Surprising Performance of Alginate Beads for the Release of Low-Molecular-Weight Drugs

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ABSTRACT: The model of low-molecular-weight drugs has been encapsulated within alginate beads hardened with calcium chloride. The drug's release kinetic using 3% (w/v) alginate has shown a surprising behavior after 2 h, where the release kinetic was shifted from Fickian to case II transport mechanism contradicting other authors like Akihiko et al. (J Control Release 1999, 58, 21). To support this finding, we studied the swelling of dried gel beads of 2 and 3% (w/v) alginate, which showed a sudden decrease in the swelling of 3% (w/v) alginate after 2 h due to a partial bursting of the beads. This sudden bursting was clearly observed using the optical microscope to emphasize the new findings. Calcium alginate beads revealed pH sensitivity, where 2% (w/v) alginate beads showed a maximum swelling of 5000% in alkaline medium at pH 7.4, compared with a negligible swelling percent of 60% in acidic medium (pH 1.2). Accordingly, it could be a good candidate for targeting smart and low-molecular-weight drugs to the intestine. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 3021–3026, 2010

Key words: biopolymer; controlled intestinal drug release; hydrogel, release kinetics; low-molecular-weight drugs

INTRODUCTION

Drug delivery systems have been extensively studied over the last years, and polymers are now being studied as a method of controlling the release of drugs.[1] Biodegradable polymers are one of the key materials for these devices and have advantages over nondegradable implants. The main advantage is that biodegradable devices degrade and are absorbed by the body during and/or after drug release; this allows us to bypass the need for surgical removal of the device.[2] It is naturally an advantage if substances that are already permitted for use in the pharmaceutical or food industries can be utilized. Hydrogels, such as alginates, carrageenans, and chitosans, are polysaccharide families belonging to this category that are commercially available, have diverse applications,[3–5] and are available at a reasonable cost. Another advantage is to use drug carriers, which are easily prepared using a simple method such as alginate. Alginate is a water-soluble linear polysaccharide extracted from brown seaweed and is composed of alternating blocks of 1–4 linked α-L-guluronic and β-D-mannuronic acid residues. Alginate has the ability to form hydrogel in the presence of multivalent cations like Ca^{2+} in aqueous medium, and it shows excellent features such as immunogenecity, biocompatibility, bioadhesion, and nontoxicity. These features make it a very attractive biomaterial for use in many types of applications such as wound dressing, scaffold for tissue engineering, and pharmaceutical industries.[6]

Although many authors[7,8] have studied Ca–Alg beads as a matrix for drug delivery system, optimization of the preparation parameters was not clear; there are many conflicts between authors, and this was the main reason for doing this work. For example, Bajpai and Rasika[9] reported that using high alginate concentration 4% (w/v) gave stable beads for drug delivery system, whereas Arica et al.[10] reported that beads prepared with 1% (w/v) alginate sustained the release of 5-fluorouracil. Also, the concentration of $CaCl_2$ as a crosslinking agent showed various results. Gåserød et al.[11] reported that by increasing the concentration of $CaCl_2$ to 3% (w/v) should increase the porosity of beads, leading to higher diffusion of entrapped drug. While Mayur et al.[12] found that using 0.5% (w/v) of $CaCl_2$ gives weak gel because of insufficient crosslinking of

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alginate. Chong-Kook and Eun-Jin[13] reported that calcium ion content in the gel beads leveled off after 6 min of curing time in $CaCl_2$ solution, and there was little variation in the release of blue dextran from alginate gel beads cured for more than 6 min.

Thus, this work is aiming to unveil the contradiction between authors in certain issues as we are limited in space by the regulations of the journal. Such investigations could be exploring the hypothesis described previously by Akihiko et al.[2] that the release of low-molecular-weight drugs depends on diffusion through the pores of polymeric matrix. As there are wide varieties of low-molecular-weight drugs and it will be almost impossible to study all ranges of molecular weights, we used in this study brilliant blue (BB) as it has been used mainly in literature as a model of low-molecular-weight drugs.

MATERIALS AND METHODS

Materials

Alginic acid sodium salt from brown algae was purchased from Fluka (Germany), and brilliant blue R 250 (BB, M_w 825) was purchased from Aldrich (USA); anhydrous calcium chloride was purchased from Gen Lab (Egypt). All other reagents were of analytical grade and used as received.

Methods

As a general rule, all experiments were carried out in triplicate and data are means ± SD ($n = 3$).

Preparation of calcium alginate beads

Sodium alginate was dissolved in bidistilled water at various concentrations 1–4% (w/v), and then BB 25% (w/w) was added and suspended thoroughly by stirring. Three milliliters of this solution was dropped into a 15 mL of gelling solution through a disposable plastic syringe using a 23G needle at a dropping rate of 1 mL/min under mild agitation for various time 30–120 min. The gelling solution contained 0.5–5% (w/v) Ca^{2+}. The formed beads were collected, washed with 20 mL bidistilled water, and dried at room temperature for 24 h or used in the wet state.

Swelling study

Swelling studies were conducted using dry beads. The term dry refers to the state of the beads that were left to dry for 24 h in air till constant weight. Swelling studies of Ca–Alg beads were carried out in simulated intestinal fluid (SIF) of 10 m*M* phosphate buffer (pH 7.4) and in simulated gastric fluid of 10 m*M* HCl buffer (pH 1.2). Accurately weighed amount of beads was incubated in 25 mL of swelling solution at 37°C under shaking at 100 rpm. At predetermined time intervals, the beads were separated from the medium using a stainless steel grid. Immediately, they were wiped gently with filter paper and weighed. The swelling percent of the beads was calculated according to the formula:

$$\text{Swelling percent } \% = [(W_s - W_i)/W_i] \times 100, \quad (1)$$

where W_s is the weight of the beads in the swollen state and W_i is the initial weight of the beads.

In vitro release study

In vitro release study was used to simulate the gastrointestinal tract physiological conditions. For dissolution and drug release studies, the US Pharmacopoeia Paddle method II was used.[14] The release studies were performed in 10 m*M* phosphate buffer, pH 7.4. Accurately weighed amount of beads was placed in conical flasks containing 25 mL of the release medium. The samples were incubated at 37°C under shaking at 100 rpm. At predetermined time intervals, samples of 3 mL were withdrawn from the release medium and were replaced with fresh phosphate buffer solution. The concentration of BB in the solution was assayed by UV–vis spectrophotometer (Shimadzu) at 590 nm.

Morphology of the beads

The surface of the beads was examined using scanning electron microscopy (SEM, S-590, HITACHI). Before observation, samples were mounted on metal grids using double-sided adhesive tape and coated by gold under vacuum.

RESULTS AND DISCUSSION

Morphology of the beads

Scanning electron micrographs of air-dried Ca–Alg beads prepared with 3% (w/v) $CaCl_2$ and 1–3% (w/v) alginate concentration are illustrated in Figure 1. At 1% (w/v) alginate, the dry beads completely lost their spherical shape [Fig. 1(A)], and the surface showed highly roughness and large cracks [Fig. 1(B)] caused by collapsing the polymer layers during dehydration due to low mechanical strength of the gel.

By increasing the alginate concentration to 2% (w/v), beads remained its spherical shape [Fig. 1(C)], and the surface morphology was improved, but still showed large cracks [Fig. 1(D)]. Further increase in alginate concentration to 3% (w/v) increases the viscosity leading to nonspherical (elongated) beads

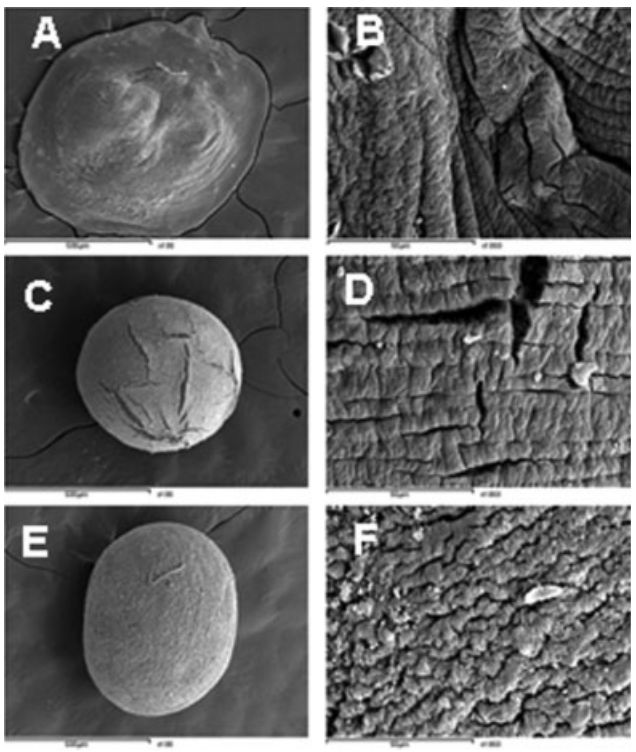

Figure 1 A–F: SEM micrographs of air-dried calcium alginate beads prepared with 3% $CaCl_2$ and different concentrations of alginate; 1% (w/v) alginate beads (A) and surface morphology (B); 2% (w/v) alginate beads (C) and surface morphology (D); 3% alginate beads (E) and surface morphology (F).

[Fig. 1(E)], which have smooth surface with smaller pores size due to the high gels beads mechanical strength [Fig. 1(F)]. These results indicate that the surface morphology of Ca–Alg beads improved by increasing alginate concentration, whereas increasing alginate concentration above 3% made preparation of the beads difficult because the solution becomes too viscous for dropping and nonspherical (elongated) beads were formed.

In vitro release study

As described previously,[2] the release of low-molecular-weight drugs depends on diffusion through pores, whereas high-molecular-weight drugs like protein release through swelling and disintegration of polymeric matrix. In our case, BB was used as model drug for low-molecular-weight drugs, so its release was expected to be controlled by diffusion mechanism; however, we noticed that the release profile obeyed another mechanism according to the preparation condition.

Effect of $CaCl_2$

Increasing Ca^{2+}concentration from 2 to 3% (w/v) and keeping the Alg concentration and curing time at 2% (w/v) and 30 min, respectively, decreased the release rate of BB in phosphate buffer, pH 7.4 (stimulated intestinal fluid), which is in agreement with the previous studies. For example, the release percent after 120 min was 66 and 84% using 3 and 2% (w/v) Ca^{2+}, respectively, as shown in Figure 2(A).

This can be explained by the fact that at higher concentration of Ca^{2+}, crosslinking of alginate was more efficient, so strong gel with smaller pores size was formed, which retarded penetration of dissolution medium into the beads, which in turn decreased the release rate. Also, the efficient crosslinking of alginate beads at higher Ca^{2+} retarded disintegration of beads, which take place due to the presence of phosphate ions in the buffer (phosphate buffer), which have a high affinity for Ca^{+2} ions. Dainty et al.[15] reported that the disruption of Ca–Alg beads occurred faster in phosphate buffer above pH 5.5 by chelating action of phosphate ions; at these higher pH values, the affinity of phosphate ions toward calcium ions is higher than that of alginate, and the solubility of calcium phosphate complex is high.

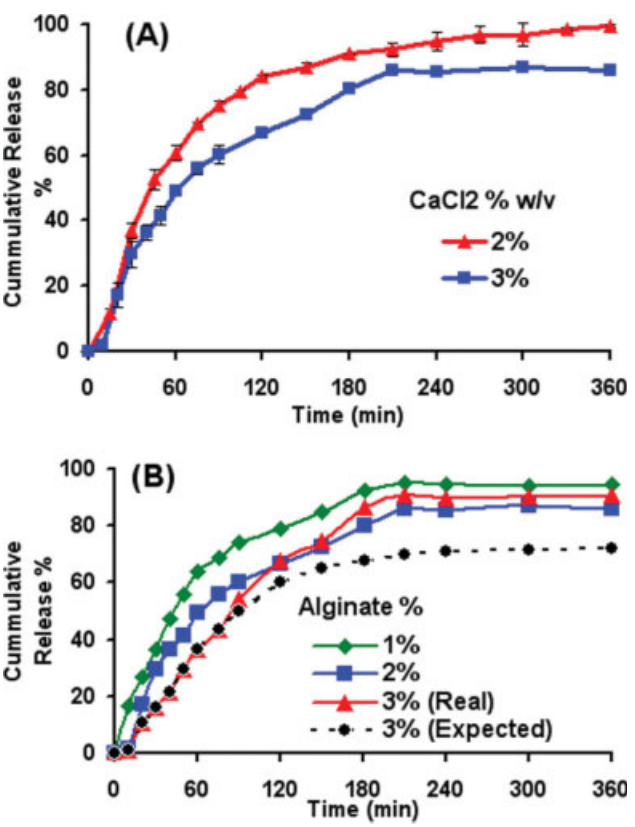

Figure 2 A, B: Effect of (A) $CaCl_2$ concentration and (B) alginate concentration on the release rate of BB in phosphate buffer, pH 7.4, at 37°C and 100 rpm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

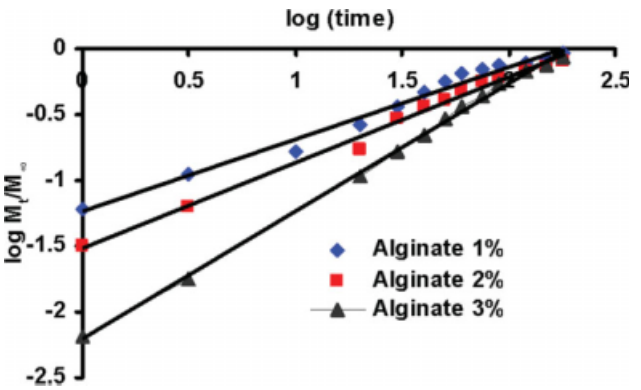

Figure 3 Release kinetics of brilliant blue from calcium alginate beads. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Effect of alginate concentration

Increasing the alginate concentration from 1 to 3% (w/v) at constant Ca^{2+} concentration and curing time of 3% (w/v) and 30 min, respectively, showed a significant effect on the release of BB in phosphate buffer, pH 7.4. The release rate could be divided into two sections: (a) before 2 h, the release of BB was found to follow this order: 1% > 2% > 3%, and (b) after 2 h, the release of BB was surprisingly as follows: 1% > 3% > 2% as shown in Figure 2(B).

For the release rate before 2 h, that is, 1% > 2% > 3%, it could be explained by the fact that increasing the alginate concentration increases the viscosity and provided more number of binding sites of alginate for Ca^{2+} ions resulting in the formation of a more stable and compact gel membrane with smaller pore size, so the penetration of dissolution medium into beads retarded and the release rate decreased.

This was also supported by studying the release kinetics as shown in Figure 3, where the release of drug from simple swellable polymeric matrix follows power law expression[16] as shown in eq. (3).

$$\mathrm{Log}[M_t/M_\infty] = \log K + n \log t, \qquad (2)$$

where $[M_t/M_\infty]$ is the drug released fraction at time t, K is a constant, and n is the release exponent.

There are three scenarios for n values:

1. If $n \leq 0.5$, the mechanism is called Fickian and the release is diffusionally controlled.
2. If $0.85 > n > 0.5$, the mechanism is called non-Fickian (anomalous), and the release is depending on both the diffusion and relaxation of the polymer.
3. If $n \geq 0.85$, the mechanism is called case II transport, and the release is depending only on the relaxation/swelling of the polymer.

The increase in Alg concentration from 1 to 3% (w/v) tended to increase the n values. Alginate concentration of 1% (w/v) showed $n = 0.55$ being close to Fickian mechanism due to large pore size and low mechanical strength of beads, which disintegrated rapidly so the release depended only on diffusion mechanism. Further increase of Alg concentration to 2% (w/v) increased n value to 0.64 following anomalous transport (non-Fickian) due to increase in the gel mechanical strength, so the release undergoes through diffusion and swelling (relaxation) of the polymer network. While using 3% (w/v) Alg, the n value increased to 0.97 shifting the release mechanism from anomalous transport to case II transport, which depended mainly on swelling/relaxation of the polymer network. These results were in contradiction of Akihiko et al.,[2] who stated that the release of low-molecular-weight drugs depends on diffusion through pores of polymeric matrix.

For the release rate after 2 h, that is, 1% > 3% > 2%, the results were unexpected as the release of 3% w/v alginate became faster than that of 2% w/v alginate. Figure 2(B) is showing that for 3% w/v alginate beads, after 240 min, the expected release was 70% and the real release was incredibly 90%. This unexpected behavior of release was elaborated by the swelling study as shown in Figure 4.

Swelling study

To understand the above unexpected release kinetics behavior, swelling study for Ca–Alg beads of 2 and 3% (w/v) Alg concentration was carried out in phosphate buffer, pH 7.4, as shown in Figure 4. Two observations were identified from the swelling study. The first was that the beads prepared with 3% (w/v) Alg showed lower swelling percent than that of 2% (w/v) Alg, which was in harmony with

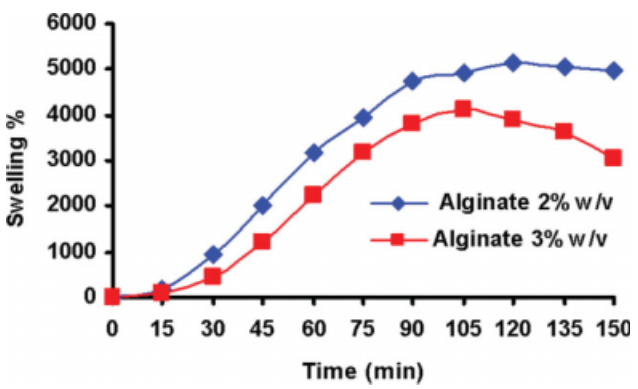

Figure 4 Effect of alginate concentration on swelling percent of dry beads in phosphate buffer, pH 7.4, at 37°C and 100 rpm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

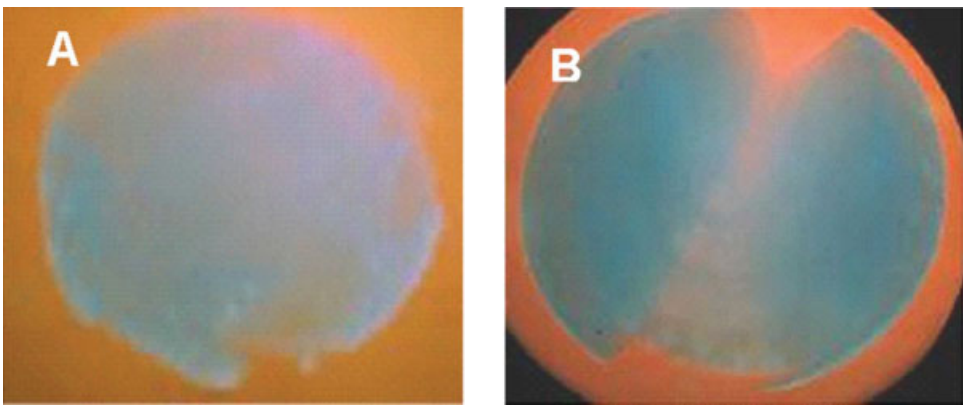

Figure 5 A, B: Optical images showing bursting of beads after incubation in SIF buffer. (A) 2% (w/v) alginate and (B) 3% (w/v) alginate. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the release results and release kinetics. The second was bursting and disruption of beads prepared with 3% (w/v) Alg after 2 h while no bursting in the case of 2% (w/v) Alg, which showed a decline in swelling percent after 2 h for 3% (w/v) Alg beads. The latter was supported by the optical images of the beads after incubation in the release medium as shown in Figure 5. This may be due to the higher osmotic pressure inside beads prepared with 3% (w/v) Alg compared to that prepared with 2% (w/v) Alg.

To target the BB to the gastrointestinal track, the swelling studies were carried out to monitor the swelling percent and disintegration of the dry beads in acidic medium, pH 1.2 in stimulated gastric fluid, and in alkaline medium, pH 7.4 in SIF, as shown in Figure 6. Calcium alginate beads exhibited nearly 5000% swelling percent in phosphate buffer, pH 7.4, at 120 min and then beads started to disintegrate. This was due to the hydration of the dried beads and the ion exchange mechanism between Na^+ and Ca^{2+} ions, which was explained previously.[17] The dried beads showed a very small swelling of 60% in HCl buffer without disintegration. This was due to the hydration of the hydrophilic groups of alginate.[9] These results indicated that Ca–Alg beads have a resistance toward acidic medium, whereas it showed highly swelling percent and disintegration in alkaline medium. This phenomenon can be exploited in targeting the release of low-molecular-weight drugs to the intestinal region.

CONCLUSIONS

The optimum conditions for preparing alginate beads based on calcium chloride were studied inclusively. The results showed that Ca–Alg beads prepared using 2% (w/v) alginate and hardened with 3% (w/v) $CaCl_2$ and cured for 30 min are showing the most suitable conditions for controlled BB release. The BB release was following anomalous mechanism, which is in contradiction to that of Akihiko et al. The release kinetic was supported by the swelling of the gel at 2 and 3% (w/v) Alg and their optical image. We concluded that the use of higher alginate concentration could (i) increase the mechanical strength of beads, (ii) increase the encapsulation efficiency, and (iii) delay the drug release. However, the use of highly viscous alginate solution may create some other complication such as (i) nonhomogenous crosslinking of beads as highly viscous medium of droplet could retard the diffusion of Ca^{+2} ions into the interior of beads, (ii) inability to use hypodermic syringe to prepare smaller beads, where the size of dried beads was increased from 544 to 638 μm with increasing Alg concentration from 2 to 3% (w/v), respectively, (iii) improper mixing of drug with alginate solution, and (iv) bursting of beads due to the high osmotic pressure. Ca–Alg beads of 2% (w/v) were found to be an excellent carrier for targeting BB to the intestine, where the swelling of the beads was 5000% in alkaline medium compared with 60% in acidic medium. Overall, this work could be a concrete guide for other authors

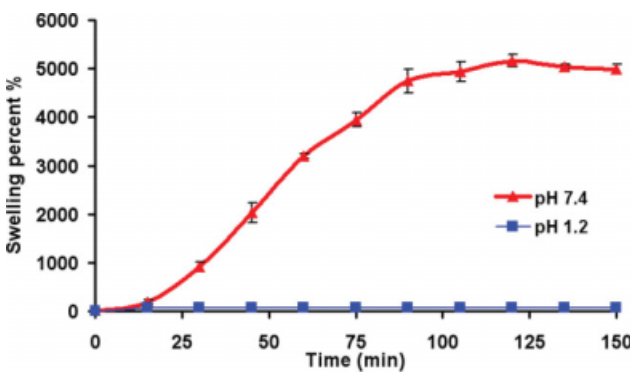

Figure 6 Swelling percent of dried beads in HCl buffer, pH 1.2, and phosphate buffer, pH 7.4, at 37°C and 100 rpm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

who are working with calcium alginate beads and the carrier, and the method of optimization could be used for targeting low-molecular-weight drugs to the intestine.

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