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PII: S0144-8617(97)00237-3

*Carbohydrate Polymers* **35** (1998) 1-6

0144-8617/98/$19.00 + 0.00

# Na- and K-alginate; effect on $Ca^{2+}$-gelation

**Kurt Ingar Draget[a], Kjersti Steinsvåg[b], Edvar Onsøyen[b], Olav Smidsrød[a]**

[a]*Norwegian Biopolymer Laboratory, Department of Biotechnology, Norwegian University of Science and Technology, N-7034 Trondheim, Norway*

[b]*Pronova Biopolymer A/S, PO Box 494, N-3002 Drammen, Norway*

(Received 11 August 1997; accepted 3 October 1997)

The effect of two different monovalent alginate counterions has been studied in $Ca^{2+}$-mediated sol/gel transitions and in $Ca^{2+}$-gels at apparent equilibrium for alginates with different chemical composition and sequence. K-alginates were generally found to undergo faster sol/gel transitions compared to the Na-alginate. At apparent equilibrium and at low $Ca^{2+}$ concentration, Na-alginate gels showed reduced elastic moduli compared to the K-alginates. The relative differences both in sol/gel transition kinetics and modulus at apparent equilibrium increased with increased content of guluronic acid residues in the alginate sample. Increasing the amount of internally released $Ca^{2+}$ from 15 to 30 mM resulted in equal elastic properties at apparent equilibrium, suggesting that dissimilarities in ion exchange reactions between $Ca^{2+}/Na^+$ and $Ca^{2+}/K^+$ is the main driving force. The specific effect of one of the counterion could be reversed by adding increasing amounts of the other. Selectivity coefficient determinations for alginates revealed a slight enhanced binding of $Na^+$ compared to $K^+$, most probably due to the guluronate residues. © 1998 Elsevier Science Limited. All rights reserved.

## INTRODUCTION

It is a well established and studied fact that alginates, being a family of unbranched binary copolymers of (1–4) linked β-D-mannuronic acid and α-L-guluronic acid (Painter, 1983), undergo a sol/gel transition in the presence of multivalent cations such as $Ca^{2+}$ (Smidsrød, 1974; Stokke *et al.*, 1991). Monovalent salts of alginic acid (with the exception of $Ag^+$) have generally only been considered as being the water soluble species of alginate. The possibility that monovalent cations may also show different affinity towards alginates which could lead to difference in gelation kinetics and final gel properties have scarcely been considered.

In a study by Seale *et al.* (1982), the binding of monovalent cations to alginates was studied by circular dichroism. It was found that c.d. changes induced by $Na^+$ were different from those obtained by other $Me^+$, and resembled the changes following selective binding of $Ca^{2+}$. Solution studies showed evidence of interchain association in the presence of $Na^+$; this effect becoming more pronounced as the content of guluronic acid residues increased (Seale *et al.*, 1982). This work was followed up by a $^{23}Na$ NMR study (Grasdalen and Kvam, 1985), but their results could be satisfactorily explained by electrostatic theories although a small specific site binding of $Na^+$ to polyguluronate sequences could not be ruled out.

The scope of this study was to study the difference between $Na^+$- and $K^+$-alginates in a $Ca^{2+}$-induced gel state. If a site specific binding of $Na^+$ resembling the binding of $Ca^{2+}$ exists, an enhanced competition for binding sites in the junction zones is expected for the ion pair $Na^+/Ca^{2+}$ compared to $K^+/Ca^{2+}$. This increased competition should, at least at low concentration of the crosslinking ion, reveal itself both in the kinetics and the equilibrium properties of $Ca^{2+}$-induced gels.

## MATERIALS AND METHODS

### Alginate samples

Three different samples of alginic acid was kindly provided

**Table 1. Chemical composition and sequence for the three different alginic acid samples used in this study**

| Alginate source | $F_G$ | $F_M$ | $F_{GG}$ | $F_{MM}$ | $F_{GM,MG}$ | $F_{GGG}$ | $F_{GGM}$ | $F_{MGM}$ | $N_{G>1}$ |
|---|---|---|---|---|---|---|---|---|---|
| *Laminaria hyperborea* (stipe) | 0.71 | 0.29 | 0.59 | 0.17 | 0.12 | 0.55 | 0.04 | 0.08 | 15.8 |
| *Laminaria hyperborea* (leaf) | 0.51 | 0.49 | 0.36 | 0.34 | 0.15 | 0.32 | 0.04 | 0.11 | 10.0 |
| *Lessonia negrescens* | 0.40 | 0.60 | 0.25 | 0.44 | 0.17 | 0.21 | 0.04 | 0.13 | 6.8 |

by Pronova Biopolymer A/S. The samples were isolated from *Lessonia negrescens* (low guluronate content), from leaves of *Laminaria hyperborea* (medium guluronate content) and stipes of *Laminaria hyperborea* (high guluronate content). These alginic acid batches were thermally degraded at 60°C to yield the same relative viscosity in a 1.0% solution as measured by a Brookfield DV-III Rheometer. After degradation, the weight average molecular weight ($M_w$) was measured by SEC-LALLS as described earlier (Draget *et al.*, 1996), and was found to be 240 kDa and identical for all three samples. Table 1 gives the chemical composition and sequence of these three alginic acid batches as determined by high-field NMR spectroscopy (Grasdalen, 1983). Finally, each of the three different batches was split into three and converted to 100% Na-alginate, Na-/K-alginate (50%/50%) and 100% K-alginate by adding the appropriate amount of the different carbonates.

### Alginate gelation

Ca-alginate gels were prepared by internal gelation as described earlier (Draget *et al.*, 1991). Briefly, an appropriate amount of solid $CaCO_3$ was added to a $15\,mg\,ml^{-1}$ alginate stock solution followed by the addition of twice the molar amount (relative to $CaCO_3$) of a freshly made solution of D-glucono-δ-lactone (GDL). Finally, the alginate concentration was adjusted to $10\,mg\,ml^{-1}$.

Gelation kinetics was followed by a Bohlin VOR general purpose rheometer using a serrated plate–plate measuring geometry. Instrumental parameters: 1 Hz frequency, 0.044 strain, 25°C, 12.15 g cm torsional bar and 1.00 mm gap.

At apparent equilibrium (24 h), longitudinal compression measurements were performed by a TA-XT2 Texture Analyser on alginate gels with geometries of 16 × 18 mm ($d \times h$). These measurements were carried out using a cylindrical P/25A probe and at $0.1\,mm\,s^{-1}$ deformation speed. Young's modulus ($E$) of the alginate gels was calculated from the initial (0–1% strain) linear part of the resulting force/deformation curve as described earlier (Smidsrød *et al.*, 1972; Draget *et al.*, 1994).

### Selectivity coefficient determination

The sodium form of the three different samples described in Table 1 was used throughout this study. 5 ml of a $15\,mg\,ml^{-1}$ solution was placed inside a dialysis tube. From 1.0 M stock solutions of KCl and NaCl, 0.25 M solutions containing different $K^+/Na^+$ ratios was prepared (ranging from 300/1 to 1/10). The dialysis bags containing the alginate solutions were placed in beakers containing 50 ml of the salt solution. This volume was exchanged three times within 24 h. After reaching salt exchange equilibrium, the alginate samples were dialysed extensively against MQ water until the conductivity of the dialysis liquid was close to that of the MQ water itself. In order to release the associated counterions, the alginate was converted to alginic acid by treating the dialysis bags with three volumes of 50 ml 0.1 M $HNO_3$ over 24 h. The $HNO_3$ fractions were analysed for $Na^+$ and $K^+$ by atomic absorption (Perkin-Elmer Spectrometer type 560). To avoid contamination, no glass equipment was used in this study; only extensively washed polystyrene beakers.

The selectivity coefficients were determined in 1:1 (v/v) water:ethanol mixtures to keep the alginate in an insoluble, swollen state in order to make possible both intra- and intermolecular binding of counterions.

## RESULTS AND DISCUSSION

Fig. 1 shows the sol/gel transition of the Na-alginate (closed symbols) and the K-alginate samples gelled with 15 mM $CaCO_3$ and 30 mM GDL. It is readily seen that the apparent equilibrium properties (15 h) of the two gels are different. The K-alginate sample exhibits more of a viscoelastic solid character compared to the Na-alginate sample with a higher dynamic storage modulus and a lower loss modulus.

The sol/gel transition kinetics of the different alginates were measured over a relative short period of time at high resolution, and the results are presented in Fig. 2(a) and 2(b). Fig. 2(a) shows the initial change in the phase angle $\delta$ ( $= \arctan G''/G'$) of the Na-, K- and Na/K-alginates isolated from *L. hyperborea* stipe (high guluronic acid content; see Table 1) gelled with 15 mM $CaCO_3$ and 30 mM GDL. The trend is that an increasing content of potassium in the alginate sample leads to an earlier drop in the phase angle, i.e. the sol/gel transition becomes faster. Fig. 2(b) shows the time from mixing the alginate solution with the gelling components to the point where the systems change to a predominately viscoelastic solid (i.e. $\delta = 45°$). Independent on the biological origin, and hence also on the content of guluronic acid residues, the results show that $\delta = 45°$ is reached first with the K-alginates.

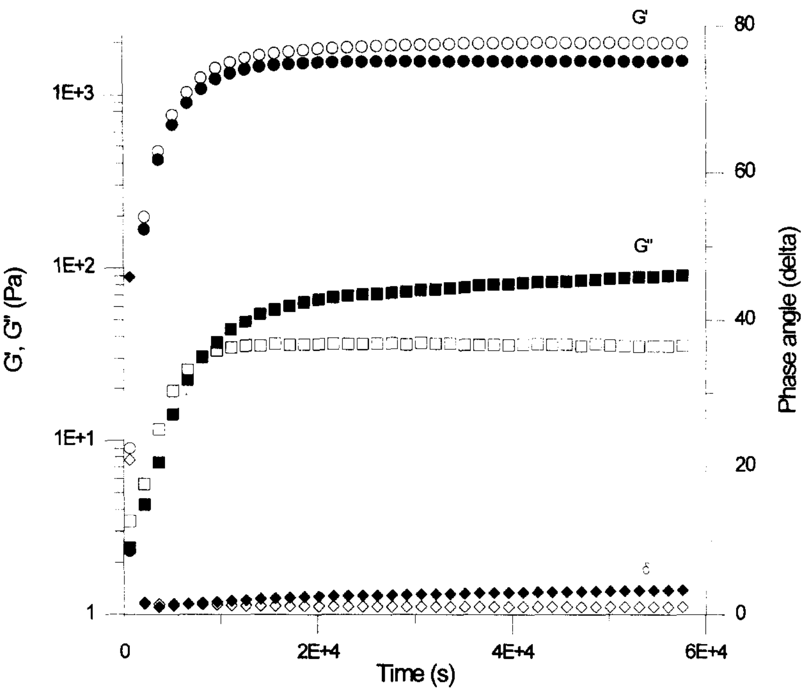

**Fig. 1.** Sol/gel transitional curves of $G'$ (circles), $G''$ (squares) and $\delta$ (diamonds) for the sodium (closed symbols) and potassium (open symbols) form of the *L. hyperborea* stipe alginate sample at 15 mM $CaCO_3$ and 30 mM D-glucono-$\delta$-lactone (GDL).

Similar trends are observed at equilibrium (Table 2). For all the different alginates samples, the potassium form showed the highest value of both the dynamic storage modulus ($G'$) and Young's modulus ($E$) compared to the sodium form after 16 h of gelation. The relative difference between gels made from alginates with the two different counterions becomes larger as the content of guluronic acid residues in the alginate increases.

The next step was to investigate how the equilibrium strength of alginate gels made from the sodium and potassium form was affected by adding different amounts of NaCl and KCl. For this purpose, the alginate with the highest content of guluronic acid residues was chosen. Fig. 3(a) and 3(b) show these results at 15 and 30 mM $Ca^{2+}$, respectively, presented as a change in Young's modulus ($E$). With no salt added and at 15 mM $Ca^{2+}$ (Fig. 3(a)), there is an initial difference as already described in Table 2. As the salt concentration increases, there is a slight increase in gel strength at low concentrations. This increase is more pronounced for the sodium sample compared to the potassium alginate, making the difference in gel strength undistinguishable. A further salt addition results in a lowering of gel strength and, independent on the type of original counter ion of the alginate sample, is more pronounced for NaCl than for KCl. Again, a specific effect of $Na^+$ becomes evident. The increase in gel strength at low salt concentrations may be explained by a non-specific shielding of the long range electrostatic repulsion between the highly negatively charged alginate molecules, thus facilitating $Ca^{2+}$ mediated interchain crosslinking. Such effects have been observed earlier (Smidsrød and Haug, 1965)). At higher salt concentrations, the effect of the added salt starts to overshadow that of the original counter ion and the gel strength becomes lower for the $Na^+$-addition. The observed results could possibly be explained by an ion exchange effect if the sodium ions compete more effectively with $Ca^{2+}$ than the potassium ions. Increasing the concentration of the crosslinking $Ca^{2+}$ ion from 15 to 30 mM (Fig. 3(b)) totally eliminates the difference between the two samples at low salt concentration. This can be attributed to a more efficient exchange of $Na^+$ and $K^+$ ions with the increased concentration of the crosslinking calcium ions, thus making the elastic properties at apparent equilibrium indistinguishable between the gels made from the two different samples. At high salt concentrations, i.e. where the effect of the added salt predominates, the same behaviour is observed as at low $Ca^{2+}$ concentration; a

**Table 2. Dynamic storage modulus ($G'$) and Young's modulus ($E$) at apparent equilibrium for gels made from the sodium and potassium form of the different alginates given in Table 1**

| Alginate | $G'$ (kPa) | $E$ (kPa) |
|---|---|---|
| *L. hyperborea* stipe (Na form) | 1.58 | 7.95 |
| *L. hyperborea* stipe (K form) | 2.01 | 10.20 |
| *L. hyperborea* leaf (Na form) | 0.58 | 2.67 |
| *L. hyperborea* leaf (K form) | 0.74 | 3.58 |
| *L. negrescens* (Na form) | 0.35 | 1.58 |
| *L. negrescens* (K form) | 0.38 | 1.66 |

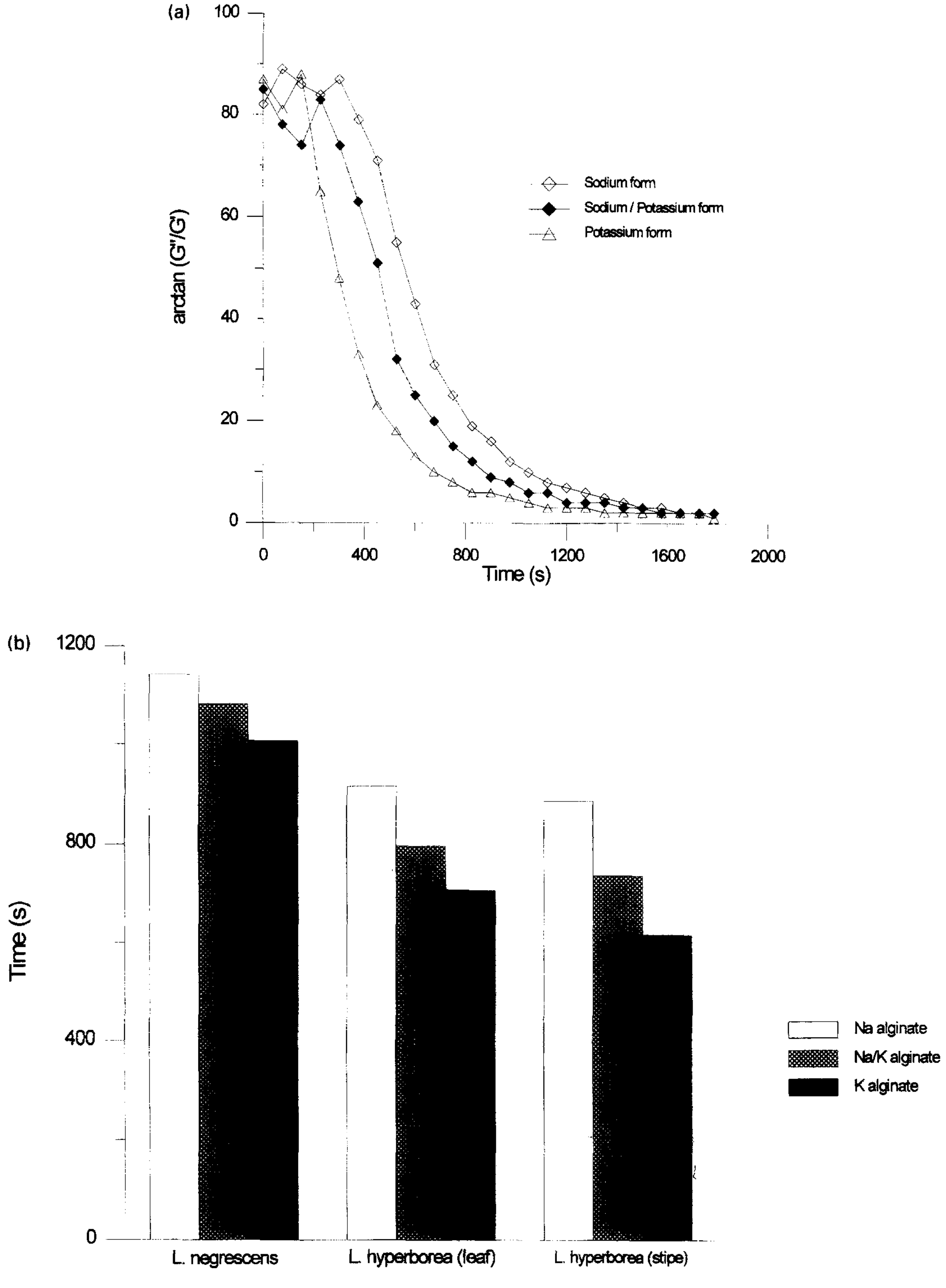

**Fig. 2.** (a) Initial sol/gel transitions at 15 mM $CaCO_3$/30 mM GDL as represented by the change in the phase angle ($\delta$ = arctan $G''/G'$) for the *L. hyperborea* stipe sample with different counterions. (b) Time where the gelling systems change from a predominately viscoelastic liquid to a viscoelastic solid ($\delta = 45^0$) at 15 mM $CaCO_3$/30 mM GDL.

$Na^+$-dependent reduction in elastic properties becomes prominent. This result is in agreement with earlier observations on the destabilization of Ca-alginate gel beads at high $Na^+/Ca^{2+}$ ratios (Martinsen *et al.*, 1989).

Fig. 4 presents the selectivity coefficients $K_K^{Na}$ determined in the $Na^+/K^+$ ion pair exchange reaction as function of the molar fraction of $Na^+$ in the salt solution. The selectivity coefficient is given by the equation:

$$K_K^{Na} = \frac{X_{Na}}{X_K} \cdot \frac{C_K}{C_{Na}}$$

where X represents the equivalent fraction of the counterions in the polymer phase and C represents the

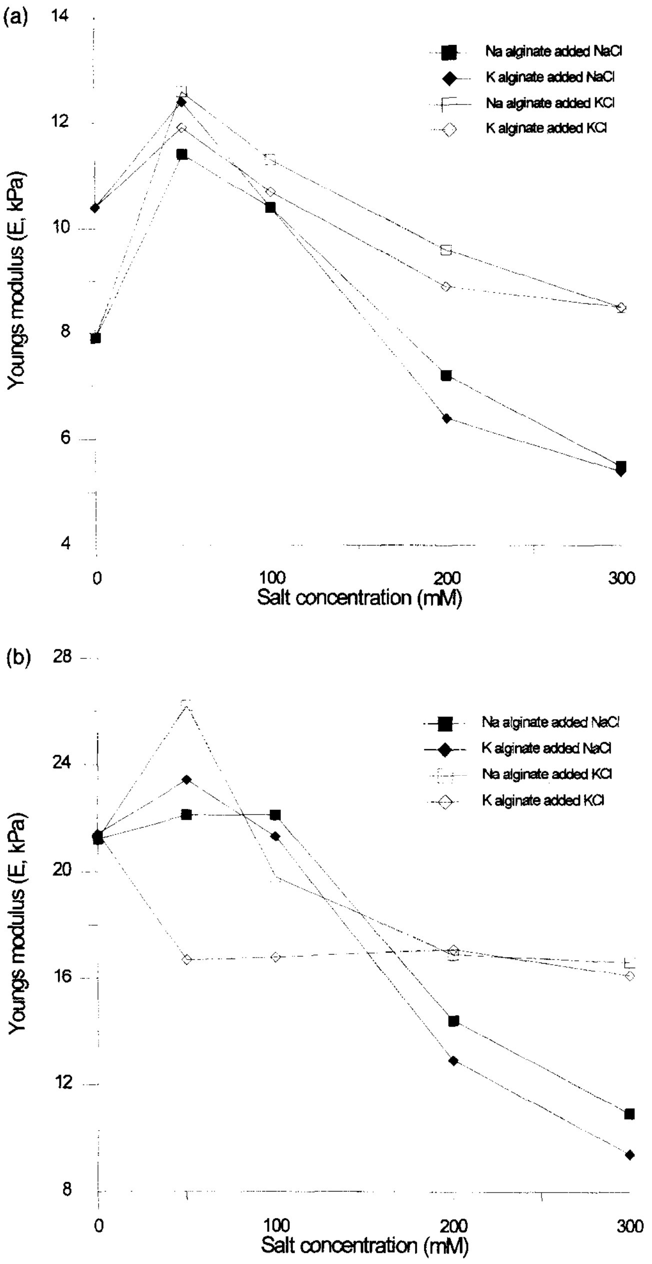

**Fig. 3.** Changes in Young's modulus of gels made with the sodium and potassium form of *L. hyperborea* stipe alginate at 15 mM $CaCO_3$/30 mM GDL (a) and at 30 mM $CaCO_3$/60 mM GDL (b) as function of added NaCl and KCl.

concentration of the ions in solution. It can be observed that a selective alginate binding of $Na^+$ becomes more pronounced as the molar fraction of $Na^+$ in the salt solution decreases. By analysing these results according to the theory of multiple equilibria (van Holde, 1971), the binding of sodium ions occurs either in a hetero (anti-) cooperative fashion or to binding sites of different selectivity in the sodium–potassium exchange reaction. The results suggest one type of site with a selectivity coefficient in the order of 10 and another type of site being almost without selectivity. With reference to earlier works (Seale *et al.*, 1982; Grasdalen and Kvam, 1985), it is reasonable to allocate the strongest binding of sodium ions to guluronate residues, although the results obtained in the present study refer to a 50:50 mixture of ethanol and water as solvent compared to pure aqueous solutions in the works cited above. All three alginate samples show very similar behaviour but a detailed correlation to uronate sequence, and inter- or intramolecular binding in these soluble, highly swollen gel particles seems premature. However, an inescapable conclusion seems to be that sodium ions would show the strongest binding also in an exchange reaction with calcium ions as in the gel experiments above. Hence, the different affinity of alginates towards $Na^+$ and $K^+$ could account for both the delayed sol/gel transition and the reduced elasticity at apparent equilibrium and at low concentrations of $Ca^{2+}$ of the $Na^+$ alginate sample, and with excess sodium salt addition.

## CONCLUSIONS

It has been shown that commercially available alginates with identical molecular weights and highly different chemical composition have a counterion dependence ($Na^+/K^+$) on $Ca^{2+}$-induced sol/gel transitional kinetics and elastic properties at apparent equilibrium. At low $Me^+/Ca^{2+}$ ratios, these differences are observed only at low concentration of the crosslinking ion, and the specific counterion dependence can be eliminated by adding a sufficient amount of the other monovalent salt. At high $Me^+/Ca^{2+}$ ratios, however, another regime is entered where a specific effect of the different $Me^+$ is observed which seems to be more or less independent on the amount of crosslinking ion. Together with an observed selective binding of $Na^+$ relative to $K^+$ in the alginate molecules, the total results point towards an explanation given by a difference in the exchange reactions between the $Na^+/Ca^{2+}$ and $K^+/Ca^{2+}$ ionic pairs.

The fact that monovalent, water soluble salts of alginic acid behave differently could be of importance in any application with internal setting at low concentration of the crosslinking ion. At medium to high content of monovalent cation content, e.g. in food applications, it is also important to realize that various end-product properties can be obtained following an addition of different salt species.

## ACKNOWLEDGEMENTS

The authors wish to thank Kristin B. Anthonsen for skilful technical assistance. This study has been financially supported by Pronova Biopolymer A/S.

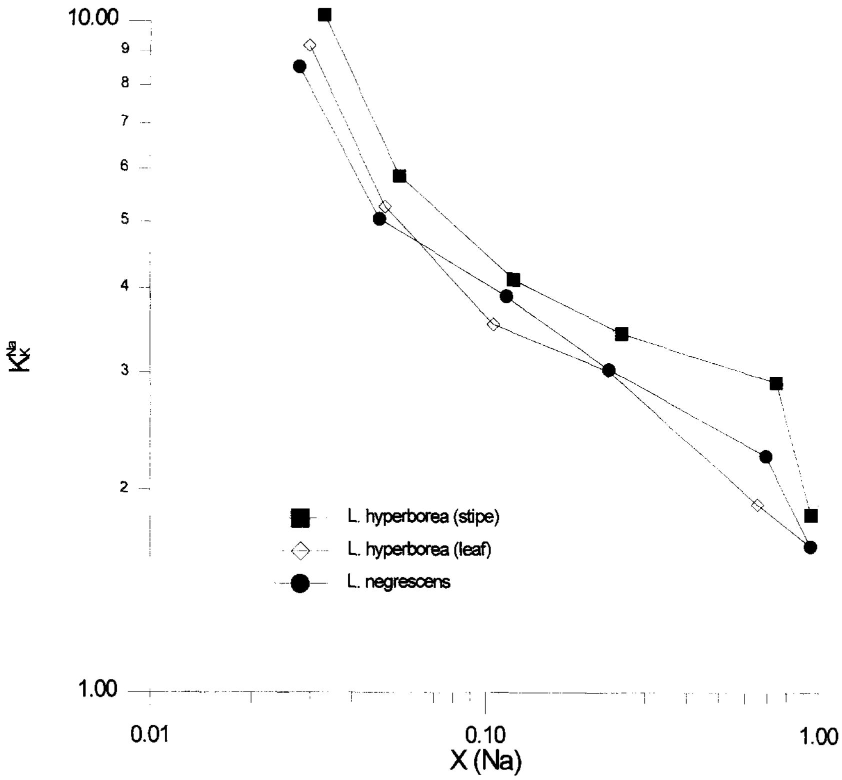

**Fig. 4.** Determination of the selectivity coefficient ($K_K^{Na}$) as function of the equivalent fraction of $Na^+$ ($X_{Na}$) bound to the alginate phase at equilibrium.

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