Calorimetric determination of the enthalpy change for the binding of xanthene dyes to polycations in aqueous solutions

I. Sardharwalla† and J. B. Lawton*
Department of Biological Sciences, University of Salford, Salford M5 4WT, UK

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Thermodynamic parameters have been determined for the interaction of two xanthene dyes, fluorescein (Fl) and eosin Y (Eo-Y) with the polycation (poly(paraxylylviologen dichloride)) (PPXVCl$_2$) and for the interaction of Fl with poly(4-vinyl pyridinium methyl chloride) (P4VPMeCl) in aqueous solutions. Enthalpy changes were determined by the use of a flow microcalorimeter. Free energy changes were calculated from the results of spectrophotometric studies. The results have been interpreted in terms of the structures of the dyes and the conformations of the polycations. Analysis of the data shows that the electrostatic interactions in these dye–polycation systems are in the order: P4VPMeCl > PPXVCl$_2$ for the polycations, and Eo-Y > Fl, for the two dyes. The free energy changes for the dye–polycation interactions are in the order: Fl–P4VPMeCl > Eo-Y–PPXVCl$_2$ > Fl–PPXVCl$_2$.

(Keywords: poly(paraxylylviologen dichoride); poly(4-vinyl pyridinium methyl chloride); eosin Y; fluorescein; thermodynamic parameters; polycation conformation)

INTRODUCTION

Extensive studies of the interaction between a variety of cationic dyes with many different polyanions in aqueous solutions have been made by a number of workers[1]. Investigations on the physicochemical behaviour of anionic dyes in aqueous solutions of polycations are, on the contrary, relatively rare.

Spectrophotometric investigations on the interactions of fluorescein and its halogenated derivatives with polymers have been reported in the literature[2–8]. In general, a red or bathochromic shift in the absorption spectra of these dyes occurs upon binding to a polymer. There are at least two hypotheses as to the spectral shift on binding: (a) the spectral shift occurs because of dye–dye interaction[3–5] (effective aggregation of the dye molecules bound on the polyelectrolyte producing what is called metachromacy); and (b) the spectral shift is due to dye–polyelectrolyte interaction[6,9].

To obtain insight into the type of dye-anion/polycation interactions and the forces involved in the process of binding, a knowledge of the enthalpic and entropic contributions to the free energy of binding are necessary. Enthalpies of binding of xanthene dyes to polymers have been calculated from the temperature dependence of the binding constants[6,10]. Direct determination of these quantities by calorimetry has been reported by Manzani *et al.*[11], who studied the binding of various drugs including fluorescein to serum albumin. Involvement of both ionic and hydrophobic interactions in the binding of these dyes to polymers was deduced from these studies.

In this paper we report measurements by direct calorimetry of the enthalpy changes associated with the binding of two xanthene dyes (fluorescein and eosin Y) to polycations (poly(paraxylylviologen dichloride) and poly(4-vinyl pyridinium methyl chloride)) in aqueous solutions. For the structures of the dyes and polycations used see *Figure 1*.

EXPERIMENTAL

Materials

Poly(*p*-xylylviologen dibromide) was prepared by the method of Factor and Heinsohn[12]. Quaternized poly(4-vinyl pyridinium methyl iodide) was prepared by the method of Igawa *et al.*[13]. Chloride forms of both polycations (PPXVCl$_2$ and P4VPMeCl) were prepared by passing a solution of polycation (0.5% w/v) through a pre-conditioned Amberlite IRA 410 ion-exchange column in chloride form, at a rate of 2 ml min^{-1}. The fractions were checked for bromide when using PPXVBr$_2$ and iodide when using P4VPMeI. The fractions containing only chloride forms of the polycations were then freeze dried and the percentage quaternization was determined to be 100%[14]. Both polycations were polydisperse.

Fluorescein (Fl) obtained from the Eastman Kodak Company was further purified by a precipitation method[15]. Purified Fl showed a single band by thin-layer chromatography[16] (t.l.c.) and had a molar extinction coefficient of 1.09×10^5 dm^3 mol^{-1} cm^{-1} at 492 nm at a concentration of 10^{-6} mol l^{-1}, pH 12. Eosin Y (Eo-Y) obtained from Eastman Kodak Company was further purified by column chromatography owing to the presence of coloured impurities detected by t.l.c.[14,16]. The column (800 mm length and 25 mm inside diameter) was packed with activated aluminium oxide (100–250 mash; pH 9.3–9.7) and the dye was eluted with a solvent mixture of 3°-butanol-n-butanol-0.2 N NH$_3$ (aq.) (40:30:30 parts

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*Author to whom correspondence should be sent. Present address: Fire Service College, Moreton-in-Marsh, Gloucestershire GL56 0RH.

†Present address: Department of Biochemistry, King's College, Strand, London WC2R 2LS, UK.

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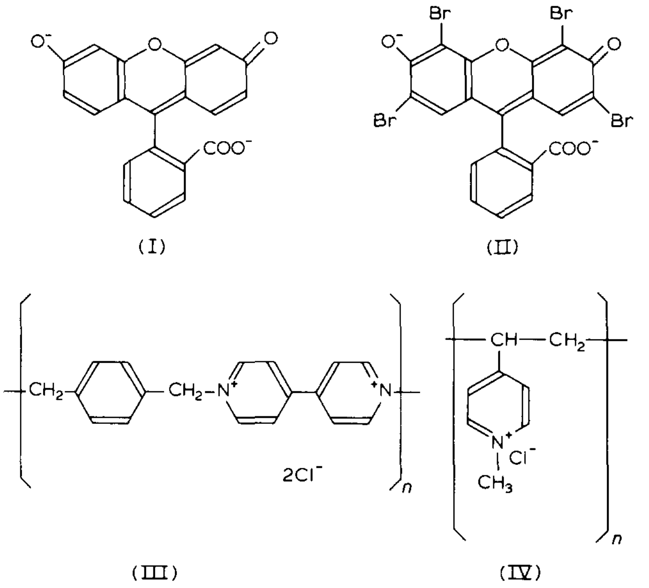

Figure 1 Structures of fluorescein (I), eosin Y (II), poly(paraxylylviologen dichloride) (III), and poly(4-vinyl-pyridinium methyl chloride) (IV)

by volume). The apparent pH of this solvent mixture was pH 9.0.[16] The dye fractions were collected and after rotary evaporation the dye was dissolved in distilled water and freeze dried. Purified Eo-Y showed a single band by t.l.c.[16] and had a molar extinction coefficient of 1.02×10^5 dm^3 mol^{-1} cm^{-1} at 517 nm at a concentration of 10^{-6} mol l^{-1}, pH 12.

All other chemicals and solvents used were of Analar Reagent Grade and were used without further purification.

Techniques

The degree of binding of anionic dyes (Fl and Eo-Y) by cationic polymers (PPXVCl$_2$ and P4VPMeCl) in water was measured by spectrophotometric and calorimetric methods. The binding was measured for solutions containing 2.5×10^{-4} bm l^{-1} of polycation; where bm l^{-1} = base mole of polycation per litre (one base mole is the mass of the polymer corresponding to one positive charge).

All solutions were made up in distilled water and adjusted to pH 7.2 with sodium hydroxide. Measurements of pH following the experiments showed no significant change in value. All experiments were made at 25°C unless otherwise stated.

Absorption spectra were recorded on a Pye–Unicam SP1800 ultra-violet spectrophotometer[17,18]. The wavelength was calibrated during each experiment, and the absorbance readings were corrected by comparison with a standard solution of potassium dichromate. After selecting an appropriate wavelength, 492 nm and 517 nm for Fl and Eo-Y, respectively, a calibration curve was plotted for absorbance against dye concentration for each dye studied. Absorption cells and all other glass ware were treated with Repelcote (BDH) to reduce dye adsorption thereon.

Calorimetric measurements were made by using an LKB 2107 flow microcalorimeter as described elsewhere[19]. The accuracy of the instrument was checked by determining the heat changes for the dilution of a standard solution of tris-HCl buffer (0.1 M; pH 7) with distilled water.

RESULTS

Spectrophotometry

Changes in the absorption spectra of dyes with polymer concentration provide a convenient procedure for calculating the extent of dye-binding or complexing. Klotz[20] has shown that the fraction of free dye, α, can be determined from the apparent molar absorbances at any convenient wavelength by the equation

$$\alpha = (\varepsilon_{\mathrm{app}} - \varepsilon_{\mathrm{b}})/(\varepsilon_{\mathrm{f}} - \varepsilon_{\mathrm{b}}) \qquad (1)$$

where $\varepsilon_{\mathrm{app}}$, ε_{f} and ε_{b} are the molar absorbancies of the actual solution, free dye and the fully bound dye, respectively.

The almost complete binding of these dye-anion/polycation systems and a shift of the absorption band (*Figures 2–4* and *Table 1*) allowed the use of this method for the determination of the quantity α. The

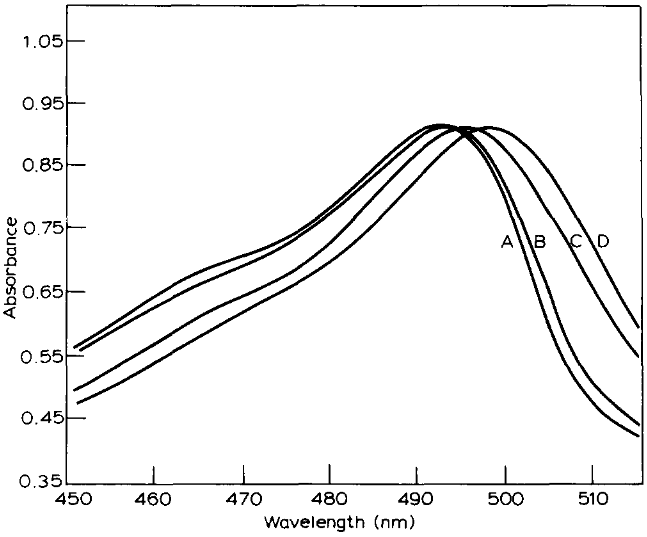

Figure 2 The effect of poly(paraxylylviologen dichloride) on the absorption spectrum of fluorescein (2×10^{-5} M) at 25°C in aqueous solutions. Polymer:dye ratios: A, 0;, B, 1.0; C, 5.0; D, 7.0

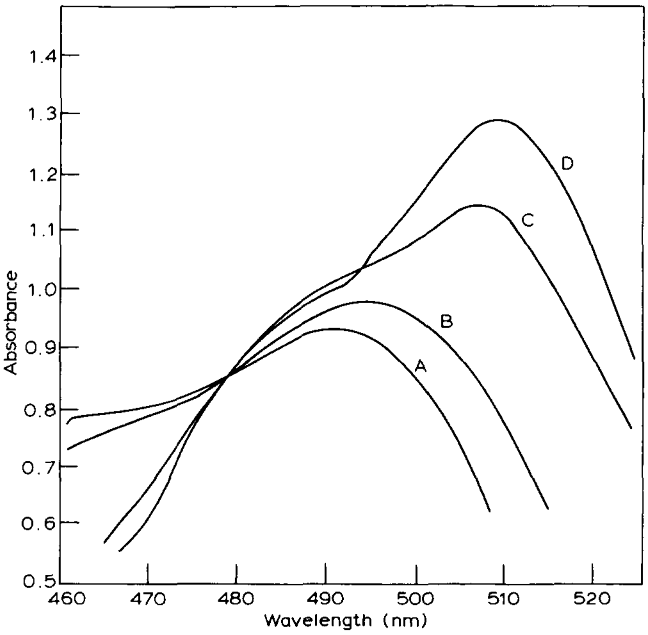

Figure 3 The effect of poly(4-vinylpyridinium methyl chloride) on the absorption spectrum of fluorescein (2×10^{-5} M) at 25°C in aqueous solutions. Polymer:dye ratios: A, 0; B, 1.0; C, 3.0; D, 5.0

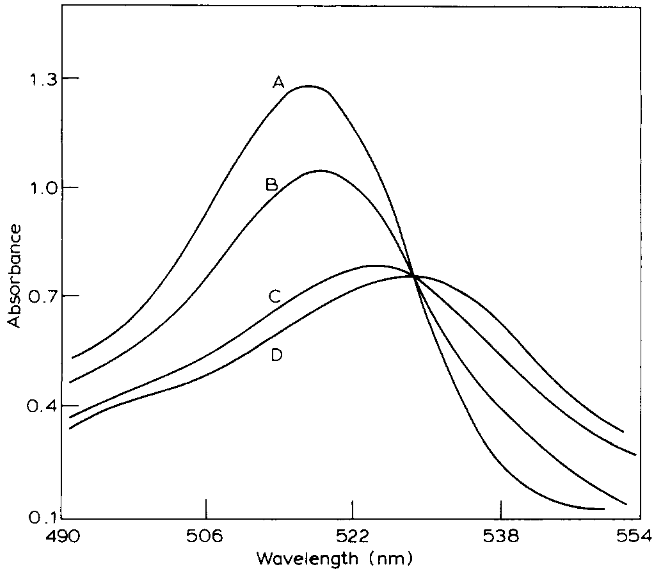

Figure 4 The effect of poly(paraxylylviologen dichloride) on the absorption spectrum of eosin Y (2×10^{-5} M) at 25°C in aqueous solutions. Polymer:dye ratios: A, 0; B, 1.0; C, 3.0; D, 5.0

Table 1 The positions of the absorption bands of dyes in the absence and in the presence of polycations in water at 25°C

Dye	Polycation	Wavelength of m-band (nm)	Wavelength of metachromatic band (nm)
Fl	$PPXVCl_2$	492	498
Fl	P4VPMeCl	492	510
Eo-Y	$PPXVCl_2$	517	525

fraction of free dye (α) was determined from the absorption changes at 485 nm and 495 nm for Fl and 510 nm and 520 nm for Eo-Y. The results at the two wavelengths, for each dye, agreed to within ±2.5%.

With knowledge of α and the total dye concentration (C_T), the concentrations of the bound dye (C_b) and the free dye (C_f) were determined[14]. The theoretical analysis of equilibrium of binding by Scatchard[21] indicates that if the binding sites are equivalent, a plot of γ/C_f *versus* γ should be linear, where γ represents the moles of bound dye per base mole of the polymer. As can be seen from *Figure 5*, this is evident in the binding of Fl and Eo-Y to the polycations studied.

These values have been used to evaluate the binding constant, K, and the number of moles of dye per base of polycation, n, for each dye-anion/polycation system. The values of K and n have been calculated by means of the least squares method. The free energy changes ($-\Delta G^\circ$) of dye-anion/polycation binding process have been arrived at from

$$\Delta G^\circ = -RT\ln K \qquad (2)$$

where R and T are the gas constant and the absolute temperature (K), respectively. The binding parameters and the free energy change for each system are presented in *Table 2*.

Calorimetry

Enthalpy of dilution. The enthalpies of dilution, ΔH_D (J mol^{-1} or J bm^{-1}), of dyes and polycations have been arrived at by the procedure described elsewhere[14,19] and are illustrated in *Figures 6* and *7*, respectively. It can be seen from *Figure 6* that the enthalpy of dilution of Fl is endothermic in nature and is in contrast to Eo-Y. The latter exhibits an exothermic enthalpy change upon dilution. For both polycations examined, however, the enthalpy of dilution is exothermic over the measured concentration range (*Figure 7*). The exothermic nature of ΔH_D (J bm^{-1}) for the polycations is in the order P4VPMeCl > $PPXVCl_2$ and the difference between the polycations is much more pronounced at higher concentrations.

Enthalpy of reaction. The enthalpies of dilution of the dyes (ΔQ_d) and the polycations (ΔQ_p) and the enthalpy of reaction (ΔQ_R), all in joules per hour, for all the dye-anion/polycation systems have been calculated by the method outlined elsewhere[14,19] and are presented in *Tables 3–5*. The observed values of the heat of reaction (ΔQ_R^{obs}) was corrected for the enthalpies of dilution of both the dye and the polycation to obtain the actual enthalpy of reaction (ΔQ_R) by the expression

$$\Delta Q_R = (\Delta Q_R^{obs}) + (\Delta Q_d) + (\Delta Q_p) \qquad (3)$$

The exothermic nature of ΔQ_R and the dependence of it on the dye concentration is apparent from these tables. The exothermicity of ΔQ_R for the dye–$PPXVCl_2$ complex formation is in the order Eo-Y > Fl and for the Fl–polycation complex formation is P4VPMeCl > $PPXVCl_2$.

Evaluation of the thermodynamics of binding. The thermodynamic parameters for the dye-anion/polycation complex formation are presented in *Tables 3–5*. The enthalpy of binding (ΔH_B (J mol^{-1})), has been calculated by the relation[14]

$$\Delta H_B = \Delta Q_R/\sigma \qquad (4)$$

where σ is the amount of dye bound in moles per hour. The value of σ has been evaluated from the spectrophotometric data by the expression[14]

$$\sigma = C_b \times n_T \qquad (5)$$

where C_b is the bound dye in moles per litre, and n_T is the total flow rate in litres per hour in the microcalorimeter.

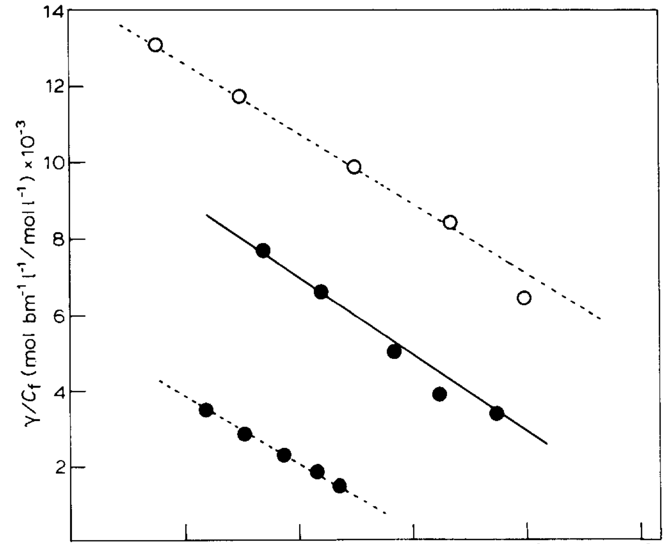

Figure 5 Scatchard plots for the binding of fluorescein (●) and eosin Y (○) to polycations at 25°C in aqueous solutions: poly(paraxylylviologen dichloride) (- - -); poly(4-vinylpyridinium methyl chloride) (——)

Table 2 Binding constants and free energy changes of dye-anion/polycation complexes at 25°C obtained by the Scatchard treatment

Dye	Polycation	C_p°* ($\times 10^4$ bm l^{-1})	K ($\times 10^{-3}$ mol^{-1} l)	$-\Delta G^{\circ}$ ($\times 10^{-3}$ J mol^{-1})	n (mol dye/ bm polycation)
Fl	PPXVCl$_2$	2.50	8.45	22.4	0.65
Fl	P4VPMeCl	2.50	11.02	23.1	1.03
Eo-Y	PPXVCl$_2$	2.50	9.94	22.8	1.47

* C_p° is the initial concentration of the polycations

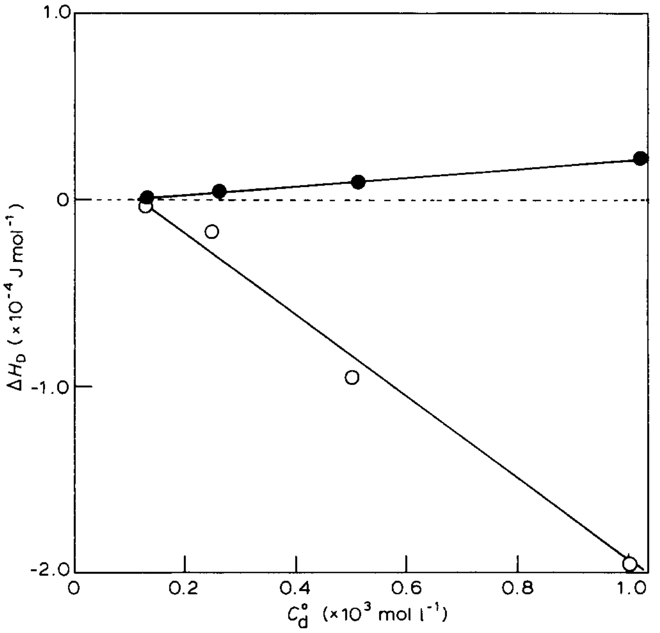

Figure 6 Enthalpies of dilution of fluorescein (●) and eosin Y (○) at 25°C to a final concentration of 6×10^{-5} mol l^{-1}

The free energy changes ($-\Delta G^{\circ}$) have been evaluated from the spectrophotometric data as described above (equation (2)). The entropy change of binding (ΔS°) for the dye-anion/polycation complex formation has been obtained by the use of the Gibbs–Helmholtz equation in the form

$$\Delta S^{\circ} = (\Delta H_B - \Delta G^{\circ})/T \qquad (6)$$

where $\Delta H_B \equiv \Delta H^{\circ}$ and T is the absolute temperature.

The values of ΔH_B, ΔG° and ΔS° are all negative and are independent of the dye concentration (*Tables 3–6*).

Standard states. In our calculations, we have assumed that in dilute solutions the activity coefficients of the dyes and the polycations are unity and the values of $\Delta H_B(\Delta H^{\circ})$, ΔG° and ΔS° have been calculated on that basis. The values of the enthalpy changes (and also the other parameters) relate to the ion exchange of one mole of the dye cation for one mole of the chloride ion at the concentrations specified, after the appropriate corrections have been made.

DISCUSSION

Enthalpies of dilution of xanthene dyes

The aggregation of fluorescein and its halogenated derivatives has been thoroughly investigated in the past[22–25]. The dimerization constants for Fl and Eo-Y have been determined in water to be 5 and 110, respectively[22]. The nature of the forces involved in aggregation and the mechanism thereof have been proposed for these dyes on the basis of the thermodynamic parameters that have been calculated by the use of the Van't Hoff Isochore. From the trend in the values of ΔH° and ΔS° it has been deduced that the process of dimerization gradually changes from an enthalpy-directed one to entropy-directed one upon increasing halo-substitution of Fl.[23] Involvement of hydrogen bonding through solvent has been proposed as the mechanism for dimerization of Fl.[23,24] Hydrophobic interactions, however, have been implicated to be the major driving force for the aggregation of Eo-Y.[22,23]

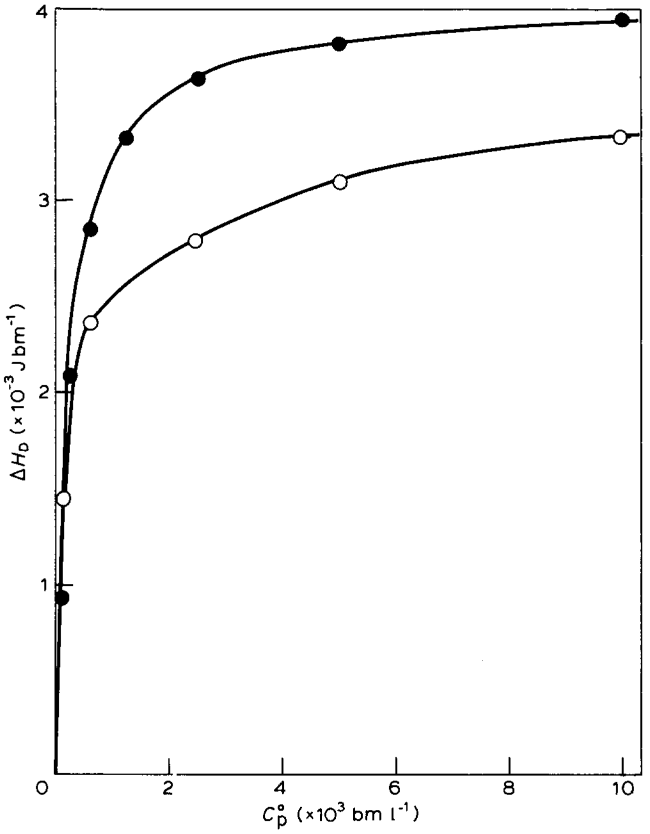

Figure 7 Enthalpies of dilution of poly(paraxylyviologen dichloride) (○) and poly(4-vinylpyridinium methyl chloride) (●) at 25°C, to a final concentration of 8×10^{-5} bm l^{-1}

It has been shown previously[19] that the overall enthalpy of dilution, ΔH_D, can be expressed in terms of at least four major factors by the relation

$$\Delta H_D = (-\Delta H_H) + (-\Delta H_e) + (-\Delta H_0) + (\Delta H_L) \qquad (7)$$

where ΔH_H is the enthalpy of hydrolysis, ΔH_e denotes the enthalpy change of electrostatic interactions, ΔH_0 that of non-electrostatic interactions (hydrophobic interactions,

Table 3 Thermodynamic parameters for the binding of fluorescein by poly(*p*-xylyl viologen) dichloride in water at 25°C

C_p* (× 10^4 bm l^{-1})	C_d* (× 10^4 mol l^{-1})	ΔQ_p (× 10^2 J h^{-1})	ΔQ_d (× 10^2 J h^{-1})	ΔQ_R (× 10^2 J h^{-1})	σ (× 10^6 M h^{-1})	ΔH_B† (× 10^{-3} J mol^{-1})	ΔG (× 10^{-3} J mol^{-1})	ΔS° (J K^{-1} mol^{-1})
2.43	5.24	−0.16	1.46	−5.08	2.08	−24.5	−22.4	−7.05
2.39	4.26	−0.16	0.93	−4.78	1.96	−24.5	−22.4	−7.05
2.46	3.37	−0.16	0.62	−4.70	1.92	−24.5	−22.4	−7.05
2.39	2.66	−0.15	0.32	−3.87	1.57	−24.6	−22.4	−7.38
2.36	1.89	−0.15	0.16	−3.35	1.37	−24.5	−22.4	−7.05
2.59	1.22	−0.18	0.09	−3.20	1.30	−24.6	−22.4	−7.38
2.68	0.47	−0.15	0.00	−2.35	0.96	−24.5	−22.4	−7.05

* C_p and C_d are the concentrations of $PPXVCl_2$ and Fl, respectively, in the flow-mixing cell of the microcalorimeter
† $\Delta H_B \equiv \Delta H^\circ$

Table 4 Thermodynamic parameters for the binding of fluorescein by poly(4-vinyl pyridine) methyl chloride in water at 25°C

C_p* (× 10^4 bm l^{-1})	C_d* (× 10^4 mol l^{-1})	ΔQ_p (× 10^2 J h^{-1})	ΔQ_d (× 10^2 J h^{-1})	ΔQ_R (× 10^2 J h^{-1})	σ (× 10^6 M h^{-1})	ΔH_B† (× 10^{-3} J mol^{-1})	ΔG° (× 10^{-3} J mol^{-1})	ΔS° (J K^{-1} mol^{-1})
2.54	5.02	−0.54	2.39	−10.00	3.69	−27.1	−23.1	−13.6
2.50	4.08	−0.53	1.35	−8.60	3.17	−27.1	−23.1	−13.6
2.52	3.29	−0.53	0.88	−7.40	2.72	−27.2	−23.1	−13.8
2.49	2.55	−0.53	0.52	−6.40	2.35	−27.2	−23.1	−13.8
2.51	1.78	−0.53	0.25	−5.00	1.84	−27.2	−23.1	−13.8
2.51	1.27	−0.53	0.13	−4.30	1.59	−27.1	−23.1	−13.6
2.50	0.51	−0.53	0.00	−3.10	1.14	−27.1	−23.1	−13.6

* C_p and C_d are the concentrations of P4VPMeCl and Fl, respectively, in the flow-mixing cell of the microcalorimeter
† $\Delta H_B \equiv \Delta H^\circ$

Table 5 Thermodynamic parameters for the binding of Eosin-Y by poly(*p*-xylyl viologen) dichloride in water at 25°C

C_p* (× 10^4 bm l^{-1})	C_d* (× 10^4 mol l^{-1})	ΔQ_p (× 10^2 J h^{-1})	ΔQ_d (× 10^2 J h^{-1})	ΔQ_R (× 10^2 J h^{-1})	σ (× 10^6 M h^{-1})	ΔH_B† (× 10^{-3} J mol^{-1})	ΔG° (× 10^{-3} J mol^{-1})	ΔS° (J K^{-1} mol^{-1})
2.43	3.32	−0.17	−0.55	−8.19	2.59	−31.6	−22.8	−29.2
2.40	2.58	−0.17	−0.36	−6.98	2.23	−31.4	−22.8	−29.0
2.42	1.80	−0.17	−0.15	−5.38	1.71	−31.4	−22.8	−29.0
2.41	1.03	−0.17	−0.05	−3.18	1.01	−31.4	−22.8	−29.0
2.44	0.51	−0.17	−0.01	−1.58	0.50	−31.6	−22.8	−29.2

* C_p and C_d are the concentrations of $PPXVCl_2$ and Eo-Y, respectively, in the flow-mixing cell of the microcalorimeter
† $\Delta H_B \equiv \Delta H^\circ$

Table 6 Thermodynamic parameters for the binding of xanthene dyes by polycations in water at 25°C

Dye	Polycation	K (× 10^{-3} mol^{-1} l)	ΔG° (× 10^{-3} J mol^{-1})	ΔH_B* (× 10^{-3} J mol^{-1})	ΔS° (J K^{-1} mol^{-1})
Fl	$PPXVCl_2$	8.45	−22.4	−24.5	−7.1
Fl	P4VPMeCl	11.02	−23.1	−27.2	−13.7
Eo-Y	$PPXVCl_2$	9.94	−22.8	−31.5	−29.1

* $\Delta H_B \equiv \Delta H^\circ$

for example) excluding the hydrolysis (ΔH_H), and ΔH_L that of London–van der Waals interactions.

Since only hydrogen bonding has been suggested to be involved in the dimerization of Fl, it would be consistent to suggest that the process of disaggregation would also involve mainly these forces. Consequently we may rule out any contributions from ΔH_0 towards the overall enthalpy of dilution, or disaggregation, of Fl. However, for Eo-Y all four enthalpic factors will contribute towards the overall enthalpy of dilution. The exothermic nature resulting from the contributions from ΔH_0 and ΔH_e in particular (*Figure 6*). This explanation for the enthalpies of dilution is consistent with the dimerization constants (K_D(Fl) = 5; K_D(Eo-Y) = 110) and the thermodynamics of dimerization for these dyes[21–23].

Enthalpy of dilution of polycations

The dependence of the enthalpy of dilution of polyelectrolytes on the concentration, charge density and temperature has been observed by Mita *et al.*[26,27]. This dependence of enthalpy change of dilution on concentration is apparent from *Figure 7*. The difference in the exothermicity of ΔH_D for the two polycations is quite significant at the higher concentrations and may be considered in terms of the charge densities. The exothermic enthalpy of dilution has been shown to increase with increasing charge density and has been explained in terms of the charge density being the function of the flexibility of the polyelectrolyte[26]. The charge density of the polyelectrolyte in solution has been estimated to be larger than that of the simple linear model, owing to the flexibility of the polymer chain. Thus greater exothermic ΔH_D would be expected for a more flexible polyelectrolyte with larger charge density. A lower charge density would be expected, on this basis, for a stiff linear polyelectrolyte such as $PPXVCl_2$. A much higher density would, however, be expected for a polycation with pendant groups such as P4VPMeCl[26]. Thus it may be deduced that the difference in the exothermic enthalpy of dilution is due to the conformation of these polyelectrolytes. That is, the expected higher exothermic enthalpy of dilution for the more flexible P4VPMeCl and lower exothermic enthalpy of dilution for a more rigid or less flexible $PPXVCl_2$ are consistent with our observations.

Enthalpies of binding of xanthene dyes by polycations

Examples of the binding of xanthene dyes to polymers such as serum albumin[3,6,7,11] and poly(vinyl pyrrolidone)[10,14] are present in the literature. Involvement of hydrophobic interactions, in addition to energetic ones, has been suggested to play a major role in the process of binding.

For the three dye-anion/polycation systems studied, it is evident from their spectral changes (*Figures 2–4*) that the type of interactions involved in the process of binding are quite different for each system. This is further substantiated by their thermodynamic parameters obtained during this investigation (*Tables 3–5*). In developing an explanation of the mechanisms of binding it is most convenient to consider first the dye-anion/$PPXVCl_2$ system and then the Fl-anion/polycation system.

Although the red shift in the absorption spectra for both dye-anion/$PPXVCl_2$ systems is similar (i.e. 6 nm for Fl and 8 nm for Eo-Y), the difference in the shape of the spectra is quite significant (*Figures 2* and *4*). For Eo-Y the intensity of absorption decreases with an increase in the polyelectrolyte concentration. However, no such drop in the intensity of absorption occurs for Fl upon increasing $PPXVCl_2$ concentration. For the latter the spectra gradually shifts to the higher wavelength without a decrease in the absorption intensity.

Spectral shifts on binding of dye-anions to polycations have been considered to occur because of dye–dye interactions[3–5] or dye–polyelectrolyte interactions[6,9]. The gradual spectral shift towards the higher wavelength, upon increasing the polyelectrolyte/dye ratio, for Fl–$PPXVCl_2$ can be interpreted in terms of the dye–polyelectrolyte interaction hypothesis. That is to say that the spectral shift is purely due to the change in the environment of the dye–anion upon binding the polycation. This suggestion is supported by two observations. First, spectral shifts, similar to the Fl–$PPXVCl_2$ system, towards higher wavelengths have been observed for Fl in ethanol[14]. An analogy can be drawn between the effect of ethanol and the binding of the dye to polyelectrolyte in changing the environment of the dye. This analogy is reinforced by the observation that ethanol quenches the fluorescence of this dye[14] just as does the polycation, $PPXVCl_2$.[28] It has been proposed that alcohol probably affects the charge centre ($-\bar{O}-Na^+$) of the chromophoric system of the dye, thereby producing a spectral shift similar to that produced when a dye is bound on a polyelectrolyte[29]. Secondly, since there is no difference between the absorption intensities of Fl in water and ethanol, it may be concluded that Fl is almost entirely monomeric in water ($k_D = 5$)[22], as no dimerization would occur in ethanol[30]. The low aggregating tendency of Fl has also been considered above, in terms of the enthalpy of dilution. Only 0.67 mol of Fl are bound to one base mole of $PPXVCl_2$ (*Table 2*), which suggests that the dye molecules are too far apart from each other for any intermolecular interactions to take place.

The dye–dye interaction hypothesis for the spectral changes does, however, explain the spectral anomalies observed for the Eo-Y anion/$PPXVCl_2$ system. Approximately 1.5 mol of Eo-Y are bound to 1 bm of $PPXVCl_2$ (*Table 2*). An enhancement of the absorption intensity is observed for Eo-Y when transferred from water to ethanol[14]. That indicates that the dye is in an aggregated state in aqueous solutions[30]. This suggests that Eo-Y must exist partially in an aggregated form ($k_D = 110$)[22] before binding the polycation. Unlike the Fl–$PPXVCl_2$ system, much more Eo-Y is bound to $PPXVCl_2$; consequently dye molecules are closer together, and the intermolecular interactions are inevitable. Furthermore, complete quenching of fluorescence was observed upon addition of $PPXVCl_2$ to Eo-Y in aqueous solution[28] and is contrary to the Eo-Y–ethanol system, where enhancement rather than quenching of fluorescence was observed[14]. Thus the reduction in the absorption intensity with a simultaneous spectral shift to the higher wavelengths upon increasing the polyelectrolyte/dye ratio, suggests that the Eo-Y molecules, after getting bound to $PPXVCl_2$, undergo some interaction between one another, due to closeness and superposition, which affects the charges of the chromophoric systems, thereby resulting in a spectral shift.

The considerations above may be taken a step further due to the availability of the thermodynamic parameters. As would be expected from the dimerization constants of these dyes (k_D(Eo-Y) = 110; k_D(Fl) = 5), the binding strength is greater for the Eo-Y–$PPXVCl_2$ system than for Fl–$PPXVCl_2$ system (*Table 2*). The entropy changes of binding for both systems are negative, which suggests that the complex formation is essentially electrostatic in nature and is verified by the high exothermic enthalpy changes of binding (*Tables 3, 5, 6*). However, the entropy changes are much less negative for the Fl–$PPXVCl_2$ system (*Tables 3* and *6*), suggesting that forces, other than electrostatic ones, are involved in stabilizing this complex. This is also evident from *Table 2* from the fact that approximately 1.5 bm $PPXVCl_2$ is involved in building 1 mol of Fl. The complex is probably stabilized by van der Waals forces between dye molecules and the pyridinium rings of $PPXVCl_2$.

The spectral changes for the Fl–P4VPMeCl system (*Figure 3*) again differ to a great extent from the Fl–$PPXVCl_2$ system (*Figure 2*). A gradual spectral shift to the higher wavelength (18 nm for P4VPMeCl and 6 nm for $PPXVCl_2$; *Table 1*) together with an increase in the absorption intensity of the metachromatic band, upon increasing the polyelectrolyte/dye concentration, have been suggested to be due to dye–dye interactions upon binding the polycation[4]. This is further established by the complete quenching of fluorescence upon binding the polycation (P4VPMeCl)[28]. A 1:1 stoichiometric complex formation for Fl–P4VPMeCl in contrast to 0.67:1 for Fl–$PPXVCl_2$ (*Table 2*) suggests that the negatively charged dye molecules are held on the positively charged sites on the former polycation mainly by coulombic attraction. The electrostatic nature of the Fl–P4VPMeCl complex formation is also apparent from its higher exergonic free energy change of binding as well as its much larger exothermic enthalpy and negative entropy changes as compared with Fl–$PPXVCl_2$ complexation (*Tables 3* and *4*).

By comparing the three systems (*Table 6*), it may be noted that although the Eo-Y–$PPXVCl_2$ system carries much larger negative entropy and enthalpy changes, the exergonic value of the free energy change of binding is lower than that of the Fl–P4VPMeCl system. This suggests that the process of binding of Eo-Y to $PPXVCl_2$, like that of Fl to $PPXVCl_2$ considered above, may involve forces other than electrostatic ones. We therefore suggest that the binding of Eo-Y to $PPXVCl_2$ involves hydrophobic interactions as well as London–van der Waals, in addition to the electrostatic ones.

CONCLUSIONS

It is concluded from the thermodynamic parameters that the binding of Fl to P4VPMeCl is mainly electrostatic in nature, as would be expected for ionic interactions. However, for $PPXVCl_2$, where the binding would also be expected to be largely electrostatic, the thermodynamic parameters show that a significant contribution to the binding comes from hydrophobic or non-electrostatic interactions. It is deduced that the aromatic rings of $PPXVCl_2$ chain are the cause of this difference in the binding parameters, which is further substantiated by the spectral anomalies that these dyes undergo in the presence of this polycation.

Both hydrophobic and electrostatic forces have also been suggested to be involved in the stabilization of the Eo-Y–$PPXVCl_2$ complex. The difference in the binding behaviour of Fl and Eo-Y to $PPXVCl_2$ is postulated to be a consequence of the higher aggregating tendency of the latter.

From the thermodynamic parameters and the spectral changes obtained for these dye–polycation systems it is deduced that: (a) dye-anion/polycation interactions are responsible for the spectral changes in the Fl–$PPXVCl_2$ system; (b) both dye–dye and dye-anion/polycation interactions are involved in the spectral anomalies for the Eo-Y–$PPXVCl_2$ and Fl–P4VPMeCl systems; (c) the electrostatic contribution to dye-anion/polycation binding process for the three systems is in the order: Fl–P4VPMeCl > Eo-Y–$PPXVCl_2$ > Fl–$PPXVCl_2$.

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