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Inhomogeneous Polysaccharide Ionic Gels

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ABSTRACT

Polysaccharide ionotropic gels formed by diffusion of calcium ions into solutions of sodium alginate or pectate exhibited various degrees of inhomogeneity, in the sense that the polymer concentration was much higher at the surface than in the center of the gels. This was in contrast to the uniform gels obtained when κ-carrageenan was dialyzed against potassium ions and gellan gum against potassium and lead ions. The non-uniform distribution of polymer in alginate and pectate depended on parameters such as polymer concentration and molecular size, and the concentration of the gel inducing ion in the outer reservoir. For alginate a high fraction of L*-guluronate residues slightly increased the inhomogeneity, whereas the presence of non-gelling cations strongly decreased it. A theory, limited to diffusion of reactants in infinite long tubes, compared with relevant experiments, suggested that the gelation was controlled by the relative rate of diffusion of polymer and gelling cations and that the gelling in the absence of non-gelling cations was irreversible and stoichiometric. A new simple technique for generating homogeneous calcium alginate gels by diffusion was also demonstrated.*

INTRODUCTION

Many charged polysaccharides form hydrogels in the presence of cations. Alginate and pectate require multivalent ions such as calcium and strontium ions (Smidsrød, *et al.*, 1972; Smidsrød 1974), while polymers such as κ-carrageenan (Rees *et al.*, 1982) and gellan-gum (Grasdalen & Smidsrød, 1987) also form gels with monovalent cations above certain concentrations. Such gels have a wide industrial use as thickeners and gelformers and have recently found biotechnological applications as immobilization materials for various types of living cells (Mattiason,

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1983; Martinsen *et al.*, in press). We have previously shown (Skjåk-Bræk *et al.*, 1986) that when a solution of sodium alginate is dialyzed exhaustively against a solution containing calcium ions, the formed gel will be non-uniform with respect to polymer concentrations by having a high alginate concentration at the interface with the calcium solution and a gradual decrease in concentration towards the center of the gel. We have also demonstrated that this phenomenon can be avoided, and homogeneous Ca-alginate gels can be formed by internal release of calcium ions from Ca:EDTA in the presence of a slow acidifier like glucono-δ-lactone (Toft, 1982; Skjåk-Bræk *et al.*, 1986). The alginate molecules are then 'locked' in a mixed H^+ Ca^{2+} gel which can be converted into the complete calcium form by dialysis against a calcium chloride solution. In the present paper we report on the heterogeneity in gels of alginate, pectate, carrageenan and gellan gum formed by diffusion of gel-inducing ions into solutions of these polymers.

MATERIALS AND METHODS

Polysaccharide samples

Commercial samples of sodium alginate isolated from *Laminaria digitata* with $F_G = 0{\cdot}41$, $\bar{N}_{G>1} = 6$ and $[\eta] = 7$ (100 ml/g) and *L. hyperborea* stipe LF 10/60, with $F_G = 0{\cdot}71$, $\bar{N}_{G>1} = 16$, $[\eta] = 5{\cdot}9$ were provided by Protan A/S Drammen. Three samples of an alginate enriched in guluronic acid with $F_G = 0{\cdot}75$, $\bar{N}_{G>1} = 18$ and $[\eta] = 3{\cdot}5$, 7 and 14, respectively, were isolated from the outer cortex of *L. hyperborea* stipes as described by Haug (1964). Another alginate sample was prepared from *Macrocystis pyrifera* with $F_G = 0{\cdot}43$, $\bar{N}_{G>1} = 6{\cdot}5$ and $[\eta] = 5{\cdot}4$ and finally one alginate sample enriched in mannuronic acid was prepared from *Ascophyllum nodosum* receptacles with $F_G = 0{\cdot}10$. A ^{3}H-labeled alginate with $F_G = 0{\cdot}45$ and $[\eta] = 2{\cdot}5$ and a specific activity of 15000 cpm/mg was prepared from *Azotobacter vinelandii* by growing the bacteria on ^{3}H-glucose as described previously (Skjåk-Bræk & Larsen, 1982).

Sodium pectate was obtained from Fluca, κ-carrageenan from Sigma Chemicals and gellan gum termed Gellrite from Kelco Division of Merck. The two latter polymers were transferred into the cold-water soluble TMA form as described previously (Grasdalen & Smidsrød, 1987). Intrinsic viscosities $[\eta]$ were determined in 0·1 M NaCl in a Cannon Ubbelohde Capillary viscometer as described by Haug & Smidsrød (1966).

NMR spectroscopy

All the alginate samples were analyzed by ^{1}H NMR spectroscopy using a Jeol FX-100 or Bruker WM-400 spectrometer. The monad frequencies F_G and F_M, the diad frequencies F_{GG}, F_{MG}, F_{GM} and F_{MM} and 'G'-centered triad frequencies F_{GGG}, F_{MGG}, F_{GGM} and F_{MGM} were determined as described previously (Grasdalen *et al.*, 1979; Grasdalen, 1983). Knowledge about these frequencies allowed us to calculate the average length of G-blocks, consisting of two or more contiguous units $\bar{N}_{G>1} = (F_G - F_{MGM})/F_{MGG}$, a parameter giving good correlation with the modulus for compression of Ca-alginate gels (Skjåk-Bræk *et al.*, 1986).

Gel-formation

Aqueous solutions of sodium alginate, sodium pectate and the TMA form of κ-carrageenan and gellan gum, were placed in plastic cylinders (diameter = 14 mm, length = 19 mm) and capped with a dialysis membrane at both ends. The cylinders were then transferred to an aqueous solution containing the gel-inducing salt or salt mixtures and dialyzed for 72 h, with a change of salt solution every 12 h. When non-gelling salts were used, these were added in the same concentration to the polysaccharide solution and the salt solution before the start of the dialysis. The gels were then taken out of the cylinders and sliced into 1 mm discs perpendicular to the cylinder axes in a self-build gel slicer as described in Fig. 1. The weight of each slice was determined immediately, and the content of alginate was determined after extensive dialysis against distilled water to remove excess salt, and then weighing after drying at 45°C for 24 h. The water content of the dried slices was determined by the Karl Fischer method, and was, within the experimental error, the same as in the starting material. In the experiments with potassium κ-carrageenan and potassium gellan gum, the dialysis step was omitted to avoid dissolution of the gel slices. The migration of the gelling zone in calcium alginate gel could be visualized by mixing the sodium alginate with Tetramethyl Murexid (Merck) which changes color from red to yellow in the presence of free calcium ions in the dialysate.

Quantitative determination of excess calcium ions in the alginate gel was achieved by dialyzing the gel slices against distilled water and analyzing the calcium concentration in known volumes of dialysates by atomic absorption in a Perkin–Elmer 560 Atomic Absorption Spectrometer.

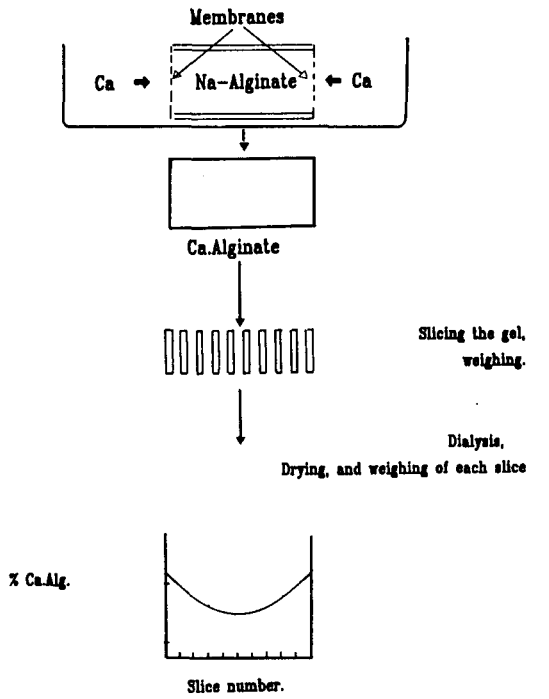

Fig. 1. Procedure for preparation of inhomogeneous alginate gels and measurement of the polymer distribution in cylindrical gel plugs.

RESULTS

Gelling with only gel-inducing ions

When sodium alginate and pectate solution (2% w/w) were dialyzed against a solution containing calcium ions, inhomogeneous gels were formed. This is clearly demonstrated in Figs 2 and 3 where the calcium alginate and calcium pectate concentration profiles in the gels are given for a range of calcium chloride concentrations. The inhomogeneity is greatest when the concentration of calcium chloride is kept lowest relative to the polymer concentration. For the lowest concentration of calcium chloride in Fig. 2 (5 mM), the concentration of calcium alginate in the center of the gel was about 0·2% compared to about 6% near the end of the gel plugs. By increasing the concentration of calcium chloride, the polymer concentration gradient in the gel decreased, but even at a concentration of 1·0 M a significant inhomogeneity was observed. The same tendency is seen in calcium pectate gels in Fig. 3, based on two calcium concentrations only.

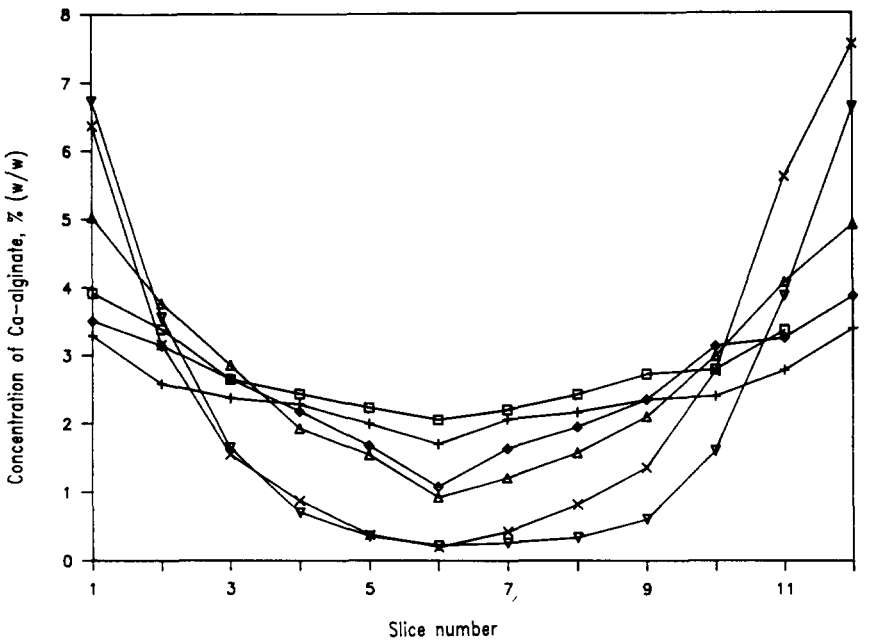

Fig. 2. Concentration profiles of calcium alginate in cylindrical gel plugs formed by dialyzing aqueous solutions of *L. hyperborea* alginates (2% w/v) against various concentrations of calcium chloride. (□) 1 M, (+) 0·34 M, (◇) 0·1 M, (△) 0·05 M, (×) 0·01 M, (▽) 0·005 M.

Gels formed by diffusing potassium chloride into solutions of TMA-κ-carrageenan and potassium chloride and lead nitrate into solutions of TMA-gellan gum by the same procedure (Fig. 4) did not give much sign of concentration gradients.

The parameters (see theoretical section) giving non-uniform alginate gels were investigated further. An increase of the concentration of polymer with respect to that of calcium ions led to increased inhomogeneity, both for pectate (Fig. 5) and for alginate (Fig. 6). When gels were formed from alginates of the same type and concentration, differing only in their molecular size, a more uniform distribution of polymer was found in gels made from the high molecular weight material (Fig. 7).

Gelling in the presence of other ions

When calcium alginate gels were formed in the presence of sodium ions on both sides of the membranes, more homogeneous gels were formed (Fig. 8) and at 0·2 M and 0·6 M sodium chloride the gels were quite uniform. When sodium ions were replaced with magnesium ions (Fig. 9),

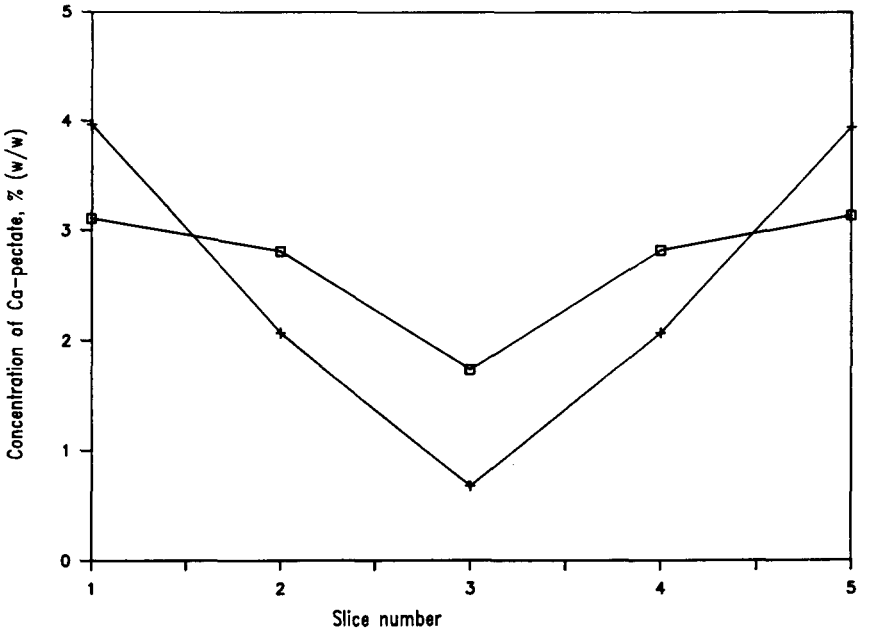

Fig. 3. Concentration profiles of calcium pectate in cylindrical gel plugs formed by dialyzing a 2% (w/v) aqueous solution of sodium pectate against two concentrations of calcium chloride. (□) 0·34 M, (+) 0·02 M.

the same effect was observed, but a lower concentration of magnesium ions than sodium ions was sufficient to form a homogeneous gel, suggesting some correlation to the competitive binding of calcium to alginate in the presence of magnesium ions and sodium ions, respectively.

Since the gel-forming properties of alginates are correlated with both the content and sequential arrangement of L-guluronic acid in the polymer chain, gels (2% w/w) were made from alginates with approximately the same intrinsic viscosities, differing only in their chemical composition. In Fig. 10 similar inhomogeneity in all the alginate gels is demonstrated, but the two gels formed from alginates rich in L-guluronic acid residues seemed to have a somewhat lower concentration in the center of the gels.

This difference is however enhanced when the gels are formed in presence of low concentration of sodium ions. This is demonstrated by comparing Fig. 8 with Fig. 11. The latter figure demonstrates the effect of sodium on the concentration profile in alginate gels formed from *L. digitata* $[\eta] = 7{\cdot}0$ in the presence of 0·05 M and 0·01 M NaCl.

Inhomogeneity apparently is favored by factors such as low concentrations of calcium ions, low molecular weight, high concentrations of alginate and high L-guluronic acid content of the alginate.

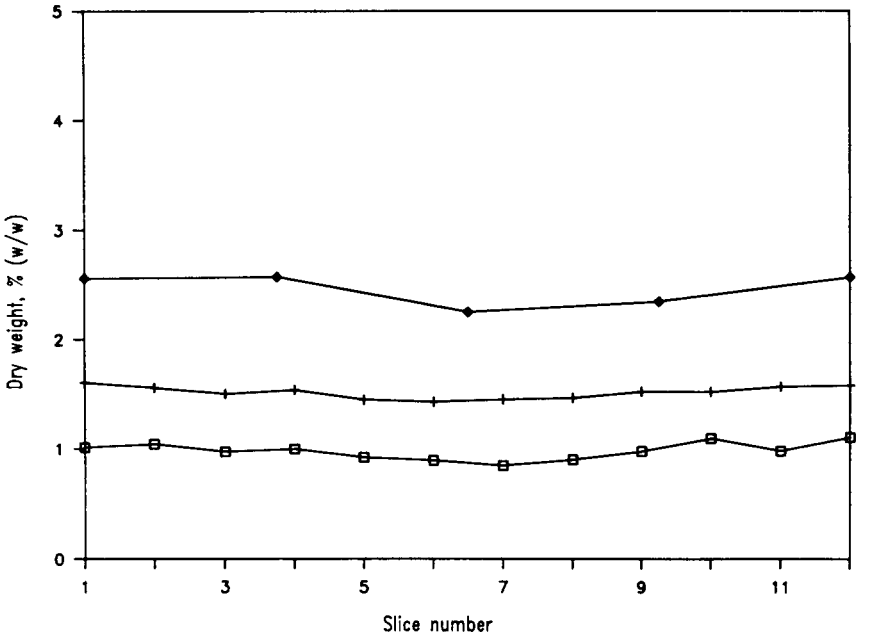

Fig. 4. Concentration profiles in cylindrical gel plugs of potassium-carrageenan and potassium and lead gellan gum formed by dialyzing aqueous solutions of TMA-κ-carrageenan (2% w/v) against 0·1 M KCl and TMA-gellan gum (1% w/v) against 0·1 M KCl and 0·05 M $Pb(NO_3)_2$ respectively. The potassium gels have not been dialyzed and consequently contain excess salt. (□) Pb-gellan gum, (+) K-gellan gum, (◇) K-κ-carrageenan.

This is demonstrated for the low molecular weight alginate from *L. hyperborea* outer cortex in a high concentration (4% w/w) dialyzed towards a solution containing 10 mM calcium. The gross inhomogeneity with respect to alginate concentration in the gel is demonstrated in Fig. 12. The concentration of polymer varies from 10% at the calcium chloride/alginate interface, to 0·2–0·5% in the center of the gel plug. Due to this low concentration the gel in the center of the plugs was very sloppy, and some loss of water during the slicing procedure gave a measured value for the concentration which was somewhat too high.

Diffusion of alginate molecules in the gel phase

To investigate if the alginate molecules can migrate from the solution into the alginate gel phase, and consequently contribute to the observed inhomogeneity, sodium alginate solution was placed in small cylinders and transferred to an aqueous calcium chloride solution. After three hours of gelling 100 μl of a solution containing ^{3}H-alginate was injected

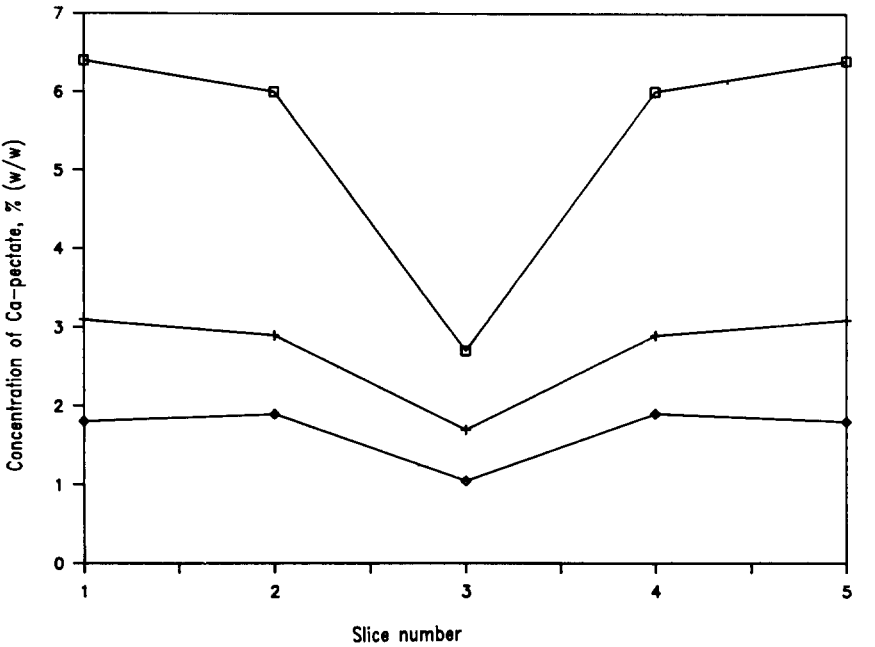

Fig. 5. Concentration profiles of calcium pectate in cylindrical gel plugs formed by dialyzing aqueous solution of sodium pectate of various concentrations against 0·34 M $CaCl_2$. (□) 5·5% (w/v), (+) 2·5% (w/v), (◇) 1·5% (w/v).

with a syringe into the alginate solution in the center of the cylinder through a hole in the cylinder wall. After gelling had been completed, the gel cylinders were sectioned into slices, and the distribution of the radioactive label was measured by a liquid scintillation counter. From Fig. 13 it is evident that the diffusion of free alginate molecules in the gel phase is severely restricted. The very large gradients in radioactivity (larger than in polymer concentration) in the part of the gel which was formed after the label was added are probably due to the low molecular size of the labeled alginate.

Compositional homogeneity in calcium alginate gels

Since alginates are known to be compositionally polydisperse, and since the composition of alginate seemed to have some impact on the heterogeneity of the calcium alginate gels (Figs 10 and 11), it was of interest to determine whether alginate gels also were inhomogeneous with respect to chemical composition. When alginate gels prepared by dialysis against 0·1 M calcium chloride was sliced and each slice analyzed by NMR spec-

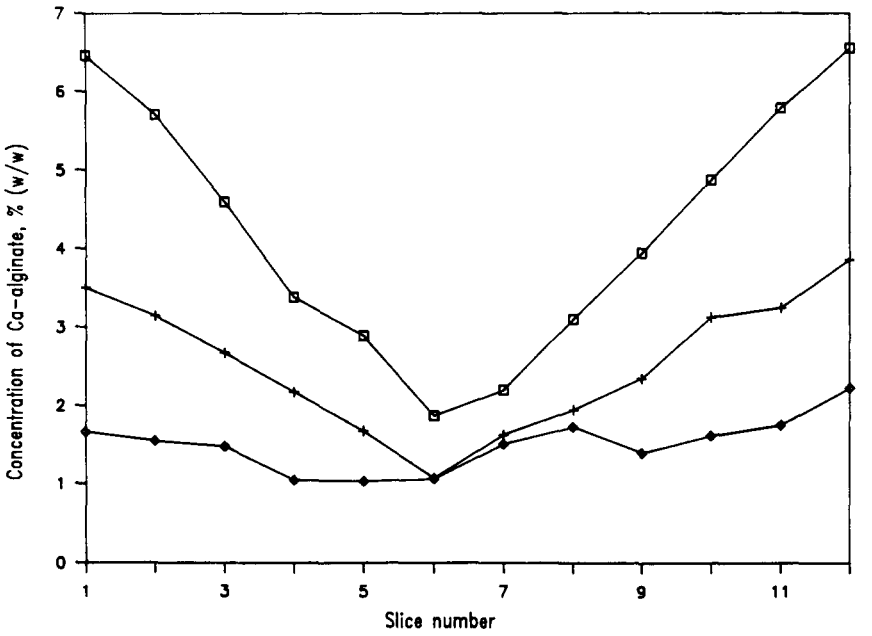

Fig. 6. Concentration profiles of calcium alginate formed when aqueous solutions of *L. hyperborea* sodium alginate in varying concentrations were dialyzed against 0·1 M $CaCl_2$. (□) 4% (w/v), (+) 2% (w/v), (◇) 1% (w/v).

troscopy no significant difference in composition could be detected in the different slices ($F_G = 0{\cdot}70 \mp 0{\cdot}02$). When gels were formed from mixtures of 50% alginate $F_G = 0{\cdot}70$ with 50% alginate with $F_G = 10\%$, only a slightly higher content of guluronic acid was detected in the outer slices ($F_G = 0{\cdot}50$) than in the center of the gel ($F_G = 0{\cdot}46$).

Gel formation by diffusion of calcium ions into sodium alginate without membranes

To investigate a possible effect on inhomogeneity of the presence of the dialysis membrane at the interface between the polymer and the salt solutions, a sodium alginate solution (2% w/w) was placed in a beaker, and a calcium chloride solution (0·5 M) was carefully layered on the top with a pasteur pipette. After a few minutes the calcium chloride solution was discarded and the beaker was filled up with an aqueous solution containing 10 mM calcium chloride. After the gelling was completed (72 h) an intact cylinder 20 mm long, was cut out of the macrogel and sliced as described previously and the distribution of Ca-alginate in the gels

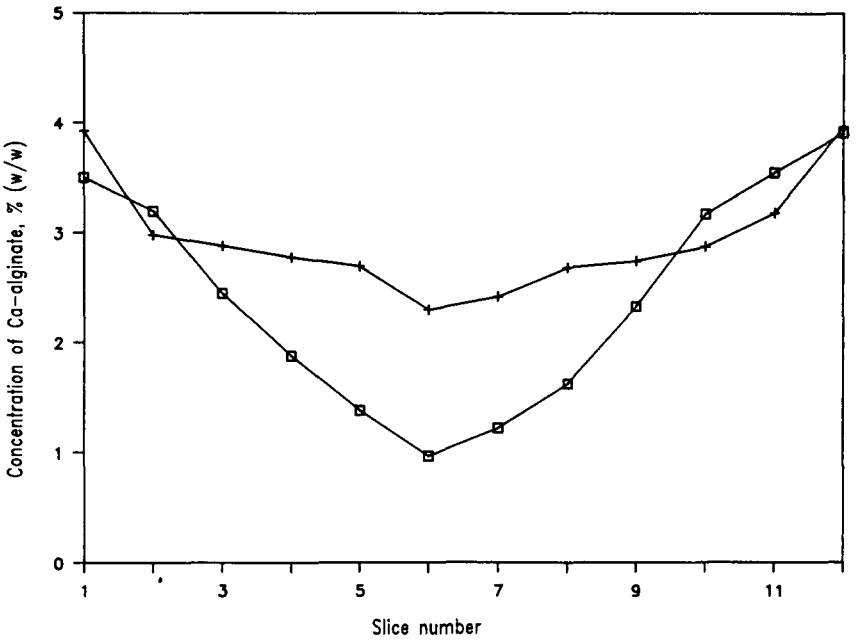

Fig. 7. Concentration profiles of calcium alginate in gels formed when 2% (w/v) aqueous solutions of *L. hyperborea* outer cortex sodium alginate with various molecular size were dialyzed against 0·1 M $CaCl_2$. (□) $[\eta] = 3$ (100 ml/g), (+) $[\eta] = 15$ (100 ml/g).

determined. The results given in Fig. 14 demonstrate the same inhomogeneity as found in the other gel plugs.

THEORY

Developing a quantitative theory from first principles for calculating the different concentration gradients of the gels above is, at the outset, very difficult. The gelling systems are clearly non-ideal with high concentration of polyelectrolytes and varying concentrations of small ions. An exact treatment of diffusion, ion binding and gel formation is hardly achievable, but some characteristic features of the system can be figured out. The binding of calcium ions to the sodium alginate chains may be considered to be stoichiometric and almost irreversible in the absence of other ions and the reaction is probably very rapid compared with the rate of diffusion of the reactants. Consequently, diffusion may be assumed to be the rate determining step in the gelation process.

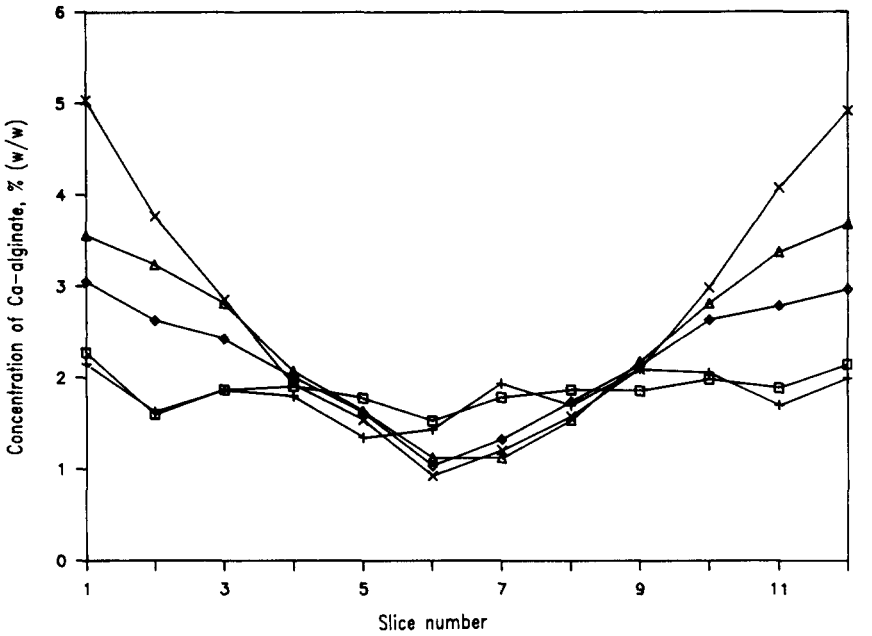

Fig. 8. Concentration profiles of calcium alginate formed when 2% (w/v) aqueous solutions of *L. hyperborea* sodium alginate were dialyzed against 0·05 M $CaCl_2$ in the presence of varying concentrations of sodium chloride. (□) 0·6 M, (+) 0·2 M, (◇) 0·05 M, (△) 0·02 M, (×) control.

A theory has been developed (Sherwood & Pigford, 1952) which describes the time course of an extremely fast second-order irreversible chemical reaction between two freely diffusing species in solution. The treated model assumes that the species diffuse towards each other from infinite reservoirs and the reaction takes place at a plane perpendicular to the direction of diffusion.

In an attempt to confront this theory with experimental facts from our system, we consider diffusion of calcium from an infinitely large Ca^{2+} reservoir of concentration $[Ca]_0$ into an infinite reservoir of alginate with a monomolar concentration of uronic acid residues of $C_{p,0}$, as depicted in Fig. 15.

Initially the concentration in the alginate reservoir is uniform. When it is exposed to the Ca^{2+} solution, Ca^{2+} will enter by diffusion, bind to unoccupied binding sites on the alginate, and form a gel state. When diffusing through an already formed gel where all the binding sites are occupied, there is no opportunity for Ca^{2+} to bind until it reaches avail-

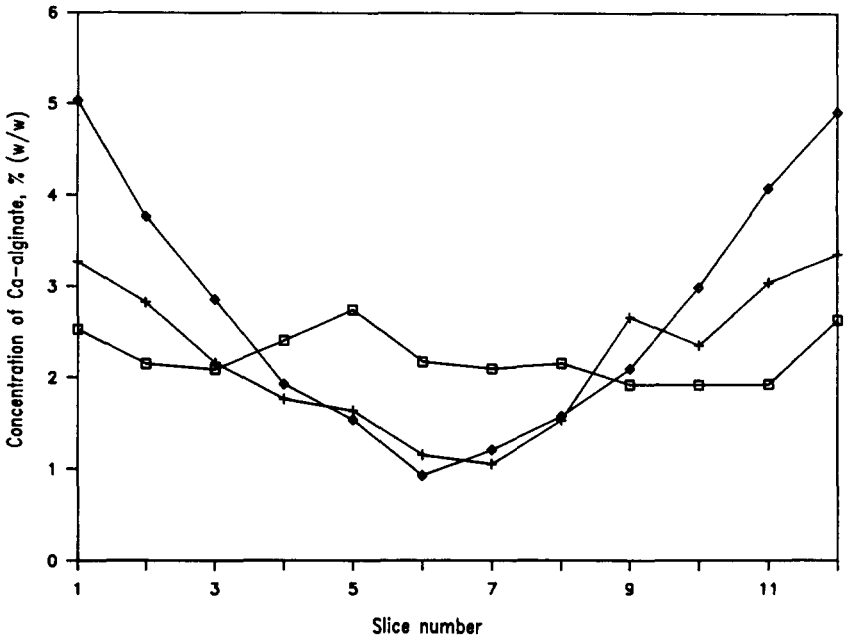

Fig. 9. Concentration profiles of calcium alginate in cylindrical gel plugs formed by dialyzing aqueous solutions of sodium alginate from *L. hyperborea* (2% w/v) against 0·05 M $CaCl_2$ in the presence of various concentrations of magnesium chloride. (□) 0·05 M, (+) 0·01 M, (◇) control.

able binding sites in the gelling zone at the gel/sol interface. The gelling zone will be nearly planar, parallel to and moving away from the $CaCl_2$/alginate interface at a distance of x from the latter. The flux of Ca^{2+} into this zone is given by:

$$-D_{Ca}\left.\frac{\partial[Ca^{2+}]}{\partial x}\right|_x$$

where D_{Ca} is the calcium ion diffusion coefficient.

The polymer diffusing into the gelling zone from the opposite direction will be immobilized immediately by calcium ions in the gelling zone, and the created concentration gradient will force diffusion of more alginate into it. The resulting local gel concentration of alginate will be determined by a stoichiometric balance between the amount of Ca^{2+} crossing a unit area in the gelling zone per unit time, and the number of binding sites on the alginate molecules present. The mass balance in the

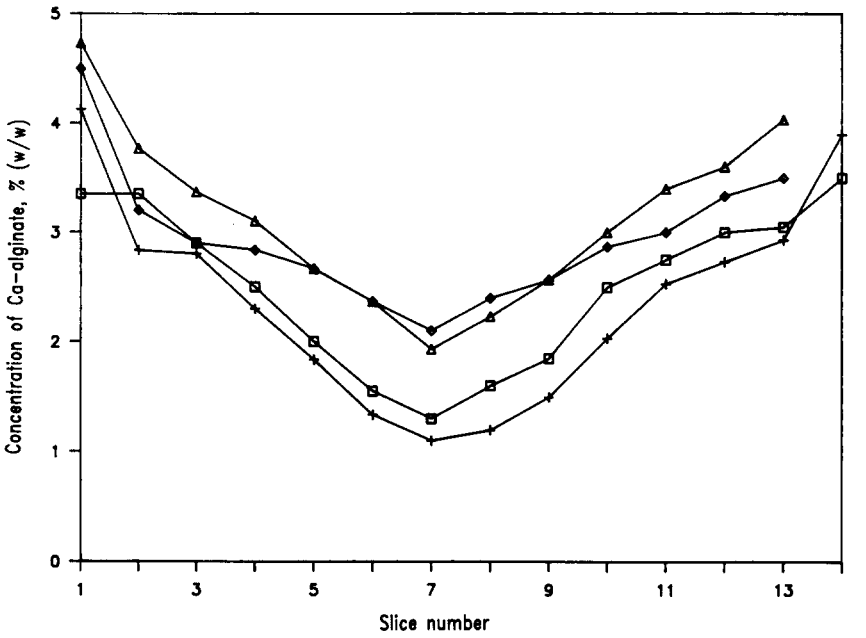

Fig. 10. Concentration profiles of calcium alginate in cylindrical gel plugs formed by dialyzing 2% (w/v) aqueous solution of sodium alginate from various sources against 0·1 M $CaCl_2$. (□) *L. hyperborea* (stipes), (+) *L. hyperborea* (outer cortex), (△) *M. pyrifera*, (◇) *L. digitata*.

gelling zone of thickness dx filled with calcium ions in a time dt can be expressed by:

$$-D_{Ca}\left.\frac{\partial[Ca^{2+}]}{\partial x}\right|_x dt = 1/2\, C_g(x)dx \qquad (1)$$

where $C_g(x)$ is the resulting local gel concentration of uronate residues. The stoichiometric factor is 1/2 and assumes that the calcium ions bind both to the guluronate and the mannuronate residues to saturation in an ion exchange reaction with sodium ions from the alginate molecules. Since the polymer diffuses considerably slower than the cations, the gelling zone will move gradually away from the $CaCl_2$/alginate interface. According to the theory of free molecular diffusion mentioned above (Sherwood & Pigford, 1952) the dependence of x on t is given by

$$x = \sqrt{4\alpha t} \qquad (2)$$

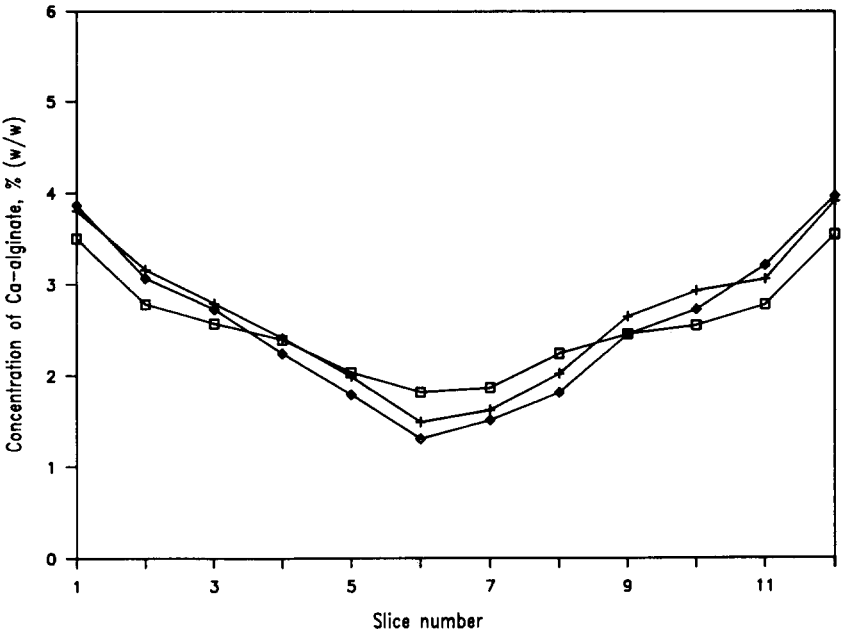

Fig. 11. Concentration profiles of calcium alginate formed when 2% (w/v) aqueous solutions of *L. digitata* sodium alginate were dialyzed against 0·05 M $CaCl_2$ in the presence of varying concentrations of sodium chloride. (□) 0·05 M, (+) 0·01 M, (◇) control.

where α is a parameter given implicitly by a relation between concentrations and diffusion constants.

To see if molecular diffusion of cations and migration of the gelling zone follow the theoretical predictions, gelation was performed in a large tube (8·5 cm) to simulate an infinite reservoir of polymer. The solid curve in Fig. 16, showing the time course of the gelling zone movement, was calculated from eqn (2) with a best fit value of $\alpha = 5{\cdot}6 \times 10^{-6}$ cm^2/s. Equation (2) gives a very good description of the experimental data in the first part of the tube where the reservoir of the alginate can still be considered as infinite. The results in Fig. 17 show concentration values of free Ca^{2+} analyzed in the gel formed after 5 h in the tube. A near linear decline in concentration from the $CaCl_2$/alginate interface was found as expected from slow diffusion of Ca^{2+} towards a plane where the ions become rapidly depleted.

These findings allow us to derive a simple expression for $C_g(x)$ in the following way. To a close degree of approximation, the concentration

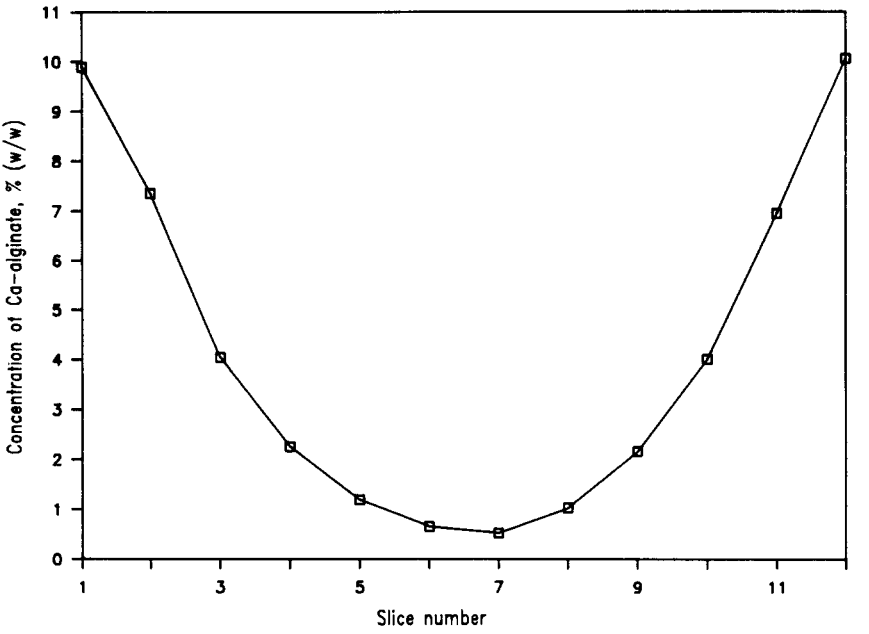

Fig. 12. Concentration profile of calcium alginate in a gel cylinder formed by dialyzing a 4% (w/v) aqueous solution of low molecular weight $[\eta] = 3$ (100 ml/g) sodium alginate from *L. hyperborea* (outer cortex) against 0·01 M $CaCl_2$.

gradients of Ca^{2+} ions in the gel are given by $-[Ca^{2+}]_0/x$. Insertion into eqn (1) yields:

$$D_{Ca} \frac{[Ca]_0}{x} dt = 1/2\, C_g(x) dx \tag{3}$$

The displacement, dx, of the gelling zone in the time dt is found from eqn (2)

$$dx = \sqrt{\alpha/t}\, dt \tag{4}$$

Combination of eqns (2)–(4) yields:

$$C_g(x) = \frac{D_{Ca}[Ca]_0}{\alpha} \tag{5}$$

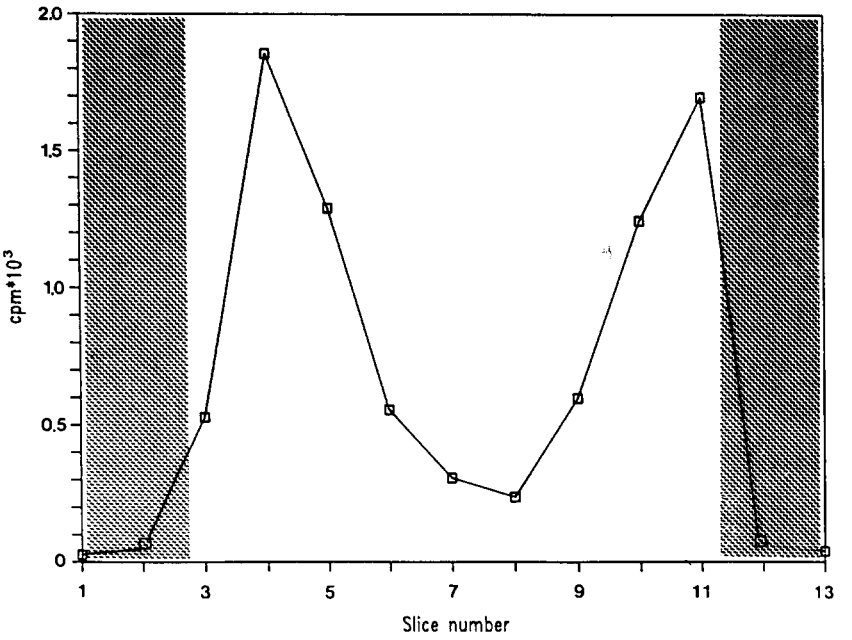

Fig. 13. Distribution of tritium labeled alginate in a calcium alginate gel. The shaded area indicates the gelled section at the time when the labeled alginate was injected into the center of the cylinder.

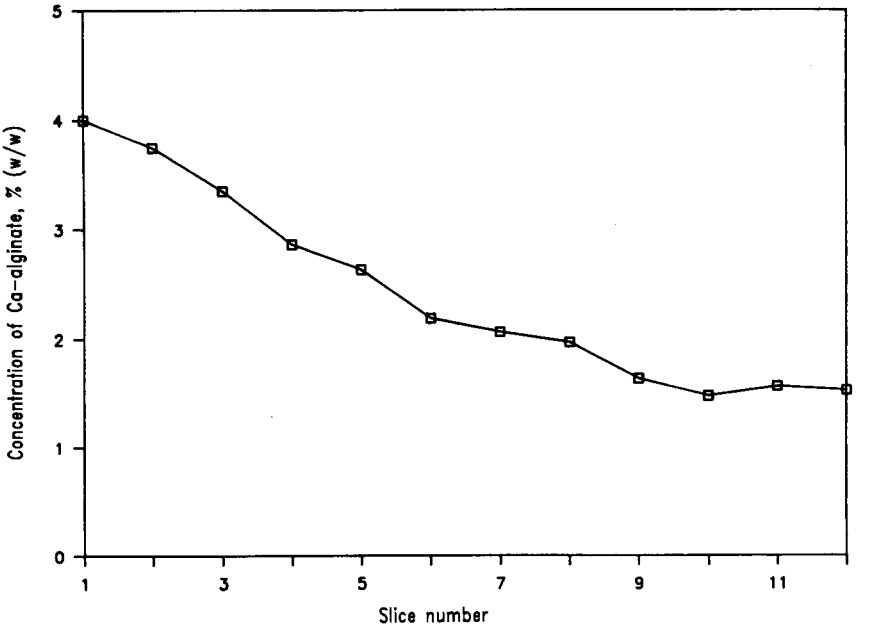

Fig. 14. Concentration profile of calcium alginate in an alginate gel dialyzed against 0·01 M $CaCl_2$ from one side without dialysis membrane.

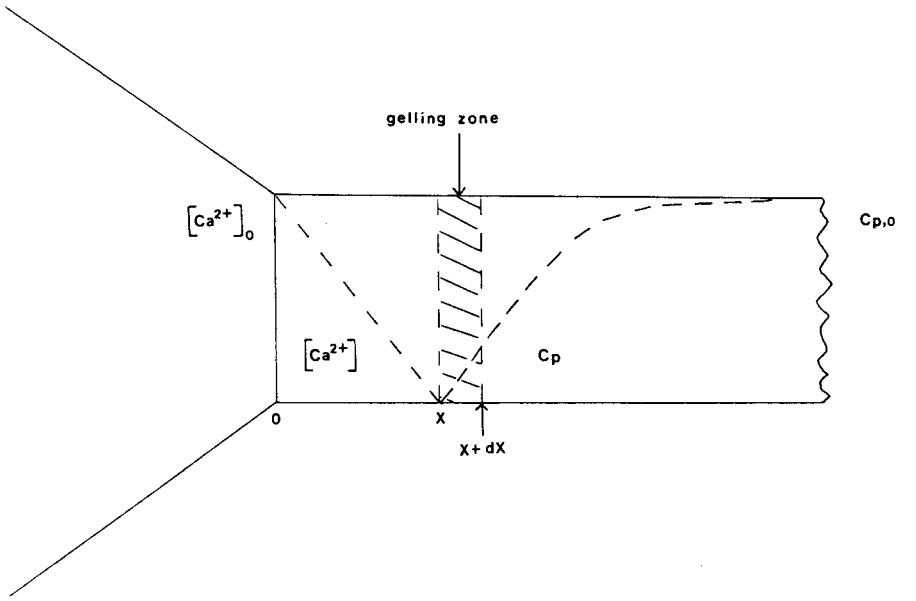

Fig. 15. Theoretical model for gel formation in an infinitely long tube with free diffusion from infinitely large reservoirs of polymer C_{p_0} and calcium ions $[Ca^{2+}]_0$.

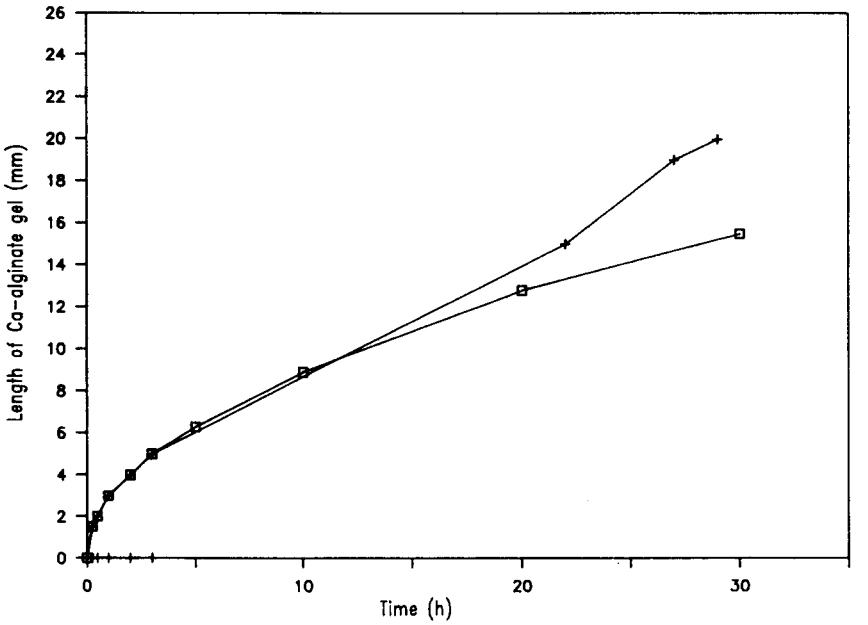

Fig. 16. Migration of the gelling zone as a function of gelling time. (+) determined experimentally, (□) estimated from $x = \sqrt{4\alpha t}$ where $\alpha = 5{\cdot}6 \times 10^{-6}$ cm^2/s.

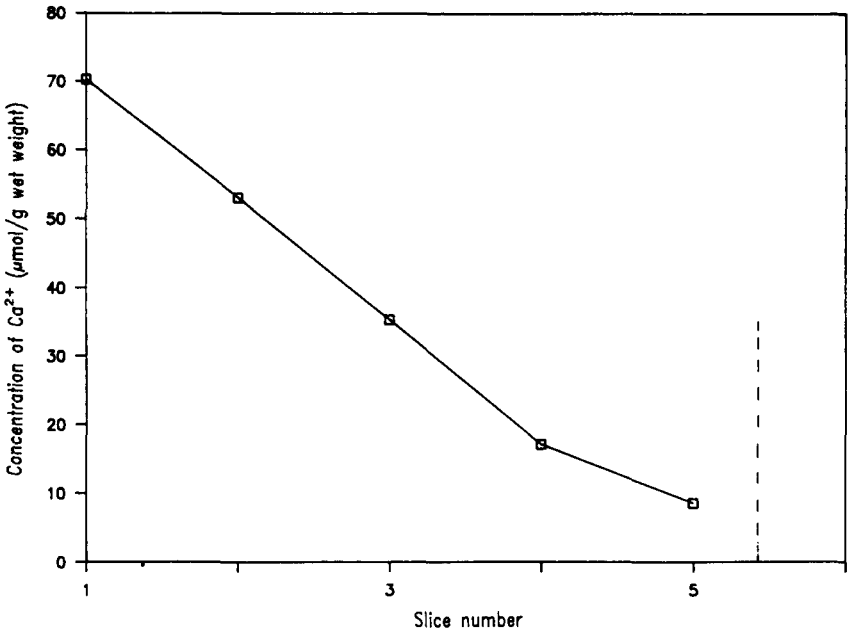

Fig. 17. Concentration gradient of excess calcium ions in calcium alginate gels, formed by 2% *L. hyperborea* alginate dialyzed against 0·1 M $CaCl_2$. The dotted line indicates the gel/sol interface. As in the other experiments the slices were taken at uniform intervals across the gel and each slice was approximately 1 mm thick.

It is seen that the predicted concentration of alginate in the gel now is independent of x in close agreement with the experimental results shown in Fig. 18. Measured values for C_g in the first 30 mm of a 300 mm long tube were constant within the experimental error, varying from 3·5 to 4·2% with an average of 3·9%. Theoretically we get a value of 3·8 from eqn (5) by using a diffusion constant $D_{Ca^{2+}} = 1 \times 10^{-9}$ m^2/s and a molecular weight of 213 for the calcium uronate residue in the chain including 1/2 Ca and one water molecule. Most probably some more water is present when the gel slices are weighed after drying. An average of 1·3 water per residue would yield 3·9 alginate in the gel. We are not aware of any measured value for $D_{Ca^{2+}}$ in an aqueous gel state. However, it is known that diffusion of ions in porous gels is only slightly (typically 10%) reduced compared to that in water. Our chosen value for $D_{Ca^{2+}}$ is 10% lower than reported in H_2O solution at 25°C and 0·1 M concentration (*Handbook of Chemistry and Physics*, 1979).

We stress that eqn (5) contains only three parameters, all of which have reliable values. The excellent quantitative agreement with theory and experiment strongly supports our model of gelation.

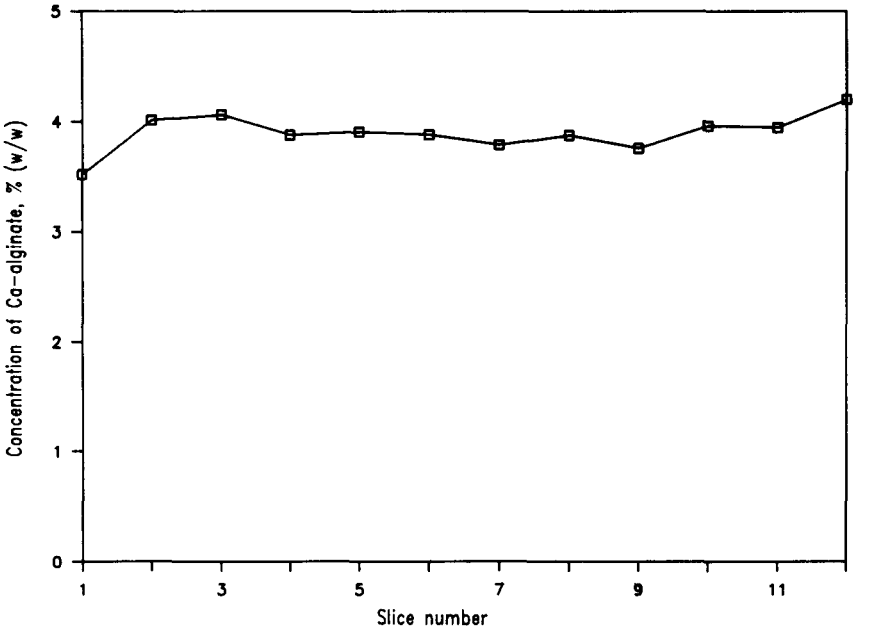

Fig. 18. Concentration profile of calcium alginate in the first 22 mm of a cylindrical gel plug formed in a 300 mm long tube by dialyzing a 2% (w/v) sodium alginate from *L. hyperborea* against 0·1 M $CaCl_2$. The tube was closed at one end.

We turn now to the inhomogeneous gels which were formed in short tubes in the absence of excess sodium or magnesium ions. Evidently the gel concentration at the $CaCl_2$/alginate interface should be independent of the magnitude of the alginate reservoir because it takes some time for the slow diffusion process to affect the polymer concentration at large. However, after some time, the shorter the tube, C_p in the finite reservoir will decrease due to extensive diffusion of alginate towards the gelling zone. The alginate becomes accumulated in the gelling zone, resulting in inhomogeneous gels with the highest concentration of polymer at the $CaCl_2$/gel interface. This is particularly well illustrated in Fig. 19, showing alginate concentration formed in tubes of different length and where Ca^{2+} ions were allowed to diffuse from one side only. The steepest concentration profile was formed in the shortest tube as expected.

DISCUSSION

Gels with a non-uniform distribution of polymers are formed when calcium ions are allowed to diffuse into finite volumes of sodium pectate

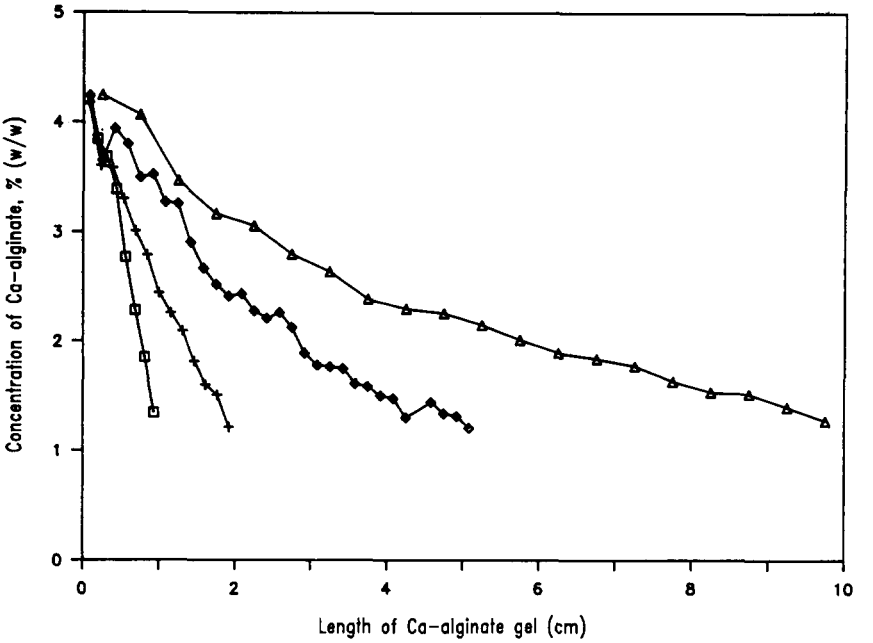

Fig. 19. Concentration profiles of calcium alginate in cylindrical gels formed in tubes of variable length by dialyzing aqueous solutions of sodium alginate from *L. hyperborea* against 0·1 M $CaCl_2$. The tubes were closed at one end. Tube length (□) 10 mm, (+) 20 mm, (◇) 50 mm, (△) 100 mm.

and sodium alginate solutions as demonstrated in Figs 2 and 3. Diffusion of gel-inducing ions into solutions of gellan gum and κ-carrageenan does not give the same effects as seen in Fig. 4.

Since the formation of inhomogeneous gels apparently is limited to alginate and pectate and is not observed for other ionic polysaccharides such as κ-carrageenans and gellan gum, the observed inhomogeneity is probably due to differences in the gelling mechanism of the two systems. Alginate and pectate have in common that they form binding sites for calcium ions and the junction in the gel network is formed by inter-chain chelation of calcium ions between blocks of L-guluronic acid in alginates (Smidsrød, 1974) and D-galacturonic acid in pectate (BeMiller, 1986). When the soluble alginate or pectate molecules diffuse towards the gelling zone their activity will drop to zero when they come into contact with free calcium ions. In the absence of other cations, binding of calcium to alginate and pectate will essentially be irreversible. A typical feature of the alginate–Ca^{2+} gel system in the absence of other ions is a very thin and distinct gel/sol interface almost as if cut by a sharp knife. Probably a steep concentration gradient of non-gelled alginate at the

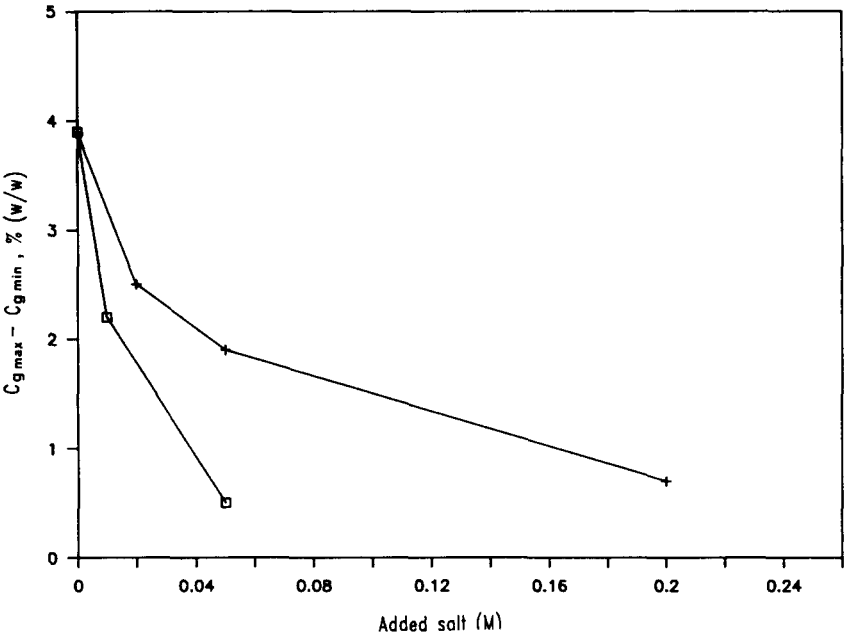

Fig. 20. Inhomogeneity in calcium alginate gels formed in the presence of non-gelling ions. The inhomogeneity is given as the difference between maximum and minimum gel concentration in alginate gel cylinders formed as described in Figs 8 and 9. (□) $MgCl_2$, (+) NaCl.

gelling zone is maintained which drives the diffusion of polymer towards the gel interface. Since there is a limited supply of soluble alginate molecules in the gel cylinder the concentration of molecules in solution will decrease as the gelling process proceeds, resulting in a non-uniform distribution of polymer in the gel. These ideas were incorporated in the theoretical model by assuming irreversible and stoichiometric binding of calcium to the polyuronate chains.

The salt-induced gelling of κ-carrageenan (Smidsrød & Grasdalen, 1984) and gellan gum (Grasdalen & Smidsrød, 1987) are very sensitive to the total ionic strength and the type of salt in the systems. However, although site binding of ions are sometimes involved, the gelling mechanisms are probably different, being thermoreversible for both κ-carrageenan and gellan gum. The gelling zone is broad and diffuse and hence the condition for a diffusional accumulation of polymer in the gelling zone is violated. This may possibly be the reason why no concentration gradients are observed in these gelling systems.

Another difference in the gelling behavior of κ-carrageenan and gellan gum as compared to gelling of polyuronides is that the former

systems have a two-step gelling mechanism. The first step involves conformational orderings with a marked increase in intrinsic viscosity (Smidsrød & Grasdalen, 1984) and hence, a lowering of the diffusion coefficient. If conformational orderings occur ahead of the gelling zone, diffusion of polymer molecules will be lowered and smaller concentration gradients will result.

The theoretical model treating gelling as an irreversible and stoichiometric process with diffusion as the rate determining step gave excellent agreement between measurement and calculated concentration of polyuronate in the first segment of an infinitely long tube provided that the rate of diffusion of calcium ions and the rate of migration of the gelling zone were known. A similar theory and data are not available for tube with finite lengths. It may, however, be inferred from the theory that the mass balance in the gelling zone and hence the concentration gradients in the gels must be governed by the relative rate of diffusion of calcium ions and polyuronate into the gelling zone. A decrease in calcium chloride concentration in the outer reservoir should decrease the rate of diffusion and consequently increase the inhomogeneity as seen for alginate in Fig. 2 and for pectate in Fig. 3. Increasing the relative rate of diffusion of polyuronate by increasing its concentration should (Nystrøm & Roots, 1982) also result in higher concentration gradients as observed for pectate (Fig. 5) and alginate (Fig. 6). By increasing the diffusion rate by degrading the alginate, increased concentration gradients are also expected, as was experimentally observed (Fig. 7).

Since the theory assumes stoichiometric binding of calcium to both L-guluronate and D-mannuronate residues in the absence of excess non-gelling cations, no effect on the concentration gradients is predicted when changing the monomer composition of the alginates, providing that their diffusion coefficients stay the same.

In Fig. 10 it is seen that the concentration gradients of alginates of different composition and similar intrinsic viscosities (and hence diffusion coefficients) are quite similar although the high G-alginate has a somewhat lower concentration in the center of the gel. This is in agreement with the prediction in the theory. However, the concentration gradients become slightly dependent on the chemical composition in the presence of sodium chloride, being highest for high G-alginate (Figs 8 and 11) which could suggest that calcium binding to the L-mannuronate is not complete in this case.

Obviously the introduction of non-gelling salts violates the conditions for effective diffusion of polymer resulting in much more homogeneous gels as shown in Figs 8 and 9. The divalent magnesium ion is seen to have a markedly stronger effect than the monovalent sodium ions. No thorough explanation of this phenomenon is given at present. The gelling

could possibly approach some equilibrium situation, giving some activity of alginate in a broad gelling zone, and thus lowering the concentration gradients that drive the diffusion of alginate into the gelling zone. In addition the salts could lower the amount of calcium necessary to gel the alginate, by a salting out effect or by competing with the calcium ions in the part of the chains containing only L-mannuronic acid residues (Haug & Smidsrød, 1965; Smidsrød & Haug, 1965) and thus increase the rate of migration of the gelling zone, leaving less time for the alginate molecules to diffuse and form concentration gradients. In both these hypotheses the divalent magnesium ions would be expected to have the strongest effect, as was observed (Fig. 20).

CONCLUSION

Calcium gels of alginate or pectate formed by diffusion of calcium into the polymer solution exhibit various degrees of inhomogeneity depending upon how the calcium ions are added. The inhomogeneity is essentially a result of an irreversible gelling mechanism characterized by strong site binding of cross-linking ions, and is further governed by the relative diffusion rate between calcium ions and the polymer molecules. More homogeneous calcium alginate gels can be formed by letting the gel formation take place in the presence of other non-gelling cations. By varying these parameters, the distribution of polymers in the gel can be controlled. This technique can also be used for making pure calcium alginate micro-capsules by diffusing calcium ions into small droplets of sodium alginate. A study of this process will be published in a subsequent paper.

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