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Swelling and partial solubilization of alginic acid gel beads in acidic buffer

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Swelling behaviour of alginic acid gel beads with different chemical composition, molecular weight and size was studied in acetate buffer at pH4. A correlation was observed between the swelling behaviour in this buffer and the equilibrium properties of alginic acid gels. High contents of long L-guluronic acid blocks (G-blocks), known to give a high acid gel strength, reduced the rate of swelling and also the amount of solubilized alginate molecules leaching out of the gel beads. Compared to the original alginate, the leaching molecules had a lower average molecular weight, higher content of mannuronic acid residues and a reduced average length of G-blocks. Swelling capacity, rate of swelling and solubility of alginic acid seemed to depend on a balance between the tendency of homopolymeric blocks to form intermolecular junction zones, and the tendency of alginate to reduce the chemical potential of water. As expected, swelling rate increased with increasing temperature and decreasing bead size. Copyright © 1996 Elsevier Science Limited.

INTRODUCTION

It is well known that alginates, being binary copolymers of (1→4) linked β-D-mannuronic acid and α-L-guluronic acid, have the ability to form gels at low pH. These acid gels are nevertheless much less studied compared to the ionic gels formed by crosslinking with calcium or other multivalent metal cations. The molecular interactions in the acid gels are less understood than in the ionic gels, although it has been proposed that they are stabilized by intermolecular hydrogen bonds (Atkins *et al.*, 1971).

In a recent work (Draget *et al.*, 1994), it was shown that as in the case of ionic gels, homopolymeric blocks of L-guluronic acid residues (G-blocks) also had the strongest tendency towards junction formation in acid gels. But in contrast to the ionic gels, the homopolymeric blocks of D-mannuronic acid also had a significant effect in supporting gel formation. Even alginates mainly composed of long stretches of strictly alternating sequences showed some gel forming ability, whereas stretches of randomly distributed MG-transitions seemed to destabilize the acid gel. The gel strength increased over a very broad range of molecular weight, especially for alginate rich in guluronic acid. Finally, and in contrast to ionically crosslinked alginate gels, alginic acid gels possess physical properties which do not depend on the history of their formation, suggesting that their gel structure was the result of a process reaching equilibrium within the time-scale of the experiments.

For certain applications of alginic acid, such as an anti-reflux agent or as a matrix in drug delivery systems, the kinetics of swelling and dissolution of the acid gel may be just as important as the equilibrium properties. In general, the kinetics of swelling must be connected to the equilibrium properties since both are results of a balance between the osmotic pressure, determined by the sum of a polymer–solvent mixing term and the Donnan equilibrium term, and the elastic resistance towards swelling caused by the network structure (Moe *et al.*, 1993). However, an important difference is that whereas the swelling capacity is determined by a difference in chemical potential of the water molecules between the inside and outside of the gel particle, the rate of swelling is determined by an inward flux of water which again depends on a gradient in chemical potential and the size and shape of the swelling particle. Since alginates are heterogeneous with respect to both chemical composition and molecular weight, some fractions may become soluble during the swelling process and evidently leak out of the gel particle. This, in turn, will reduce the degree of both the swelling rate and swelling capacity in an unpredictable way.

The object of the present paper is to study the effect of chemical composition and molecular weight on the swelling behaviour of standard sized, spherical alginic

Table 1. Chemical composition and molecular sequence of the different alginate samples used in this study as characterized by high field ^{1}H NMR spectroscopy (Grasdalen, 1983). *F* assigns the different fractions of monomers and monomer sequences found in these samples. $N_{G>1}$ = average length of guluronic acid blocks larger than 1

Sample	F_G	F_M	F_{GG}	F_{MM}	$F_{GM.MG}$	F_{GGG}	F_{GGM}	F_{MGM}	$N_{G>1}$
L. hyperborea stipe	0.68	0.32	0.54	0.18	0.14	0.50	0.04	0.10	14.5
L. hyperborea leaf	0.50	0.50	0.33	0.33	0.17	0.29	0.04	0.13	9.3

acid gel beads in a defined buffer medium. In addition, the molecular properties of the leaching material will be characterized in order to obtain information on the gel structure.

MATERIALS AND METHODS

Alginates from two different sources, i.e. *Laminaria hyperborea* stipe and leaf, have been used in this study. These are non-commercial samples isolated in this laboratory as described earlier (Haug, 1964), and the chemical composition and sequence are presented in Table 1. By partial acid hydrolysis (Grasdalen, 1983), these samples were depolymerized to give five different molecular weights each. The intrinsic viscosities ([η]) of the depolymerized samples are given in Table 2.

Swelling as function of chemical composition and molecular weight

Alginic acid gel beads were made from 2.0% Ca-alginate gel beads, made as described earlier (Martinsen *et al.*, 1989). Both the alginate solution and the $CaCl_2$ gelling bath contained 0.2M NaCl to give homogeneous beads (Skjåk-Bræk *et al.*, 1989a). These Ca-alginate beads were converted to alginic acid by a 2×3h incubation in 0.1M HCl followed by an incubation overnight in the same acid at 4°C. This treatment led to a 25–35% volume reduction of the beads. The amount of Ca^{2+} remaining after this treatment was estimated by atomic absorption (Skjåk-Bræk *et al.*, 1989b) to be less than 0.05%.

Table 2. Intrinsic viscosity ([η]) in 0.1 M NaCl of samples obtained from partial acid hydrolysis of the alginates presented in Table 1

Sample # and source	[η] (ml/g)
1. *L. hyperborea* stipe	320
2. *L. hyperborea* stipe	440
3. *L. hyperborea* stipe	510
4. *L. hyperborea* stipe	610
5. *L. hyperborea* stipe	870
6. *L. hyperborea* leaf	440
7. *L. hyperborea* leaf	460
8. *L. hyperborea* leaf	620
9. *L. hyperborea* leaf	800
10. *L. hyperborea* leaf	1000

Swelling was monitored by filling 10ml alginic acid beads (mean diameter = 2.5±0.1mm) into a 100ml measuring cylinder and adding 90ml 0.1M acetate buffer (I = 0.1, pH = 4.0). The cylinders were sealed with rubber stoppers, and stored at 4°C. Every 24h, the volume of the beads was monitored, the cylinders were inverted once and a sample of the buffer was put aside for a quantitative determination of leached alginate by the phenol–sulphuric acid method (Haug, 1964). New buffer was added. It should be stressed that these experiments were performed without any stirring of the solution outside the gel beads. This means that the observed swelling rates will not reflect the actual performance properties of e.g. alginate-based drug delivery systems which will take place under different conditions, but rather express the relative differences in swelling rates based on differences in molecular weight and chemical composition of the alginate samples investigated.

The incubation buffer used for the alginic acid beads made from alginate samples 3 and 7 (Table 2) was collected. Different buffer batches were mixed depending on the amount of leached alginate found in the different volumes. Table 3 shows the buffer batches from which the alginate material was isolated. The resulting alginate samples were examined for chemical composition and sequence by high field ^{1}H NMR spectroscopy (Grasdalen, 1983).

Analytical SEC-LALLS experiments on the same fractions of leached alginate material were performed using two serially connected columns (TSK G6000-

Table 3. Description of the different incubation buffer batches which were collected and mixed before isolation of leached alginate and characterization by NMR and SEC-LALLS

Fraction #	Source and number of batches collected
1/3	*L. hyperborea* stipe days 1–8
2/3	*L. hyperborea* stipe days 9–20
1/7	*L. hyperborea* leaf days 1–4
2/7	*L. hyperborea* leaf days 5–8
3/7	*L. hyperborea* leaf days 9–12
4/7	*L. hyperborea* leaf days 13–20

PWXL and TSK G5000-PWXL) and eluted (Spectra-Physics IsoChrom LC-pump, 0.4mlmin^{-1}) at ambient temperature with 0.05M Na_2SO_4 containing 0.01M Na_2EDTA (pH adjusted to 6.0). The samples were filtered through a 0.45μm filter (on-line) prior to injection. Injection volumes were 100, 200 or 400μl with polysaccharide concentrations in the range 0.4–0.6mg/ml. Light scattering data (Chromatix KMX-6 light scattering photometer equipped with the standard HPLC flow cell) and refractive index data (Shodex RI SE-61 detector) were collected and analyzed by the PCLALLS software, yielding the molecular weight distributions and the number and weight average molecular weights (M_n and M_w). The software also controlled the auto-injector (Shimadzu SIL-10A).

Swelling as a function of bead size and temperature

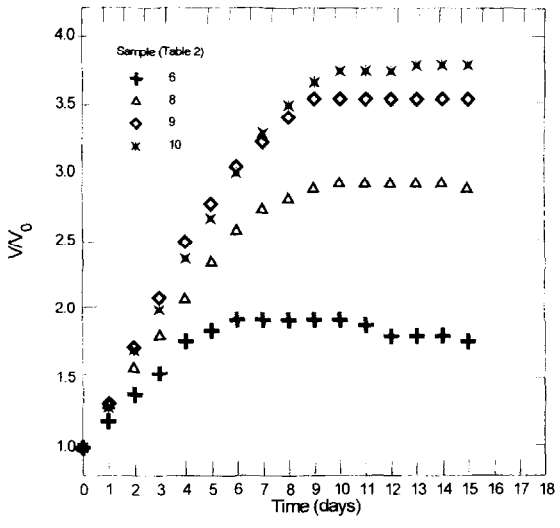
Alginic acid beads in this experiment were made from samples 4 and 8 (Table 2). The alginate flow rate was controlled by a syringe pump. The 900μm beads were prepared by using a 0.45mm needle (inner diameter) and the size of the droplets was controlled by applying a coaxial airstream as described earlier (Martinsen *et al.*, 1989). However, in the preparation of beads with diameters of 200 and 500μm, the size of the droplets was reduced by applying an electrostatic voltage (6.5kV) between the needle and the gelling solution (Dorian & Cochrum, 1994), instead of the coaxial airstream. The inner diameter of this needle was 0.1mm, and bead size was adjusted by using a higher alginate flow rate for 500μm beads than for 200μm beads; 0.9 and 0.2ml/min, respectively. The distance between the needle tip and the gelling solution was kept constant at 8mm. The diameter of the beads was calculated as the average of 20 beads measured in a Nikon Inverted Microscope, Diaphot-TMD, and the standard deviation of the bead diameters was found to be 3–6% relative to the average. Conversion to alginic acid beads was performed as described above. Swelling was monitored, as described above, at both 4 and 37°C.

RESULTS AND DISCUSSION

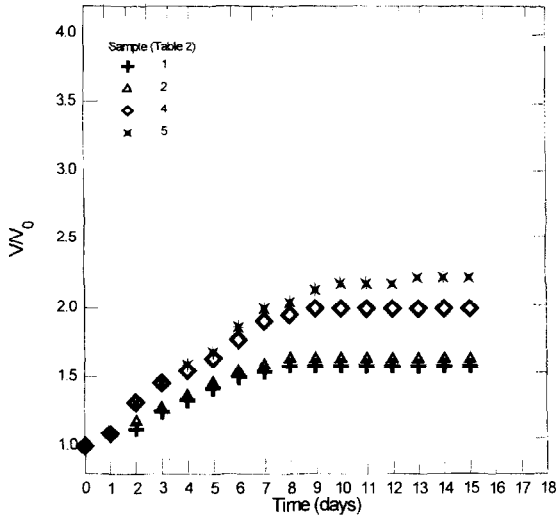
Swelling as a function of chemical composition and molecular weight

Initial studies showed that no swelling occurred at pH2 and below where the alginate is fully protonized, whereas solubilization completely overshadowed swelling at pH4.5 and above; i.e. where the alginate is almost fully charged (data not presented).

Figure (a) and (b) shows the swelling at pH4 of alginic acid beads, made from *Laminaria hyperborea* leaf and stipe alginates respectively, in terms of relative volume change with time. The swelling capacity of alginic acid gels at this pH clearly decreases with increasing amount of guluronic acid residues. Earlier observations showing that G-blocks are the most important stabilizing, junction forming structures of alginic acid gels under equilibrium conditions (Draget *et al.*, 1994) seems therefore also to be reflected under non-equilibrium conditions.



(a)



(b)

Fig. 1. Swelling behaviour of alginic acid gel beads made from *Laminaria hyperborea* leaf (a) and stipe (b) alginates incubated in 0.1M acetate buffer at pH4.

In both cases, the maximum swelling capacity increases with increasing molecular weight. This result suggests that the major part of the observed swelling behaviour can be explained by diffusion and leaching of low molecular weight alginate fragments. This, in turn, leads to a decrease in the uneven distribution of mobile ions between the swelling gel and the buffer, reducing the difference in the chemical potential of water between the inside and the outside of the gel bead. The overall effect becomes a reduced osmotic driving force of the

swelling process with decreasing molecular weight of the starting material.

To evaluate the correlation between swelling and leaching of alginate fragments, the amount of free alginate in the incubation buffer was recorded. Figure 2(a) and (b) presents these results over the same period of time. The total amount of leached alginate during this period of 16days was estimated to be in the range from 32–42% in the case of *L. hyperborea* leaf alginate (Fig. 2(a)), and 9–20% from beads made of stipe alginate (Fig. 2(b)). In the latter case, there is an inverse relation between leaching and molecular weight, i.e. the opposite of what is observed for swelling. In this case, the proposed dependence between swelling and the maintenance of the osmotic pressure seems to be valid.

In the case of *L. hyperborea* leaf alginate, the relationship between swelling (Fig. 1(a)) and the osmotic pressure is not so obvious since there is no clear dependence between molecular weight and amount of leached alginate (Fig. 2(a)). Additionally, these gels exhibit a high degree of both swelling capacity and leaching simultaneously, and can therefore not be explained by changes in the osmotic pressure alone. This behaviour is most likely to be explained by the difference in chemical composition between the two types of alginate. *L. hyperborea* leaf alginate contains higher fractions of poly-mannuronic and poly-alternating sequences and shorter poly-guluronic acid blocks compared to the stipe alginate (Table 1). Altogether, this suggests that acid gels made from this alginate will be less stable than acid gels made from alginates rich in guluronic acid residues. At the given pH conditions, this will increase both the amount of leached alginate and the average length of the stabilizing elastic segments between junction sites and thus allow further swelling.

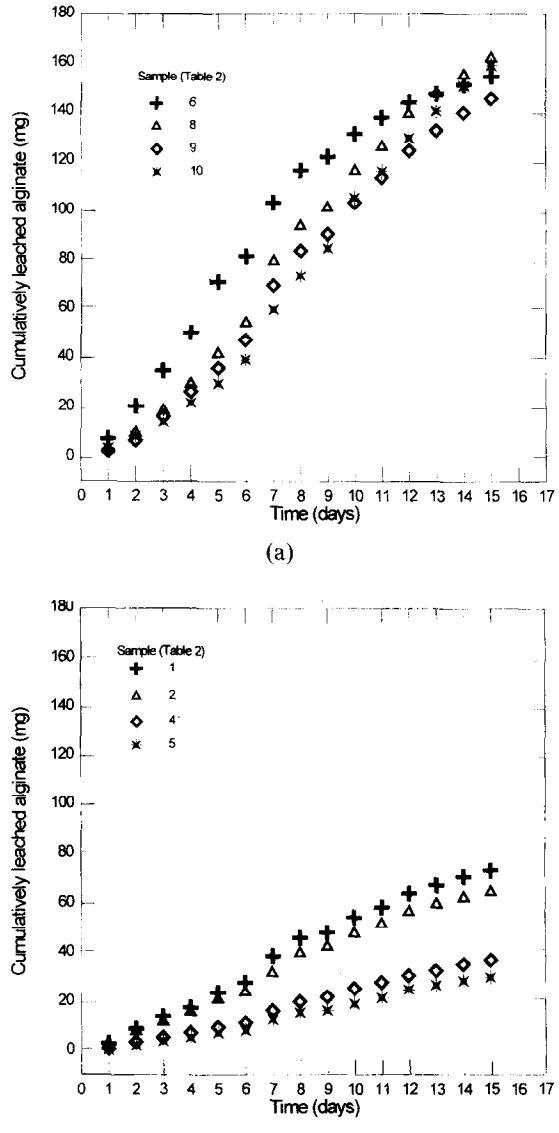


Fig. 2. Cumulatively leached alginate from alginic acid gel beads from *L. hyperborea* leaf (a) and stipe (b) alginates incubated in 0.1 M acetate buffer at pH 4.

Molecular weight and molecular weight distribution of the leached alginate material from beads made from samples 3 and 7 are summarized in Table 4. Based on the properties of the starting material, it can be seen that both molecular weight and degree of polydispersity (M_w/M_n) are considerably more reduced for leached fragments from *L. hyperborea* stipe beads compared to the leached material from leaf alginate beads. The molecular weight of leached stipe alginate is initially 20% of the original, whereas the initially leached leaf alginate only drops to around 50% of the starting material. And whereas leached leaf alginate rapidly approaches the molecular weight of the starting material, leached stipe alginate does not reach 30% of the original molecular weight even at the end of the incubation period. Evidently, the reason for this behaviour must be found in the different chemical composition. Since G-blocks give the most stable crosslinks within the alginic acid gel, these results show an expected dependence between the average G-block length and the molecular weight of the solubilized alginate.

Table 4. Weight average molecular weight and degree of polydispersity (M_w/M_n) of leached alginate from alginic acid gel beads made from samples 3 and 7 (Table 2) as obtained by combined size exclusion chromatography and low angle light scattering (SEC-LALLS). Fractions refer to Table 3

Fraction #	M_w (kDa)	M_w/M_n
Alginate sample 3 (starting material)	126.2	2.8
1/3	25.3	1.2
2/3	35.4	1.4
Alginate sample 7 (starting material)	96.9	2.6
1/7	43.5	1.8
2/7	58.6	1.8
3/7	59.7	1.7
4/7	80.7	1.7

Table 5 presents the chemical composition and sequence of the leached alginate from the same fractions. The general trend is identical in both cases. Compared to the starting material, the leached fragments show a decrease in the amount of guluronate and an increase in mannuronate and alternating sequences. The decrease in the amount of guluronate leads to a pronounced reduction in the sequence of this monomer (doublets and triplets). In both cases, determination of $N_{G>1}$ shows that the average length is reduced below 50% of the original value. With time, it seems that $N_{G>1}$ approaches $N_{G>1}$ of the starting material faster for leaching material from leaf alginate beads compared to alginate from stipe. This result is consistent with earlier observations on equilibrium properties of alginic acid gels (Draget *et al.*, 1994) and may be explained by cooperative forces involved in G-block junctions. A simple model for a quantitative description of the leaching process would be to assume that molecules are free to diffuse out of the gel beads if they do not contain any stretches of homopolymeric G-blocks above a certain critical length. The fraction of such material, i.e. that which is not linked to the network structure, would be higher for alginate from *L. hyperborea* leaf compared to stipe as shown earlier for Ca^{2+} crosslinked alginate gels (Stokke *et al.*, 1991).

Swelling as a function of bead size and temperature

Table 6 shows the average size of the beads, made from samples 8 and 4 (Table 2), at different experimental stages. The swelling behaviour at 4°C of these different sized alginic acid beads is presented in Fig. 3(a) and (b). Compared to the results obtained with larger beads (average diameter = 2.5mm; Fig. 1), a higher swelling rate and a more rapid apparent equilibrium is observed with decreasing bead size. This is an obvious effect resulting from a change in bead size leading to shorter diffusion distances. Figure 4(a) and (b) presents the data from the same experimental set-up at 37°C, and the results show an amplification of what was observed at 4°C. In Fig. 4(a), one can easily observe a rapid decrease of bead volume after an initial burst of swelling, suggesting that at these temperatures and low content of guluronic acid residues, solubilization of alginate fragments starts to overshadow swelling at an early stage.

CONCLUSIONS

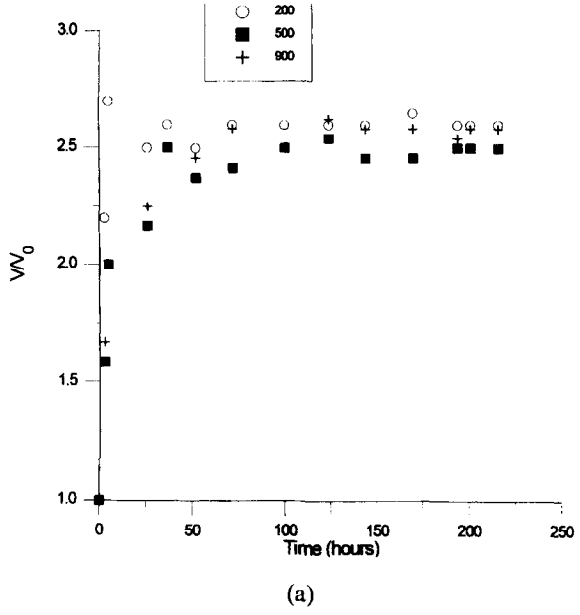
It has been shown that the rate of swelling and leaching of fragments from alginic acid gels at pH4 can be explained by changes in the swelling capacity (osmotic pressure) combined with an understanding of the stability of the gel network in terms of chemical composition and molecular weight of the alginate molecules. For alginic acid gels with a high content of guluronic acid, both molecular weight and bead particle size seem to be of major importance for the swelling kinetics. These effects become less pronounced as the content of mannuronic acid increases, reflecting the importance of guluronic acid blocks in the stabilization of the alginic acid gel.

Table 5. NMR determination of chemical composition and molecular sequence of leached alginate material from alginic acid beads made from alginate samples 3 and 7

Fraction #	F_G	F_M	F_{GG}	F_{MM}	$F_{GM,MG}$	F_{GGG}	F_{GGM}	F_{MGM}	$N_{G>1}$
Starting material (sample #3)	0.68	0.32	0.54	0.18	0.14	0.50	0.04	0.10	14.5
1/3	0.54	0.46	0.33	0.25	0.21	0.27	0.06	0.15	6.5
2/3	0.57	0.43	0.42	0.28	0.15	0.35	0.07	0.08	7.0
Starting material (sample #7)	0.50	0.50	0.33	0.33	0.17	0.29	0.04	0.13	9.3
1/7	0.31	0.69	0.13	0.51	0.18	0.09	0.04	0.14	4.3
2/7	0.42	0.58	0.21	0.37	0.21	0.17	0.04	0.17	6.3
3/7	0.45	0.55	0.24	0.34	0.21	0.20	0.04	0.17	7.0
4/7	0.45	0.55	0.25	0.35	0.20	0.21	0.04	0.16	7.3

Table 6. Size (in μm) of alginic acid beads at different experimental stages. Data presented is an average of 20 beads±STD. The beads were made from samples 4 and 8 (Table 2). Sizes of the swelled beads were monitored at the end of the experimental period (200 h)

Experimental stage	*L. hyperborea* stipe (sample #4)			*L. hyperborea* leaf (sample #8)		
	'200'-series	'500'-series	'900'-series	'200'-series	'500'-series	'900'-series
Ca-alginate	230±13	578±25	961±32	215±10	515±19	859±37
Acid gel	180±12	464±17	708±42	171±10	407±17	701±34
Swelled (4°C)	224±18	567±28	949±30	260±15	591±24	978±29
Swelled (37°C)	209±12	518±31	840±34	216±12	503±23	901±55



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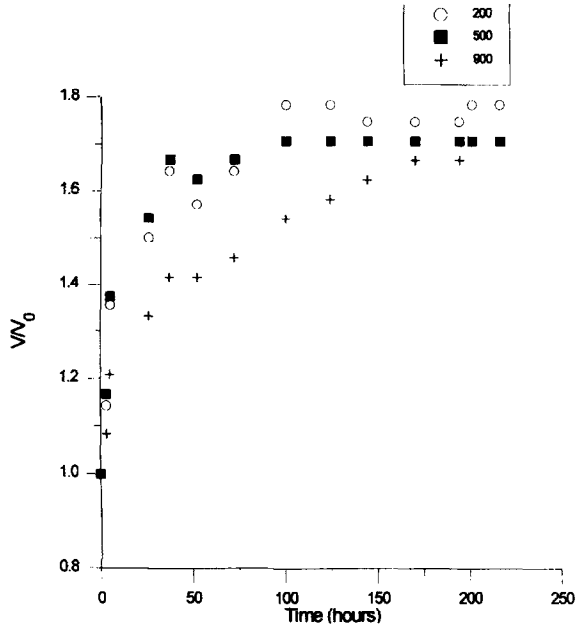
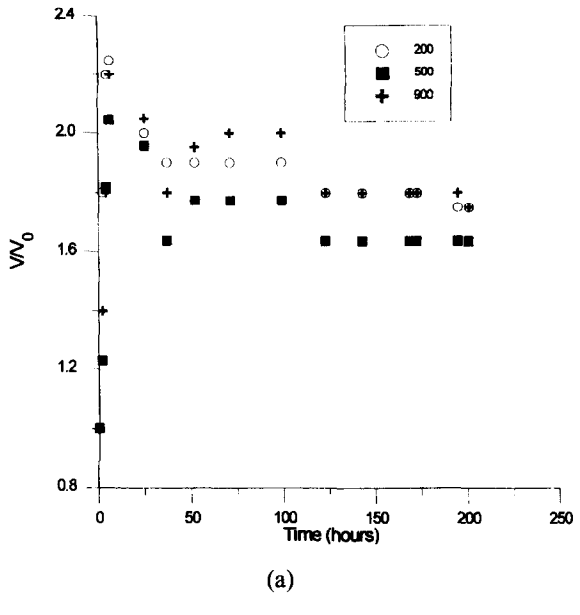


(b)

Fig. 3. Swelling behaviour of alginic acid beads of different sizes made from *L. hyperborea* leaf alginate (sample 8, Fig. 3(a)) and *L. hyperborea* stipe alginate (sample 4, Fig. 3(b)) at 4°C.



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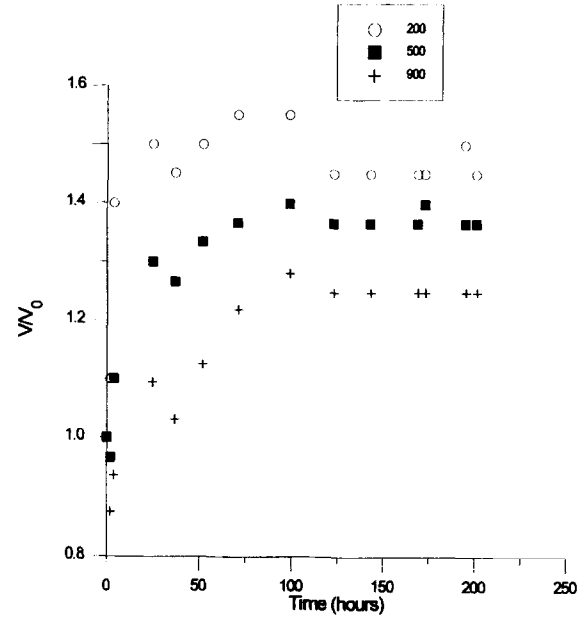

(b)

Fig. 4. Swelling behaviour of alginic acid beads of different sizes made from *L. hyperborea* leaf alginate (sample 8, Fig. 4(a)) and *L. hyperborea* stipe alginate (sample 4, Fig. 4(b)) at 37°C.

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REFERENCES

Atkins, E. D. T., Mackie, W., Parker, K. D. & Smolko, E. E. (1971). Crystalline structures of poly-D-mannuronic and poly-L-guluronic acids. *Polym. Lett.*, **9**, 311–316.

Dorian, R. & Cochrum, K. (1994). Novel electrostatic process for manufacturing coated transplants and products. International Patent #WO 94/13266.

Draget, K. I., Skjåk-Bræk, G. & Smidsrød, O. (1994). Alginic acid gels: the effect of alginate chemical composition and molecular weight. *Carbohydr. Polym.*, **25**, 31–38.

Grasdalen, H. (1983). High-field ^{1}H-n.m.r. spectroscopy of alginate: Sequential structure and linkage conformations. *Carbohydr. Res.*, **118**, 255–260.

Haug, A. (1964). Composition and properties of alginates. Thesis. Norwegian Institute of Technology, Trondheim.

Martinsen, A., Skjåk-Bræk, G. & Smidsrød, O. (1989). Alginate as immobilization material. I. Correlation between chemical and physical properties of alginate gel beads. *Biotech. Bioeng.* **33**, 79–89.

Moe, S. T., Skjåk-Bræk, G., Elgsæter, A. & Smidsrød, O. (1993). Swelling of covalently crosslinked alginate gels: Influence of ionic solutes and nonpolar solvents. *Macromolecules*, **26**, 3589–3597.

Skjåk-Bræk, G., Grasdalen, H. & Smidsrød, O. (1989a). Inhomogeneous polysaccharide ionic gels. *Carbohydr. Polym.*, **10**, 31–54.

Skjåk-Bræk, G., Zanetti, F. & Paoletti, S. (1989b). Effect of acetylation on some solution and gelling properties of alginate. *Carbohydr. Res.*, **185**, 131–138.

Stokke, B. T., Smidsrød, O., Bruheim, P. & Skjåk-Bræk, G. (1991). Distribution of uronate residues in alginate chains in relation to alginate gelling properties. *Macromolecules*, **24**, 4637–4645.