Polyanions and their Complexes. Part 10.[1] Mechanism of Dye Binding to Chemically Different Sites

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Thermodynamic parameters have been determined for the ion-exchange of Methylene Blue cations with sodium ions, or potassium ions on cellulosic polyanions. These polyanions resemble biological polyelectrolytes with respect to their ion binding characteristics. The chemical nature of the site determines the free energy change for the binding of Methylene Blue in the order $SO_3^- > CO_2^- > PO_2^- > CH_2CO_2^-$. The mechanism of dye binding is discussed and an explanation is advanced for the influence of the site on the order of binding. It is shown that the affinity of cationic dyes for a polyanion may be predicted from a knowledge of their tendency to aggregate in aqueous solution.

RECENTLY [1] it has been shown that the interaction between Methylene Blue and heparin is reduced by the ions of both the alkali and alkaline earth metals with an effectiveness that depends upon the hydrated radius of the ion used. Consideration of the known polyanions of biological origin shows that the acid groups present are limited to one or more of the following: SO_3^-, $NHSO_3^-$, PO_3^{2-},PO_3H^-, PO_2^-, $NHCO_2^-$, and CO_2^-, (heparin contains 3 types, *viz*, SO_3^-, $NHSO_3^-$, and CO_2^-). For naturally occurring polyanions the order of affinity for dye cations has usually been given as sulphate > phosphate > carboxylate.[2] Although we have advanced a possible explanation [3] for the order sulphate > carboxylate, the role of the anionic site in dye binding has not yet been clearly defined.

We have reported previously [3] the use of synthetic cellulosic derivatives as models for the study of the dye-binding properties of biological polyanions. In our present study we have extended this work to include oxycelluloses in which certain of the primary alcohol groups have been oxidised by dry dinitrogen tetraoxide to carboxylic acid groups. Thus treated, the oxycelluloses and their salts show features which resemble several biologically important polymers, such as hyaluronates, alginates, and pectinates.

To clarify the role of the site in the phenomenon of dye binding, we have compared the behaviour of the oxycelluloses with that of a new series of cellulose sulphates and a cellulose phosphate using the dye Methylene Blue.

EXPERIMENTAL

Materials.—Cotton yarn supplied by U.S. Department of Agriculture, was of the Deltapine variety and was purified by a method [4] based on that of Kettering and Conrad.[5] The purified material had the same degree of polymerisation as yarn purified according to ref. 5, but the final product was cleaner, had a higher reflectivity, and contained fewer carboxy-groups (0.22mM per 100 g compared with 0.60mM per 100 g).

Cellulosic polyanions containing phosphate (CP) and sulphate (CS) were prepared by the methods described previously.[3] One particular cellulose sulphate (CSW) was prepared by the method of Schofield and Lawton [6] using a solution of urea in aqueous sulphuric acid. Additionally, a cellulose sulphate (CSP) was prepared from air-dry cotton yarn (7.2% moisture) which had been pretreated with pyridine before reacting with chlorosulphonic acid in pyridine.

Oxycelluloses were prepared by a method based on that

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[1] Part 9, F. Joojahdeh, J. S. Moore, G. O. Phillips, and J. V. Davies, *J.C.S. Perkin II*, 1974, 1468.

[2] J. E. Scott, 'Solution Properties of Natural Polymers,' Chem. Soc. Special Publ. No. 23, 1968,

[3] P. J. Baugh, J. B. Lawton, and G. O. Phillips, *J. Phys. Chem.*, 1972, **76**, 688.

[4] J. B. Lawton, Ph.D. Thesis, University of Salford, 1972.

[5] J. H. Kettering and C. M. Conrad, *Ind. and Eng. Chem.* (*Analyt. Edn.*), 1942, **14**, 432.

[6] A. Schofield and J. B. Lawton, B.P. 1,096,736/1965.

of Nevell[7] using a dry cotton and anhydrous dinitrogen tetraoxide at room temperature. Under these conditions only the primary alcohol group is oxidised to carboxy.[8,9] The reaction was stopped after various times by immersing the yarn in deionised water (OC1,OC3). One sample (OC2) was additionally immersed in sodium carbonate solution (10 g l^{-1}; room temperature) and then washed in deionised water.

The degree of substitution (d.s.) and the degree of polymerisation of the materials were determined as described previously.[3] In addition, the d.s. was checked by a radiotracer method[4] based on the work of Maroudas and Thomas.[10] In the method, a solution of sodium veronal (pH 8) containing ^{22}Na (β^+, γ, half-life 2.56 years) was equilibrated with the sodium salt of the polyanion and the uptake of radioactive sodium determined. It was found that some of the cellulosic polyanions contained a small amount of a soluble sodium salt (from 0.21×10^{-4} mol g^{-1} for CS1 to 0.80×10^{-4} mol g^{-1} for CSP1). This was allowed for in relevant calculations. Sodium was determined by use of an EEL flame photometer, model A.

The sulphur content of the cellulose sulphates was determined by the oxygen method of Schoniger,[11] as modified by Beswick and Johnson.[12] Phosphorus was estimated by the wet digestion method of Basch and Wasserman.[13]

Methylene Blue was obtained from G. T. Gurr Ltd. The sample was used in all experiments and was found to be chromatographically homogeneous by the method of Bergmann and O'Konski[14] using a column of alumina eluted with ethanol. The dye content of the sample (87.74%) was determined by the method of Ferrey.[15] The moisture content (12.36%) was measured by drying at 105 °C. The molar extinction coefficient of a 9.6×10^{-7}M solution was calculated to be 9×10^4 at 665 nm by comparison with standard copper sulphate solution.[16] The t.l.c. method of Loach[17] showed the presence of a trace of trimethylthionine in the dye.

Polyanion–Dye Equilibria.—These were studied by the method described previously.[3] The conditions were chosen to give equilibrium dye concentrations in the range 3—9×10^{-5}M; this allowed a study to be made over a wide range of composition of the dye–polyanion complexes. Adsorption isotherms, at pH 8, have shown that for equilibrium dye concentrations $>$*ca.* 10^{-4}M, in the absence of salt, virtually all sites are occupied by dye cations. For the cellulose sulphates the concentration of salt (NaCl or KCl) used was extended from 0.15 to 0.9M in order to displace almost all the dye from the polyanion.

RESULTS

The experimental data for equilibrium (1) ($A^+ = Na^+$ or K^+, and MB^+ is the Methylene Blue cation) were analysed

$$CA + MB^+ = CMB + A^+ \qquad (1)$$

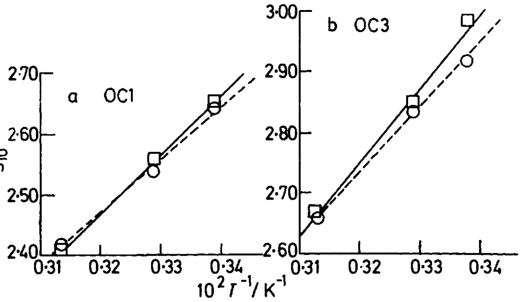
by our previous method.[3] Details may be found elsewhere.[4] In an alternative treatment we used the concentrations and the activity coefficients of monomeric Methylene Blue calculated[4] from the results of Mukerjee and Ghosh[18] for solutions having ionic strengths up to 0.15. Table 1 shows the results, with the subscripts M and T relating to calculations with monomeric and total dye cation concentrations respectively. The agreement between $\log K_M$ and $\log K_T$ is very good, and only in two instances do the values differ by $>1\%$ (OC3, Na^+, 23 °C by 2.3%, and OC3, K^+, 25.6 °C by 1.3%). These variations are only slightly outside the experimental error, which is estimated to give an error in $\log_{10}K$ of $\pm 0.3\%$.

TABLE 1

A comparison of equilibrium constants calculated by different methods for the ion exchange of Methylene Blue with sodium or potassium ions on cellulose polyanions

Polyanion	Counter-ion	Temp. (°C)	$\log K_M$	$\log K_T$
OC1	Na^+	22.9	2.649	2.636
OC1	Na^+	30.9	2.516	2.538
OC1	Na^+	46.0	2.405	2.412
OC1	K^+	25.6	2.562	2.547
OC2	Na^+	25.9	2.704	2.689
OC2	Na^+	37.9	2.574	2.591
OC2	K^+	27.0	2.492	2.469
OC2	K^+	38.4	2.309	2.334
OC3	Na^+	23.0	2.986	2.920
OC3	Na^+	30.9	2.487	2.836
OC3	Na^+	46.4	2.669	2.660
OC3	K^+	25.6	2.678	2.645
CP7	Na^+	25.9	2.470	2.452
CP7	Na^+	37.8	2.264	2.252
CP7	K^+	26.8	2.222	2.213
CP7	K^+	37.7	2.172	2.188
CS1	Na^+	24.7	*	3.679
CS1	Na^+	35.8	*	3.552
CS2	Na^+	25.6	*	3.736
CS2	Na^+	36.8	*	3.524
CS2	K^+	24.6	*	3.494
CS2	K^+	36.0	*	3.203
CSP1	Na^+	25.5	*	3.624
CSP1	Na^+	36.9	*	3.498
CSW1	Na^+	24.9	*	2.783
CSW1	Na^+	36.0	*	2.594

* Data on activity co-efficients of monomeric dye not available at ionic strengths >0.15M.



Evaluation of enthalpy changes from plots of the van't Hoff isochore, for the ion exchange of Methylene Blue and sodium ions on oxycelluloses: ○, K_T(ΔH: a, 17.6; b, 23.2 kJ mol^{-1}); □, K_M(ΔH: a, 18.5; b, 24.1 kJ mol^{-1})

For the cellulose sulphates the ionic strengths of the

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[12] G. Beswick and R. M. Johnson, *Talanta*, 1970, **39**, 709.

[13] A. Basch and T. Wasserman, *Textile Res. J.*, 1969, **39**, 679.

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[18] P. Mukerjee and A. K. Ghosh, *J. Amer. Chem. Soc.*, 1970, **92**, 6413.

solutions at equilibrium were >0.15M, and thus calculations could be made using only total dye concentrations. For OC1 and OC3 van't Hoff plots are shown in the Figure for the variation of $\log_{10}K_T$, or $\log_{10}K_M$ with temperature. Table 2 summarises the thermodynamic parameters calculated from the values of $\log_{10}K_T$ in Table 1 by application of the reaction isotherm, the van't Hoff isochore and the Gibbs–Helmholtz equation. Due to the form of the van't Hoff isochore, a small error in $\log_{10}K_T$ causes a considerable change in the value of ΔH° (and hence also in ΔS°). Calculations show that the maximum errors in ΔH° range from $\pm 7\%$ for CS2 (K^+, Table 3) to $\pm 16\%$ for OC2 (Na^+, Table 2) with a possible error of $\pm 53\%$ for CP7 (K^+, Table 3). Thus, even in the worst case (CP7, K^+) the sign of ΔH° is not affected, but the magnitude of ΔH° is subject to such uncertainty that it is not safe to base any reasoned argument on variations in ΔH° or ΔS° at present.

TABLE 2

Thermodynamic parameters for the exchange of Methylene Blue and sodium ions

Polyanion	Temp. (°C)	ΔG°/ kJ mol^{-1}	ΔH°/ kJ mol^{-1}	ΔS°/ J K^{-1} mol^{-}	d.s.
OC1	22.9	−15.0	−18	−9	0.007
OC1	30.9	−14.8			
OC1	46.0	−14.7			
OC2	25.9	−15.4	−15	+3	0.019
OC2	37.9	−15.4			
OC3	23.0	−16.6	−20	−12	0.049
OC3	30.9	−16.5			
OC3	46.4	−16.3			
CP1	25.9	−14.0	−30	−53	0.011
CP1	37.8	−13.4			
CS1	24.7	−21.0	−20	+3	0.009 6
CS1	35.8	−21.0			
CS2	25.6	−21.4	−33	−41	0.010
CS2	36.8	−20.9			
CSP1	25.5	−20.7	−20	+3	0.158
CSP1	36.9	−20.8			
CSW1	24.9	−15.9	−30	−47	0.017 8
CSW1	36.0	−15.4			

TABLE 3

Thermodynamic parameters for the exchange of Methylene Blue and potassium ions

Polyanion	Temp. (°C)	ΔG°/ kJ mol^{-1}	ΔH°/ kJ mol^{-1}	ΔS°/ J K^{-1} mol^{-1}	d.s.
OC1	25.6	−14.6			0.007
OC2	27.0	−14.2	−21	−23	0.019
OC2	38.4	−13.9			
OC3	25.6	−15.1			0.049
CP1	26.8	−12.7	−4	+29	0.011
CP1	37.7	−13.0			
CS2	24.6	−19.9	−45	−85	0.010
CS2	36.0	−19.0			

Thus, we prefer to base our subsequent discussion on the values of the free energy changes for the reactions. We note here that all the exchanges studied are exothermic when represented by equation (1).

DISCUSSION

Dye Binding to Oxycellulose.—The previously observed influence of site density [3] on the value of ΔG° is evident for the carboxy-group (Table 2). The data also show that the order of dye binding of the acid groups on polyanions of similar degrees of substitution (CS3, OC2, CP1) to be $SO_3^- > CO_2^- > PO_2^-$. This trend agrees with the data in Table 3 and taken in conjunction with earlier results [3] establishes the strength of dye binding for the four groups studied to be: $SO_3^- > CO_2^- > PO_2^- > CH_2CO_2^-$.

Binding Properties of the Sulphate Group.—The results for CS2 and CSW1 (Table 2) show that the same polyanion (here polysulphate) prepared by different methods can exhibit very different binding properties due to differences in distribution of the anionic group within the polymer. To elucidate the role of intersite distance (as distinct from the degree of substitution), three types of cellulose sulphate have been studied. Using the microfibrillar model for cellulose postulated by Jefferies *et al.*,[19] the anhydrous, non-swelling conditions used in the preparation of CS1 and CS2 would ensure that the sulphate groups were located at more localised fibrillar surfaces, or junctions in the cellulose matrix, than the groups in CSP1, which was prepared under conditions which ensured that the cellulose had a more 'open' structure. The sites in CSW1 would be even more widely dispersed, since *ca.* 25% of the hydroxy-groups in cellulose are accessible to aqueous solutions, whereas only 13% can be reached by non-swelling solvents such as pyridine.[19] Thus, for a given DS, the magnitudes of the intersite distances would vary CSW > CSP > CS. Table 2 shows the free energy change for the binding of methylene blue to be CSW < CSP < CS, despite the fact that CSP1 has the highest DS which would contribute to stronger binding.

The distribution of the sites (*i.e.* the intersite distance) is, therefore, almost as important as the nature of site in controlling the interaction of Methylene Blue with a given polyanion. The affinity of the dye for CSW1 (Table 2) is comparable with that for OC2 (similar DS), but dye binding remains $SO_3^- > CO_2^-$. Thus the dye-binding behaviour of a given polyanion should be interpreted with caution, since the properties of any given chemical site will be considerably influenced by the distribution of the sites and the consequent intersite distance.

Mechanism of Ion Binding.—The free energy changes associated with dye binding can be calculated on the basis of a simple model.[3] Such calculations [4] serve to give quantitative estimates of the factors that appear to be of crucial importance to dye-binding.

We have chosen to represent the site as a symmetrical, polarisable sphere of charge Ze (Z = valency, e = electric charge). The dye ion (A^+), and the alkali metal, or other competing ion (B^+) are represented as polarisable spheres of effective radii r_A and r_B.

The experimentally determined free energy changes may be regarded as arising from two main sources: (A) ion–site interactions, and (B) interaction of the ions (and sites) with the solvent and with each other.

(A) *Ion–site interactions*. When a polarisable cation approaches a polarisable anionic site, several forces of attraction are operative: coulombic attraction of the ions, interaction of the ions with both induced and perma-

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nent dipoles, dipole–dipole interactions, and multipolar interactions. We have not been able to make exact estimates of each of these due to lack of data, but the considerations which now follow show that the coulombic interaction is almost an order of magnitude greater than the other effects.

(i) Free energy change due to coulombic interactions. Using previous nomenclature,[3] the coulombic or electrostatic component of the free energy change, $\Delta G_{AB}{}^{E}$ kJ mol^{-1}, for the exchange reaction (2) is given by (3) where

$$A^+ + BC \rightleftharpoons AC + B^+ \qquad (2)$$

$$\Delta G_{AB}{}^{E} = \frac{138.9}{D}\left[\frac{1}{r_e + r_A} - \frac{1}{r_e + r_B}\right] \qquad (3)$$

the radii r_A, r_B, and r_e are measured in nm, and D is the effective dielectric constant of the medium separating an ion from the site.

Some estimates are available about the value of D. Rice and Nagasawa[20] have suggested that the aqueous phase trapped within a polyanion will have a dielectric constant of 30. However, if the ions approach very closely (<0.2 nm) the D value decreases to 2.[21] The bulk dielectric constant of water (78.54) is only reached at a distance of *ca.* 1.0 nm from the polyanion.[22]

Estimating the radii of the sites and the cations presents some difficulty, and these will be considered separately.

Radii of the anionic sites. For the carboxy-group Ling[23] considered that the negative charge might be considered to reside on one of the oxygen atoms. The effective radius of such a negative site is determined by the inductive effects of adjacent groups in the polyanion. On the Bronsted–Lowry definition, an acid is a proton donor, and the strength of such an acid is conveniently measured by the pK value. However, for polyacids the pK value has no simple meaning. Nevertheless under standard conditions, comparison of the pK values of different polyacids can distinguish one type of site from another, as illustrated in Table 4.

A site of small effective radius will attract protons strongly, and will thus have a high pK value and *vice versa*. Eisenman[24] has given an empirical relationship between pK and the effective radius of a site, r_e, as: $r_e = -0.044\text{p}K + 1.5$ where r_e is in units of 10^{-10} m.

On this basis, using the data in Table 4, the r_e values for the sites ($\times 10^{-10}$ m) may be taken as: SO_3^- 1.50; CO_2^- 1.36; $-CH_2CO_2^-$ 1.35. The r_e value for the PO_2^- group cannot be estimated at present due to lack of data.

Radii of the metal cations. Pauley[25] suggested that the radii of the ions in an exchanger were the same as the radii of the hydrated ions in aqueous solution. In terms of equation (2), potassium ions should, therefore, always bind more strongly than sodium ions ($\Delta G_{K,Na}{}^{E}$ will always be negative). This order is found to be true for sulphate groups,[25,26] but not for carboxy-groups[27,28] where sodium is the preferred ion. It is possible that the controlling factor is the more powerful attraction of the carboxy-group for cations, which appear to lose more of their water of hydration when bound to this grouping. This possibility is supported by the ultrasonic absorption studies of Tondre and Zana.[29]

TABLE 4

p*K* Values of some polyacids

Ionisation of polyanion	p*K*	Reference
$Cell{-}OC_2H_5SO_3H \rightleftharpoons Cell{-}OC_2H_5SO_3{}^+ + H^+$	0.5	*a*
$Cell{-}CO_2H \rightleftharpoons Cell{-}CO_2{}^- + H^+$	3.24	*b*
$Cell{-}CO_2H \rightleftharpoons Cell{-}CO_2{}^- + H^+$	3.1	*c*
Glucuronic acid	3.24	*b*
$Cell{-}PO_3H_2 \rightleftharpoons Cell{-}PO_3H^- + H^+$	1.5	*a*
$Cell{-}PO_3H^+ \rightleftharpoons Cell{-}PO_3{}^{2-} + H^+$	7.5	*a*
$Cell{=}PO_2H \rightleftharpoons Cell{=}PO_2{}^- + H^+$	†	
$Cell{-}OCH_2CO_2H \rightleftharpoons Cell{-}OCH_2CO_2{}^- + H^+$	3.65	*a*
$Cell{-}OCH_2CO_2H \rightleftharpoons Cell{-}OCH_2CO_2{}^- + H^+$	3.37 *	*d*
$Cell{-}OCH_2CO_2H \rightleftharpoons Cell{-}OCH_2CO_2{}^- + H^+$	3.40 *	*c*
$CH_3CO_2H \rightleftharpoons CH_3CO_2{}^- + H^+$	4.75	*e*
$HCO_2H \rightleftharpoons H\,CO_2{}^- + H^+$	3.75	*e*
$Cl_3CCO_2H \rightleftharpoons Cl_3CCO_2{}^- + H^+$	0.70	*e*

* Water soluble forms. † Relevant data is not available at present.

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Thus, the effective radius of the sodium ion in the presence of polysulphates will resemble its hydrated radius of 0.216 nm.[30] In contrast, in the presence of polycarboxylates the effective radius will be nearer to the crystal radius of 0.095 nm.[31]

Radius of the dye cation. The Methylene Blue cation will be only feebly hydrated in solution due to its hydrophobic nature and the delocalisation of the positive charge over the structure. It has been shown[32] that the sulphur atom of the central ring does not carry any positive charge so that the charge must reside elsewhere. Traditionally it has been taken to reside on the dimethylamino nitrogen atoms, and if this picture is correct, the effective radius should be similar to that of the tetramethylammonium ion which is 0.347 nm.[30]

Estimation of the actual value of the coulombic contribution to dye binding. On the basis of equation (1), using D 30, with the assumptions that the effective radius of the dye cation is the same at all sites whilst that of the sodium ion is 0.216 nm at a sulphate group (S), and 0.095

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[32] Y. Takahshi, K. Yabe, and T. Sato, *Bull. Chem. Soc. Japan*, 1969, **42**, 2707.

nm at a carboxy-group (C), the free energy changes due to coulombic interactions are $\Delta G_S{}^E$ +1.7 and $\Delta G_C{}^E$ +5.2 kJ mol^{-1}. (If the hydrated radius of the sodium ion is used, $\Delta G_C{}^E$ becomes +2.1 kJ mol^{-1}.)

The experimentally observed free energy changes for CS1 and OC1 are −21 and −15 kJ mol^{-1} (Table 2). It is clear that even if considerable changes were made in the parameters previously discussed, the endergonic coulombic interactions will not, in themselves, promote the preferential binding of the dye cation that is observed experimentally.

(ii) Free energy changes due to dipolar interactions. Due to lack of data it is only possible to estimate the order of magnitude of these interactions.

Making simple assumptions about the polarisabilities, and dipole moments of the ions and the sites, and using equations given by Bottcher,[33] the contributions of dipole–dipole, ion–dipole, and ion-induced dipole interactions have been estimated to be −0.68 kJ mol^{-1} at a sulphate group and −0.75 J mol^{-1} at a carboxy-group. Multipolar effects will be of lower magnitude and have been neglected.

Thus ion–site interactions appear to be of minor importance in dye-binding, since sodium would be predicted to be the preferred ion at both types of site.

By comparison with the data in Table 2 for CS1 and OC1 the free energy changes to be accounted for in terms of other types of interactions are 22 and 20 kJ mol^{-1}, respectively. These interactions will now be considered.

(B) *Free energy changes due to the rearrangement of water and other interactions during dye binding.* In concentrated aqueous solutions Methylene Blue has been shown to form dimers and higher polymers.[34] Spectral changes during dye binding [1,35] show that similar interactions between the dye ions occur when the dye is bound to a polyanion. For methylene blue in aqueous solution Mukerjee and Ghosh [34] determined the free energy change for dimerisation to be 19 kJ mol^{-1}. This is remarkably near the energy changes that have to be accounted for in our present study of dye binding.

In most dye–polyanion aggregates there are no data relating to the dye–dye distance, or the dye–polymer orientation. In the case of Methylene Blue–oxycellulose complexes, however, detailed *X*-ray crystallographic studies have been made [36,37] and Warwicker [37] has shown that the Methylene Blue cations were oriented perpendicularly to the cellulose chains. The cations were in pairs with a dye–dye distance of 0.35 nm and a distance between pairs of form 0.4—1.0 nm. (The dye–dye distance agrees well with that of 0.34 nm proposed for the Acridine Orange dimer by Kurucsev and Strauss.[38]) Warwicker's picture is supported by the work of Patel [39] who studied the dichroism of oxycellulose fibres dyed with Methylene Blue. He showed that at low dye concentrations the dye cations tended to have their long axes parallel to the fibre axis, with some dimers. At higher dye concentrations (>6mM dye per 100 g oxycellulose) the dye appeared to be present mainly as dimers, perpendicular to the fibre axis. Thus, as dimers have been found in cellulosic polyanions, and the part of the total free energy change yet to be accounted for is almost equal to that resulting from dimerisation, we conclude that the forces causing dye binding are substantially the same as those causing aggregation of the dye in aqueous solutions. Padday [40] has considered these in detail and has suggested that dimerisation may be represented by three steps: (i) the separation of two dye–water interfaces, (ii) the combination of the two water surfaces, and (iii) the combination of the two dye surfaces (to form a dimer). Calculations [40] showed the dominant force to be the mutual attraction of the water molecules, more than equal in itself to the dye–water attractions and the mutual repulsion of the dye cations. The London–van der Waals attraction of the dye cations for each other also promotes dimerisation. Mukerjee and Ghosh [34] have shown that ' hydrophobic bonding ' also makes a small contribution to the dimerisation of Methylene Blue. In the case of dye ions bound monomerically,[1] only hydrophobic bonding will be important, and one would expect such dye cations to be comparatively weakly bound. It must be noted that the dye ions have little affinity for cellulose itself,[41] so that whilst favouring dye binding, dye–cellulose interactions will be negligible.

Mechanism of Dye Binding.—The question of the essential role of the polyanion in dye aggregation arises, since it has long been noted that dyes aggregate readily in the presence of polyanions. As suggested by Schiebe and Zanker,[42] the polyanion must in some way behave as a template which allows the dye cations to interact with one another more readily than in a pure dye solution, possibly due to the partial screening of their positive charges by the negative charge on the polyion. This would agree with the energetic considerations discussed here. It would appear that since both dye aggregation and dye binding are dynamic processes, the basic role of the polyanion is to reduce the rate of disaggregation relative to the rate of aggregation. (We hope to investigate this aspect of the interaction using soluble polyanions.)

On the basis of the present model one would predict that dyes that aggregate strongly in aqueous solution will bind strongly to polyanions, and the order of binding should be the same as that of the dimerisation constants

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(since $\Delta G_d^0 = -RT\ln K_d$). This is in fact supported by published data. The order of dye binding has been given by Pal and Schubert [41] and Stone [43] as Acridine Orange > Methylene Blue > proflavin. The dimerisation constants are 1.5×10^4,[44] 3.5×10^3,[34] and 395 l mol^{-1},[45] respectively.

The present approach also allows an explanation of the often reported fact [2,3,43,46] that dyes bind more strongly to polysulphates than to polycarboxylates (as we have observed also in the present experiments). It is apparent that this is a result of the smaller effective radius of the carboxy-site. The sodium ions are bound there more strongly than at a sulphate group, thus reducing the tendency of a dye cation to bind. However, we do not consider that this is a complete explanation, since our present calculations have shown that the free energy changes that have to be accounted for in terms of dye–dye and dye–solvent interactions, were 22 kJ mol^{-1} for the polysulphate (CS1) and 20 kJ mol^{-1} for the polycarboxylate (OC1). We believe this difference to be due to the more powerful attraction of the carboxy-group for a dye cation. Thus, we postulate that the dye cations are freer to move towards each other and so interact more strongly in the presence of a polysulphate than in the presence of a polycarboxylate. This agrees with the observed metachromatic properties of the dye in the presence of the polyions.[43,47] Other evidence that points to the validity of this postulate is that the metachromatic colour change is lost when the dye–polyion complexes are dehydrated.[47] Under these conditions, dye–site attraction will be a maximum (up to 30 times greater than in water) and will reduce dye–dye interactions to a minimum. This shows in an extreme form the effect of dye–site attraction on the interaction between the bound dye cations.

Finally, in the light of the ideas used so far it is interesting to look at a puzzling aspect of the present results. That is, the greater affinity of the carboxy-group for potassium than for sodium (Tables 2 and 3). As already noted, some reported studies show that the affinities are $Na^+ > K^+$ at carboxy-groups and $K^+ > Na^+$ at sulphate groups. Now the potassium ion is a ' structure breaker ' and thus the water–water interactions that promote dye binding will tend to be weaker in solutions of potassium salts. Thus, when dye binding is used to compare the affinities of Na^+ and K^+ the order will invariably be $K^+ > Na^+$ (Tables 2 and 3).

To summarise, therefore, the largest contribution to the free energy change when dye cations exchange with sodium ions at an anionic site, arises from the interaction of the dye cations with each other and with the solvent. Theses effects counteract the energetically unfavourable ion–site interactions and are responsible for the high affinity of the dye for the polyanion, which appears to play the role of a template against which the dye ions can stack together with reduced mutual repulsion. The role of the anionic site in dye binding is two-fold. First it has been shown that relative attraction of a site for the dye ion and the counterion depends on the effective radius of the site and secondly it is postulated that the dye–site attraction determines the strength of the dye–dye interaction. Thus, polyions with sites of large effective radii will display the greatest affinity for dye ions. The order of binding ($SO_3^- > CO_2^- > -CH_2CO_2^-$) is in agreement with the order of decreasing site radius as measured by the pK value of the polyanion.

We thank Dr. P. J. Baugh for helpful discussions. We also thank Mrs. B. J. Brain for sulphur determinations and Dr. A. G. Bradbury for estimation of phosphorus.

[6/287 *Received*, 11*th February*, 1976]

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