Polyanions and their Complexes. Binding Affinities of Inorganic Ions to Carrageenans and Carboxymethylcellulose

By **William R. Ashton, Geoffrey Pass,* Glyn O. Phillips, and David J. Wedlock,** Department of Chemistry and Applied Chemistry, University of Salford, Salford M5 4WT, Lancashire

The effect of inorganic ions on the interaction between Methylene Blue and κ and λ carrageenan fractions and carboxymethylcellulose (CMC) has been investigated using visible absorption spectroscopy. The concentration of ions required to remove completely Methylene Blue (MB) from the MB–polyanion complex *i.e.* the limiting salt concentration, has been determined and found to decrease in the order $Li^+ > Na^+ > K^+ > Cs^+$, and $Mg^{2+} > Ca^{2+} > Sr^{2+}$ for carrageenans and $K^+ > Na^+$ and $Mg^{2+} > Ca^{2+} > Sr^{2+}$ for CMC. Comparing ions of the same valency, the strength of binding is greatest for the smallest hydrated ion, and decreases in proportion to the hydrated ion size, except for the alkali ions with CMC, when the opposite is the case.

THE degree of specificity exhibited when inorganic cations bind to or condense on polyanions has been the subject of a number of investigations.[1-9] The carrageenans are naturally occurring sulphated polyanions [10,11] and their interaction with alkali metal cations has been investigated by e.m.f. methods, using cation selective electrodes.[3,9] However since the reported results are contradictory, an alternative method of studying the interaction is required. One possibility is the dye displacement technique which has been used to study quantitatively the binding affinities of inorganic ions to heparin,[2] which is also a sulphate containing polysaccharide.

Interaction of a cationic dye with a polyanion leads to a distinct change in the absorption spectrum of the dye. This effect is termed metachromasia. Addition of simple electrolyte destroys the dye–polyanion interaction and reverses the metachromatic effect.[2,5,12-15] For each polyanion there is a concentration of simple electrolyte above which only minimal amounts of dye–polyanion complex exist. This simple electrolyte concentration is called the critical electrolyte concentration, and depends on the size and valency of the cation,[2] the molecular weight,[12] and the charge density and ionic substituents of the polyanion.[15]

The interaction between Methylene Blue and carrageenans or sodium carboxymethylcellulose (CMC) produces metachromasia. By monitoring the change in the visible absorption spectrum which occurs when simple electrolyte is added to a solution of the dye–polyanion complex, the limiting salt concentration (l.s.c.) required to destroy the complex can be determined. In the present study the relative strength of binding of inorganic cations from Groups IA and IIA to κ and λ carrageenans and to sodium carboxymethylcellulose has been determined.

EXPERIMENTAL

The κ and λ carrageenans used were obtained from Copenhagen Pectin Factory S.A. The sodium salt forms were prepared by ion exchange on a 0.4% w/v solution using a sodium form Amberlite IR 120 column, in five fold excess. The solution was concentrated by rotary evaporation then freeze dried. Equivalent weights of the sodium salt forms were determined as sodium sulphate by evaporating concentrated sulphuric acid (AnalaR) from the sample in a silica crucible, using ammonium carbonate to convert any pyrosulphate to sulphate. Sodium carboxymethylcellulose was supplied by Hercules Powder Company Limited, its degree of substitution was 0.7, corresponding to an average of 7 carboxylate groups for every 10 anhydroglucose units in the polymer. Methylene Blue was supplied by E. Gurr Limited. AnalaR grade lithium, sodium, potassium, caesium, magnesium, calcium, and strontium chlorides were used as simple electrolytes. The dye–polyanion complex was made with a total dye concentration sufficiently low (1×10^{-5}M) that (*a*) the optical density of the free dye could be measured accurately and (*b*) precipitation of the dye–polyanion complex was prevented. A gradual increase in polyanion concentration (site : dye ratio) causes metachromasia of Methylene Blue. The lowest site : dye ratios were selected which gave

TABLE 1

Equivalent weights of sodium carrageenans

Carrageenan (Na salt)	κ	λ
Equivalent mass	294	209

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[12] J. S. Moore, G. O. Phillips, and R. E. Morgan, *J. Histochem. Cytochem.*, 1972, **20**, 831.

[13] J. E. Scott, Chem. Soc. Spec. Publ. No. 23, 'Solution Properties of Natural Polymers.'

[14] J. E. Scott and J. Dorling, *Histochemie*, 1965, **5**, 221.

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minimum optical density at 665 nm, to maximise the quantity of dye liberated when simple electrolyte was added. This value was 2 : 1 for both carrageenan fractions and 5 : 1 for CMC. All visible absorption spectra were recorded using a Pye–Unicam SP 500 spectrophotometer.

RESULTS

The visible absorption spectra of carrageenan–Methylene Blue complexes for the λ fraction at varying site : dye ratios are shown in Figure 1. The percentage absorbance at 665 nm compared with the free dye, with change in the site : dye ratio is shown in Figure 2 for κ and λ carrageenan. On addition of the simple electrolyte, the visible absorption spectrum of the carrageenan–Methylene Blue complex reverts from a trace intermediate between (d) and (e) in Figure 1 (since the site to dye ratio of the complex is 2 : 1), and approaches trace (a), the original dye spectrum. Reversion to the original dye spectrum is not obtained exactly, because of the influence of the increased ionic strength of the solution on the dye spectrum, causing a slight enhancement of the shoulder and a depression of the main peak. The progressive reversal of metachromasia as indicated by the increase in peak height at 665 nm is shown in Figures 3—5 for λ carrageenan, κ carrageenan, and CMC, as a function of simple electrolyte concentration.

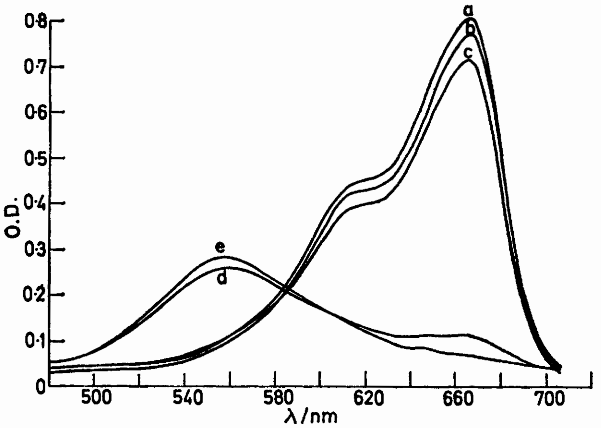

FIGURE 1 Effect of varying site : dye ratio of a λ carrageenan–Methylene Blue complex: a, free dye 1×10^{-5}M; b, complex, site : dye ratio (S : D) 1/100; c, complex, S : D 1/10; d, complex, S : D 1/1; e, complex, S : D 10/1

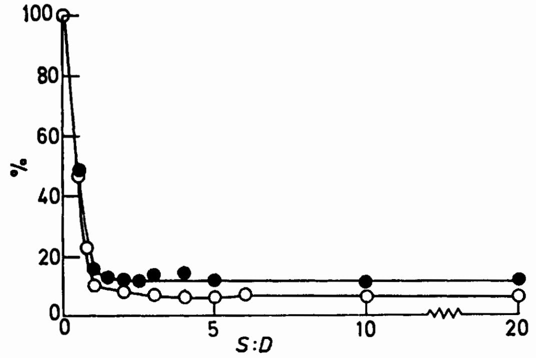

FIGURE 2 Effect on percentage free dye of varying site to dye ratio using ○, λ carrageenan and ●, κ carrageenan

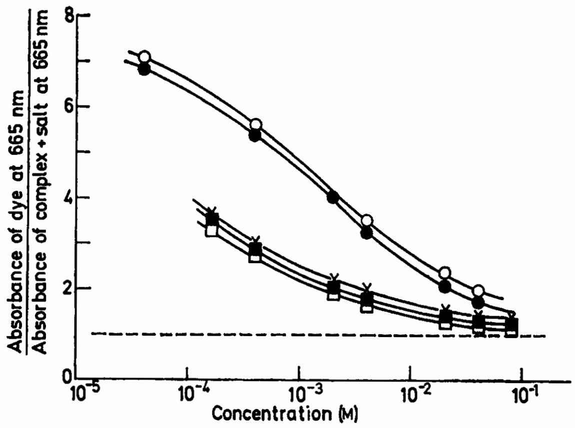

FIGURE 3 Effect of alkali metal chlorides and alkaline earth metal chlorides on the absorption at 665 nm of κ carrageenan–Methylene Blue solutions (S : D 2 : 1): ○, LiCl; ●, NaCl; ×, $MgCl_2$; ■, $CaCl_2$; □, $SrCl_2$. The broken line represents the absorption when all dye has been liberated. Extrapolation of curves to this line gives the l.s.c. value

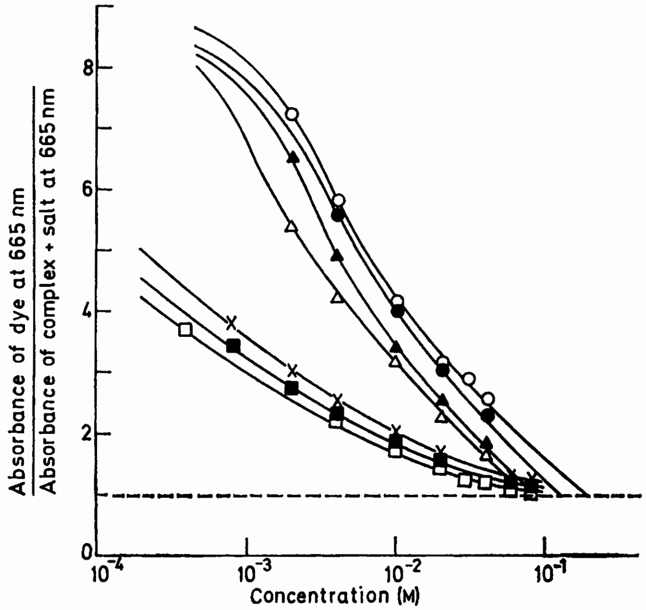

FIGURE 4 Effect of alkali metal chlorides and alkaline earth metal chlorides on the absorption at 665 nm of λ carrageenan–Methylene Blue solutions (S : D 2 : 2): ○, LiCl; ●, NaCl; ▲, KCl; △, CsCl; ×, $MgCl_2$; ■, $CaCl_2$; □, $SrCl_2$

K^+ and Cs^+ salts were not added to κ carrageenan because of the risk of gellation observed under such conditions.[16] The limiting salt concentrations for reversal of metachromasia are given in Tables 2 and 3 respectively for the carrageenans and CMC.

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DISCUSSION

The limiting salt concentrations for reversal of metachromasia in the Methylene Blue–carrageenan complexes follows the order $Mg^{2+} > Ca^{2+} > Sr^{2+}$, and $Li^+ > Na^+ > K^+ > Cs^+$, where appropriate. Thus for the alkali metal ions binding to either carrageenan fraction, the relative order of binding affinity is $Cs^+ > K^+ > Na^+ > Li^+$, and for alkaline earth metal ions binding to either fraction of carrageenan, the order is $Sr^{2+} > Ca^{2+} > Mg^{2+}$. This is identical to the results for Methylene Blue–heparin complexes,[2] using both visible absorption spectrophotometry and pulse radiolysis to detect free dye. The same relative order of binding strength of metal ions is reported for other sulphated polysaccharides,[6,7] well illustrated by dextran sulphate where by measuring counterion activity coefficients in solution, it was shown that the order of preference for the alkali metals was $K^+ > Na^+ > Li^+$.

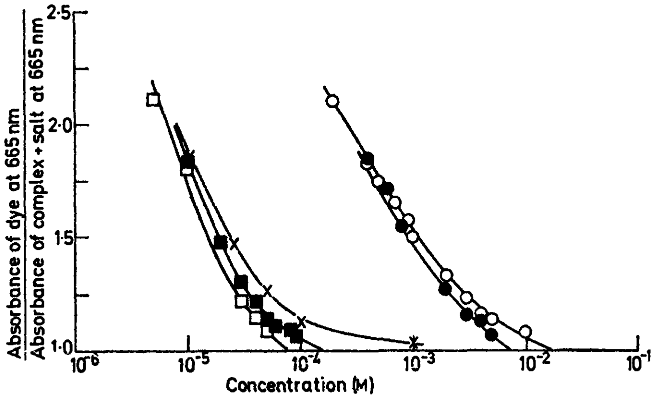

FIGURE 5 Effect of alkali metal chlorides and alkaline earth metal chlorides on the absorption at 665 nm of CMC–Methylene Blue solutions ($S:D$ 5 : 1): ●, NaCl; ○, KCl; ×, $MgCl_2$; ■, $CaCl_2$; □, $SrCl_2$

TABLE 2

Effect of inorganic ions on carrageenan–Methylene Blue interactions (site : dye ratio 2 : 1)

λ Carrageenan	Li	Na	K	Cs
10^{-2} L.s.c./M	18.20	12.20	8.32	7.59
	Mg	Ca	Sr	
10^{-2} L.s.c./M	11.50	8.75	6.03	
κ Carrageenan	Li	Na	K	
10^{-2} L.s.c./M	24.40	14.45		
	Mg	Ca	Sr	
10^{-2} L.s.c./M	19.95	13.18	8.13	

TABLE 3

Effect of inorganic ions on CMC–Methylene Blue interactions (site : dye ratio 5 : 1)

CMC	Na	K	
10^{-3} L.s.c./M	6.92	16.60	
	Mg	Ca	Sr
10^{-4} L.s.c./M	10.00	1.91	0.79

The limiting salt concentrations for reversal of metachromasia of Methylene Blue–carboxymethylcellulose follow the order $Mg^{2+} > Ca^{2+} > Sr^{2+}$ and $K^+ > Na^+$, where the results for the alkali metal ions are in agreement with the results of ultrasonic relaxation studies in which sodium was found to bind more strongly than potassium.[17]

Thus for displacement of Methylene Blue from the carrageenans, a correlation between limiting salt concentration and the hydrated cation size[18] is shown in Figure 6. The interaction is one that is primarily electrostatic binding of the hydrated cation, not binding of the cation with loss of co-ordinated water. If the interaction had been of the latter type, the relative strength of binding would be dependent upon the crystallographic radius of the cation, the cation with the smallest crystallographic radius binding most strongly. However with CMC, the relative order of alkali metal ion binding strength $Na^+ > K^+$ indicates that the cation with the smallest crystallographic radius binds most strongly.

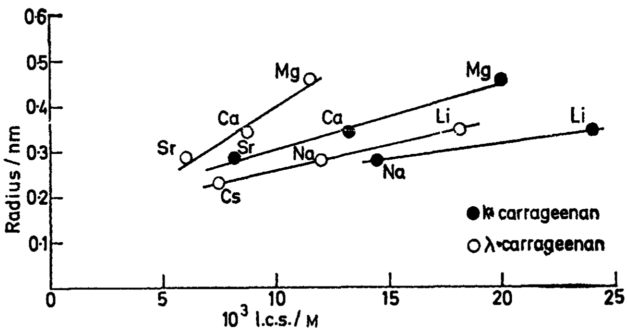

FIGURE 6 Hydrated ion radius *versus* limiting salt concentrations for λ carrageenan–Methylene Blue and κ carrageenan–Methylene Blue complexes

The reversal of charge phenomenon on sulphate and carboxylate polyanions using inorganic cations varies with the nature of the cation and the anionic substituent groups.[19] The respective concentrations of inorganic cations for charge reversal with sulphate polyanions is comparable with the order for dye–polyanion complex destruction now found with the carrageenans *i.e.* $Li^+ > Na^+ > K^+ > Cs^+$, and $Mg^{2+} > Ca^{2+} > Sr^{2+}$. The charge reversal effects have been explained in terms of polarisability, where the polarisability of the anionic groups takes the order $CO_2^- > PO_3^{2-} > H_2O > SO_4^-$. Although it is energetically unfavourable for an SO_4^- group to displace water from around a cation, as it is less polarisable than the water it would displace, it is energetically favourable for a PO_3^{2-} or CO_2^- group to displace water of hydration from around a cation. Consequently it is generally found that sulphated

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polyanions show counterion specificity of binding with the smallest hydrated ion binding most strongly. Carboxylate and phosphate polyanions show a preference for the counterion with the smallest crystallographic radius, since binding takes place usually, with water displacement from the cation. Since for a given group of cations, such as the alkali metals, the sequence of hydrated ion radii is the reverse of the sequence of the crystallographic radii, this results in the reversal of the sequence of binding affinities where sulphate and carboxylate polyanions are compared. This is now found to be the case for the alkali metal cations but not for the alkaline earth cations, where apparently the cation is bound as the hydrated ion to both sulphate and carboxylate polyanions.

The dye displacement technique requires low polyanion concentrations, *ca.* 2×10^{-5}M, because the sensitivity in obtaining dye release and accurate measurement of optical densities of the released dye dictated these low site : dye ratios and polyanion concentrations. Under these conditions, the ordered double helical conformation associated with κ carrageenan [20] would be diluted out. The present study did not include destruction of the dye–κ carrageenan complex with added KCl or CsCl, because of the possibility of gel formation.[21] However we have measured single ion activity coefficients [16] of the counterions of the sodium and potassium salts of κ carrageenan, with no added simple electrolyte, in the higher concentration range of 5×10^{-3}—5×10^{-2} equivalents kg^{-1}. Even under these conditions which should favour the formation of double helices and site binding of the cation, the results showed that the binding process could be fully explained in terms of electrostatic binding of an hydrated cation. Namely, K^+ was more firmly bound than Na^+.

We conclude that in view of the good agreement given by the dye displacement method with the previously reported results for the same or similar systems, a comparison with the e.m.f. results [3,9,16] for the carrageenans would seem to be well justified. Such a comparison supports the order of binding strength $K^+ > Na^+$ reported by Wedlock.[16]

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