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Short communication

Effect of sodium fluorescein on release characteristics of a macromolecule from calcium alginate gel beads

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ABSTRACT

This study describes a detailed investigation of swelling and release behavior of a macromolecule from calcium alginate gel (CAG) beads in the presence of a small molecule, sodium fluorescein (SF). Blue dextran (BD) was used as a model macromolecule. The bead diameter was slightly different after soaking in various concentrations of SF although the SF uptake into calcium gel beads is markedly different. The swelling kinetics of CAG beads showed the rapid hydration and reached a maximum within 6 h. It is thought that the effect of SF, which is predominant in the swelling of CAG beads without BD, was hindered by the entanglement of BD. It appeared that the SF concentration has an effect on the BD release; the higher the SF concentration, the higher amount of BD released from the CAG beads. The release from this system basically follows Fick's law (Higuchi's expression). The increase in SF concentration decreased the Higuchi release coefficient. It was observed that the effect of small molecule is quite obvious, since the difference in Higuchi release coefficients of the CAG beads soaked in 1000-μg SF is less than non-soaked beads, about 25%.

1. Introduction

The next generations of drug substances are likely to include potent therapeutic macromolecules. Although many of those drugs will first reach the market as an injection, significant continued commercial success will often depend on developing a more conveniently administered dosage form. Gels, especially those formed from biopolymers, are useful matrices to entrap therapeutic macromolecules and prolong their release (Graham & McNeill, 1984). 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. This understanding will assist in the manipulation and control of the release characteristics of the drug from the gel (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). Calcium ions form cross-links primarily between the poly-G blocks (Braccini & Perez, 2001), although the MG blocks do contribute somewhat to cross-linking with calcium (Morch, Donati, Strand, & Skjaak-Braek, 2006). It has been known for some time that calcium alginate beads have potential for use in the development of controlled drug delivery technologies for the gastrointestinal administration of proteins (e.g., Gombotz & Wee, 1998; Rasmussen, Snabe, & Pedersen, 2003), and drug molecules (e.g., Aslani & Kennedy, 1996; Fernandez-Hervas, Holgado, Fini, & Fell, 1998).

Diffusion of a molecule through a three-dimensional polymer matrix requires co-operative movement of several segments of the polymer chain. The effective mesh size of the gel, and therefore molecular diffusion in the gel, are influenced by many factors including the molecular flexibility of the polysaccharide (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 (e.g., Kudela, 1987). The diffusion of any solute in gels will also depend 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 mobility

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Table 1
Composition of BD-loaded gel beads prepared from 3% sodium alginate.

Concentration of SF ($\mu g\,mL^{-1}$)	BD nominal loading (%)	Designation	SF uptake ($\mu g\,mg^{-1}$ of bead)	BD content ($\mu g\,mg^{-1}$ of bead)
0	5	A3BD-0	–	66.78 ± 1.13
250	5	A3BD-250	0.35 ± 0.18	68.23 ± 4.14
500	5	A3BD-500	1.24 ± 0.13	66.89 ± 0.67
1000	5	A3BD-1000	3.76 ± 0.19	64.34 ± 1.47

of a labeled model macromolecule (a dextran) should reflect the degree of entrapment and entanglement of the macromolecules. Subsequently, this will influence the diffusion properties of the model macromolecule. Most applications of these gels in drug delivery will require that the gel is dried and stored for some time prior to administration to a patient after which time the dry gel will have to rehydrate.

We have found that the small molecule, sodium fluorescein (SF) affects the swelling properties of calcium polysaccharides gel beads (Sriamornsak & Kennedy, 2010). Thus, the aim of this research is to further investigate the effect of SF on the release characteristics of a model macromolecule from selected calcium alginate gel beads.

2. Materials and methods

2.1. Materials

Medium viscosity sodium alginate obtained from *Macrocystis pyrifera*, blue dextran (molecular weight 2×10^6 Da) and sodium fluorescein (referred as SF) were purchased from Sigma Chemical Company (USA). Calcium chloride was purchased from Merck (Germany). All other chemicals were pharmaceutical grade or analytical grade.

2.2. Rheology measurement of sodium alginate

The rheology of a 2% w/w solution of sodium alginate was measured using a Brookfield DVIII Ultra Rheometer (Brookfield Engineering Laboratories, USA) with SC25 small sample spindle at 22 °C and the data were analyzed using Rheocalc software by Brookfield. Flow curves were modelled according to the Herschel–Bulkley rheological model, as described mathematically as follows:

$$\tau = \tau_0 + K(\gamma)^n \quad (1)$$

where τ is shear stress, is τ_0 yield stress, K is consistency index, γ is shear rate, and n is power law exponent. The Herschel–Bulkley equation is preferred to power law or Bingham relationships because it results in more accurate models of rheological behavior when adequate experimental data are available.

2.3. Preparation of calcium alginate gel beads

The gel beads containing a model macromolecule, i.e., blue dextran (BD), were prepared as described in the previous paper (Sriamornsak & Kennedy, 2010). Briefly, the BD (500 mg) was dissolved in 10 g of 3% w/w sodium alginate and then extruded as droplets through a plastic needle (inner diameter of 1.0 mm) into a continuously stirred solution of 0.34 M calcium chloride at 25 °C. The needle was positioned 5 cm above the surface of calcium chloride solution. The rate of extrusion of sodium alginate solution is approximately 3 mL min^{-1}. The beads formed were stirred continuously in the aqueous solution for 4 h, separated and washed with 200 mL of distilled water (2 min) for 3 times and consequently left in water overnight. The beads were separated by filtration, soaked in different concentrations of SF solution (i.e., 0, 250, 500 and 1000 $\mu g\,mL^{-1}$) for 6 h and then dried at 25 °C for 72 h. The composition of BD-loaded gel beads is summarized in Table 1.

2.4. Study of particle size and morphology of CaPG beads

The mean Feret diameter and morphology of the wet, dried and rehydrated CaPG beads ($n = 10$) were determined with a stereomicroscope (Olympus SZ-40, Olympus Co., Japan). Under the same optical conditions, an image of a linear scale was used for calibration purposes.

2.5. Swelling and BD release studies

Ten dried beads were weighed and then placed in a 20-mL screw cap vial with 10 mL of deionized water, and were shaken in an orbital shaker incubator (Ratek Instruments, Australia) at a constant temperature of 37 °C. Over a period of time, up to about 17 days, the degree of swelling during rehydration and the release of macromolecule, i.e., BD, were monitored at suitable frequent intervals. The supernatant was removed and the release of BD was monitored by visible spectroscopy at 609 nm. The swelling/rehydration was measured gravimetrically after carefully removing water adhering to the external surface with a filter paper. The relative weight change can be calculated from W_t/W_o, where W_t is the weight at any specific time and W_o is the initial weight. The swelling behavior of the CaPG beads was also monitored by the measuring the size changes during release ($n = 10$). The release and swelling studies were continued after replacement with 10 mL of fresh medium. Each study was performed in triplicate.

2.6. Analysis of release data

The mechanism of drug release from CAG beads during dissolution tests in water was determined using Higuchi and exponential equation (Korsmeyer–Peppas equation). The Higuchi equation is used to describe the drug release from an inert matrix (Higuchi, 1963):

$$\frac{M_t}{M_f} = k \cdot \sqrt{t} \quad (2)$$

where k is a Higuchi release coefficient and M_t/M_f represents the drug dissolved fraction at time t. Eq. (2) yields a straight line when M_t/M_f is plotted against square root of time. The Korsmeyer–Peppas equation is often used to describe the drug release behavior from polymeric systems (Korsmeyer et al., 1983):

$$\frac{M_t}{M_f} = k' \cdot t^n \quad (3)$$

where k' is a constant incorporating the structural and geometric characteristics of the matrix pellets, n is the release exponent, indicative of the drug release mechanism. The release profiles between 10% and 60% release were used for analysis. To clarify the release exponent for different batches of CAG beads, the log value of percentage drug released was plotted against log time for each

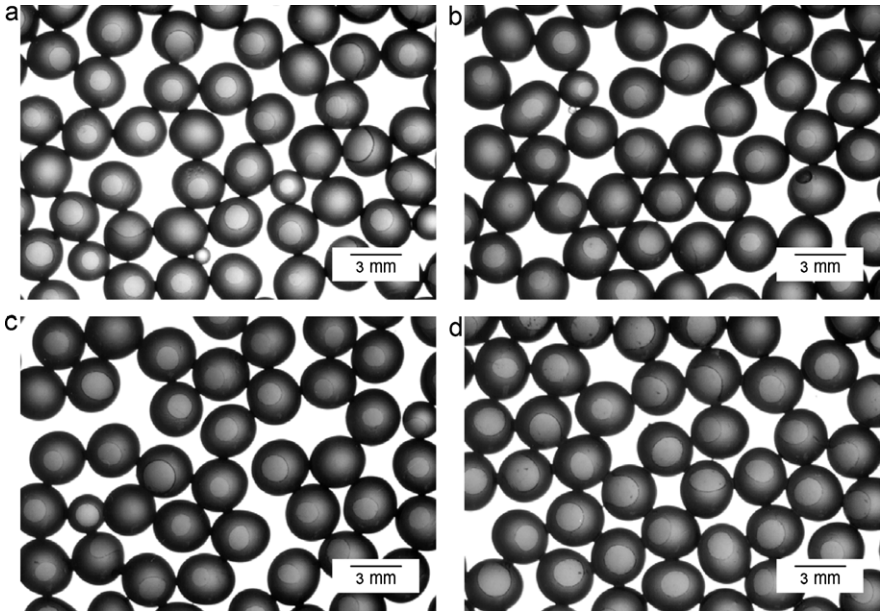

Fig. 1. Photomicrographs of BD-loaded calcium alginate gel beads, (a) after soaking in water, (b) soaking in sodium fluorescein 250 μg mL^{-1}, (c) 500 μg mL^{-1}, and (d) 1000 μg mL^{-1} for 6 h.

batch according to Eq. (4).

$$\log\left[\frac{M_t}{M_f}\right] = \log k' + n \log t \quad (4)$$

In the case of Fickian release (diffusion-controlled release), n has a limiting value of 0.43 for release from spheres. In the case of Case II transport or relaxation-controlled delivery, the exponent n is 0.85. The non-Fickian release or anomalous transport of drug occurred when the n values fell between the limiting values of Fickian and Case II transport. The non-Fickian kinetics corresponds to coupled diffusion/polymer relaxation. Occasionally, values of $n > 0.85$ have been observed and considered to be Super Case II kinetics.

2.7. Statistical analysis of data

One way analysis of variance was performed with Minitab 16 (Minitab Inc., USA). *Post hoc* testing ($p < 0.05$) of multiple comparisons was performed with Fisher's Least Significant Difference test.

3. Results and discussion

Using the Herschel–Bulkley model, the estimated viscosity of sodium alginate (2%, w/w) at a shear rate of 1 s^{-1} was 745 m Pa s and the flow behavior index was 0.99. The correlation coefficient for the linear regression was 0.994. A flow behavior index of 1 implies Newtonian flow behavior. Therefore, this solution displayed a Newtonian viscosity of 745 m Pa s.

The CAG beads containing a model macromolecule, BD, were prepared by ionotropic gelation method (e.g., Aslani & Kennedy, 1996; Sriamornsak & Nunthanid, 1998), in which BD was dissolved in sodium alginate solution prior to extruding into 0.34 M calcium chloride solution. The beads formed were separated, washed, left in water overnight, and then soaked in different concentrations of SF solution before drying. The swelling behavior of the dried beads was investigated gravimetrically and dimensionally. The CAG beads showed only a slight difference in the swollen bead diameter after soaking in various concentrations of SF (Figs. 1 and 2) although the SF uptake into calcium gel beads was markedly different (Table 1). However, it is thought that the swelling induced by the SF (as reported by Sriamornsak & Kennedy, 2010) may have been masked by the additional macromolecular entanglements due to the BD that was also loaded into these CAG beads. The diameters of all of the dried beads, were very similar, i.e., about 1.10–1.11 mm.

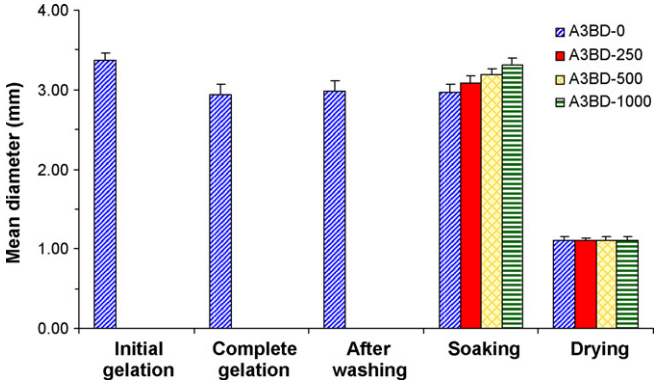

Fig. 2. The mean diameter of BD-loaded calcium alginate gel beads at the different stages of manufacturing ($n = 10$).

The swelling of CAG beads occurred rapidly, and reached a maximum within the first 6 h. The swelling behavior evaluated by measuring relative weight changes during release of BD (see Fig. 3) does not show the pronounced degrees of swelling reported previously (Sriamornsak & Kennedy, 2010) for CAG beads without added BD. In our previous report, SF induced a large increase in the swelling of CAG beads. It is possible that the calcium ions cross-linked in the polyguluronate sequences undergo ion-exchange with sodium ions and that the associations between alginate chains are further disrupted and weakened by entrapment of the large polyaromatic fluorescein molecules (Fig. 4). CAG beads containing BD showed less swelling even though the beads were also exposed to SF. This is probably because the physical entanglement of dextran molecules with alginate molecules during gel formation might hinder swelling and expansion during rehydration (Fig. 4), and would be expected to hinder the diffusion of BD and therefore lead to a slow and sustained release of BD.

The release of BD from CAG beads was monitored for 17 days. Fig. 5 shows the effect of SF concentration on the percentage of

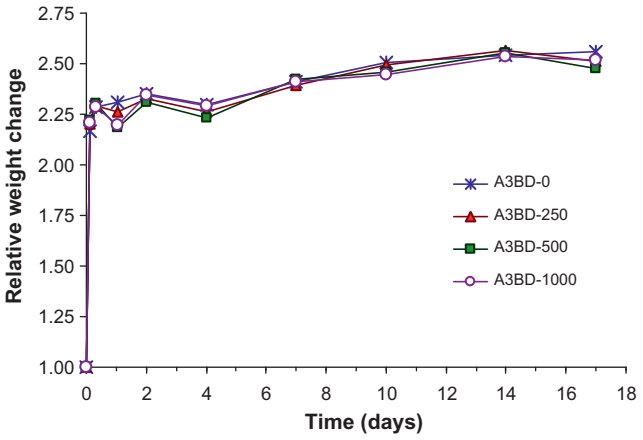

Fig. 3. The swelling kinetics of calcium alginate gel beads investigated by measuring the relative weight change during release.

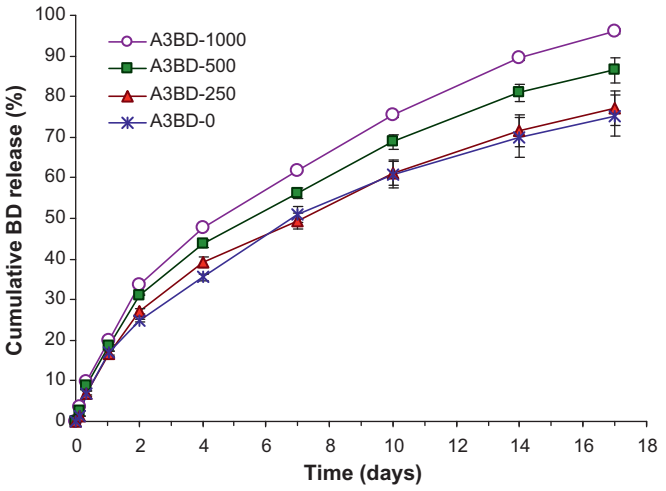

Fig. 5. Effect of SF concentration on the percentage of cumulative blue dextran release from calcium alginate gel beads ($n = 3$; error bars = SD).

cumulative BD release from CAG beads. After about 10–20% of BD was released, the entanglement of BD molecules may have been sufficiently decreased, that the effect of SF on swelling was more apparent and therefore BD release was enhanced by higher concentrations of SF. Since BD was homogeneously dissolved throughout the polysaccharide mass, the monolithic solution model best described this system (Higuchi, 1963). The release from this system basically follows Fick's law in which the percentage release is linear with the square root of time. A typical plot of cumulative percentage release of BD versus the square root of time is shown in Fig. 6. This is different from the previous report by Kim and Lee (1992) in which the BD release from alginate beads in pH 6.8 buffer showed nearly zero-order kinetics. The difference in release patterns of BD may result from the effect of SF and also because at pH 6.8 achieved by a phosphate buffer there would be substantial erosion of the beads leading to alterations of the mechanism of release.

In order to compare quantitatively the release profile from different formulations of CAG beads, the Higuchi and Korsmeyer–Peppas equations were used and are shown in Table 2. The drug release data of CAG beads showed a good fit into both Higuchi equation and Korsmeyer–Peppas equation. Higuchi model is applicable if the release of drug is largely governed by diffusion through water-filled pores in the inert matrix. An increase in SF con-

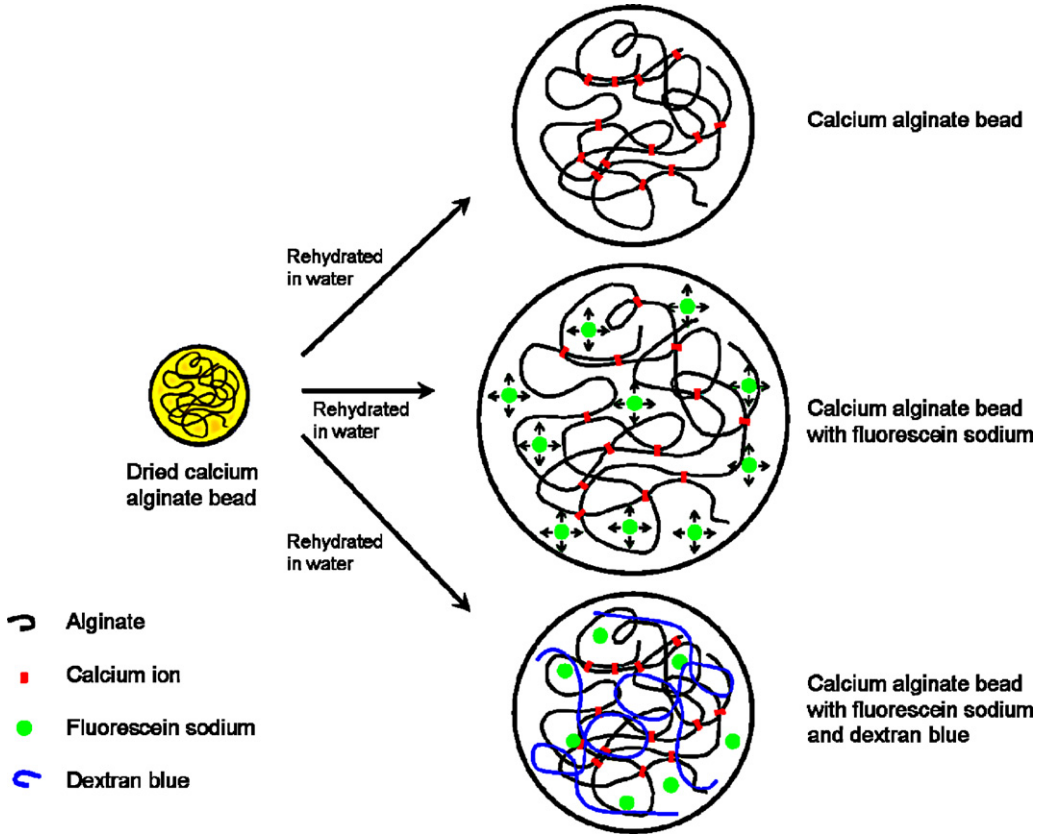

Fig. 4. Diagram showing the swelling mechanism of different calcium alginate gel beads.

Table 2
Mathematic modeling and release kinetics of BD from calcium alginate gel beads.[a]

Formulation	Higuchi model		Korsmeyer–Peppas model			
	Correlation coefficient (r^2)	Rate constant, k	Correlation coefficient (r^2)	Kinetic constant, k'	Diffusional exponent, n	Order of release
A3BD-0	0.9974	19.88 ± 1.60	0.9985	0.166 ± 0.001	0.56 ± 0.03	Non-Fickian
A3BD-250	0.9965	20.13 ± 1.19	0.9898	0.173 ± 0.005	0.56 ± 0.01	Non-Fickian
A3BD-500	0.9956	22.47 ± 1.02	0.9813	0.193 ± 0.001	0.57 ± 0.02	Non-Fickian
A3BD-1000	0.9990	24.87 ± 0.29	0.9815	0.207 ± 0.004	0.58 ± 0.01	Non-Fickian

[a] Analyzed by the regression coefficient method.

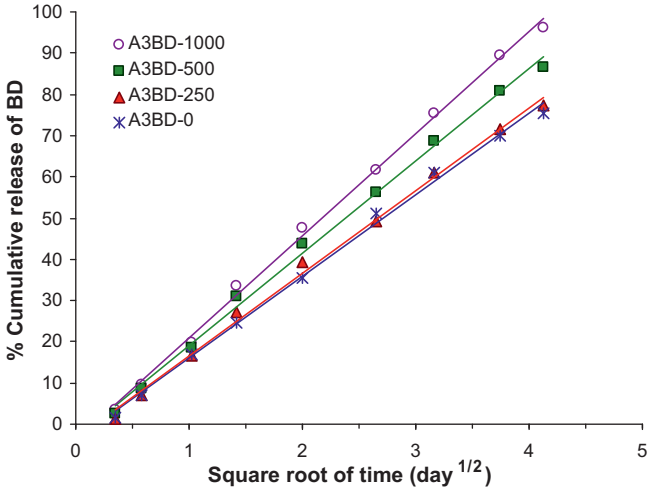

Fig. 6. A typical plot of the percentage blue dextran release from calcium alginate gel beads versus square root of time ($n = 3$; error bars = SD).

centration above 500 μg mL^{-1}, significantly increased the Higuchi release coefficient, ultimately by about 25%. The statistically significant differences in the Higuchi release coefficients are summarized below; CAG systems within brackets "{}" are not different significantly, whereas those separated by "<" are significantly different ($p < 0.05$).

{A3BD-0 ≈ A3BD-250} < A3BD-500 < A3BD-1000

A good fit to Korsmeyer–Peppas equation indicated combined effect of diffusion and erosion mechanisms for drug release (Korsmeyer et al.,1983). The value of release exponent 'n' determined from various formulations ranged from 0.56 to 0.58. The k value ranged from 0.166 to 0.207 (Table 2). The value of 'n' and k was found to increase with the increase in SF concentration. The CAG beads exhibited an anomalous (non-Fickian) diffusion controlled release.

In summary, the swellable CAG beads have been prepared and the effect of SF, a small molecule, on the diffusion and swelling properties of those beads was investigated. The increase in the concentration of SF in the soaking solution, above a threshold concentration of around 500 μg mL^{-1}, increased the release of BD but does not greatly affect the swelling of BD-loaded CAG beads. The release kinetics of BD from CAG beads followed the diffusion model and fit well to Higuchi's expression. The release rate from the beads can be slightly moderated by altering the concentration of SF. However, we think that the swelling behavior of the BD-loaded CAG beads was masked by the macromolecule. Therefore, more studies on the effect of macromolecule, itself, on the diffusion and swelling behaviors must be undertaken. These studies are in progress.

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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.
Fernandez-Hervas, M. J., Holgado, M. A., Fini, A., & Fell, J. T. (1998). In vitro evaluation of alginate beads of a diclofenac salt. *International Journal of Pharmaceutics*, *163*, 23–34.
Gombotz, W. R., & Wee, S. F. (1998). Protein release from alginate matrices. *Advanced Drug Delivery Review*, *31*, 267–285.
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.
Higuchi, T. (1963). Mechanism of sustained-action medication: Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *Journal of Pharmaceutical Science*, *52*, 1145–1149.
Kim, C. K., & Lee, E. J. (1992). The controlled release of blue dextran in from alginate beads. *International Journal of Pharmaceutics*, *79*, 11–19.
Korsmeyer, R. W., Gurny, R., Docler, E., Buri, P., & Peppas, N. A. (1983). Mechanism of solute release from porous hydrophilic polymers. *International Journal of Pharmaceutics*, *15*, 25–35.
Kudela, V. (1987). Hydrogels. *Encyclopedia of Polymer Science and Engineering* (pp. 703–807). New York: Wiley.
Morch, Y. A., Donati, I., Strand, B. L., & Skjaak-Braek, G. (2006). Effect of Ca^{2+}, Ba^{2+}, and Sr^{2+} on alginate microbeads. *Biomacromolecules*, *7*, 1471–1480.
Rasmussen, M. R., Snabe, T., & Pedersen, L. H. (2003). Numerical modelling of insulin and amyloglucosidase release from swelling Ca-alginate beads. *Journal of Controlled Release*, *91*, 395–405.
Sriamornsak, P., & Kennedy, R. A. (2010). Effect of a small molecule on diffusion and swelling properties of selected polysaccharide gel beads. *Carbohydrate Polymers*, *79*, 219–223.
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.