

## Dependence upon Uronic Acid Composition of Some Ion-Exchange Properties of Alginates

OLAV SMIDSRØD and ARNE HAUG

*Norwegian Institute of Seaweed Research N.T.H., Trondheim, Norway*

The ion-exchange selectivity coefficients of a number of alginates and alginate fragments were determined by equilibrium dialysis. By assigning two different selectivity coefficients to the two uronic acid residues it was possible to account both for the variation in the selectivity coefficient of a given alginate sample with the ionic composition of the polymer phase, and for the effect of variations in uronic acid composition between samples.

For the exchange reactions Ca—Mg, Ca—Sr, Sr—Mg, and Co—Ca, the mannuronic acid residues had selectivity coefficients close to unity, while those due to the guluronic acid residues varied from 150 (Sr—Mg) to 0.17 (Co—Ca); the selectivity in these cases was therefore due almost entirely to the guluronic acid residues. On the other hand, in the Cu—Ca reaction, both types of uronic acid residue exhibited marked selectivity for Cu.

The high selectivity of guluronic acid residues in the alkaline earth exchange-reactions appeared to be dependent upon the existence of the alginate in the insoluble, gel state.

The ion-exchange properties of alginates have attracted considerable attention in recent years, owing to their bearing upon some practical uses for these materials.<sup>1</sup> Such uses include the preparation of gels by exchange of alkali-metal ions for those of calcium<sup>2</sup> or other divalent metals,<sup>3</sup> and the selective inhibition of strontium absorption by the gut.<sup>4-7</sup>

Ion binding properties of alginates have been investigated from several different viewpoints. Counterion activities have been measured with permselective membranes,<sup>8</sup> osmotic and Donnan equilibria<sup>9</sup> and cation sensitive electrodes.<sup>10</sup> The relative capacities of divalent cations to bring about gelation of alginates have been investigated by several authors,<sup>3,11-14</sup> and the relative affinities of the divalent cations to alginates have also been determined.<sup>15</sup> The sequence of divalent metals based on gel-forming ability and on affinity were significantly different,<sup>3</sup> and also depended upon the uronic acid composition of the sample.<sup>15</sup>

The sodium-(potassium)-calcium exchange reaction has been investigated in some detail,<sup>16,17</sup> with particular regard to factors influencing the physical

properties of calcium alginate fibers.<sup>18</sup> The influence of uronic acid composition upon this ion exchange reaction has been investigated in this laboratory,<sup>19,20</sup> and both in this case and in the case of other divalent-monovalent ion exchange reactions,<sup>3</sup> the affinity of alginate for the divalent cation increased with increasing content of guluronic acid residues. Also in the case of the calcium-magnesium<sup>21</sup> and the strontium-calcium<sup>22</sup> ion exchange reaction a marked increase in the selectivity coefficients with increasing content of guluronic acid residues in the polymer was observed.

These earlier investigations of the correlation between uronic acid composition and ion exchange properties of alginates were limited in scope by the limited range of uronic acid compositions found in naturally occurring, whole alginate samples. Recent work in this laboratory<sup>23-25</sup> has shown that the mannuronic and guluronic acid residues in alginate are not uniformly distributed along the polymer chains, and that, after limited degradation with acid, polymeric fragments consisting almost entirely of residues of mannuronic acid only, or of guluronic acid only, can be isolated. This has made it possible to study the ion-exchange behaviour of polymannuronic acid and polyguluronic acid separately, and thereby to assess more accurately the relative contributions of the two types of unit to the total selectivity of a whole alginate sample.

In the present paper, only exchange reactions between divalent metals are considered, since these are considerably simpler than systems in which the ions are of different valency. This is because, in systems of the latter type, shift of the exchange equilibrium occurs when the total concentration of ions in the medium is changed, whereas this does not occur, in dilute solutions, when the two ionic species have the same valency.<sup>26</sup>

## RESULTS

a) *Methods.* The ion exchange properties of alginates have previously been examined in this laboratory by preparing threads of calcium alginate (or other divalent metal alginates) and equilibrating these threads against solutions containing sodium, potassium,<sup>19,20</sup> or magnesium ions.<sup>21</sup> This procedure was originally introduced by Mongar and Wassermann,<sup>16</sup> and is restricted to conditions where the alginate forms a firm gel. In order to carry out selectivity determinations also outside this range of conditions, a dialysis procedure was adopted. Solutions of sodium alginate were dialysed first against large volumes of salt solutions of suitable composition, and then against water. The ionic composition of the residual polymer solution was determined by a final dialysis against acid followed by analysis of the cations liberated into the acidic solution. A detailed description of the procedure is given in the experimental section.

The ion exchange properties of polymeric materials are most commonly described by a selectivity coefficient, defined for the exchange of the two cations A and B as

$$k_A^B = \frac{X_B \cdot C_A}{X_A \cdot C_B}$$

where  $X_A$  and  $X_B$  represent the equivalent fractions of the counterions in the polymer phase and  $C_A$  and  $C_B$  the concentrations of the same ions in the solution. It has been shown for several anionic polymers,<sup>27</sup> including alginate,<sup>9</sup> that the activity coefficients of the free ions in the solution are determined solely by the concentration of the free ions and are not influenced by the presence of the charged polymers. In the present work the ionic strength of the salt solution was the same in all experiments. Even if the ratio between the activity coefficients of the cations should deviate significantly from 1 in the original concentrated salt solution, the ratio approaches 1 when the solution is diluted during the dialysis against water. It has been shown for alginate that divalent metals are mainly bound as ion pairs.<sup>9</sup> The charge of the polymer is therefore negligible, and the Donnan equilibrium will not cause a significant difference in the concentration of free ions inside and outside the dialysis bag.

The selectivity coefficient may, therefore, be given as

$$k_A^B = \frac{X_B}{X_A} \cdot R_B^A \quad (1)$$

where  $R_B^A$  represents the ratio between the concentrations of the two cations in the external salt solution.

b) *Variation of the selectivity coefficient with varying ionic composition of polymer phase.* As it is defined above, the selectivity coefficient will depend upon the activity coefficients of the two ions in the polymer phase. If the ratio between the two activity coefficients depends upon the ionic composition of the polymer, a variation in the selectivity should be observed. In the case of alginate, the polymer contains two different types of anionic sites, with different selectivity for the ions. In such a case, the selectivity coefficient  $k_A^B$  must decrease with increasing  $X_B$ . If it is assumed that the two monomers can be characterized by two different selectivity coefficients,  $(k_A^B)_M$  and  $(k_A^B)_G$ , which are independent of the ionic composition of the polymeric phase, this effect can be expressed quantitatively.

If M and G represent the mole fractions of the two monomers in the polymer

$$X_B = (1-M)X_{BG} + MX_{BM} \quad (2)$$

where  $X_{BM}$  and  $X_{BG}$  represent the fractions of the monomers of type M and G, respectively, which are associated with counterion B. For the two monomers separately, eqn. (1) leads to

$$(k_A^B)_G = \frac{X_{BG}}{1-X_{BG}} \cdot R_B^A, \text{ and}$$

$$(k_A^B)_M = \frac{X_{BM}}{1-X_{BM}} \cdot R_B^A,$$

which, when combined with eqn. (2), gives

$$X_B = (1-M) \frac{(k_A^B)_G}{(k_A^B)_G + R_B^A} + M \frac{(k_A^B)_M}{(k_A^B)_M + R_B^A} \quad (3)$$

and for the observed average selectivity coefficient of the polymer

$$k_{A^B} = \frac{\frac{(1-M)(k_{A^B})_G}{(k_{A^B})_G + R_B^A} + \frac{M(k_{A^B})_M}{(k_{A^B})_M + R_B^A}}{1 - \frac{(1-M)(k_{A^B})_G}{(k_{A^B})_G + R_B^A} - \frac{M(k_{A^B})_M}{(k_{A^B})_M + R_B^A}} \cdot R_B^A \quad (4)$$

The average selectivity coefficients of two alginate samples were determined for different values of  $X_{Ca}$ . The results are given in Fig. 1. The curves drawn in the figure are calculated from eqns. (3) and (4), using  $(k_{Mg^{Ca}})_M = 1.8$  and  $(k_{Mg^{Ca}})_G = 40$ , which were found by trial and error. A reasonably good agreement between the calculated curves and the experimental results was demonstrated. Three alginate fragments and an alginate sample approaching homopolymeric composition were also included in the experiment. At high values of  $X_{Ca}$  a good agreement between calculated and experimental results was observed also in this case. At low values of  $X_{Ca}$ , however, the selectivity coefficients found by experiment were much lower than those calculated. While the alginate samples usually forms a gel by dialysis against the calcium-magnesium chloride solution, in these cases the samples were found to be partly or completely soluble. This is to be expected when the alginates occur mainly as magnesium salt, particularly for the low molecular weight fragments. This observation, therefore, indicates that the ion-selectivity of alginates depends very much upon whether the polysaccharide is in solution or in the gel state. For alginate gels, the results in Fig. 1 show that the correlation between the observed selectivity coefficient for the calcium-magnesium exchange reaction is satisfactorily described by formulae (3) and (4), using the selectivity coefficients of the individual monomeric residues given above.

A few experiments with strontium-magnesium ion exchange were also carried out using alginate from *Laminaria hyperborea* stipes (27.5 % mannanuronic acid). The results are given in Table 1. Again the agreement between

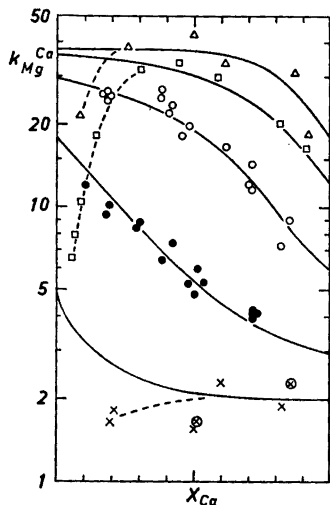


Fig. 1. Selectivity coefficients as a function of  $X_{Ca}$  for Ca—Mg exchange. Alginate from  $\circ$  *L. hyperborea* stipes, 27.5% mannanuronic acid,  $\bullet$  *L. digitata*, 61.5% mannanuronic acid,  $\otimes$  *Ascophyllum nodosum*, receptacles, 91% mannanuronic acid,  $\Delta$  Alginate fragment, preparation  $C_2$ , 6.8% mannanuronic acid,  $\square$  preparation  $C_1$ , 13.6% mannanuronic acid,  $\times$  preparation B, 91% mannanuronic acid.

The curves are calculated from formulae (3) and (4) for preparations with the uronic acid composition given above, with  $(k_{Mg^{Ca}})_G = 40$  and  $(k_{Mg^{Ca}})_M = 1.8$ .

Table 1. Comparison between observed and calculated values of  $k_{Mg}^{Sr}$  for alginate sample (27.5 % mannuronic acid).

$X_{Sr}$	Observed	$k_{Mg}^{Sr}$	Calculated
0.3	92		90
0.46	60		67
0.7	18.5		19

observed and calculated values was good. The values of the monomeric selectivity coefficients giving the best fit were  $(k_{Mg}^{Sr})_M=1.2$  and  $(k_{Mg}^{Sr})_G=150$ .

c) *Variation of the selectivity coefficients with varying uronic acid composition of the alginates.* In order to compare ion exchange properties of alginates with different uronic acid composition, conditions must be chosen so that the values of  $X_B$  in all cases are approximately the same. The selectivity coefficients

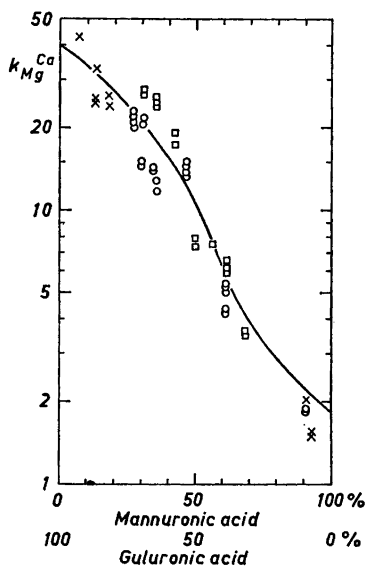


Fig. 2. Selectivity coefficients as a function of uronic acid composition for Ca-Mg exchange.

× Alginate fragments, dialysis technique,  
 ○ alginate samples, dialysis technique,  
 □ alginate samples, calcium thread technique. The curve is calculated from formulae (3) and (4).

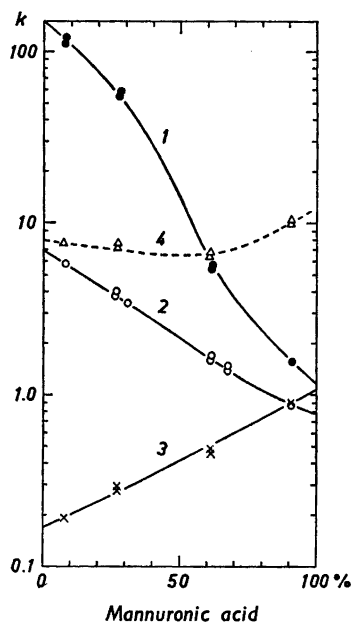


Fig. 3. Selectivity coefficients as a function of uronic acid composition. 1) Sr-Mg, 2) Sr-Ca, 3) Co-Ca, 4) Cu-Ca.

Table 2. Selectivity coefficients determined by extrapolation (Fig. 3).

Ion exchange reaction	$k_M$	$k_G$
Sr—Mg	1.2	150
Ca—Mg	1.8	40
Sr—Ca	0.8	7
Co—Ca	1.1	0.17
Cu—Ca	12	8

$k_{Mg}^{Ca}$  of a number of alginate samples have been determined under conditions giving  $X_{Ca}=0.5\pm 0.05$ . The results are given in Fig. 2. By using the same values of  $(k_{Mg}^{Ca})_M$  and  $(k_{Mg}^{Ca})_G$  as above (1.8 and 40,<sup>1</sup> respectively) in formulae (3) and (4) a series of corresponding values of  $k_{Mg}^{Ca}$  and  $X_{Ca}$  were obtained for alginates of different uronic acid composition. The curve in Fig. 2 gives the calculated values of  $k_{Mg}^{Ca}$  corresponding to  $X_{Ca}=0.5$ . Also in this case, a satisfactory agreement between calculated and observed results was obtained. Some of the results given in Fig. 2 were obtained by equilibrating calcium alginate threads against magnesium chloride solutions as described previously;<sup>19</sup> no significant disagreement between the results obtained by this method and by the dialysis technique was observed.

The most convenient method for determining  $k_M$  and  $k_G$  of a given exchange reaction was to determine the selectivity coefficients at  $X_{Me}=0.5\pm 0.05$  for alginates with different uronic acid composition and to extrapolate the curves to 100 % guluronic and 100 % mannuronic acid. In order to make this extrapolation as reliable as possible it is important to include preparations with a composition approaching homopolymers. This procedure has been used for the ion exchange reactions Sr—Ca, Sr—Mg, Cu—Ca and Co—Ca. The results are given in Table 2 and Fig. 3. For comparison, the results of the reaction Ca—Mg are also given in the table.

Figs. 1, 2, and 3 include results obtained both with high molecular weight alginates ( $P_w \geq 2000$ ) and alginate fragments approaching a homopolymer composition ( $P_n$  between 20 and 60). One of the alginate samples also ap-

Table 3. Selectivity coefficients of an alginate fragment containing mainly alternating mannuronic-guluronic acid residues compared with whole alginate of the same uronic acid composition (62 % mannuronic acid).

	Alginate	Alginate fragment
$k_{Mg}^{Ca}$	5	4.5
$k_{Mg}^{Sr}$	5.5	3.1
$k_{Ca}^{Sr}$	1.6	1.02
$k_{Ca}^{Co}$	0.5	0.7
$k_{Ca}^{Cu}$	8.5	7

proaches in composition a polymannuronic acid, while the other alginate samples consist of both homopolymer blocks and parts with a predominantly alternating sequence. The fact that the results obtained with the fragments and with whole alginate can be described by the same curve indicates strongly that neither the molecular weight (within the range tested here) nor the sequence can be of prime importance for the selectivity. The possible influence of the sequence was further investigated by determining the selectivity coefficients for an alginate fragment containing predominantly alternating mannanuronic-guluronic acid residues (Preparation A). The results are given in Table 3, and indicate a somewhat lower selectivity of this fraction than of a sample of undegraded alginate of the same composition. It is clearly demonstrated, however, that the guluronic acid residues still retain a marked selectivity also when they occur in an alternating sequence.

#### DISCUSSION

The results of this investigation confirm that the ion exchange properties of alginates depend upon their uronic acid composition. By ascribing two different selectivity coefficients,  $k_M$  and  $k_G$ , to the two different monomers, both the variation of the observed selectivity coefficients with varying ionic composition of the polymer (Fig. 1), and the difference in selectivity between alginate samples of different uronic acid composition (Fig. 2), were satisfactorily described for the calcium-magnesium ion exchange reaction. This means that the alginate behaves like a physical mixture of two substances of different selectivity. It should be clearly understood, however, that the selectivity of the isolated monomers most probably are quite different from the values of  $k_M$  and  $k_G$ . The dependence of the selectivity on degree of polymerization for monomers and low molecular weight oligomers remains to be investigated. It should also be kept in mind that the selectivities reported in this work refer to alginate in the gel phase, and that the results indicate (Fig. 1) that the selectivity properties of alginate in solution may be significantly different from that of alginate gels. The values of  $k_M$  and  $k_G$  for a given ion exchange reaction can be found by determining the selectivity coefficients of a series of alginate samples of different uronic acid compositions under conditions giving approximately equal amounts of the two ions bound to the polymer.

The selectivity coefficients  $k_M$  and  $k_G$  were determined for the ion exchange reactions strontium-magnesium, calcium-magnesium, strontium-calcium, cobalt-calcium, and copper-calcium. With the exception of the last reaction, the selectivity coefficients of the mannuronic acid residues,  $k_M$ , were in all cases close to one; while the corresponding values of the guluronic acid residues,  $k_G$ , varied from 0.17 to 150.

Several different theories have been proposed to explain the selectivity of ion exchange resins. Those which at present seem most commonly accepted<sup>26</sup> relate mainly to the exchange of alkali metal ions. Due to the high electrostatic potential of the resin, the most important factor in determining the degree of binding is supposed to be the distance of closest approach between the ion and the fixed group. Different degrees of hydration of the counter-

ions, therefore, lead to selectivity even for a purely electrostatic binding. The energy necessary for dehydration of the ions must also be taken into account. The different selectivity of sulfonate groups and carboxyl groups, for example, is considered to be connected with the difference in field strength around the two types of fixed groups, leading to a different degree of dehydration of the counterions.

Even if the possibility should not be completely excluded, it is unlikely that such marked differences in selectivity as observed in this case between monomers so closely related as mannuronic and guluronic acid residues can be due to different degrees of dehydration of the counterions. It is more reasonable to conclude that the differences are due to the different stereochemistry of the two uronic acids.

From a practical point of view, the differences between the two monomers with respect to ion selectivity should be taken into account when alginate preparations with special ion exchange properties are needed. The importance of this point has been effectively illustrated recently by the comparison of the effect of different alginate preparations in the prevention of strontium absorption.<sup>6,28,29</sup> The effect was clearly correlated to the guluronic acid content of the alginate, and a guluronic rich fragment<sup>30</sup> similar to preparations C<sub>1</sub> and C<sub>2</sub> used in this work, and prepared by methods developed in this laboratory,<sup>21,23,25</sup> was found to be particularly effective.

Biologically, the existence of highly selective polyelectrolytes like alginate rich in guluronic acid residues may be of considerable importance. The close agreement between the "selectivity coefficient" calculated for brown algae growing in seawater, and the selectivity coefficients of the alginate they contain<sup>31</sup> showed the prime importance of alginate in the accumulation of alkaline earth metals in these plants. It seems likely that a further understanding of the ionic selectivity of naturally occurring polyelectrolytes may be in many cases valuable for the understanding of ionic accumulation in marine organisms.

## EXPERIMENTAL

*Materials.* Samples of alginate were prepared as described previously<sup>20</sup> and the uronic acid composition determined according to Haug and Larsen.<sup>32</sup> A very mannuronic acid rich alginate was prepared by soaking unripe receptacles from *Ascophyllum nodosum* in distilled water, squeezing out the viscous liquid and preparing the alginate by acid precipitation. The intrinsic viscosity of the samples were in all cases above 10, corresponding to weight average degree of polymerization of more than 2500.<sup>33</sup> Alginate fragments were prepared by methods described previously.<sup>24</sup> Four different alginate fragments were investigated:

	$P_n$	% Mannuronic acid
Preparation A	30	65
» B	30	91
» C <sub>1</sub>	20	13.6
» C <sub>2</sub>	60	6.8

Preparation C<sub>2</sub> was prepared by fractionating the guluronic rich fragments by precipitation with calcium and magnesium ions.<sup>24</sup>

Determination of exchange equilibrium: Solutions of sodium alginate (1 %, 2 ml) were dialysed against 50 ml of a solution containing salts of the two cations to be inves-



tigated in a total concentration of 0.22 M. Different combinations of  $MgCl_2$ ,  $CaCl_2$ ,  $SrCl_2$ ,  $CoCl_2$ , and  $Cu(NO_3)_2$  were used. The solution containing the dialysis bag was gently shaken, and the salt solution was changed 3 times with intervals of 8–16 h. The alginate solution was then dialysed against distilled water (50 ml, changed 3 times). Finally the dialysis bag was transferred to 20 ml 0.1 M hydrochloric acid. The acid solution was changed twice, the solutions pooled, and the amount of the two cations determined by atomic absorption. Some experiments were also carried out by preparing calcium alginate threads and equilibrating the threads against solutions containing magnesium ions, as previously described for the determination of the sodium-calcium ion exchange equilibrium.<sup>19</sup>

*Acknowledgement.* We are indebted to Miss Lillian Sivertsen for skilful technical assistance and to siv. ing. Asbjørn Øien and Knut Smidsrød, Statens Jordundersøkelser, Norges Landbrukshøgskole, Vollebekk, for atomic absorption analyses.

## REFERENCES

1. Percival, E. and McDowell, R. *Chemistry and Enzymology of Marine Algal Polysaccharides*, Academic, London and New York 1967.
2. Smidsrød, O. and Haug, A. *Acta Chem. Scand.* **19** (1965) 329.
3. Haug, A. and Smidsrød, O. *Acta Chem. Scand.* **19** (1965) 341.
4. Paul, T. M., Waldron-Edward, D. and Skoryna, S. L. *Can. Med. Assoc. J.* **91** (1964) 553.
5. Hesp, R. and Ramsbottom, B. *Nature* **208** (1965) 1341.
6. Harrison, G. E., Humphreys, E. R., Sutton, A. and Shepherd, H. *Science* **152** (1966) 655.
7. van der Borcht, O., Colard, J., van Puymbroeck, S. and Kirchmann, R. *Radioecological Concentration Processes*, Pergamon, Oxford 1966.
8. Hardisty, D. R. and Neale, S. M. *J. Polymer Sci.* **46** (1960) 195.
9. Katchalsky, A., Cooper, R. E., Upadhyay, J. and Wassermann, A. *J. Chem. Soc.* **1961** 5198.
10. Smidsrød, O. and Haug, A. *J. Polymer Sci. C* **16** (1967) 1587.
11. Thiele, H. and Hallick, K. *Kolloid-Z.* **151** (1957) 1.
12. Thiele, H. and Schacht, E. *Z. Physik. Chem. (Leipzig)* **208** (1957) 42.
13. Thiele, H. and Schacht, E. *Kolloid-Z* **161** (1958) 120.
14. Schweiger, R. G. *Kolloid-Z.* **196** (1964) 47.
15. Haug, A. *Acta Chem. Scand.* **15** (1961) 1794.
16. Mongar, J. L. and Wassermann, A. *Discussions Faraday Soc.* **7** (1949) 118.
17. Mongar, J. L. and Wassermann, A. *J. Chem. Soc.* **1952** 492.
18. Mongar, J. L. and Wassermann, A. *J. Chem. Soc.* **1952** 500.
19. Haug, A. *Acta Chem. Scand.* **13** (1959) 1250.
20. Haug, A. Report No. 30, Norwegian Institute of Seaweed Research, Trondheim 1964.
21. Haug, A. and Smidsrød, O. *Acta Chem. Scand.* **19** (1965) 1221.
22. Haug, A. and Smidsrød, O. *Nature* **215** (1967) 757.
23. Haug, A., Larsen, B. and Smidsrød, O. *Acta Chem. Scand.* **20** (1966) 183.
24. Haug, A., Larsen, B. and Smidsrød, O. *Acta Chem. Scand.* **21** (1967) 691.
25. Haug, A., Myklestad, S., Larsen, B. and Smidsrød, O. *Acta Chem. Scand.* **21** (1967) 768.
26. Reichenberg, D. In Marinsky, J. A. *Ion Exchange*, Marcel Dekker, New York 1966.
27. Katchalsky, A., Alexandrowicz, Z. and Kedem, O. In Conway, B. E. and Barradas, R. G. *Chemical Physics of Ionic Solutions*, Wiley, New York 1966.
28. Patrick, G., Carr, T. E. F. and Humphreys, E. R. *Intern. J. Radiation Biol.* **12** (1967) 427.
29. Sutton, A. *Nature* **216** (1967) 1005.
30. Humphreys, E. R. *Carbohydr. Res.* **4** (1967) 507.
31. Haug, A. and Smidsrød, O. *Nature* **215** (1967) 1167.
32. Haug, A. and Larsen, B. *Acta Chem. Scand.* **16** (1962) 1908.
33. Smidsrød, O. and Haug, A. *Acta Chem. Scand.* **22** (1968) 797.

Received January 19, 1968.