# Selectivity of Some Anionic Polymers for Divalent Metal Ions

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The ion-exchange properties of a number of polyanions were investigated by determining the change in pH by addition of salts of divalent metals to half-neutralized samples, and by determining selectivity coefficients by dialysis. The cases where a significant selectivity was observed fell into two groups: 1) The selectivity for copper compared to calcium, which seems to be a characteristic feature of all carboxylate-containing polymers and of polyphosphate, while no such selectivity was observed for sulphated polysaccharides and 2) The selectivity of polyuronides in exchange between alkaline-earth ions. In this group, polyguluronate is unique in having selectivity coefficients in exchange between $Mg^{2+}$ and the other alkaline earth ions about one order of magnitude larger than any of those of the other polyuronides and in distinguishing between $Ca^{2+}$ and $Sr^{2+}$.

A previous paper [1] reported that the selectivity of alginates for some divalent metal ions could be expressed quantitatively in terms of selectivity coefficients, one for the D-mannuronic acid residues and the other for the L-guluronic acid residues. Whereas the selectivity coefficients of the L-guluronic acid residues in the ion-exchange reactions $Sr^{2+}—Mg^{2+}$, $Ca^{2+}—Mg^{2+}$, $Sr^{2+}—Ca^{2+}$, and $Co^{2+}—Ca^{2+}$ were 150, 40, 7, and 0.17 respectively, the corresponding coefficients of the D-mannuronic acid residues varied only between 0.8 and 1.8. The two uronic acid residues are C(5) epimers, and the remarkable difference in selectivity between two so closely related substances prompted an examination of other anionic polymers. The present paper compares the ion-exchange properties of polyguluronate with those of some other polyanions, and attempts to correlate these properties with chemical composition and structure.

The selectivity of polyanions such as polystyrenesulphonate and polymethacrylate for monovalent cations is thought to be satisfactory explained by purely electrostatic forces.[2] With divalent metal ions, the situation is complicated by the fact that the cation is associated with more than one negatively charged group on the polymer. The detailed steric arrangement of the fixed groups on the polymer should therefore be more important with multivalent cations than with monovalent cations. Morawetz, Kotlar and Mark [3] investigated the binding of alkaline earth ions to a copolymer formed

from maleic acid and a neutral monomer. They considered that the ions were bound to two adjacent carboxylate groups as a chelate complex and found higher formation constants for $Ca^{2+}$ and $Mg^{2+}$ than for $Ba^{2+}$ and $Sr^{2+}$. Morawetz [4] reported that for polymethacrylate the stereoregularity of the polymer is of importance for the binding of divalent cations, and interpreted this as evidence for chelate formation involving carboxylate groups kept at a well defined spacing from one another. On the other hand, Rice and Nagasawa [5] considered on stereochemical grounds that binding to two neighbouring carboxylate groups is unlikely.

Gregor, Luttinger and Loeb [7] studied the binding of divalent metal ions to polyacrylate, and found that the complex formation constant for $Cu^{2+}$ was much higher than those for $Mn^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Ca^{2+}$ and $Mg^{2+}$, which themselves decreased in the stated order. For polymethacrylate, Mandel and Leyte [8] reported that the formation constants decrease in the order $Cu^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Co^{2+}$, and $Mg^{2+}$.

The binding of polyvalent ions to mucopolysaccharides was investigated by Gilbert and Myers,[8] who found that $Ca^{2+}$, $Y^{3+}$, and $La^{3+}$ were weakly bound to chondroitin sulphate, the association constants being of the same order of magnitude as those with acetate as the anion, and they concluded that the ions were bound by single site association. Dunstone [9] compared the binding of alkaline earth ions to different types of mucopolysaccharides. He found significant differences between the different polysaccharides, e.g. a selectivity in the order $Mg < Ca < Sr < Ba$ for chondroitin sulphate $A$ and no selectivity for dermatan sulphate. He described the binding as being of a non-specific, electrostatic type. Mathews [10] and Buddecke and Drzenick [11] both reported results which, in contrast to those of Dunstone, indicated a stronger binding of multivalent cations to dermatan sulphate than to chondroitin sulphate. This was attributed to the different stereochemical arrangement of the carboxylate group in the two types of uronic acid residues (L-iduronic and D-glucuronic acid respectively), which, together with the sulphate groups, could give rise to different electrostatic attractions.

It is recognized that the presence in the molecule of other polar groups in addition to the charged sites can have an important influence upon ion binding and ion selectivity. For example, Morawetz and Sammak [12] showed that $Cu^{2+}$ were bound to poly($\varepsilon$-methacrylyl-L-lysine), by two amino acid groups in the same chain, involving the carboxyl group and the $\alpha$-amino group of each residue. The synthesis, properties and uses of chelating ion exchange resins, among which derivatives of iminodiacetic acid play a prominent part, have recently been reviewed.[13,14]

## RESULTS

1) *Change in pH by addition of salts of divalent metal to half-neutralized samples of polyanions.* When a solution of a metal salt is added to a half-neutralized sample of an anionic polymer containing carboxylate groups, the pH decreases; the magnitude of the decrease depends upon the affinity between the polyanion and the metal ion. This method provides a qualitative evaluation of the relative affinities of the metal ions to the different polyanions.

It has previously been applied in this laboratory [15] to alginates containing different proportions of mannuronic and guluronic acid residues.

Table 1 gives the results for a polymeric fragment of alginate containing 92 % guluronic acid, a sample of whole alginate containing 92 % mannuronic

*Table 1.* Mequiv. $H^+$ ($\times 10^3$) liberated per ml by addition of metal salts (0.02 mequiv./ml) to solutions containing 0.026—0.028 mequiv. half-neutralized polyanions per ml.

|  | Metal | Alginate fragment 92 % guluronic acid | Alginate, 92 % mannuronic acid | Pectate | Oxidised cellulose | CMC | Poly-acrylate |
|---|---|---|---|---|---|---|---|
| I | Pb | 2.4 | 2.8 | 3.1 | 1.93 | 1.5 | 0.07 |
| | Cu | 1.2 | 1.8 | 3.0 | 1.11 | 0.9 | 0.05 |
| | Cd | 0.35 | 1.2 | 0.85 | 0.52 | 0.3 | 0.01 |
| II | Ni | 0.08 | 0.6 | 0.75 | 0.33 | 0.15 | <0.003 |
| | Zn | 0.10 | 0.4 | 0.61 | 0.26 | 0.14 | <0.003· |
| | Co | 0.05 | 0.4 | 0.36 | 0.22 | 0.11 | <0.003 |
| | Mn | 0.05 | 0.4 | 0.21 | 0.29 | 0.09 | <0.003 |
| III | Ba | 1.00 | 0.66 | 0.63 | 0.40 | 0.13 | <0.002 |
| | Sr | 0.84 | 0.50 | 0.52 | 0.34 | 0.12 | <0.002 |
| | Ca | 0.43 | 0.53 | 0.55 | 0.31 | 0.10 | <0.002 |
| | Mg | 0.07 | 0.48 | 0.18 | 0.31 | 0.09 | <0.002 |

acid, pectate, (6)-oxidised cellulose, carboxymethyl cellulose, and poly-acrylate. The results are expressed as mequiv. protons per ml liberated by addition of salt to a concentration of 0.02 M to the half-neutralised polyanion at a concentration of 0.026—0.028 mequiv./ml (*i.e.* containing 0.013—0.014 mequiv. undissociated carboxyl groups per ml).

The results permit division of the metals into three groups as indicated (Table 1). Group I includes Pb, Cu, and Cd and, with the exception of the guluronic-acid rich alginate fragment, these three ions exhibit a markedly higher affinity for the polyanions than do the other ions investigated. The guluronic acid rich alginate fragment is unique in having a much higher affinity for $Ba^{2+}$, $Sr^{2+}$, and $Ca^{2+}$ than for the elements in group II, and in showing selectivity between $Sr^{2+}$ and $Ca^{2+}$. A marked difference between the affinities for $Ca^{2+}$ and $Mg^{2+}$ was observed both with the guluronic acid rich fragment and with pectate. With the exception of pectate, the anions showed only small differences in affinity for the elements in group II.

2) *Determination of selectivity coefficients by dialysis.* We have previously [1] determined selectivity coefficients of alginates and alginate fragments by dialysis against solutions containing the two cations in suitable proportions. The selectivity coefficients attributed to the two different uronic acid residues were determined by extrapolation to uronic acid compositions of 100 % mannuronic and 100 % guluronic acid residues, and are in the following

described as the selectivity coefficients of polymannuronate and poly-
guluronate, respectively.

The selectivity coefficients for the $Ba^{2+}-Mg^{2+}$ exchange reaction of some
alginates and alginate fragments are given in Fig. 1. The ionic composition
of the polymer phase was in all cases kept between 45 and 55 % Ba. For
comparison, the selectivity coefficients for the $Sr^{2+}-Mg^{2+}$ exchange, deter-
mined previously,[1] are given in the same figure. The results show that the selec-
tivity coefficient for the exchange reaction $Ba^{2+}-Mg^{2+}$ of polymannuronate
is about 10, and therefore differs significantly from the coefficients of this
material for exchange reactions between the other alkaline earth ions.

Parenthetically it should be mentioned that the clear correlation between
the average uronic acid composition and the selectivity coefficient observed
for the $Sr^{2+}-Mg^{2+}$ exchange does not seem to hold for the $Ba^{2+}-Mg^{2+}$
exchange reaction. In this connection it should be remembered that the
alginate chain consists of three types of block, two homopolymeric, and one
consisting of an alternating sequence of guluronic and mannuronic acid resi-
dues.[15] A curve of the type shown in Fig. 1 for the $Sr^{2+}-Mg^{2+}$ exchange reac-
tion indicates that a guluronic acid residue has a selectivity which is indepen-



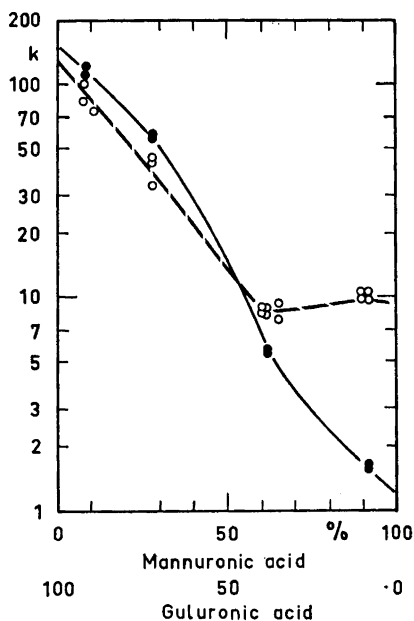Fig. 1. Selectivity coefficients for alginates
of different uronic acid composition.
$\bullet = k_{Mg}{}^{Sr}$;   $O = k_{Mg}{}^{Ba}$.
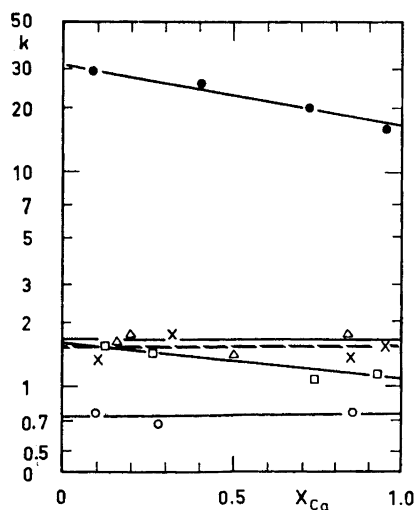
Fig. 2. Selectivity coefficients at different
mole fractions of calcium in the polymer
phase.
$\bullet = k_{Ca}{}^{Cu}$-CMC;   $\triangle = k_{Mg}{}^{Ca}$-alginate frag-
ment (92 % M);   $\times = k_{Mg}{}^{Ca}$-dextran sul-
phate;   $\square = k_{Mg}{}^{Ca}$-CMC;   $O = k_{Ca}{}^{Cu}$-dextran
sulphate.

dent of on the identity of the uronic acid residues situated next to it in the chain.[1] That this may not be the case for the $Ba^{2+}-Mg^{2+}$ exchange is indicated by the fact that one of the points lying on the curve in Fig. 1 (corresponding to 60.0 % mannuronic acid) represents a fraction of alginate in which the alternating sequence predominates and no contiguous guluronic acid residues are present. The alternating sequence in this exchange reaction, therefore seems to give a selectivity very similar to that of polymannuronate, rather than to that of a physical mixture of polymannuronate and polyguluronate of equivalent monomeric composition.

The variation of the net selectivity coefficient of alginates with varying cationic composition can, as previously discussed,[1] be explained by assuming two selectivity coefficients, one for each type of site, these being individually independent of the ionic composition. For polyanions with only one kind of site, the selectivity coefficient would therefore be expected not to vary significantly with varying ionic composition of the polymer. Fig. 2 gives the selectivity coefficients for the $Ca^{2+}-Mg^{2+}$ exchange of an alginate with a composition closely approaching polymannuronic acid. No significant variation with varying ionic composition was observed. The same figure also gives the selectivity coefficients for the $Ca^{2+}-Mg^{2+}$ and $Cu^{2+}-Ca^{2+}$ exchange reactions of dextran sulphate and carboxymethyl cellulose. In these substances the charged groups are not uniformly distributed because of a lack of specificity of the

*Table 2.* Selectivity coefficients of anionic polymers. The values for polyguluronate and polymannuronate are determined by extrapolations, those for pectate correspond to a cationic composition of approximately 50 % of each of the two ions bound to the pectate, while the rest of the figures are determined with equivalent amounts of the two ions in solution.

| Anionic group | Polymer | $k_{Mg}^{Ca}$ | $k_{Mg}^{Sr}$ | $k_{Mg}^{Ba}$ | $k_{Ca}^{Co}$ | $k_{Ca}^{Cu}$ |
|---|---|---|---|---|---|---|
| | Polyguluronate | 40 | 150 | 130 | 0.17 | 8 |
| | Polymannuronate | 1.8 | 1.2 | 10 | 1.1 | 12 |
| | Alginate, 92 % mannuronic acid | 1.6 | 1.4 | 13 | 1.0 | 11 |
| | Acetylated polyguluronate | 1.0 | 1.0 | | 1.2 | 12.0 |
| —COOH | Pectate | 7.0 | 9.6 | 10.1 | 0.7 | 32 |
| | Oxidized cellulose | 1.1 | 1.5 | 3.5 | 1.1 | 11 |
| | Carboxymethyl dextran | 1.8 | 1.4 | 1.3 | 1.2 | 10 |
| | Carboxymethyl cellulose | 1.4 | 1.3 | 1.6 | 1.3 | 17 |
| | Polyacrylate | 1.1 | 0.7 | 3.0 | 1.7 | 25 |
| | Hyaluronate | 0.9 | | | | 3.0 |
| —COOH and —SO₃H | Chondroitin sulphate | 1.1 | 0.95 | 1.3 | | 2.4 |
| | Ascophyllan | 1.1 | 1.3 | 2.1 | 0.9 | 7.0 |
| —SO₃H | Fucoidan | 1.3 | 1.6 | 2.9 | 1.0 | 1.5 |
| | Dextransulphate | 1.1 | 2.0 | 7.0 | 1.2 | 0.9 |
| | Carrageenan | 1.2 | 1.4 | 1.6 | 1.2 | 1.2 |
| —OP(OH)₂O | Polyphosphate | 0.9 | 1.1 | 6.0 | 1.2 | 8.0 |

substitution reactions, and sites of different selectivities might be expected to occur. The results show, however, that the variation of the selectivity coefficient with varying ionic composition is small.

Fig. 3 gives the selectivity coefficients of pectate for the exchange reactions $Ca^{2+}-Mg^{2+}$ and $Cu^{2+}-Ca^{2+}$ as a function of the ionic composition in the polymer phase. In this case, only one charged monomer is present, D-galacturonic acid, and one of the preparations (sunflower head pectate) contains more than 90 % of this monomer. The selectivity coefficient of the $Ca^{2+}-Mg^{2+}$ exchange varies, however, very much with the ionic composition of the polymer, in a way which suggests the presence of two sites of very different selectivities. The selectivity coefficients of the exchange reaction $Cu^{2+}-Ca^{2+}$ are on the other hand independent of the ionic composition of the polymer.

The selectivity coefficients of a number of different polyanions are given in Table 2. Those of polyguluronate and polymannuronate were determined by extrapolation as stated above, and those of pectate are given for equimolar amounts of the two ions in the polymer phase. For the rest of the polyanions in Table 2, the selectivity coefficients were determined for equimolar amounts of the two cations in the solution.

The results (Table 2) indicate that, with a few exceptions like hyaluronate and chondroitin sulphate, a high selectivity for copper compared to calcium is a characteristic feature of carboxylate containing polyanions in general. A similar type of selectivity was found for polyphosphate, while the selectivity coefficients for the $Cu^{2+}-Ca^{2+}$ exchange reaction of sulphate polysaccharides were close to unity. The observation that the $Cu^{2+}-Ca^{2+}$ selectivity coefficients is about the same for polymannuronate as for polyguluronate, while these substances show very different selectivities in the $Ca^{2+}-Mg^{2+}$ exchange reaction should indicate a marked difference in $Cu^{2+}-Mg^{2+}$ selectivity. That this actually is the case is shown in Table 3.

Table 3. Selectivity coefficients in the Cu—Mg exchange reaction.

|  | $k_{Mg}^{Cu}$ |
|---|---|
| Alginate fragment (92 % guluronic acid) | 600 |
| Alginate fragment (92 %) mannuronic acid) | 30 |
| Dextran sulphate | 1 |

Although the selectivity coefficients of polyguluronate in exchange between $Mg^{2+}$ and the other alkaline earth ions are about one order of magnitude larger than any of those of the other polyanions, significant selectivities of polymannuronate and pectate (polygalacturonate) were also observed. Table 4 gives some additional selectivity coefficients of polyuronides for exchange reactions involving alkaline earth ions. It is clear from this and Table 2 that polymannuronate has a higher selectivity for the largest of the four ions, $Ba^{2+}$, than for the other three, while pectate, under the conditions used, has approximately the same affinity for the three larger ions, but has a significantly lower affinity for $Mg^{2+}$. Polyguluronate is the only one of the polyuronides
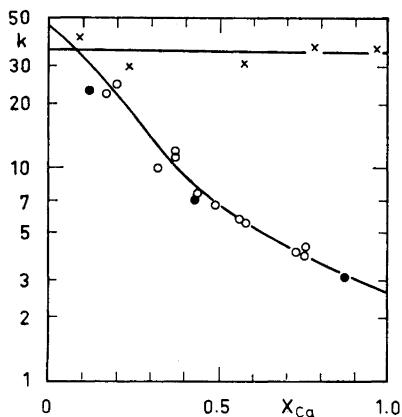
*Fig. 3.* Selectivity coefficients for pectate at different mole fractions of calcium in the polymer phase.
$\times = k_{Ca}^{Cu}$-apple pectate; $O = k_{Mg}^{Ca}$-apple pectate; $\bullet = k_{Mg}^{Ca}$-sunflower-head pectate.
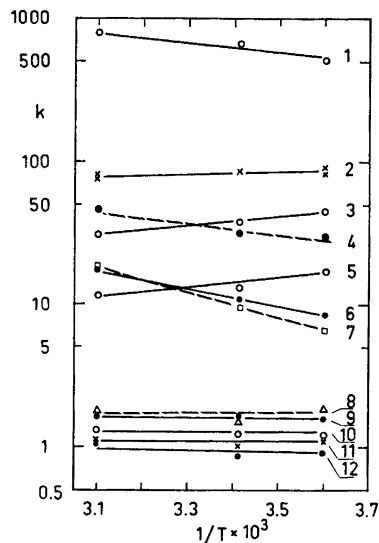
*Fig. 4.* Selectivity coefficients at different temperatures. (Alginate fragment = a.f.)
Curve 1: $k_{Mg}^{Cu}$-a.f. (92 % G); 2: $k_{Mg}^{Ba}$-a.f. (92 % G); 3: $k_{Mg}^{Ca}$-a.f. (92 % G); 4: $k_{Mg}^{Cu}$-a.f. (92 % M); 5: $k_{Mg}^{Ba}$-a.f. (92 % M); 6: $k_{Ca}^{Cu}$-a.f. (92 % M); 7: $k_{Ca}^{Cu}$-a.f. (92 % G); 8: $k_{Mg}^{Ca}$-a.f. (92 % M); 9: $k_{Mg}^{Ba}$-CMC; 10: $k_{Mg}^{Ca}$-CMC; 11: $k_{Mg}^{Ca}$-dextran sulphate; 12: $k_{Ca}^{Cu}$-dextran sulphate.

*Table 4.* Selectivity coefficients for the exchange reactions Ba—Sr and Sr—Ca for poly-uronides.

|  | $k_{Sr}^{Ba}$ | $k_{Ca}^{Sr}$ |
|---|---|---|
| Alginate fragment (92 % guluronic acid) | 1.8 | 7.0 |
| Alginate fragment (92 % mannuronic acid) | 6.5 | 1.0 |
| Pectate | 1.4 | 1.2 |

investigated which selects between ions of intermediate sizes, $Ca^{2+}$ and $Sr^{2+}$. On the other hand, this substance has no significant selectivity in the $Sr^{2+}-Ba^{2+}$ exchange reaction.

3) *Temperature effects.* Some experiments were carried out with the dialysis technique at three different temperatures. The concentrations in the soluble phase were chosen so as to give equimolar amounts of the two ions in the polymer phase at 20°. In Fig. 4 the observed selectivity coefficients are plotted as a function of $1/T$. The results indicate that the reactions may be classified

into three main groups: 1) Those in which the selectivity coefficients are approximately independent of temperature. These reactions include the $Ca^{2+}-Mg^{2+}$ exchange for carboxymethylcellulose, dextran sulphate, and polymannuronic acid, the $Ba^{2+}-Mg^{2+}$ exchange for carboxymethylcellulose and the $Cu^{2+}-Ca^{2+}$ exchange for dextran sulphate. For all these reactions the selectivity coefficients are close to unity. 2) Those with a negative enthalpy change (a positive slope in Fig. 4). These are reactions between polyuronates and alkaline earth ions with selectivity coefficients significantly higher than 2. 3) Those with a positive enthalpy change (a negative slope in Fig. 3). All these reactions involve $Cu^{2+}$ and alkaline earth ions and have selectivity coefficients above 7. The positive enthalpy change indicates that the driving force in this group of exchange reactions must be a favourable entropic change.

## DISCUSSION

In this work only selectivities have been determined; *i.e.* the affinity of a polyanion to a metal ion relative to the affinity to another metal ion, and no attempt have been made to determine the absolute strength of association between the cations and the polyanions. A very high accuracy has not been sought, and only selectivity coefficients higher than 2 or lower than 0.5 will be considered as indicating a significant selectivity (Table 2). It should also be kept in mind that selectivity coefficients may vary with the ionic composition of the polymer phase; this is to be expected in cases such as alginate where two sites with different affinities are present, but it has also been demonstrated for pectate with only one type of charged monomer. The selectivity coefficients in Table 2, determined with equimolar amounts of the two ions in solution, refer to the corresponding ionic composition of the polymers only, and considerable variations with varying ionic composition may occur.

In many cases the polyanions form gels with divalent cations. This is the case with guluronic acid rich alginates and pectates, and all carboxylate containing polymers in the presence of copper. There appears to be a correlation between the affinity of a polyanion for a certain cation and the capacity of the cation to cause gel formation, but certain exceptions to this general rule may exist.[16] On the other hand, some observations reported in the previous paper [1] indicated that the selectivities may be different in the gel state and when the polymer is in solution. This point is the subject of a special investigation which will be reported later. In the present work the results refer to the state of the polyanions under the conditions described in experimental section, and it should be kept in mind that other conditions (*e.g.* different polyanion concentration, introduction of crosslinks by chemical treatment *etc.*) might change the gelforming tendency of the polyanion and thereby possibly affect the selectivity.

With the above-mentioned limitations taken into account, the results in Table 2 illustrate some general features of polyanion selectivity. With a few exceptions (a slight selectivity for $Ba^{2+}$ in the $Ba^{2+}-Mg^{2+}$ exchange reaction of polyacrylate and some sulphated polysaccharides) the cases where

selectivity has been observed fall into two groups; 1) The selectivity for copper compared to calcium, which seems to be a characteristic feature of all carboxylate containing polymers and of polyphosphate, while no such selectivity was observed for sulphated polysaccharides and 2) The selectivities of polyuronides in exchange reactions between alkaline earth ions.

Characteristic of the reactions in group 1 is the lack of importance of the detailed structure of the polyanion, and particularly significant is the observation that this selectivity is not destroyed by acetylation. This implies that the binding mechanism that is responsible for the higher affinity of copper relative to calcium ions in carboxylate-containing polyanions is independent of the presence of free hydroxyl groups in the polymer and of the detailed steric arrangements of the carboxylate groups. The low $Cu^{2+}-Ca^{2+}$ selectivity of chondroitin sulphate and hyaluronate might indicate a lower affinity of $Cu^{2+}$ compared to $Ca^{2+}$ of polysaccharides containing acetamido groups, but this needs further investigation. The exchange reactions of group 1 are associated with an unfavourable enthalpy change and the driving force of the $Cu^{2+}-Ca^{2+}$ exchange reaction must therefore be a relatively large positive entropic change connected with the binding of copper ions and liberation of calcium ions from the polyanion. It seems possible that this entropic change is caused by the copper ions having a larger ordering effect upon the water molecules than calcium ions and that the hydration of the ions is destroyed when they are bound to the polymer. The difference between polyanions with carboxylate groups and with sulphate groups may be due to the different effect of the two types of fixed groups in destroying the hydration of the copper ions.

The exchange reactions in group 2 depend very much upon the detailed structure of the polymer, and any selectivity is completely destroyed by acetylation of the hydroxyl groups. In all cases where a significant selectivity within the alkaline earth ions has been observed, the larger ion has been preferentially bound. The three polyuronides investigated seem, however, to behave quite differently with respect to the different alkaline earth ions. Polymannuronate distinguishes only between the large barium ions and the other three, pectate between the small magnesium ion and the other three, and polyguluronate is unique in distinguishing between the alkaline earth ions with intermediate sizes, namely the calcium and strontium ions. Polyguluronate, however, has no significant selectivity in the $Ba^{2+}-Sr^{2+}$ exchange reaction. The reactions within this group seem to be associated with a favourable enthalpy change for binding of the larger ions.

The mechanism of binding of divalent ions to alginate and pectate have been discussed by Schweiger [17] who postulated that the ions were bound by chelate bonds involving two carboxyl groups of one macromolecule and two vicinal hydroxyl groups of another. Kohn and Furda [18] investigated the binding of calcium ions to acetylated pectate and concluded that the binding was mainly caused by electrostatic forces. In a recent study of thin layer chromatography of divalent metal ions, with alginic acid and acetylated alginic acid as the stationary phase, Cozzi, Desideri and Lepri [18] demonstrated the importance of free hydroxyl groups for the selectivity of alginic acid. When the

hydroxyl groups are blocked, the selectivity of alginic acid decreases, and approaches that of carboxymethylcellulose.

The importance of the stereochemistry of the polyuronides and the indication that sites of different dimensions are involved in polymannuronate, polyguluronate, and pectate makes it reasonable to assume that the alkaline earth ions must be bound at two or more points in the same molecule, that these points are sufficiently far removed from each other to give the best fit for the larger ions, and that the distance between them increases in the order pectate-polyguluronate-polymannuronate. The fact that acetylation destroys this kind of selectivity indicates that the hydroxyl groups are involved in the binding, but the possibility that this destruction may be due to some steric effect of the bulky acetyl group cannot be completely excluded. It would follow, however, that this steric effect then must be assumed to be of no importance for the $Cu^{2+}-Ca^{2+}$ selectivity.

In order to determine in detail the mechanism of the binding of alkaline earth ions to polyuronides, the exact dimensions in the molecule should be known. In the case of alginate this is not yet the case. X-Ray-investigations by Frei and Preston [20] may be interpreted as indicating that the x-ray diagram previously described by Astbury [21] for alginic acid actually is caused by the blocks of guluronic acid residues present in the polymer, and that the poly-mannuronic acid has a distinctly different x-ray diagram. This may again indicate that the two uronic acid residues possibly occur in different conformations.

The fact that the $Cu^{2+}-Ca^{2+}$ selectivity seems to be caused by a mechanism different from that governing the selectivities for the alkaline earth ions, does not mean that the copper ions cannot be bound to polyuronides by the same mechanism as are the calcium ions. On the contrary, the remarkable constancy of $Cu^{2+}-Ca^{2+}$ selectivity coefficients indicates strongly that, when the structure of the polyuronide allows a preferential binding of $Ca^{2+}$ compared to $Mg^{2+}$, also $Cu^{2+}$ is bound preferentially (see Table 3), probably by the same mechanism. In addition, the mechanism making the binding of $Cu^{2+}$ to carboxylate groups energetically more favourable than the binding of $Ca^{2+}$, is operative.

## EXPERIMENTAL

*Materials.* The alginates and alginate fragments used are those described previously.[1] Two samples of pectic acid have been used, one prepared from apple pectin as described previously,[22] and another prepared from sunflower head and described by Zitko and Bishop.[23] Ascophyllan and fucoidan were both prepared from *Ascophyllum nodosum*, the former as described by Larsen, Haug and Painter [24] and the latter by the procedure of Larsen and Haug.[25] The carrageenan was prepared from *Gigartina acicularis* and its preparation [26] and properties [27] are described elsewhere.

Oxidized cellulose was prepared as follows: Cotton cellulose (5 g) was macerated with syrupy 85 % (w/w) orthophosphoric acid (150 ml) and ground at room temperature with a pestle and mortar until it had dissolved. Sodium nitrite (3 g) was dispersed in the viscous mass by grinding, and this addition was repeated at hourly intervals for 5 h. The mixture was poured into icecold water (1.5 l), and aqueous sodium hydroxide (30 % w/w) was added slowly with ice-cooling until the pH reached 7. The resultant mixture was stirred overnight at room temperature to allow dissolution of most of the gelatinous particles of oxycellulose, filtered and dialysed against water. The solution was then concentrated

to 500 ml, made 2 % with respect to sodium chloride, and poured with stirring into ethanol (2 l). The precipitated product was collected by filtration, washed with ethanol and ether and dried. The product (3.5 g) had an equivalent weight of 330.

Guluronic acid rich alginate fragments (1 g) were acetylated by suspension in its acid form in glacial acetic acid (20 ml). The material was recovered by centrifugation and resuspended in acetic acid three times. It was finally suspended in a mixture of glacial acetic acid (5 ml) and acetic acid anhydride (10 ml). Three drops of 60 % aqueous perchloric acid were added with stirring and the mixture was stirred for 4.5 h. After 20 h, water was added, and the mixture was neutralized, dialysed and freeze-dried. The yield was 0.9 g and the degree of acetylation 1.4.

The carboxymethyl dextran (Pharmacia, degree of substitution 0.7), the polyacrylate (Fluka), the hyaluronate (Fluka), the chondroitin sulphate (Sigma, whale and shark cartilage), the dextran sulphate (Pharmacia, degree of substitution 1.6) and the polyphosphate were all commercial products.

The change in pH upon addition of divalent metal salts to half-neutralized samples was determined as follows: The amount of sodium hydroxide necessary to give half-neutralized polyanions was first determined by dissolving a sample of polyanion in its acidic form (25 mg) in an amount of 0.02 M sodium hydroxide that was estimated to be a slight excess. After 5 h the mixture was titrated with 0.01 M hydrochloric acid, with phenolphthalein as an indicator. Samples of polyanions (25 mg) were then suspended in half the equivalent amount of 0.02 M sodium hydroxide and the mixture was diluted to 5 ml with water. After 5 h the pH was determined, using a Radiometer pH meter 4, equipped with a glass electrode (G 200 B). Salt solutions (0.5 ml, 0.1 M) were added and the pH was redetermined after 20 h.

The technique for determination of selectivity coefficients by dialysis has been described previously.[1]

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