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Short communication

Effect of a small molecule on diffusion and swelling properties of selected polysaccharide gel beads

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ABSTRACT

The effect of a small molecule (e.g., sodium fluorescein, SF) on the swelling properties of and diffusion from calcium polysaccharide (alginate or pectin) gel beads was investigated. The gel beads were prepared by ionotropic gelation, soaked in different concentrations of SF solution, and then dried. The swelling behavior and release of SF from the dried beads were investigated. After soaking in SF, the beads swelled to sizes that depended on the initial concentration of SF. However, the size of the dried beads was independent of the SF concentration. The swelling of the beads occurred quite rapidly and reached a maximum within 2 h. Although most beads swelled to a size which was less than their original size of wet beads, some of them swelled much more than their original wet size. Higher concentration of SF and lower concentration of sodium alginate provided a greater increase in weight. The release profile of SF from dried gel beads in water consists of a burst or a very rapid release phase during the first 60 min followed by a much slower release phase. The similarity of the relative weight increase and release profiles of SF, suggests that swelling might contribute to release of SF, particularly during the burst phase.

1. Introduction

Gels, especially those formed from biopolymers, are useful matrices to entrap therapeutic macromolecules and prolong their release (Graham & McNeill, 1984). The conditions required for the fabrication of gels from polysaccharides is relatively benign. However, before widespread use can be made of polysaccharide gels, there is a vital need for a better understanding of the diffusion of macromolecules in the gels and an improved understanding of how the properties of the gel may be altered, both during manufacture and by the physiological environment after administration, so that their release characteristics of the drug from the gel can be manipulated (Graham & McNeill, 1984; Kudela, 1987).

Alginates, a non-toxic group of anionic polysaccharides extracted from seaweed, are linear polysaccharides containing (1 → 4)-β-D-mannuronic acid (M) and (1 → 4)-α-L-guluronic acid (G), arranged as homopolymeric blocks (poly-M and poly-G) and as mixed blocks (MG) (Haug, Larsen, & Smidsrod, 1967). The primary mode of calcium binding within alginate gels is the formation of cross-links between the poly-G blocks (Braccini & Perez, 2001). However, although the poly-M are not involved with calcium binding, there is a contribution made by the binding of calcium ions to the poly-MG blocks (Mørch, Donati, Strand, & Skjåk-Bræk, 2006). Furthermore, recent studies using isothermal titration microcalorimetry, viscometry and measurements using a calcium-selective electrode, have shown that binding of calcium to alginates follows a three-step process (Fang et al., 2007). They concluded that the first step was the complexation of the calcium ions with the poly-G blocks of a single chain, followed by chain dimerization (i.e., in accordance with the well accepted egg-box model), and finally there was lateral association between the dimerized chains.

Pectin is an inexpensive, non-toxic polysaccharide extracted from higher plant cell walls, that has been used as a food additive, a thickening agent and a gelling agent (May, 1990; Rolin, 1993). Pectin has a very complex structure which depends on both its source and the extraction process. Numerous studies have contributed to elucidate the structure of pectin. Basically, it is a partial methyl ester of α-D-galacturonic acid (poly-Gal) interrupted with (1 → 2)-α-L-rhamnose units and other neutral sugars. The poly-Gal is almost a mirror image of poly-G; the difference is the configuration of the OH at C3 (Rolin, 1993). Low methoxy pectin with a degree of esterification less than 50%, can form rigid gels by the action of calcium ions or multivalent cations, which crosslink the

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galacturonic acid chains (Rolin, 1993). However, Braccini and Perez (2001) have pointed out that an alginate-like calcium binding model is not the most favorable state energetically for pectin. Fang et al. (2008) directly compared the association of calcium ions with pectin and alginate. They observed that low methoxy pectin appeared to undergo complexation between calcium ions and a single chain followed by dimerization, but did not appear to show the lateral association between the dimerized chains. They suggested that the differences are probably due to the different arrangement of calcium binding sites, namely the block pattern in alginate and the random distribution in pectin.

Diffusion of a molecule through a three-dimensional polymer matrix requires co-operative movement of several segments of the polymer chain. The molecular flexibility (which depends on the type and composition of the polysaccharide), the type and extent of cross-linking and the degree of hydration of the polysaccharide matrix, significantly influence the effective mesh size of the gel and therefore molecular diffusion in the gel. Alginate and pectins can be gelled at low pH or crosslinked by calcium. Calcium pectinate hydrogels are stable in low pH solution, and have been investigated as a carrier material in different controlled release systems (e.g., Sriamornsak & Nunthanid, 1998, 1999). The diffusion of any solute in gels also depends on the site of entrapment, namely within the more mobile non-crosslinked regions (e.g., poly-M zones in alginate) or perhaps tangled in the crosslink zones.

The aim of this research is to investigate the effect of a small molecule (i.e., sodium fluorescein) on the swelling properties of calcium gel beads made from sodium alginate and pectin and on the self-diffusion of the sodium fluorescein from the beads.

2. Materials and methods

2.1. Materials

Medium viscosity sodium alginates obtained from *Macrocystis pyrifera* were purchased from Sigma Chemical Company (USA) and are referred to as 'A'. Low methoxy pectin with degree of esterification (DE) of 28% (GENUpectin type LM-104 AS-FS) was the generous gift of CP Kelco (Denmark) and is referred to as 'P'. Sodium fluorescein (referred as SF) was purchased from Sigma Chemical Company (USA) and calcium chloride was purchased from Merck (Germany). All other chemicals were standard pharmaceutical grade or analytical grade.

2.2. Preparation of calcium polysaccharide gel (CaPG) beads

The CaPG beads of either sodium alginate or pectin were prepared by extruding a solution of the polysaccharide as droplets through a needle into a continuously stirred solution of calcium chloride (5% w/v). The beads formed were stirred continuously in the calcium chloride solution for 4 h, separated and washed three times with 200 mL of distilled water (each wash was 2 min) and consequently left in water overnight. The beads were filtered, soaked in different concentrations of SF solution (i.e., 0, 250, 500, 1000, and 2000 μg/mL) for 6 h at 25 °C and then dried for 72 h at 25 °C. A number of different variables were investigated and are summarized in Table 1.

2.3. Study of the size and morphology of CaPG beads

Mean diameter ($n = 10$) and morphology of the wet, dried and rehydrated (in distilled water) CaPG beads were determined and photographed (using transmitted light) using a stereomicroscope (Olympus SZ-40, Olympus Co., Japan). Under the same optical conditions, an image of a linear scale was used for calibration.

Table 1
Designation of calcium polysaccharide gel beads.

Type of polysaccharides	Concentration of SF (μg/mL)	Designation
1.5% Sodium alginate (A1)	0	A1-0
	250	A1-250
	500	A1-500
	1000	A1-1000
	2000	A1-2000
3.0% Sodium alginate (A3)	0	A3-0
	250	A3-250
	500	A3-500
	1000	A3-1000
	2000	A3-2000
5.0% Sodium alginate (A5)	0	A5-0
	250	A5-250
	500	A5-500
	1000	A5-1000
	2000	A5-2000
5.0% Pectin (P5)	0	P5-0
	250	P5-250
	500	P5-500
	1000	P5-1000
	2000	P5-2000

2.4. SF release and swelling studies

Ten dried CaPG beads were weighed and then placed in a 20-mL vial with 10 mL of water, and were shaken in an orbital shaker incubator (Ratek Instruments, Australia) at a constant temperature of 37 °C. The agitation technique ensured that SF release and swelling could occur three dimensionally. Over a period of time, the release of SF and the degree of swelling due to rehydration were monitored at frequent intervals. The release of SF was monitored by visible spectroscopy at 489 nm. The swelling/rehydration was measured gravimetrically after carefully removing water adhering to the external surface with a filter paper. The percentage relative weight change can be calculated from $(W_t/W_0)\cdot 100$, where W_t is the weight at any specific time and W_0 is the initial weight. The swelling behavior of the CaPG beads was also monitored by the measuring the size changes during release (as described in Section 2.3).

2.5. Statistical analysis

Analysis of variance (ANOVA) and Levene's test for homogeneity of variance were performed using SPSS version 10.0 for Windows (SPSS Inc., Chicago, USA). *Post hoc* testing ($p < 0.05$) of the multiple comparisons was performed by either the Scheffé or Games-Howell test depending on whether Levene's test was insignificant or significant, respectively.

3. Results and discussion

The CaPG beads were successfully prepared by dropping the solution of either sodium alginate or pectin into the solution of calcium chloride. The droplets instantaneously formed gelled spheres by ionotropic gelation (Aslani & Kennedy, 1996; Sriamornsak & Nunthanid, 1998, 1999). The CaPG beads were soaked in different concentrations of SF and then dried. Samples were taken for morphological examination. Some typical images of wet and dried CaPG beads prepared from 3% sodium alginate, after soaking in water or various concentrations of SF for 6 h, are shown in Fig. 1. The CaPG beads were slightly condensed after complete gelation process, i.e., the size of the beads were about 20% smaller, compared to that after initial gelation (Fig. 2). This was due to water loss caused by gel syneresis. After soaking, the CaPG beads swelled to different sizes. Although the mean size of soaked beads was sig-

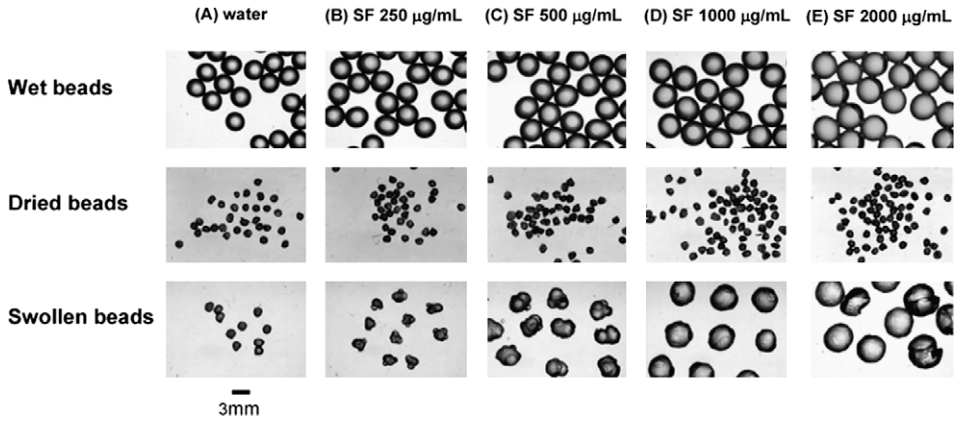

Fig. 1. Photomicrographs of wet, dried and swollen (in water) calcium polysaccharide gel beads prepared from 3% sodium alginate, after soaking in (A) water, (B) SF 250 μg/mL, (C) SF 500 μg/mL, (D) SF 1000 μg/mL, and (E) SF 2000 μg/mL for 6 h.

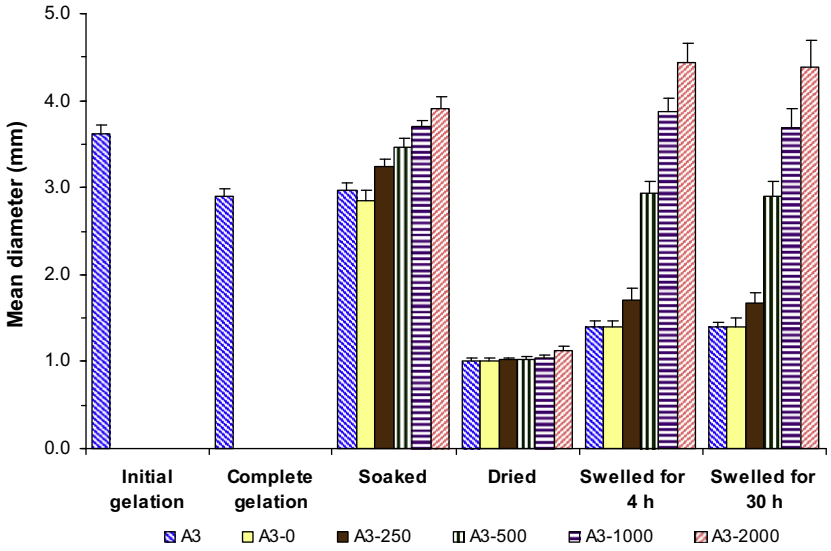

Fig. 2. Changes in the mean diameter of calcium polysaccharide gel beads prepared from 3% sodium alginate (A3), during the different stages of manufacturing and study ($n = 10$).

nificantly statistically different ($p < 0.05$) depending on the concentration of SF, the dried beads were almost the same size irrespective of the SF concentration (Fig. 2). Our preliminary results showed that the diameter of the CaPG beads (prepared from 3% sodium alginate) soaked in 0.6 mM of SF (i.e., 250 μg/mL) for 6 h was significantly greater than those soaked in 0.6 mM KCl or NaCl. The diameters of the beads soaked in 0.6 mM KCl and NaCl were 1.10 and 1.14 times larger than beads soaked in DI water. However, the diameter of the beads soaked in 0.6 mM SF were 1.5 times larger than beads soaked in water. This suggested that the ionic strength of the ionic species was a minor effect, compared to that of SF.

The swelling of dried CaPG beads occurred quite rapidly, and reached a maximum within 2 h. The swelling or rehydration of the beads could be evaluated by measuring the size changes during release. The size of the swollen beads made of 3% sodium alginate at 4 and 30 h of those beads can be seen in Fig. 2. It is apparent that as the SF concentration employed during bead soaking increased, the beads swelled to larger sizes. It is possible that the ion exchange between sodium ions from SF and calcium ions in the beads occurred during the soaking. The partial formation of soluble sodium alginate or sodium pectinate would be expected to induce water uptake in the dehydrated beads. Sriamornsak and Kennedy (2008) reported the amount of calcium remaining in swollen gel films after 4 h in the medium was about 10–30%, which was apparently sufficient to prevent total breakdown of the gel structures. The gel film could maintain its integrity for 24 h. Most beads swelled/rehydrated to a size which was less than their original wet size. However, some of gel beads, (i.e., A1 and A3, soaked in SF 2000 μg/mL) swelled much more than their original wet size and some split at 4 h (Fig. 1) or by the end of study.

The swelling behavior of dried beads can also be investigated by measuring the relative weight change. Fig. 3a shows the kinetics of swelling of dried CaPG beads measured by the relative weight change versus time. In general, the kinetic profile of the weight change of dried CaPG beads consists of a rapid swelling period which correlates to the burst phase of SF release (discussed later) and only slight changes after 2 h. To assess the swelling properties, the relative weight increase at 4 h was used as a response parameter. Fig. 3b shows that there was a statistically significant difference in relative weight increase after 4 h-swelling between the blank beads (without SF) and the SF-soaked beads. It is also seen that the higher the concentration of SF, the greater the increase in relative weight. The concentration of polysaccharide also af-

fected the swelling behavior, i.e., the lowest concentration of sodium alginate gave the largest increase in weight, i.e., the highest relative weight increase (Fig. 3b). This may be due to a decrease in number of cross-links between calcium and available polysaccharide or a possible decrease in entanglement at lower concentration.

The amount of SF taken into CaPG beads soaked in different concentrations of SF on the payload of SF in the dry beads is shown in Fig. 4. It is apparent that the payload increased as the concentration of SF used during soaking was increased. In addition, at each concentration of SF, the payload decreased as the alginate concentration increased. At least two factors may contribute to this observation. After equilibration in the soaking solution, there would be a certain concentration of SF within the aqueous domain of the gel beads. When the beads are dried, the SF would remain in the beads, but the total mass of the beads would increase to an extent that depended on the gel concentration. Therefore, if the beads acquired similar amounts of SF within them by passive diffusion during soaking, but the dry mass of the beads increased as the gel concentration was increased, the payload would be expected to decrease as the gel concentration increased. Secondly, the higher gel concentrations may exhibit greater molecular tortuosity and this may impede the passive diffusion of the SF into the beads during soaking.

Fig. 5 shows the amount of SF released from CaPG beads made of 3% sodium alginate versus time. The results for the other polysaccharides were similar (data not shown). Generally, the SF release from dried CaPG beads in water was complete within 1 h. Almost 100% of SF was released in the first hour in all formulations. The similarity of the relative weight increase and release profiles of SF, suggests that swelling might contribute to release of SF, particularly during the burst phase. This is presumably due to the good water solubility of SF, the presence of a favorable diffusion medium in the swollen beads and reduction in the impediments to diffusion in the swollen beads.

The results indicated that the addition of different concentrations of a small molecule SF, influenced the swelling properties of CaPG beads and the self-diffusion of SF from the beads. The question that arises is whether the use of SF treatment of CaPG beads would influence the diffusion of other molecules in the same beads. In particular, the use of SF treatment has potential application in being employed as a means to alter the release profile of macromolecules entrapped in the CaPG beads. This work is currently in progress.

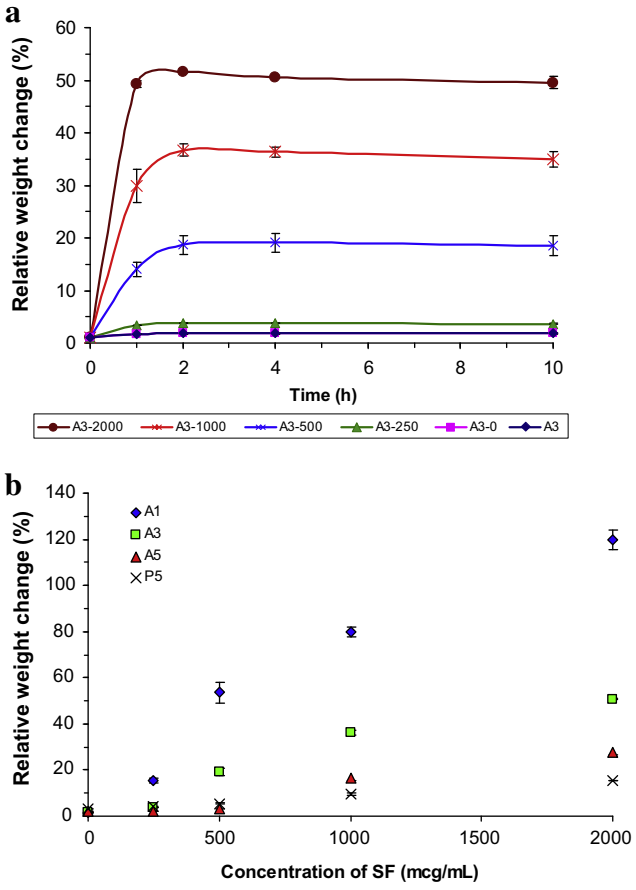

Fig. 3. Swelling kinetics of calcium polysaccharide gel beads, investigated by measuring the relative weight changes (a) during release (of only beads prepared from 3% sodium alginate) and (b) after 4-h swelling. The average of 10 measurements was shown. *Note:* 'A1, A3 and A5' represent the beads prepared from 1%, 3% and 5% sodium alginate, respectively, whereas 'P5' represents the beads prepared from 5% pectin.

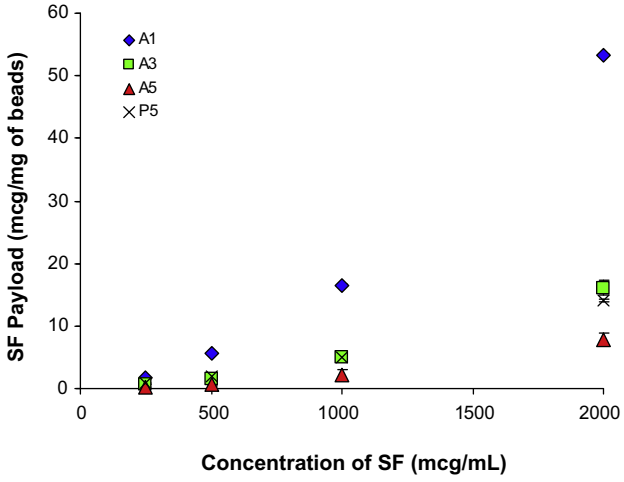

Fig. 4. The amount of sodium fluorescein uptake (payload) into calcium polysaccharide gel beads, on a dry weight basis ($n = 3$). *Note:* 'A1, A3 and A5' represent the beads prepared from 1%, 3% and 5% sodium alginate, respectively, whereas 'P5' represents the beads prepared from 5% pectin.

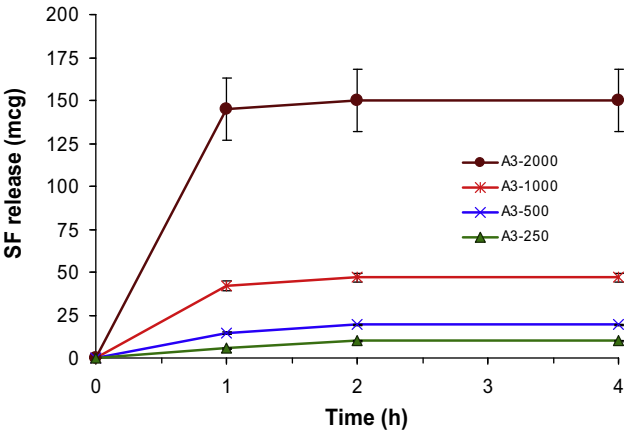

Fig. 5. The amount of sodium fluorescein release from calcium polysaccharide gel beads prepared from 3% sodium alginate (A3) ($n = 3$).

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References

Aslani, P., & Kennedy, R. A. (1996). Effect of gelation conditions and dissolution media on the release of paracetamol from alginate gel beads. *Journal of Microencapsulation, 13*, 601–614.

Braccini, I., & Perez, S. (2001). Molecular basis of Ca^{2+}-induced gelation in alginates and pectins: The egg-box model revisited. *Biomacromolecules, 2*, 1089–1096.

Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K., Funami, T., Williams, P. A., et al. (2007). Multiple steps and critical behaviors of the binding of calcium to alginate. *Journal of Physical Chemistry B, 111*, 2456–2462.

Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K., Funami, T., & Williams, P. A. (2008). Binding behavior of calcium to polyuronates: Comparison of pectin with alginate. *Carbohydrate Polymers, 72*, 334–341.

Graham, N. B., & McNeill, M. E. (1984). Hydrogels for controlled drug delivery. *Biomaterials, 5*, 27–36.

Haug, A., Larsen, B., & Smidsrod, O. (1967). Studies on the sequence of uronic acid residues in alginic acid. *Acta Chemica Scandinavica, 21*, 691–704.

Kudela, V. (1987). Hydrogels. In *Encyclopedia of polymer science and engineering* (Vol. 7, pp. 703–807). New York: Wiley.

May, C. D. (1990). Industrial pectins: Sources, production and applications. *Carbohydrate Polymers, 12*, 79–99.

Mørch, Y. A., Donati, I., Strand, B. L., & Skjåk-Bræk, G. (2006). Effect of Ca^{2+}, Ba^{2+} and Sr^{2+} on alginate microbeads. *Biomacromolecules, 7*, 1471–1480.

Rolin, C. (1993). Pectin. In R. L. Whistler & J. N. Bemiller (Eds.), *Industrial gums: Polysaccharides and their derivatives* (pp. 257–293). New York: Academic Press.

Sriamornsak, P., & Kennedy, R. A. (2008). Swelling and diffusion studies of calcium polysaccharide gels intended for film coating. *International Journal of Pharmaceutics, 358*, 205–213.

Sriamornsak, P., & Nunthanid, J. (1998). Calcium pectinate gel beads for controlled release drug delivery. I. Preparation and in vitro release studies. *International Journal of Pharmaceutics, 160*, 207–212.

Sriamornsak, P., & Nunthanid, J. (1999). Calcium pectinate gel beads for controlled release drug delivery. II. Effect of formulation and processing variables on drug release. *Journal of Microencapsulation, 16*, 303–313.