions.[5–9)] The molar absorption coefficient, ε, was defined as before.[5,8)]

Results and Discussion

Pure Spectra of Bound-dye Species and Equilibrium Constants. Figures 1a—d show the typical absorption spectra of dyes in the presence and the absence of polymers. In each dye–polymer system, optical titrations were carried out to obtain eight to nine experimental spectra over the P/D range 0—*ca.* 1, where P and D are the concentrations of a polymer in the residue unit and a dye respectively.[3)] (Not all spectra were drawn in each figure, but details are the same as were shown in Refs. 5—8.) Each family of spectra shows the presence of isosbestic points, which are independent of the concentration of added NaCl (*cf.* caption of Fig. 1), indicating that the dye–polymer solution consists of two light-absorbing components, probably, free- and bound-dye species. The result of the EPCA procedure applied to the experimental spectra revealed that each dye–polymer system, which contains either 1 mM or 0.1 mM NaCl, is composed

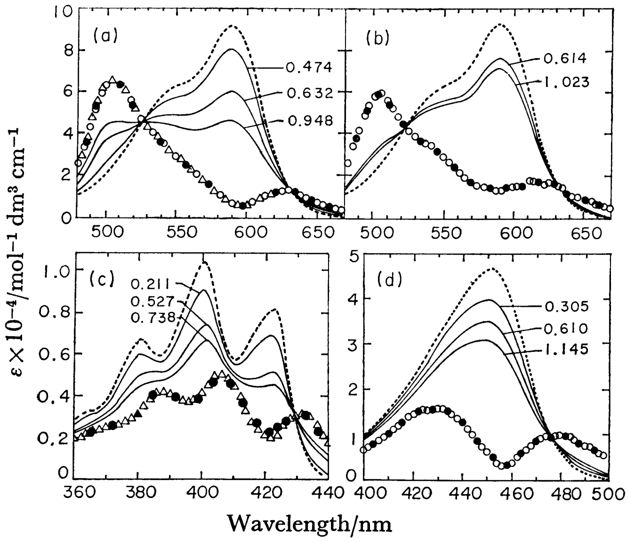

Fig. 1. Absorption spectra of CV–, AA–, and TF–polymer systems in the presence of NaCl and the corresponding pure spectra of dye species bound to the polymers. The experimental conditions are: (a) CV–NaPP with P/D 0—1.26 in 0.1 mM NaCl, (b) CV–NaPES with P/D 0—1.023 in 0.1 mM NaCl, (c) AA–NaPP with P/D 0—0.738 in 1 mM NaCl (precipitates appeared at the concentration of added NaCl higher than 5 mM), and (d) TF–NaPES with P/D 0—1.145 in 0.1 mM NaCl. The initial dye concentrations in μM are (a) 8.13, (b) 7.04, (c) 44.3, and (d) 9.43. Isosbestic points in nm are: (a) 639 and 529, (b) 641 and 526, (c) 429, (d) 476. In each figure, the spectrum of the polymer-free dye solution is shown by the dotted curve and the numerals indicate the P/D values. The bound spectra are denoted by either (○) in 0.1 mM or (△) in 1 mM NaCl and by (●) in NaCl-free solutions, the latter being cited from Ref. 5 for (a), (b), and (d) and from Ref. 8 for (c) for comparison.

Table 1. Effect of added NaCl on the empirical parameter α and the equilibrium constant K of dye–polymer systems at 25 °C

Dye–polymer	NaCl[a)]	α	K[b)]	K'[c)]
CV–NaPP	0.1	2.1(1.5)[d)]	1.0×10^{11}	1.4×10^{5}
CV–NaPP	1.0	1.9(1.5)	1.8×10^{9}	4.7×10^{4}
CV–NaPES	0.1	1.5(0.6)	5.2×10^{7}	1.4×10^{5}
AA–NaPP	1.0	1.6(1.2)	1.2×10^{8}	2.8×10^{5}
TF–NaPES	0.1	1.2(0.9)	1.5×10^{6}	1.5×10^{5}

a) The concentration in 10^{-3} mol/dm^3. b) The dimension in [dm^3/mol]$^\alpha$. c) $K'=K[\mathrm{P}]^{\alpha-1}$ at $P/D=1$. d) The α values in parentheses are for NaCl-free systems and are taken from Ref. 5 or 8 for comparison.

of two absorbing species. The pure spectra of dye species bound to NaPP and NaPES were thus evaluated and are shown in Figs. 1a—d, together with those in NaCl-free solutions. Interestingly, the bound-dye spectra in the presence or the absence of added NaCl are essentially identical with each other.

Fractions of Bound-dye Species. The scheme for the binding reaction includes an empirical parameter, α, in such a way that $K=[\mathrm{DP^*}]([\mathrm{D}][\mathrm{P}]^\alpha)^{-1}$. The notations are all the same as before.[5,8)] Both α and K were estimated by the EPCA procedure and are given in Table 1. The values of α are larger than unity in all cases where NaCl is added, indicating that the optical titration curves change sigmoidally with P/D.[5,6)] The values of α are also larger in the presence of NaCl than in its absence. It is thus clear that the empirical parameter α reflects the effect of ionic strength on the binding reaction.

The fraction of bound-dye species in each solution, f_b, may be calculated with the estimated α and K values and also directly from the EPCA method.[5–8)] The results are shown in Fig. 2, where the fraction of free-dye species remaining in solution, $1-f_b$, is plotted against P/D. The amount of CV bound to NaPP decreases most markedly with the increase in NaCl (Fig. 2a), *i.e.*, only 20% of CV remain bound even at $P/D=1$ in 1 mM NaCl, while 50% of CV are bound in the salt-free solution. In contrast, the effect of added NaCl was negligible for the AA–NaPP system (Fig. 2c), *i.e.*, 75% of AA remain bound at $P/D=1$ in 1 mM NaCl. Similarly, CV bound to NaPES is affected more than TF by added NaCl (Figs. 2b and d). It is clear that, as compared with acridine dyes (TF and AA), CV dissociates from the polymer to a remarkable extent, probably as a result of competitive inhibition by Na$^+$ ions.[10)] Our result is in accord with the cases of Proflavine–poly(glutamic acid),[10)] CV–poly(methacrylic acid),[11)] and Acridine Orange–poly(p-styrenesulfonate).[12)]

In summary, we have clearly shown that the spectra of bound-dye species are not affected by added NaCl but the Na$^+$ ion competes with the bound dye for the polymer site from which the dye is forced to dissociate. These results imply several possibilities to be resolved: (1) If the dye molecules are stacked on a polymer chain, they remain so even when over 80% of them are liberated into the solution as free dye. (2) If the added salt enhances the degree of stacking[12)] or aggregation of dye,[13)] the bound-dye spectra may be so insensitive as not to reflect such changes of the bound-dye species. (3) The bound-dye species may exist in an unstacked state regardless of the amount of NaCl present in a solution.

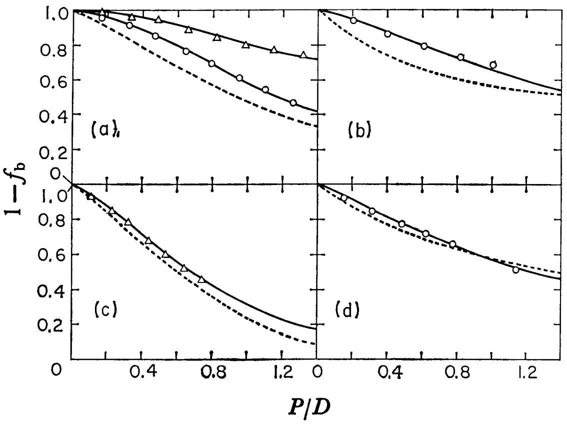

Fig. 2. The P/D dependence of the fraction of free-dye species remaining in solution, $1-f_b$, for four dye–polymer systems. (a) CV–NaPP, (b) CV–NaPES, (c) AA–NaPP, and (d) TF–NaPES. The solid curves were calculated from the values of α and K in Table 1 (*cf.* Eq. 2 in Ref. 6), while each point [(○) in 0.1 mM NaCl or (△) in 1 mM NaCl] was calculated by the EPCA method with 56 to 40 selected wavelengths (*cf.* Eq. 4 in Ref. 6). The dotted curves are the binding curves for NaCl-free systems for comparison (taken from Ref. 5 for (a), (b), and (d) and from Ref. 8 for (c)).

We thank Mr. Kinya Nakata for his technical assistance.

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