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ScienceDirect
Carbohydrate Polymers 71 (2008) 566–573
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Characterization of calcium alginate and chitosan-treated calcium alginate gel beads entrapping allyl isothiocyanate

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Received 31 March 2007; received in revised form 17 June 2007; accepted 29 June 2007
Available online 15 August 2007

Abstract

Calcium alginate (CA), chitosan-coated calcium alginate (CCA-I), and chitosan–calcium alginate complex (CCA-II) gel beads, in which an oil-in-water emulsion containing allyl isothiocyanate (AITC) was entrapped, were prepared and characterized for efficient oral delivery of AITC. The AITC entrapment efficiency was 81% for CA gel beads, whereas about 30% lower values were determined for the chitosan-treated gel beads. Swelling studies showed that all the gel beads suddenly shrunk in simulated gastric fluid (pH 1.2). In simulated intestinal fluid (pH 7.4), CA and CCA-I gel beads rapidly disintegrated, whereas CCA-II gel beads highly swelled without degradation probably due to the strong chitosan–alginate complexation. Release studies revealed that most entrapped AITC was released during the shrinkage, degradation, or swelling of the gel beads, and the chitosan treatments, especially the chitosan–alginate complexation, were effective in suppressing the release. CCA-II gel beads showed the highest bead stability and AITC retention under simulated gastrointestinal pH conditions.

Keywords: Calcium alginate; Chitosan; Allyl isothiocyanate; Swelling; Release

1. Introduction

Ally isothiocyanate (AITC), a major essential oil component of cruciferous plants such as cabbage, broccoli, mustard, and horseradish, has long been used as a pungent food flavoring agent (Pecháček, Velíšek, & Hrabcová, 1997). In addition to its outstanding antimicrobial activity (Delaquis & Sholberg, 1997), AITC has been known to possess a high chemopreventive activity (Keum, Jeong, & Kong, 2004; Zhang, Li, & Tang, 2005). Recent studies showed that AITC can inhibit the proliferation of human prostate cancer cells by inducing apoptosis (Xiao et al., 2003) and suppress metastasis in human hepatoma cells (Hwang & Lee, 2006). These suggest that AITC could be used as a key component in the formulation of a cancer-preventive nutraceutical food product. However, the application of AITC in food systems has been limited because of its high volatility, strong pungency, poor water-solubility, and easy decomposition by reactions with many natural food nucleophiles such as amines, amino acids, proteins, alcohols, water, and sulfites (Cejpek, Urban, Velíšek, & Hrabcová, 1998, 2000; Chacon, Buffo, & Holley, 2006; Li, Jin, & Wang, 2007).

These limitations could be effectively overcome by physically entrapping AITC molecules within an inert biopolymer matrix. The inclusion complexation of AITC with α- and β-cyclodextrins was reported to retard the decomposition of AITC in aqueous solutions (Ohta, Matsui, Osawa, & Kawakishi, 2004; Ohta, Takatani, & Kawakishi, 1999, 2004). The release of AITC from dry powders of the inclusion complexes into air was also examined at different relative humidities (Li et al., 2007). Chacon et al. (2006) incorporated AITC within a gum acacia matrix using a traditional flavor microencapsulation technique to suppress its high volatility and pungency. For a nutraceutical

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doi:10.1016/j.carbpol.2007.06.028

product, a gel bead system based on natural polysaccharides, which has been widely accepted as a safe and inert oral drug dosage form, could be employed as an effective matrix. Such polysaccharide gel bead system could not only reduce the limitations associated with the application of AITC, but also release the entrapped AITC into digestive track in a controlled manner so that AITC can be more efficiently absorbed by human body. However, little progress has been reported on the AITC-entrapped polysaccharide gel bead system.

Calcium alginate gel beads, formed by calcium-induced ionotropic gelation of alginate, have been extensively used for the oral delivery of a wide range of bioactive proteins and drugs (Bajpai & Tankhiwale, 2006; Pasparakis & Bouropoulos, 2006; Wong, Chan, Kho, & Heng, 2002). Alginate, a polyanionic linear copolymer of 1,4-linked-α-L-guluronic acid and β-D-mannuronic acid residues found in brown seaweeds, has been regarded as an excellent polysaccharide for gel bead system because of its unique features such as biocompatibility, biodegradability, immunogenecity, and non-toxicity. The main drawbacks of calcium alginate gel beads are their macroporous structure and possible rapid dissolution at intestinal pH, which may cause low entrapment efficiency and sudden release of core substances (George & Abraham, 2006; Wong et al., 2002). In order to improve the permeability and stability of calcium alginate gel beads, chitosan, which is insoluble at intestinal pH, has been intensively employed as a supporting polysaccharide (Bajpai & Tankhiwale, 2006; Pasparakis & Bouropoulos, 2006; Peniche, Howland, Carrillo, Zaldívar, & Argüelles-Monal, 2004; Shu & Zhu, 2002; Wong et al., 2002). Chitosan, a polycationic linear copolymer of β-(1-4)-linked *N*-acetylglucosamine and glucosamine, is also biocompatible, biodegradable, and non-toxic. Chitosan has been either coated on the surface of calcium alginate gel beads or complexed with alginate for the bulk modification of bead structure via electrostatic interaction between its amino terminals and the carboxyl residues of alginate.

The objective of this study was to explore the potential of calcium alginate and chitosan-based gel bead system as a device for efficient oral delivery of AITC. Three types of gel beads, calcium alginate, chitosan-coated calcium alginate, and chitosan–calcium alginate complex gel beads, were prepared and characterized by examining and comparing their AITC entrapment efficiencies, swelling properties, and AITC release behaviors.

2. Materials and methods

2.1. Materials

Allyl isothiocyanate (AITC, $CH_2{=}CH{-}CH_2{-}N{=}C{=}S$, molecular weight = 99.16, >98% purity) was purchased from Fluka (Steinheim, Germany). Sodium alginate of about 0.8 mannuronic acid to guluronic acid ratio and chitosan of 75–85% deacetylation degree were supplied by Kanto Chemical Co., Inc. (Tokyo, Japan) and Showa Chemical Co. Ltd. (Tokyo, Japan), respectively. The viscosity average molecular weights (M_v) of alginate and chitosan were determined using the following Mark–Houwink equation according to Mancini, Moresi, and Sappino (1996) and Basavaraju, Damappa, and Rai (2006), respectively.

$$[\eta] = KM_v^{\alpha} \quad (1)$$

where $[\eta]$ is the intrinsic viscosity of biopolymer, $K = 1.228 \times 10^{-4}$ for alginate and 3.5×10^{-4} for chitosan, and $\alpha = 0.963$ for alginate and 0.76 for chitosan. The determined M_v values were 6.6 and 9.6 kDa for alginate and chitosan, respectively. Twin 80 and Tris–base were obtained from Bio Science Inc. (East Markham, Ontario, Canada). Sodium tripolyphosphate and olive oil were purchased from Junsei Chemical Co. Ltd. (Tokyo, Japan) and Sam Chun Chemical Co. Ltd. (Gyeonggi, Korea), respectively. All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of gel beads

Olive oil containing 2.5% (w/w) of AITC was mixed with an aqueous solution of sodium alginate in a gas-tight bottle to form an oil-in-water emulsion consisting of 20% (w/w) olive oil, 0.5% (w/w) Tween 80, and 1% (w/w) sodium alginate. The emulsion was heated at 60 °C for 10 min, cooled to room temperature, and homogenized at 10,000 rpm for 1 min. Calcium alginate (CA) gel beads were formed by adding 30 mL of the emulsion dropwise into 90 mL of 0.5 M $CaCl_2$ solution through a 21 gauge needle using a peristaltic pump (Masterflex pump drive 7520-57, pump head 77200-60, Cole Parmer Instrument Co., Vernon Hills, IL, USA) at a drop rate of 4 mL/min under mild agitation at room temperature. The CA gel beads were cured in the $CaCl_2$ solution with gentle stirring for 30 min and then washed with distilled water by suction filtration.

Chitosan-coated calcium alginate (CCA-I) gel beads were prepared according to the modified method of Peniche et al. (2004). The CA gel beads prepared above were immersed in 30 mL of 0.5% (w/w) chitosan solution in 0.5% (v/v) aqueous acetic acid under gentle stirring for 10 min at room temperature. The beads were then transferred into 30 mL of 0.5 M $CaCl_2$ solution and mildly agitated for another 10 min to remove the unbounded chitosan from the bead surface without calcium loss. The CCA-I gel beads obtained were washed with distilled water by suction filtration.

Chitosan–calcium alginate complex (CCA-II) gel beads were prepared using the method described by Wong et al. (2002) with slight modifications. A volume of 30 mL of the AITC-contained oil-in-water emulsion prepared as described above was dropped into 90 mL of 0.5% acetic acid solution containing 0.5% chitosan and 0.5 M $CaCl_2$ using the peristaltic pump system. After 30-min curing, the formed beads were washed with distilled water,

followed by 4% (w/v) sodium tripolyphosphate (TPP) solution, and finally with distilled water by suction filtration. TPP, a non-toxic polyanion, was employed because it is one of the commonly used cross-linkers for the fast ionotripic gelation of chitosan (George & Abraham, 2006). The mean diameter of the beads was determined by measuring 20 beads using an optical microscopic system (model SZ-ST, Olympus, Tokyo, Japan) equipped with a digital camera (model 303, Olympus, Tokyo, Japan). The moisture content (wet-basis) of the beads was determined according to the method of AOAC (1995).

2.3. Determination of entrapment efficiency

Accurately weighed amounts (about 0.5 g) of the beads were dispersed and then dissolved by stirring in the mixture of 20 mL of 0.1 M phosphate-buffered saline (PBS, pH 7.4) and 5 mL of hexane in a 40 mL gas-tight vial for 5 h at room temperature, followed by heating for 15 min at 60 °C in a water bath. The mixture was placed at refrigerated temperature for 6 h until complete phase separation. The hexane fraction of the mixture was analyzed for AITC with a gas chromatograph (Clarus 500, Perkin-Elmer Inc., Las Vegas, NV, USA) equipped with a flame ionization detector (FID) and a HP-Innowax capillary column (30 m × 0.32 mm i.d., 0.5-μm film thickness, Agilent Technologies, Inc., Palo Alto, CA, USA). The oven temperature was programmed from 50 to 250 °C at a rate of 15 °C/min with initial and final holding times of 2 and 5 min, respectively. The flow rate of nitrogen carrier gas and the split ratio were 3.5 mL/min and 6:1, respectively. The temperatures of injection port and detector were 250 and 260 °C, respectively. The standard solutions of AITC were prepared in the mixture of 0.1 M PBS/hexane (5:1, v/v). The AITC entrapment efficiency of the beads was determined as follows:

$$\text{Entrapment efficiency } (\%) = \frac{M_a}{M_{th}} \times 100 \quad (2)$$

where M_a is the actual amount of AITC entrapped in the beads and M_{th} is the theoretical amount of AITC entrapped in the beads, calculated by assuming that all AITC molecules are entrapped within the beads without any loss during the preparation procedure.

2.4. Swelling studies

Swelling studies of the beads were carried out with three aqueous media: 0.1 M HCl solution of pH 1.2 as a simulated gastric fluid (SGF), 0.1 M Tris–HCl/PBS solution of pH 7.4 as a simulated intestinal fluid (SIF), and 0.1 M NaCl solution adjusted to pH 7.4. Accurately weighed amounts (about 0.5 g) of the beads were immersed in 20 mL of each medium in 40 mL gas-tight vials and incubated at 37 °C in a shaking water bath at 60 rpm. The incubated vials were sampled periodically and the beads in the vials were weighed after suction filtration. The weight change of the beads with respect to time was determined as follows:

$$\%\ \text{Weight change} = \frac{W_t - W_0}{W_0} \times 100 \quad (3)$$

where W_0 is the initial mean weight of the beads and W_t is the mean weight of the beads at a given time t. For the investigation of bead swelling in simulated gastrointestinal pH conditions, the beads were immersed in SGF for 4 h and treated subsequently with SIF for 8 h at 37 °C and 60 rpm according to Peniche et al. (2004).

The morphology of the gel beads treated with SGF for 4 h, SIF for 10 min, or 0.1 M NaCl solution for 6 h at 37 °C as well as of untreated beads was examined with a scanning electron microscope (SEM, model S-300N, Hitachi Science System Ltd., Tokyo, Japan). The sample beads were carefully freeze-dried up to the point where the beads were acceptably maintained in their original shape and dehydrated enough to be micrographed by SEM, and then mounted on a sample holder. The surfaces of the beads were coated with gold to a thickness of about 30 nm using a sputter coater (model E-1010, Hitachi Science System Ltd., Tokyo, Japan).

2.5. Release studies

After being accurately weighed (about 0.5 g), the beads were placed in 40 mL gas-tight vials containing 20 mL of SGF or SIF, followed by incubation at 37 °C in a shaking water bath at 60 rpm. The incubated vials were sampled periodically and the media in the vials were replaced by the mixture of 20 mL of 0.1 M PBS and 5 mL of hexane. The beads were completely dissolved in the mixture by agitation for 5 h at room temperature, followed by heating for 15 min at 60 °C in a water bath. The mixture was kept at refrigerated temperature for 6 h until complete phase separation. The hexane layer of the mixture was analyzed for AITC with the gas chromatographic system to measure the amount of AITC remaining in the beads. The fractional release of AITC with respect to time was determined as follows:

$$\text{Fractional release } (\%) = \frac{M_0 - M_t}{M_0} \times 100 \quad (4)$$

where M_0 is the amount of AITC initially entrapped in the beads and M_t is the amount of AITC remaining in the beads at a given time t. The release study was also carried out under the same varying pH conditions as described for the swelling study.

2.6. Statistical analysis

All the experiments were performed in triplicate. All data were expressed as means ± standard deviation (SD). The significance of differences in AITC entrapment efficiency and initial bead properties among the three types of beads was determined by one-way analysis of variance

(ANOVA), followed by Duncan's multiple comparison test ($p \leqslant 0.05$).

3. Results and discussion

3.1. Entrapment efficiency and initial properties of beads

The entrapment efficiency, moisture content, mean weight, and mean diameter of CA, CCA-I, and CCA-II gel beads were determined (Table 1). CA gel beads showed about 1.4-fold higher AITC entrapment efficiency (81%) than the chitosan-treated CCA-I and CCA-II gel beads (about 56%). More AITC might be lost during the preparation of CCA-I and CCA-II gel beads, compared to CA gel beads, because of the longer preparation procedures employed for the chitosan-treated gel beads. Furthermore, CCA-I and CCA-II gel beads may have less void volume available for the entrapment than CA gel beads due to their denser network structures. The bead densities calculated from the mean weights and diameters in Table 1 by assuming spherical bead morphology are 0.74, 0.79, and 0.78 mg/mm^3 for CA, CCA-I, and CCA-II gel beads, respectively, indicating that CCA-I and CCA-II gel beads are denser than CA gel beads. The electrostatic interaction between chitosan and alginate might render the network structure of CCA-I and CCA-II gel beads more entangled (Pasparakis & Bouropoulos, 2006). Interestingly, the moisture contents (about 62%) of the gel beads were determined similar regardless of bead density (Table 1), suggesting that the oil phase of entrapped emulsion was more squeezed out of the denser chitosan-treated gel beads than out of CA gel beads. The stability of the emulsion prepared in the present study is likely diminished during the entrapment processes due to the squeezing pressure generated by the formation of gel structure as well as different compatibilities of oil and water phases with the hydrophilic polysaccharides. This might cause the oil phase, which is incompatible with the biopolymers, to be more pressed from the denser gel structure. Since most AITC molecules are dissolved in oil phase, the above explanation would also support why the denser gel beads had lower AITC entrapment efficiency.

3.2. Swelling of beads

The swelling behavior of the gel beads in SGF (pH 1.2), SIF (pH 7.4), and 0.1 M NaCl solution (pH 7.4) at the physiological temperature of 37 °C is shown in Fig. 1. In the acidic environment of SGF, the weights of all types of beads suddenly decreased by about 30% during the initial 30-min incubation while decreased marginally during the subsequent period of incubation (Fig. 1a). The SEM micrographs of the gel beads treated in SGF for 4 h at 37 °C show that all the three types of gel beads shrunk in a similar spherical shape without notable degradation of bead matrix (Fig. 2). The results indicate that the weight reduction of the gel beads in SGF is mainly caused by the shrinkage of bead matrix. When CA gel beads are exposed to pH less than 4.0, the cross-linking calcium ions are displaced from the alginate network and the carboxyl residues of alginate are protonized to form water-insoluble alginic acid (Østberg, Lund, & Graffner, 1994; Pasparakis & Bouropoulos, 2006). This may lead to the disruption of ionic linkages with little electrostatic repulsion, resulting in the shrinkage of CA gel bead matrix. The chitosan on the surface of CCA-I gel beads becomes highly soluble in acidic SGF, and its positively charged amino groups weakly interact with the protonated carboxyl groups of alginate (Pasparakis & Bouropoulos, 2006). This may cause the dissolution of the chitosan coating layer of CCA-I gel beads during the incubation, and thus the shrinkage of alginate matrix is favored. The gel matrix of CCA-II gel beads is formed mainly via three types of ionic interactions; (1) calcium-mediated cross-linking of alginate, (2) electrostatic interaction between chitosan and alginate, and (3) TPP-mediated cross-linking of chitosan. Under acidic condition, the calcium alginate linkage and the chitosan–alginate interaction are disrupted due to the reasons described above, and the electrostatic repulsion between the positively charged amino residues of chitosan may be weak because of the presence of TPP. This is probably why shrinking behavior is also predominant in the CCA-II gel beads exposed to SGF.

In the alkaline environment of SIF, the weights of CA and CCA-I gel beads increased by about 27% and 60%, respectively, for the initial 30 min, and then were sharply reduced to about 10% and 30% of their initial values, respectively, for the next 1.5-h incubation (Fig. 1b). On the other hand, the weight of CCA-II gel beads increased by about 110% and reached a plateau after 2-h incubation. It is seen from the SEM micrographs taken after treating the beads in SIF for 10 min at 37 °C that CA gel beads were seriously destroyed and CCA-I gel beads were dam-

Table 1
Entrapment efficiency, moisture content, mean weight, and mean diameter of CA, CCA-I, and CCA-II gel beads[a]

	CA gel beads[b]	CCA-I gel beads[c]	CCA-II gel beads[d]
Entrapment efficiency (%)	80.9 ± 5.2 a	56.6 ± 4.3 b	56.2 ± 4.8 b
Moisture content (%)	62.8 ± 1.3 a	62.4 ± 1.8 a	61.7 ± 1.1 a
Mean weight (mg/bead)	7.74 ± 0.12 a, b	7.96 ± 0.22 a	7.55 ± 0.25 b
Mean diameter (mm)	2.71 ± 0.10 a	2.68 ± 0.07 a	2.64 ± 0.08 a

[a] Different letters within a row indicate significant differences ($p \leqslant 0.05$).
[b] Calcium alginate gel beads.
[c] Chitosan-coated calcium alginate gel beads.
[d] Chitosan–calcium alginate complex gel beads.

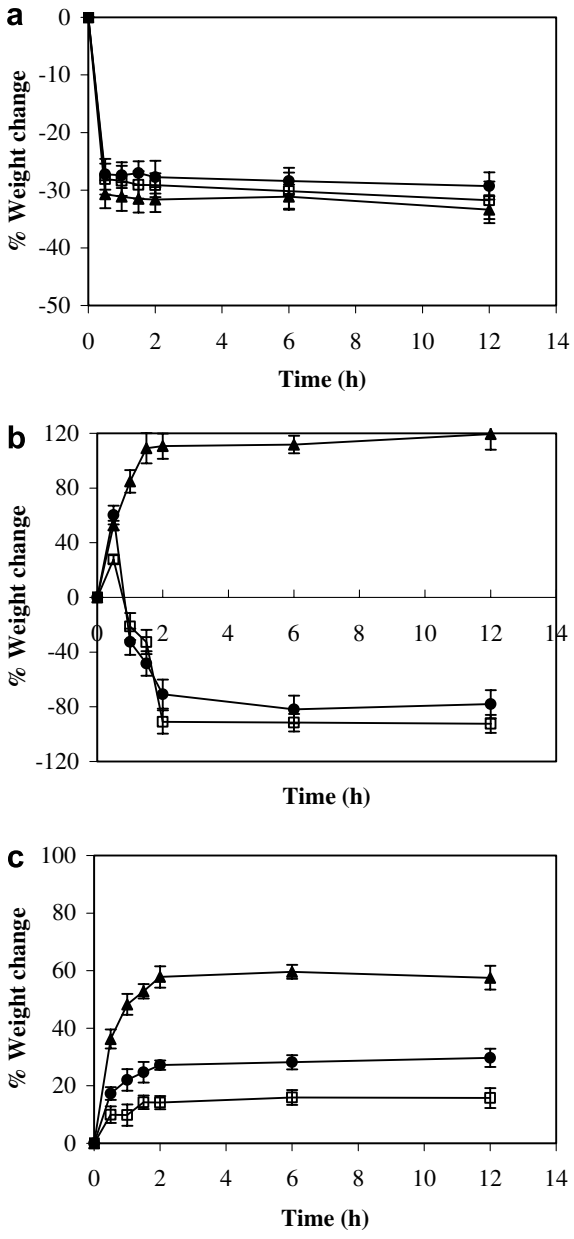

Fig. 1. Swelling of CA (□), CCA-I (●), and CCA-II (▲) gel beads in SGF of pH 1.2 (a), SIF of pH 7.4 (b), and 0.1 M NaCl solution of pH 7.4 (c) at 37 °C. Values are means ± standard deviation of three experiments.

aged and stuck together (Fig. 2). For CCA-II gel beads, no notable disintegration was observed in the micrographs, and it was visually observed that the spherical shape of CCA-II gel beads was well retained even after 12-h incubation. The results indicate that the CA and CCA-I gel beads exposed to SIF initially swell, although bead degradation occurs simultaneously, and then rapidly disintegrate. The swelling and subsequent degradation of CA gel beads in SIF are mainly caused by two reasons; (1) displacement of cross-linking calcium ions by sodium ions and (2) sequestering effect of phosphate on calcium ions, which render the calcium alginate gel structure loose and soluble (Bajpai & Sharma, 2004; Østberg et al., 1994). The twofold larger initial swelling of CCA-I gel beads compared to CA gel beads, observed after 30-min incubation, is probably attributed to the shielding effect of insoluble chitosan coating layer on swollen calcium alginate core matrix. However, the effect was not strong enough to prevent or retard bead degradation. The matrix of CCA-II gel beads becomes loose in SIF because of the following reasons; (1) the calcium-mediated alginate cross-linking is disrupted due to the calcium leakage, (2) the electrostatic interaction between chitosan and alginate is weak at pH 7.4 because the pK_a of alginate is from 3.38 to 3.65 and the pK_a of chitosan is around 6.3 (Bajpai & Tankhiwale, 2006; Liu et al., 2004; Pasparakis & Bouropoulos, 2006), and (3) the TPP-mediated chitosan cross-linking may be weak due to the deprotonation of chitosan amino groups. In spite of the looseness of bead structure, the alginate chains of CCA-II gel beads seem to be strongly entangled with the insoluble chitosan chains so that CCA-II gel beads can highly swell in SIF without degradation.

The beads exposed to 0.1 M NaCl solution of pH 7.4 showed different swelling profiles compared to those in SIF of the same pH. The weights of CA and CCA-I gel beads did not decrease but increased by 14% and 27%, respectively, during the initial 2-h incubation, and then remained almost constant (Fig. 1c). The weight gain of CCA-II gel beads for the initial 2 h was 58%, which was the half of the value measured in SIF. The SEM micrographs taken after 6-h incubation of the beads in the NaCl solution at 37 °C show that all types of beads swelled without degradation (Fig. 2). The results indicate that the beads are more stable in the NaCl solution than in SIF at the same pH of 7.4. This may be because the leakage of cross-linking calcium ions is less in the NaCl solution than in SIF due to the absence of calcium-chelating phosphate.

3.3. Release of AITC

Fig. 3 shows the release profiles of entrapped AITC in SGF and SIF at 37 °C. In acidic SGF, all types of beads showed a similar mode of release, in which the release was initially rapid, slowed down, and then reached a plateau after 2 h, although the level of release differed depending on the type of beads (Fig. 3a). The comparison of Fig. 3a with Fig. 1a reveals that the release is almost coincident with the bead shrinkage, suggesting that the primary driving force for the AITC release in SGF is the squeezing pressure induced by bead shrinkage. The cease of release after 2 h of incubation is probably because the matrix of shrunken beads is dense enough to prevent the migration of AITC molecules. The low water-solubility of AITC (Chacon et al., 2006) may also limit its diffusion out of the hydrophilic bead matrix into aqueous SGF. At any time of incubation in SGF, the level of fractional release was higher in the order of CA, CCA-II, and CCA-I gel beads for which the plateau release levels were measured to be 58%, 45%, and 32%, respectively (Fig. 3a). The lower release levels for CCA-I and CCA-II gel beads compared to CA gel beads indicate that the release from plain calcium

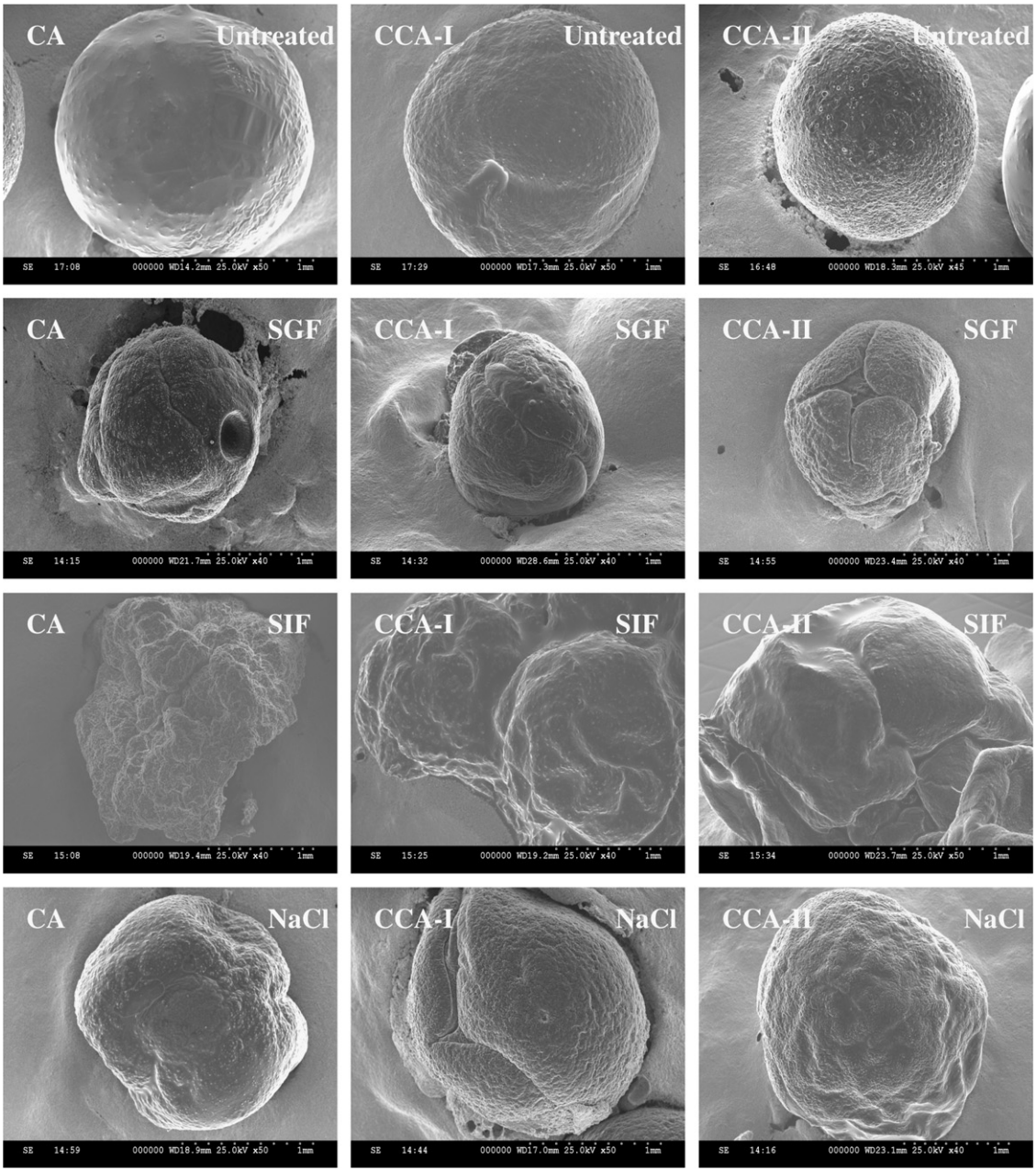

Fig. 2. SEM micrographs of CA, CCA-I, and CCA-II gel beads treated with SGF (pH 1.2) for 4 h, SIF (pH 7.4) for 10 min, or 0.1 M NaCl solution (pH 7.4) for 6 h at 37 °C.

alginate gel beads can be further suppressed by coating the bead surface with chitosan or modifying the bulk bead structure via chitosan–alginate complexation. However, the chitosan treatments were not effective enough to retard the rapid release occurring at the early stage of incubation. The lower release level for CCA-I gel beads compared to CCA-II gel beads could be attributed to the slightly less shrinkage of CCA-I gel beads in SGF (Fig. 1a).

In alkaline SIF, the three types of beads showed completely different patterns of release (Fig. 3b). It is seen from the comparison of Fig. 3b with Figs. 1b and 2 that the release in SIF is also closely related to the swelling behavior of the beads. From CA gel beads, most of entrapped AITC was released in 2 h because of the almost complete bead degradation in SIF. From CCA-I gel beads, about 70% of entrapped AITC was released in 2 h due to the degradation of core alginate gel matrix. However, only about 15% of entrapped AITC was further released for the next 10 h of incubation and complete release was not attained, probably because of the retention of AITC molecules within the matrix of insoluble chitosan shell. The release from CCA-II gel beads was markedly lower than those from the other two types of beads. CCA-II gel beads exposed to SIF did not disintegrate but highly swelled especially for the initial 2 h, as shown in Figs. 1b and 2, during which about 20% of entrapped AITC migrated out of the porous

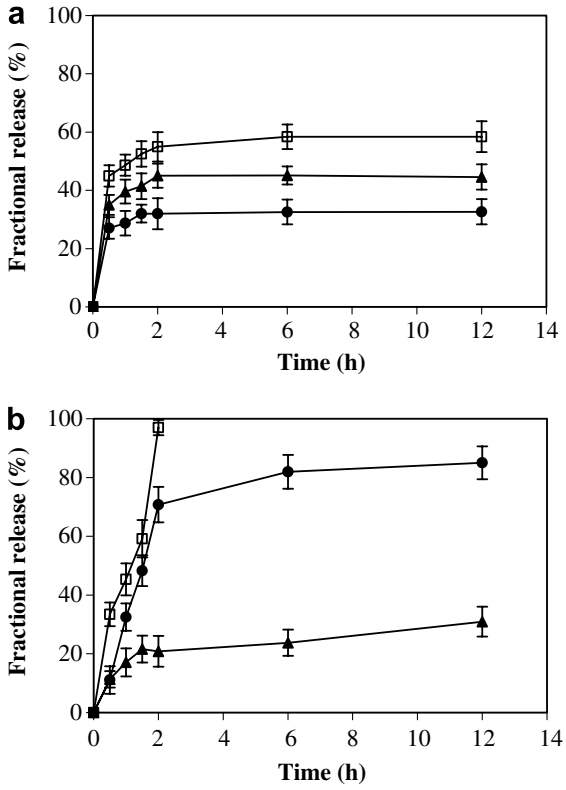

Fig. 3. Release of AITC from CA (□), CCA-I (●), and CCA-II (▲) gel beads in SGF of pH 1.2 (a) and SIF of pH 7.4 (b) at 37 °C. Values are means ± standard deviation of three experiments.

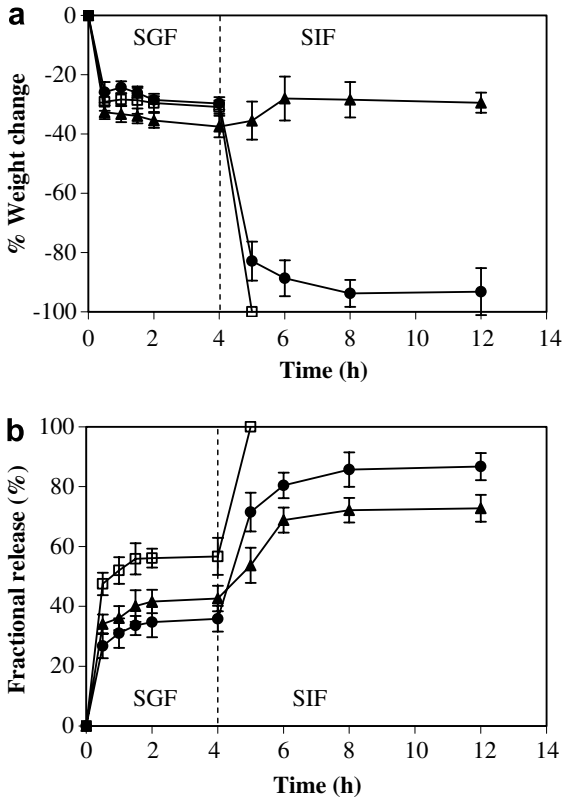

Fig. 4. Swelling (a) and AITC release (b) for CA (□), CCA-I (●), and CCA-II (▲) gel beads exposed to SGF (pH 1.2) for 4 h followed by SIF (pH 7.4) for 8 h at 37 °C. Values are means ± standard deviation of three experiments.

swollen beads. For the subsequent 10 h, the bead swelling was minimal and only about 10% of entrapped AITC was further released.

3.4. Bead swelling and AITC release under varying pH conditions

Fig. 4a shows the swelling profiles of the gel beads in simulated gastrointestinal pH conditions at 37 °C. When being transferred into SIF after 4-h incubation in SGF, CA gel beads disintegrated completely within only 1 h, while the beads treated only with SIF initially swelled and then mostly degraded after 2 h (Fig. 1b). This indicates that the CA gel beads pretreated with SGF are more susceptible to degradation in SIF than the untreated CA gel beads. The alginate molecules forming the gel beads undergo proton-catalyzed hydrolysis in acidic SGF and thus the molecular weight of alginate is reduced, resulting in faster degradation when the beads are reequilibrated in alkaline SIF (Gombotz & Wee, 1998; Mumper, Hoffman, Puolakkainen, Bouchard, & Gombotz, 1994). The proton-catalyzed hydrolysis of alginate could be also responsible for the faster and larger degradation of SGF-pretreated CCA-I gel beads in SIF compared to the untreated CCA-I gel beads. However, complete bead degradation was not observed, suggesting that the chitosan coating is not fully dissolved during the 4-h incubation in SGF. Interestingly, the shrunken CCA-II gel beads in SGF swelled by about 10% in 2 h after being transferred into SIF, and then remained without degradation. In spite of the alginate hydrolysis in SGF, the entanglement of alginate and chitosan chains in the matrix of shrunken CCA-II gel beads appears to be strong enough to prevent the bead degradation in SIF.

Fig. 4b illustrates the release profiles of entrapped AITC under the same varying pH conditions. The comparison of Fig. 4a and b shows that the release is also closely associated with the bead swelling behavior. The CA gel beads transferred from SGF to SIF quickly released all the remaining 40% of entrapped AITC in 1 h because of their fast and complete disintegration in SIF. When CCA-I gel beads were transferred from SGF into SIF, the fractional release rapidly increased from about 35% to 70% for 1 h due to the fast bead degradation and then slowly increased up to 87%. Complete release was not attained probably because some AITC molecules were entrapped in the remaining chitosan matrix. The fractional release for CCA-II gel beads increased from 43% to 69% for 2 h after the transfer into SIF, during which time the swelling of beads occurred, followed by slight increase up to 73%. The results indicate that AITC molecules were better retained in CCA-II gel beads than in CA or CCA-I gel beads under the current simulated gastrointestinal pH conditions.

4. Conclusions

The entrapment efficiency of AITC was determined to be 81%, 57%, and 56% for CA, CCA-I, and CCA-II gel beads, respectively. The longer preparation procedure and denser network structure of CCA-I and CCA-II gel beads compared to CA gel beads may be responsible for their lower entrapment efficiency. The chitosan treatments had little effect on preventing the sudden initial shrinkage of gel beads in acidic SGF. The degradation of gel beads in alkaline SIF was hardly prevented or retarded by the chitosan coating, whereas completely prevented by the chitosan–alginate complexation although a high bead swelling was induced. Most of the release of entrapped AITC occurred during the shrinkage, degradation, or swelling of the gel beads, and the diffusional release of AITC out of the shrunken or swollen beads was relatively small, suggesting that the bead swelling behavior is the primary cause of the AITC release. The chitosan treatments, especially the chitosan–alginate complexation, were effective in suppressing the release in both SGF and SIF. CCA-II gel beads showed the highest bead stability and AITC retention under simulated gastrointestinal pH conditions. It should be stressed that the sudden release mode needs to be improved to a more sustained one by modifying the bead swelling behavior in order to achieve more efficient oral delivery of AITC.

Acknowledgements

This work was supported partly by Grant No. RTI05-01-02 from the Regional Technology Innovation Program of the Korean Ministry of Commerce, Industry, and Energy, and partly by 2003 Scientific Research Promoting Grant from the Kangnung National University, Korea. Won-Tae Kim is a recipient of a graduate fellowship from the Brain Korea 21 program of the Korean Ministry of Education and Human Resources Development.

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