ELSEVIER

PII: S0032-3861(97)00597-1

Polymer Vol. 39 No. 13, pp. 2759–2764, 1998
© 1998 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0032-3861/98/$19.00+0.00

Calcium alginate gels: formation and stability in the presence of an inert electrolyte

Xingwu Wang and H. Garth Spencer*
Department of Chemistry, Clemson University, Clemson, SC 29634-1905, USA
(Received 5 May 1997; accepted 15 July 1997)

The effects of a simple salt (1:1 electrolyte, KCl) on the calcium alginate gel formation and the gel stability of alginates varying in molecular weight and mannuronate/guluronate (M/G) ratio were investigated by an equilibrium dialysis technique. There was no significant dependence of the calcium binding capacity among the alginates. However, the G residues exhibited a stronger affinity for Ca^{2+} than M residues. The addition of a simple salt to the dialysis solution resulted in displacement of bound Ca^{2+} and swelling of the calcium alginate gel that increased with concentration of the simple salt. Even the Ca^{2+} bound with the G block was displaced by monovalent cations at high salt concentration. © 1998 Elsevier Science Ltd. All rights reserved.

(Keywords: calcium alginate; polyelectrolyte gels; swelling)

INTRODUCTION

Alginate is a polysaccharide obtained from marine algae, and is a linear copolymer of 1,4-linked β-D-mannuronate (M) and α-L-guluronate (G) residues[1]. The polymer chain of alginate can be considered as composed of mannuronate diads (MM), guluronate diads (GG), and hetero-diads (GM). The exact composition depends on the source from which the alginate has been extracted. With one carboxylate group in each M or G unit, alginate is a highly negatively charged polyelectrolyte at neutral or basic pH. The interaction between alginate and divalent cations, such as Ca^{2+}, Cu^{2+}, or Zn^{2+}, provides alginate the ability to form gels in aqueous solution in the presence of low concentrations of these divalent cations[2–4]. Alginate has been widely used as a gelling or thickening agent in the food, textile and pharmaceutical industries[5,6]. Some new applications have recently been exploited for alginate[7–9]. For instance, alginate has been used to form dynamic ultrafiltration or microfiltration membranes on porous substrates for the separation of sugars from proteins and particulates. The membrane stability, which depends on the alginate gel stability, is critical to the membrane performance[9,10].

The formation and properties of calcium alginate gels have been studied extensively and by several methods, such as, ion-exchange reactions[11,12], CD spectroscopy[13–15], osmotic pressure measurements[16], light scattering[16,17], viscosity measurements[15,18,19], n.m.r. spectroscopy[20–22], d.s.c.[22], and others[23,24]. Generally, it is believed that the GG diad has higher selectivity for Ca^{2+} than the MM diad or GM diad due to the differences of the chain conformations in these blocks. The gel network is induced by cooperative bonding of Ca^{2+} with the alginate residues; with both G and M units involved in the Ca^{2+} bonding. Although there have been investigations of 1:1 electrolyte influence on the calcium alginate gel formation[3,25], no research has been reported on the stability of the calcium-induced gel in the presence of a simple electrolyte. However, knowledge of this stability is needed for applications of gel membranes in separation systems since the stability and the dimensional change of the gel in the salt solution are directly related to the membrane performance and its limitations in many applications.

In this work, the effect of molecular weight and M/G ratio on the equilibrium bound fraction of calcium ion in the calcium alginate gel; the effect of the concentration of an added simple 1:1 electrolyte in the external solution on the gel stability, i.e., the crosslink density of the gel, and the equilibrium volume swelling ratio were investigated using equilibrium dialysis procedures.

EXPERIMENTAL

Materials

Four sodium alginates were used in the study. KELTONE HV and KELTON LV were obtained from Kelco Division of Merck and Co. Inc. (Rathway, NJ), and MANUGEL DMB and MANUGEL GHB, were the product of Kelco Division of London (England). The alginates were purified with ethanol washing and then dried under 60°C to a constant weight. All other chemicals used in the research were analytical reagent grade. All the water used in the experiments was filtered by an ultrafiltration membrane and deionized to a specific conductivity of less than 4×10^{-5} S/m.

The uronic residue composition in the sodium alginates was determined by ^{13}C-n.m.r. spectroscopy (BRUKER CA300, HITACHI) using a method similar to that described by Grasdalen *et al.*[26,27]. The fractions of mannuronic acid (F_M) and guluronic acid (F_G) residues and the fractions of GG (F_{GG}), MM (F_{MM}) and MG/GM (F_{MG}) diads in the sodium alginates were determined from the n.m.r. results. The average molecular weights were measured with a capillary viscometer at 25°C in a 0.1 N NaCl solution, using the constants in the Mark–Houwink equation determined by light scattering[28]. The results of the molecular weight and monomer composition of the alginates are provided in *Table 1*.

* To whom correspondence should be addressed

Table 1 Molecular weight and monomer composition of the sodium alginate samples

Sample	M_w(kDa)	M/G	f_M	f_G	f_{MM}	f_{GG}	f_{MG}
HV	233	1.5	0.60	0.40	0.36	0.18	0.46
LV	137	1.5	0.60	0.40	0.36	0.18	0.46
DMB	220	1.0	0.50	0.50	0.28	0.28	0.44
GHB	169	1.0	0.50	0.50	0.28	0.28	0.44

Methods

The formation of calcium alginate gel and the determination of the gel stability were investigated by equilibrium dialysis using cellulose acetate dialysis tubes with a molecular weight cut-off of 6000–8000 Da (Spectrum Medical Industries, Inc., LA). The sodium alginates were dissolved in deionized water at a polymer concentration of 4.2–4.5 g/L. A 10.00 mL solution of sodium alginate was introduced in the membrane tubing and then dialyzed against a large volume, 9.00 L, of a $CaCl_2$ solution, equipped with a magnetic stirrer set at the same rate each time. The Ca^{2+} concentrations in the dialysis solutions, $[Ca^{2+}]_s$, and in the calcium alginate gels (include bound and free Ca^{2+}), $[Ca^{2+}]_t$, were determined by titration with a 0.02 M EDTA (ethylenediaminetetraacetic acid disodium salt) solution in ammonium buffer (pH 10) using a calcium sensitive electrode (Orion, calcium electrode model 93-20) and an Accumet pH Meter (Fisher, Model 810) as described by Anderson *et al.*[29,30] The stability of each calcium alginate gel was investigated by dialyzing it against a large volume (15 L) of solution containing selected concentrations of $CaCl_2$ and KCl. The dependence of the gel volume and the equilibrium concentration of bound calcium ($[Ca^{2+}]_b$) was determined as a function of the concentration of KCl. The gel volume was measured by water displacement in a graduated cylinder. The gels were strong enough to be removed mechanically from the dialysis tubing. Most of the dialyses and titrations were carried out more than two times. The relative experimental errors for the calcium ion concentrations were less than 1% and for the gel volume were less than 2%. All the dialyses, volume measurements and titration experiments were performed at 22.0 ± 0.5°C and the pH of the dialysis solutions was 7.0 ± 0.2.

The equilibrium volume swelling ratio, Q_{eq}, which is the ratio of the volume of the swollen gel to the volume of the dry polymer, was determined by the equation:

$$Q_{eq} = \frac{1000 V_g \rho}{V_s C_p} \quad (1)$$

where V_g is the resulting gel volume (mL), V_s is the volume of sodium alginate solution dialyzed to form the gel (10.00 mL), C_p is the concentration of the sodium alginate solution (g/L), and ρ (g/cm^3) is the density of the dry sodium alginate measured with a pycometer at 25°C with pure ethanol as fill liquid.

The thermal stability of the calcium alginate gels formed from the dialysis were studied by differential scanning calorimetry (d.s.c.) (d.s.c. 910, TA Instruments) in the D.I. water and formation solution environments using Perkin-Elmer large volume capsule high pressure d.s.c. cells.

RESULTS AND DISCUSSION

Dialysis equilibrium

Figure 1 is a plot of the total calcium ion concentration in the gel ($[Ca^{2+}]_t$) *versus* time during the dialysis of the sodium alginate solution in 10 mM $CaCl_2$ solution to form the gel. It shows that the dialysis equilibrium is reached after 16 hours at the experimental conditions and there is no significant difference detected in the rate of calcium inclusion in the gel among the four sodium alginate samples whose M/G ratio and molecular weight are different. The rate could simply have been determined by the rate of transport across the dialysis membrane or diffusion inside the gel and not by the rate of Ca^{2+} binding. At the dialysis equilibrium, the electrochemical potentials of all ions in the system, both inside and outside the gel, should be in the equality (μ_i(gel) = μ_i(sol)). Therefore, Donnan equilibrium is applicable to the mobile ions in the system. With the assumption that the ratios of activity coefficients of the free ions in the gel and in the external solution are unity under conditions of high gel swelling, the Donnan equilibrium can be established.

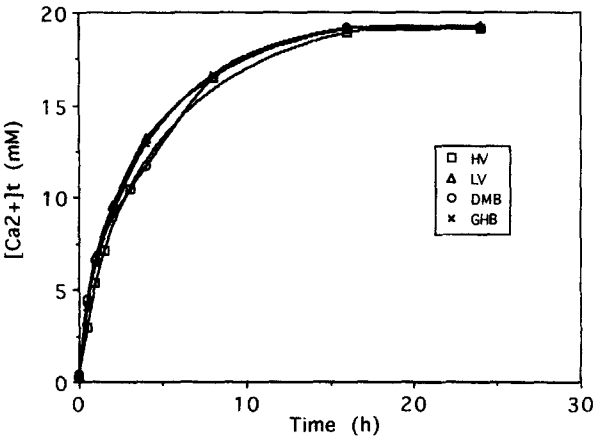

Figure 1 Total Ca^{2+} concentration in the alginate sol–gel system during dialysis of a sodium alginate solution in 10 mM $CaCl_2$ solution

$$[Cl^-]_f^2[Ca^{2+}]_f = [Cl^-]_s^2[Ca^{2+}]_s \quad (2)$$

where the subscript f refers to the free ions in the gel and s refers to the external solution. Due to electroneutrality, the following relation is given, neglecting the H^+ and OH^- concentrations at pH 7.0.

$$[COO^-]_f + [Cl^-]_f = 2[Ca^{2+}]_f \quad (3)$$

Since $[COO^-]_t = [COO^-]_f + [COO^-]_b$, $[Ca^{2+}]]_t = [Ca^{2+}]_f + [Ca^{2+}]_b$, and $[COO^-]_b = 2[Ca^{2+}]_b$, where t refers to the total concentration in the gel and b refers to the bound species, equation (4) can be obtained from equations (2) and (3) for calculating the concentration of the equilibrium bound Ca^{2+} in the calcium alginate gel formed by dialysis of the sodium alginate solution in a large volume of $CaCl_2$ solution at pH 7.

$$[Ca^{2+}]_b = [Ca^{2+}]_t - \frac{[Cl^-]_s^2[Ca^{2+}]_s}{(2[Ca^{2+}]_t - [COO^-]_t)^2} \quad (4)$$

Owing to the existence of the dissociation equilibrium of the carboxylic acid groups in the gel,

$$\text{—COOH} \rightleftharpoons \text{—COO}^- + H^+ \quad (5)$$

$$K_a = \frac{[H^+][COO^-]_f}{[COOH]}, \quad (6)$$

and $[Mon] = [COO^-]_t + [COOH]$, $[COO^-]_t$ can be presented as equation (6),

$$[COO^-]_t = \frac{K_a[Mon] + 2[H^+][Ca^{2+}]_t}{K_a + [H^+]}, \quad (7)$$

Since $[H^+] \ll K_a$ at pH 7 (pK_a is about 5 for the carboxylic group on the alginic acid), the $[COO^-]_t$ in equation (7) is approximately equal to [Mon], which is the concentration of the monomer in the gel and can be calculated from the gel volume and the dry weight of sodium alginate in the gel. Assuming complete dissociation of $CaCl_2$ in the external solution, equation (4) can be written as:

$$[Ca^{2+}]_b = [Ca^{2+}]_t - \frac{4[CaCl_2]_s^3}{(2[Ca^{2+}]_t - [Mon])^2} \quad (8)$$

where $[CaCl_2]_s$ is the concentration of the external solution, $[Ca^{2+}]_t$ can be obtained from EDTA titration.

The fraction of the stoichiometric amount of Ca^{2+} bound to the gel, $F(Ca^{2+})_b$, is calculated by dividing the concentration of bound calcium ions, $[Ca^{2+}]_b$, by one-half the monomer concentration, 0.5*[Mon], which is the total binding sites in the gel, at the dialysis equilibrium,

$$F(Ca^{2+})_b = \frac{[Ca^{2+}]_b}{0.5 * [Mon]} \quad (9)$$

The maximum fraction of bound Ca^{2+} is about 0.95 for all the four calcium alginate gels. This lack of dependence of capacity to bind Ca^{2+} on molecular weight or M/G ratio is similar to the conclusions obtained by Yokoyama *et al.*[31] using an inductively coupled plasma atomic emission spectrometer, which means that M and G units have the same Ca^{2+} binding capacity at the dialysis equilibrium, and the molecular weight range from 137 to 233 kDa has essentially no effect on the Ca^{2+} binding capacity at this high $F(Ca^{2+})_b$.

The Ca^{2+} binding constant for the alginates, K, defined by

$$K = \frac{[COO^-]_b}{[COO^-]_f [Ca^{2+}]_f}, \quad (10)$$

which can be rewritten as

$$K = \frac{F(Ca^{2+})_b}{[Ca^{2+}]_f (1 - F(Ca^{2+})_b)}, \quad (11)$$

is determined by dialyzing sodium alginate solutions against $CaCl_2$ solutions with selected concentrations. *Figure 2* indicates that, in the $[Ca^{2+}]_s$ concentration range of 0.5 to 100 mM, the fraction of sites with bound Ca^{2+} is almost constant and independent of the M/G ratio. The value of $F(Ca^{2+})_b$, 0.81 at $[Ca^{2+}]_f = 0.5$ mM, suggests that the alginates have a very strong Ca^{2+} binding constant. This constant is calculated according to the binding reaction to be about 8.0×10^3 M^{-1} by equation (11), which is comparable to the result obtained by Mayer *et al.*[21], 5000 M^{-1} for polyguluronate and 1000 M^{-1} for polymannuronate using 2D NOESY n.m.r. spectra at 40°C and low bound fraction. The rate of 8.0×10^3 M^{-1} was determined at 22°C and high bound fraction. At the high fraction of bound Ca^{2+} no difference in binding constants for GG and MM diads was indicated.

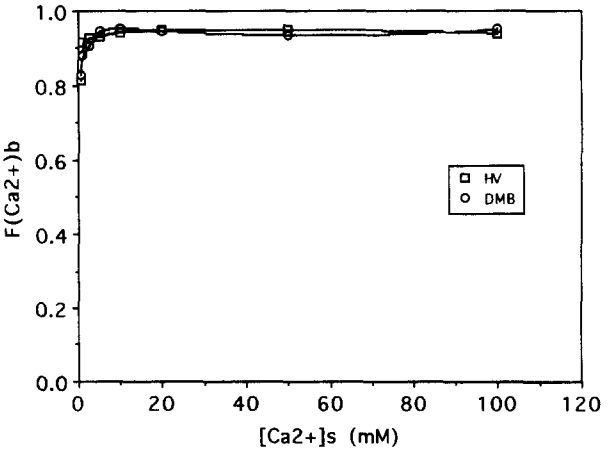

Figure 2 Equilibrium fraction of bound Ca^{2+} in the calcium alginate gel as a function of concentration of Ca^{2+} in the external dialysis solution

Table 2 Equilibrium bound fraction, $F(Ca^{2+})_b$, at pH values of 3.0 and 7.0 with and without citric acid for alginate KELTONE HV

$[Citric\ acid]/[Ca^{2+}]_s$	0	1.4
pH 3	0.78	0.77
pH 7	0.92	0.09

Table 2 reports the equilibrium dialysis data obtained from dialyzing sodium alginate solutions against $CaCl_2$ or $CaCl_2$/citric acid mixtures, $[Ca^{2+}]_s = 10$ mM and [citric acid]$_s = 14$ mM, at pH 3.0 and 7.0. The bound Ca^{2+} values at pH 3.0, $[Ca^{2+}]_b$, were calculated from equation (12), which was derived from the Donnan equilibrium and electroneutrality for the system considering the presence of H^+.

$$[Ca^{2+}]_b = [Ca^{2+}]_t - \frac{4[CaCl_2]_s^3}{(2[Ca^{2+}]_t + [H^+] - [COOO^-]_t)^2}, \quad (12)$$

where $[COO^-]_t$ is calculated by equation (7). The table indicates that the fraction of bound Ca^{2+} in the alginate is not affected significantly by the pH within the investigated range of 3 to 7, whereas the Ca^{2+} binding ability of citric acid, which contains carboxylic acid and alcohol groups, is very dependent on the pH. Citric acid has a stronger Ca^{2+} binding ability than alginate at pH 7.0, but does not compete with the binding ability of the alginate at pH 3.0. These results demonstrate the cooperative interaction of the alginate in binding the Ca^{2+} counterions.

To further investigate the existence of cooperative interaction of alginate with Ca^{2+} counterions and the gel formation properties in the presence of the inert electrolyte, KCl, sodium alginate solutions were dialyzed against mixed solutions of potassium and calcium chloride with fixed 10 mM calcium concentration and both the $[Ca^{2+}]_t$ and the gel volume (V_g) at the equilibrium were determined. $[Ca^{2+}]_b$ was calculated by equation (13), which was derived similarly as equation (4) with the consideration of the inert salt ions, Cl^- and K^+.

$$[Cl^-]_s \left(\frac{[CA^{2+}]_s}{[Ca^{2+}]_t - [Ca^{2+}]_b} \right)^{1/2} + [COO^-]_t = 2[Ca^{2+} + [K^+]_s \left(\frac{[Ca^{2+}]_t - [Ca^{2+}]_b}{[Ca^{2+}]_s} \right)^{1/2} \quad (13)$$

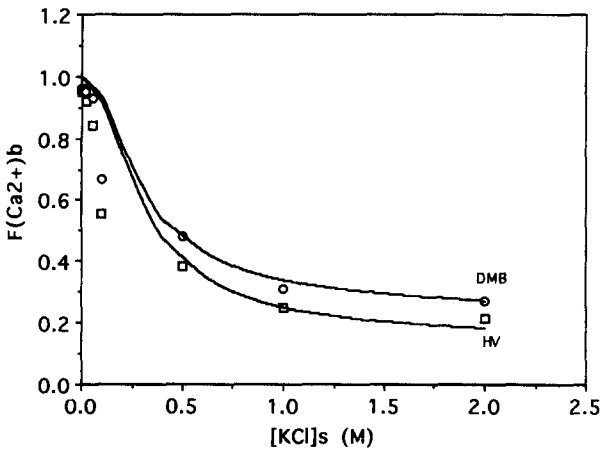

Figure 3 Alginate was dialyzed in 10 mM $CaCl_2$ solution with the addition of KCl. Equilibrium fraction of bound Ca^{2+} in the calcium alginate gel was plotted as function of the concentration of KCl. The line curves were the theoretical prediction from equation (28)

$F(Ca^{2+})_b$ were calculated and are shown in *Figure 3* as a function of $[KCl]_s$. *Figure 3* shows that the amount of bound Ca^{2+} decreased monotonically as the concentration of K^+ increased until the $[K^+]/[Ca^{2+}]$ ratio reached 100:1, and remained constant up to 200:1. For the both alginates, HV and DMB, the fractions of bound Ca^{2+} resistant to the displacement by K^+ in a solution with $[Ca^{2+}]_s = 10$ mM shown in *Figure 3* are close to the F_{GG} for both the HV and DMB alginates, i.e. 0.18 and 0.28, respectively. This result indicates a preferential chelation of calcium ions between GG diads to form dimeric junction zones, which is consistent with the results of Morris *et al.*[13] using sodium as competitive-ions of calcium.

To consider the differences in the binding of Ca^{2+} to the different diads, assume there are three types of Ca^{2+} binding reactions,

$$GGK_2 + Ca^{2+} = GGCa + 2K^+, \quad (14)$$

$$GMK_2 + Ca^{2+} = GMCa + 2K^+, \quad (15)$$

$$MMK_2 + Ca^{2+} = MMCa + 2K^+, \quad (16)$$

where K_{GG}, K_{GM}, and K_{MM} are the three different exchange constants, which can be expressed as:

$$K_{GG} = \frac{[GGCa]\cdot[K^+]_s^2}{[GGK_2]\cdot[Ca^{2+}]_s} \quad (17)$$

$$K_{GM} = \frac{[GMCa]\cdot[K^+]_s^2}{[GMK_2]\cdot[Ca^{2+}]_s} \quad (18)$$

$$K_{MM} = \frac{[MMCa]\cdot[K^+]_s^2}{[MMK_2]\cdot[Ca^{2+}]_s} \quad (19)$$

By defining [UU] as the total concentration of the uronic diads and [UUCa] as the concentration of calcium bound uronic diads, the following equations can be established:

$$[UUCa] = [GGCa] + [GMCa] + [MMCa] \quad (20)$$

$$[GGK_2] + [GGCa] = f_{GG}[UU] \quad (21)$$

$$[GMK_2] + [GMCa] = f_{GM}[UU] \quad (22)$$

$$[MMK_2] + [MMCa] = f_{MM}[UU] \quad (23)$$

where f_{GG}, f_{GM}, and f_{MM} are the fractions of the uronic diads. Combining equations (21)–(23) with equations (17)–(19) respectively, equations (24)–(26) are obtained,

$$[GGCa] = \frac{f_{GG}[UU]}{1 + \dfrac{[K^+]_s^2}{K_{GG}[Ca^{2+}]_s}} \quad (24)$$

$$[GMCa] = \frac{f_{GM}[UU]}{1 + \dfrac{[K^+]_s^2}{K_{GM}[Ca^{2+}]_s}} \quad (25)$$

$$[MMCa] = \frac{f_{MM}[UU]}{1 + \dfrac{[K^+]_s^2}{K_{MM}[Ca^{2+}]_s}} \quad (26)$$

The fraction of Ca^{2+} bound to the gel can also be defined as

$$F(Ca^{2+})_b = [UUCa]/[UU] \quad (27)$$

Combining equations (15)–(17) with equation (10) and assuming $K_{GM} \cong K_{MM}$, the following expression is obtained

$$F(Ca^{2+}) = \left[\frac{1}{\left(1 + \dfrac{[K^+]_s^2}{K_{MM}\cdot[Ca^{2+}]_s}\right)}\right] + \left[\frac{1}{\left(1 + \dfrac{[K^+]_s^2}{K_{GG}\cdot[Ca^{2+}]_s}\right)} - \frac{1}{\left(1 + \dfrac{[K^+]_s^2}{K_{MM}\cdot[Ca^{2+}]_s}\right)}\right]\cdot f_{GG} \quad (28)$$

The data from the dialysis experiments are inputted into equation (28) at $[Ca^{2+}]_s = 10$ mM and with f_{GG} values of 0.18 and 0.28 respectively for HV and DMB. The data are fit best by $K_{GG} = 2000$ M and $K_{MM} = 20$ M, so at low equilibrium fraction of bound Ca^{2+}, $F(Ca^{2+})_b \sim 0.2$, a difference in exchange constant for GG and MM diads is determined. The theoretical dependence of $F(Ca^{2+})_b$ on $[K^+]_s$ according to equation (28), the curves, is consistent with the experimental results, the points, shown in *Figure 3*, which supports the ion exchange reaction mechanism proposed above.

The stability of the calcium alginate gels was further examined by dialyzing the calcium induced gels against potassium chloride solutions in the absence of Ca^{2+}. *Figure 4* shows the rate of decrease of bound Ca^{2+} against a 20 mM KCl solution. The ion exchange reaction reaches equilibrium after 48 hours. *Figure 5* shows that with increasing $[K^+]_s$, nearly all the bound Ca^{2+} in the gel can be displaced by K^+, i.e., even the Ca^{2+} associated with the GG diads is replaced by potassium ions in 0.2 M KCl. The gel was converted to sol in the process of K^+ displacement at $[K^+]_s = 0.2$ M. However, the gels did not display thermal reversibility when examined by d.s.c. up to 250°K, which suggested that calcium polyelectrolyte gels should still be classified as a chemical gel[32].

Equilibrium gel swelling

In general, the swelling equilibrium of polyelectrolyte gels is determined by the balance of three major forces:

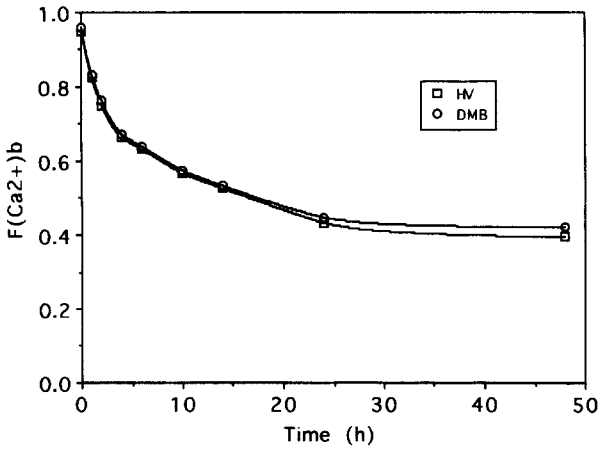

Figure 4 Calcium alginate gel was formed in 10 mM $CaCl_2$ solution, then dialyzed in 20 mM KCl solution without Ca^{2+}. Bound fraction of Ca^{2+} is plotted as a function of dialysis time

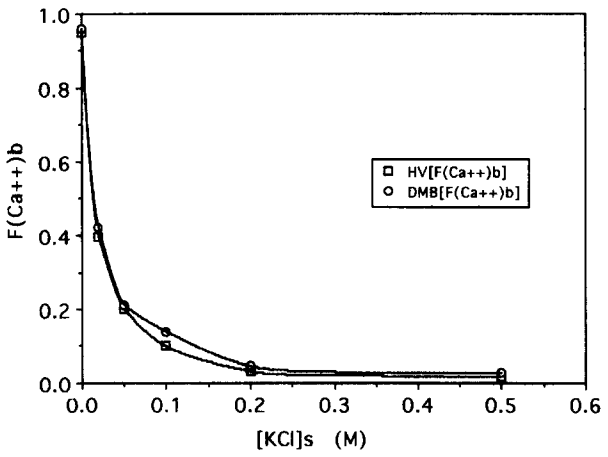

Figure 5 Calcium alginate gel was formed in 10 mM $CaCl_2$ solution, then dialyzed in KCl solution of varying concentration without Ca^{2+}. Equilibrium fraction of bound Ca^{2+} is plotted as a function of KCl concentration

(1) the free energy of mixing of the polymer chains with a swelling medium, (2) the elastic-retractive force exerted on the network, and (3) the ionic osmotic pressure generated by the mobile counterions to the charged groups in the network, which can be expressed as[33–35]:

$$\Delta G = \Delta G_{mix} + \Delta G_{el} + \Delta G_{ion} \quad (29)$$

The increase in solution strength will decrease the ionic osmotic pressure difference between the gel and solution, which results in a reduction in swelling[36,37]. However, the equilibrium swelling ratios, Q_{eq}, shown in *Figure 6* indicate that the volume of the gel increased with increasing ionic strength in the external solution. It should be noted that the crosslink density of the calcium alginate gel, unlike that of chemically crosslinked hydrogels, was reduced as the ionic strength increased as discussed above. Therefore, the effects of the ionic strength are more complicated in the calcium alginate gel than in a gel containing a fixed number of crosslinks. On one hand, the increase of ionic strength in the solution reduced the difference of the total concentration of mobile ions inside gel and outside solution, which lowered the osmotic pressure. However, the increase of ionic strength in the solution increased the number of the charged groups in the gel, which contributed the osmotic

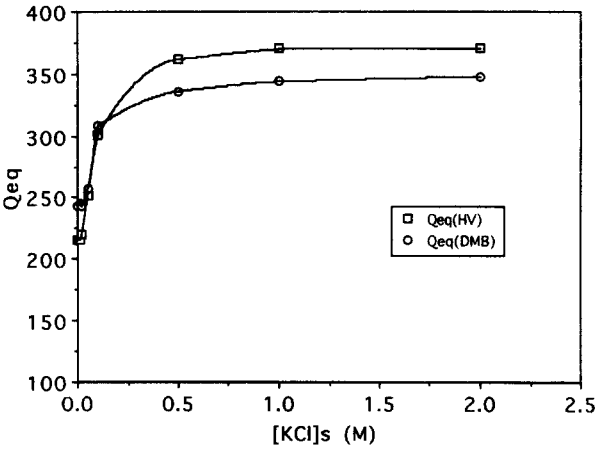

Figure 6 Equilibrium volume swelling ratios, Q_{eq}, as function of added KCl concentration in dialysis solution

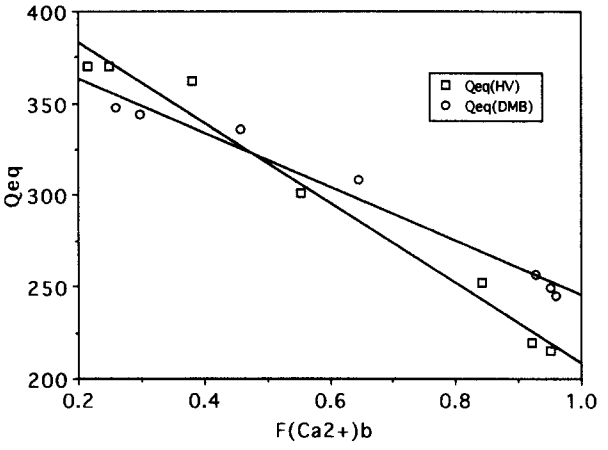

Figure 7 Equilibrium volume swelling ratios, Q_{eq}, as function of equilibrium fraction of bound Ca^{2+}

pressure in the gel. Furthermore, the increase of the charged groups in the gel increased the overall hydrophilicity of the network that enhanced the amount of water imbibed (ΔG_{mix}) and the decrease of crosslink density increased the chain length between the crosslinks that resulted in a increase of network swelling pressure (ΔG_{el})[35,38]. Overall, an inverse dependence of the equilibrium swelling ratio on the fraction of bound Ca^{2+} was observed for both alginates as shown in *Figure 7*. However, the alginate with lower M/G ratio exhibits a smaller gel volume at $F(Ca^{2+})_b \leq 0.5$ and a larger gel volume at $F(Ca^{2+})_b \geq 0.5$ than the alginate with higher M/G ratio. The result implies that the alginate with lower M/G ratio interacts with the initial amount of Ca^{2+} more strongly than does the one with the higher M/G ratio; behaviour consistent with $K_{GG} \gg K_{MM}$.

CONCLUSIONS

The M/G ratios and the molecular weights of the alginates do not affect the calcium binding capacity. However, the G residues retain more bound Ca^{2+} than the M residues under conditions of exchange with K^+. The $Ca^{2+} - K^+$ exchange constant K_{GG} is approximately 100 times greater than the constant K_{MM} at low fraction of bound Ca^{2+}. The presence of added simple electrolyte reduces the level of bound Ca^{2+} in the alginates due to the K^+ competition with Ca^{2+} on the M residues. The gel volume change is another

indicator of the crosslink density in the gel at low bound calcium fractions. Moreover, the stability of the calcium alginate gel is significantly weakened in simple salt solution containing no Ca^{2+} and the gel can be converted to a sol at salt concentrations greater than approximately 0.2 M.

REFERENCES

1. Aspinall, G. O., *The Polysaccharides*, Vol. 2. Academic Press, Orlando, FL, 1983.
2. Clark, A. H. and Roos-Murphy, S. B., *Advances in Polymer Science, 83. Biopolymers*. Springer, Berlin, 1987.
3. Smidsrod, O. and Haug, A., *Acta Chem. Scand.*, 1965, **19**, 329.
4. Schlemmer, U., *Food Chem.*, 1989, **32**, 223.
5. King, A. H., Eur. Pat. 1981, Appl. 81302015.3.
6. McDowell, R. H., *Rev. Pure Appl. Chem.*, 1960, **10**, 1.
7. Moe, S., Gudmund, S., Smidsrod, O. and Ichijo, H., *J. Appl. Polym. Sci.*, 1994, **51**, 1771.
8. Mochizuki, A., Amiya, S., Sato, Y., Ogawara, H. and Yamashita, S., *J. Appl. Polym. Sci.*, 1990, **40**, 385.
9. Zhang, L., Selective separation of proteins by microfiltration with formed-in-place membranes. Dissertation, Clemson University, 1992.
10. Wang, X. and Spencer, G. H. J., *Appl. Polym. Sci.*, 1996, **61**, 827.
11. Huag, A., *Acta Chem. Scand.*, 1959, **13**, 1250.
12. Smidsrod, O. and Haug, A., *Acta Chem. Scand.*, 1972, **26**, 2063.
13. Grant, G.T., Morris, E.R., Rees, D.A., Smith, P.J.C. and Thom, D., *FEBS Lett.*, 1973, **32**, 195.
14. Morris, E.R., Ress, D.A., Thom, D. and Body, J., *Carbohydr. Res.*, 1978, **66**, 145.
15. Gamini, A., Civitarese, G., Cesaro, A., Delben, F. and Paoletti, S., *Makromol. Chem., Macromol. Symp.*, 1990, **39**, 143.
16. Kohn, R. and Larsen, B., *Acta Chem. Scand.*, 1972, **22**, 2455.
17. Mackie, W., Noy, R. and Sellen, D.B., *Biopolymers*, 1980, **19**, 1839.
18. Matsumoto, T. and Mashiko, K., *Biopolymers*, 1990, **29**, 1707.
19. Wang, Z. Y., Zhang, Q. Z., Konmo, M. and Saito, S., *Biopolymers*, 1994, **34**, 737.
20. Wang, Z.Y., Zhang, Q.Z., Konno, M. and Saito, S., *Biopolymers*, 1993, **33**, 703.
21. Steginsky, C. A., Beale, J. M., Floss, H. G. and Mayer, R. M., *Carbohydr. Res.*, 1992, **225**, 11.
22. Barfod, N. M., Gums Stabiliser, Food Industry, 1988, 4th edn., 285 pp.
23. Bergstrom, E., Goodall, D. M. and Norton, I. T., Gums Stabiliser, Food Industry, 1990, 5th edn., 501 pp.
24. Cesaro, A., Delben, F. and Paoletti, S., Thermal Analysis: Proceedings of the 7th International Conference on Thermal Analysis, ed. M. Bernard, 1982, 815.
25. Nilsson, S., *Biopolymers,*, 1992, **32**, 1311.
26. Grasdalen, H., Larsen, B. and Smidsrod, O., *Carbohydr. Res.*, 1981, **89**, 179.
27. Grasdalen, H., Larsen, B. and Smidsrod, O., *Carbohydr. Res.*, 1977, **56**, C11–C15.
28. Martinsen, A., Skjak-Brek, G., Smidsrod, O., Zanetti, F. and Paoletti, S., *Carbohydr. Polym.*, 1991, **15**, 171.
29. Whitfield, M., Leyendekkers, J.V. and Kerr, J.D., *Anal. Chim. Acta*, 1969, **45**, 399.
30. Tackett, S.L., *Anal. Chem.*, 1969, **41**, 1703.
31. Yokoyama, F., Achife, C. E., Takahira, K., Yamashita, Y. and Monobe, K., *J. Macromol. Sci.-Phys.*, 1992, **B31**, 463.
32. Guenet, J. M., *Thermoreversible Gelation of Polymers and Biopolymers*. Academic Press, New York, 1992.
33. Flory, P. J., *Principles of Polymer Chemistry*. Cornell, Ithaca, NY, 1953.
34. Tanaka, T., Fillmore, D., Sun, S., Nishio, I., Swislow, G. and Shah, A., *Phys. Lett.*, 1980, **45**, 1636.
35. Brannon-Peppas, L. and Peppas, N. A., in *The Equilibrium Swelling Behavior of Porous and Non-Porous Hygrogels, Absorbent Polymer Technology*, ed. L. Brannon-Peppas and R. S. Harland. Elsevier, 1990, p. 67.
36. Shibayama, M., Ikkai, F., Inamoto, S., Nomura, S. and Han, C. C., *J. Chem. Phys.*, 1996, **105**, 4358.
37. Sasaki, S., Ojima, H., Yataki, K. and Maeda, H., *J. Chem. Phys.*, 1995, **102**, 9694.
38. Brannon-Peppas, L., in *Preparation and Characterization of Crosslinked Hydrophilic Networks, Absorbent Polymer Technology*, ed. L. Brannon-Peppas and R. S. Harland. Elsevier, 1990, p. 45.