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Research paper

The release behavior of brilliant blue from calcium–alginate gel beads coated by chitosan: the preparation method effect

X.Z. Shu, K.J. Zhu*

Department of Polymer Science and Engineering, Zhejiang University, Hangzhou, People's Republic of China

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Abstract

The aim of this study is to reveal how the release behavior of a model drug (brilliant blue, BB) from chitosan coating calcium–alginate gel beads (CCAGB) was influenced by the preparation methods. The CCAGB were prepared by dropping alginate solution into $CaCl_2$/chitosan solution (method 1(a)), or into chitosan solution then gelled by $CaCl_2$ (method 1(b)), or into $CaCl_2$ solution then coated by chitosan (method 2). Scanning electron microscopy was used for morphology observation, and elemental analysis was applied to determine the chitosan content bound on calcium–alginate gel beads (CAGB). Compared to CAGB, the dried CCAGB had poorer shape and rougher surface morphology especially in methods 1(a) and (b); moreover, CCAGB was found to be more instable in 0.9% NaCl and serious burst of beads occurred when high concentration of alginate (3.0 and 5.0% w/v) was used. The influence on BB release from the beads by chitosan coating was not only related to the chitosan density on bead surface, but also preparation method and other factors. Under un-dried bead state in method 1(a), the increase of chitosan content prolonged BB release in 0.9% (w/v) NaCl; while in method 2, the increase of chitosan concentration over 0.1% (w/v) (3.0% (w/v) alginate concentration was used) resulted in more serious burst of beads and hence facilitated BB release. Furthermore, in both methods 1(a) and 2, the increase of alginate from 1.5 to 3.0 or 5.0% (w/v) usually resulted in the significant burst of beads and accelerated BB release when 0.3 or 0.5% (w/v) chitosan was used for coating. Drying process greatly influenced BB release profile due to the destroying of alginate–chitosan film. The acceleration of BB release from CCAGB by drying process was more significant in the case of method 1 than of method 2. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Chitosan coating; Calcium–alginate gel beads; Preparation methods; Brilliant blue; Controlled drug release

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1. Introduction

Recently, the use of natural polymers in the design of drug delivery formulation has received much attention due to their excellent biocompatibility and biodegradability [1]. Among them, alginate and chitosan are very promising and have been widely exploited in pharmaceutical industry for controlled drug release [2,3].

Alginate is a natural polyacid, and has a unique property of gel-formation in the presence of multivalent cations such as calcium ions in aqueous media, which takes place mainly at junctions in the G–G sequence rich chain region known as the 'egg box junctions' [4,5]. Chitosan is a naturally occurring polysaccharide comprising glucoamine and *N*-acetylglucoamine with unique polycation characteristics [3,6]. The polycationic nature of chitosan leads to a strong interaction with negatively charged alginate. When alginate is dropped into chitosan solution, the electrostatic interaction of carboxylic groups of alginate with the amine groups of chitosan results in the formation of a membrane. This process has been widely used in the preparation of alginate–chitosan membranes with a solid calcium–alginate gel core [7,8]. There are many advantages of the chitosan coating, such as the improvement of drug payload and bioadhesive property, as well as the prolonged drug release properties etc.

Many results have been reported concerning the use of chitosan coating calcium–alginate gel beads (CCAGB) as drug delivery formulation. Some factors, such as concentration and molecular weight of alginate (or chitosan), *N*-acetylation degree of chitosan, the mannuronic/guluronic acid ratio of alginate, pH value in preparation and release media etc. that influenced the drug release behavior from the beads, were extensively studied [9–21]. However, the results are rather complicated. In general, the prolongation of drug release from the beads coating with chitosan was observed, but Polk et al. showed that the release rate of bovine serum albumin was accelerated on increasing the

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* Corresponding author. Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, People's Republic of China. Tel.: +86-571-795-2046.

E-mail address: kjzhu@ipsm.zju.edu.cn (K.J. Zhu).

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chitosan concentration from 0.1% to 0.2% (w/v) [9]. Lee et al. also indicated that the more significant swelling occurred when the beads were coated with chitosan [17]. These contrary results may be related to the variation in the preparation procedures [7,22], as well as other factors.

In this paper, we aimed to investigate the influence of chitosan coating on drug release properties from calcium–alginate gel beads (CAGB) prepared by three methods, i.e. by dropping alginate solution into $CaCl_2$/chitosan solution, or into chitosan solution then gelled by $CaCl_2$, or into $CaCl_2$ solution then coated by chitosan. The morphology of beads, the chitosan content of beads surface, the swelling and stability of beads in release media were also studied.

2. Materials and methods

2.1. Materials

Sodium alginate (ca. 250 cps for 2% solution at 25°C) was purchased from Sigma (St. Louis, MO, USA). Chitosan was obtained from Tianbao Chitosan Co. Ltd (Ninbo, China), and refined twice according to the literature [23], the degree of deacetylation was 86%, Mv was 460,000. Coomassie brilliant blue R250 (BB, Mw 825) was purchased from Fluka A.G. (Switzerland) and used after sieving (less than 50 μm). All other reagents were commercially available and used as received.

2.2. Preparation of CCAGB

The preparation of CCAGB by one-stage procedure was dropping alginate solution into an aqueous solution of chitosan containing calcium ions [13]. We named this procedure as method 1(a) for simplification. Sodium alginate was dissolved in double-distilled water at a certain concentration (1.5–5.0% w/v), and then BB (drug/alginate 0.25, w/w) was added and suspended thoroughly by stirring. Five milliliters of this solution was dropped into a 250-ml gelling solution through a 0.45-mm syringe needle at a dropping rate of 1.0 ml/min under mild agitation. The gelling solution contained 1.0% $CaCl_2$ (w/v) and chitosan (concentration 0.1–0.5% w/v, pH 5.0). The spherical beads formed were cured for 1 h in the gelling solution at room temperature with gentle stirring, then collected by filtration and washed twice with 100 ml double-distilled water, consecutively. Then the beads were dried at room temperature in vacuum or used in the following experiments directly.

The only difference of method 1(b) from method 1(a) was that alginate solution was dropped into chitosan solution at first, and after 1.0 h solid $CaCl_2$ (1.0% w/v) was added into gelling solution under mild stirring, and the beads were further cured for 1.0 h.

In the two-stage procedure (method 2) [7], CAGB were prepared by dropping alginate solution into 1.0% (w/v) $CaCl_2$ solution (method 1(a)) at first. Then these wet CAGB were transferred into chitosan solution (concentration 0.1–0.5% w/v, pH 5.0) containing 1.0% $CaCl_2$ (w/v). After 1.0 h, the formed CCAGB were collected and washed twice with 100 ml double-distilled water, consecutively. Then the beads were dried at room temperature in vacuum or used in the following experiments directly.

The bead diameters were determined using an optical microscope, and the average values were taken for at least 20 beads.

In all cases, usually ca. 80% BB was loaded in CAGB, and more than 95% BB was in CCAGB in preparation procedure.

2.3. Morphology observation

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

2.4. Elemental analysis

Elemental analysis of CCAGB was performed using an elemental analyzer (model EA 1110, CE). The chitosan contents in blank CCAGB were calculated from the nitrogen content of the elemental analysis. The chitosan density on the bead surface was defined as chitosan content/bead surface area in the wet state [7].

2.5. Swelling ratio measurements

The swelling ratio of CCAGB or CAGB in 0.9% (w/v) NaCl was estimated by measuring the bead weight and expressed as W_t/W_0, where W_t was the bead weight at release time t, while W_0 was the initial bead weight. The test conditions were identical to those described for the drug release studies in the following part, and at least 20 beads were measured and the mean value was reported.

2.6. Bead stability – 'explosion assay'

The beads may be burst when the mechanical strength of beads cannot afford the osmotic swelling pressure inside beads. This effect was exploited by an explosion assay similar to the method described by Thu et al. [25]. CCAGB or CAGB (100 beads) were suspended in 500 ml 0.9% (w/v) NaCl at 37°C, 50 rpm. The percent of burst beads was defined as: the number of burst beads/the number of beads given × 100%.

2.7. BB release studies

CCAGB or CAGB (20 beads) were put in a glass bottle containing 100 ml dissolution media (0.9% NaCl or 0.1 M phosphate buffered saline (PBS) pH 7.4) and incubated in a shaking water-bath at 37°C, 50 rpm. At scheduled time intervals, 4 ml solution was withdrawn, and the BB content

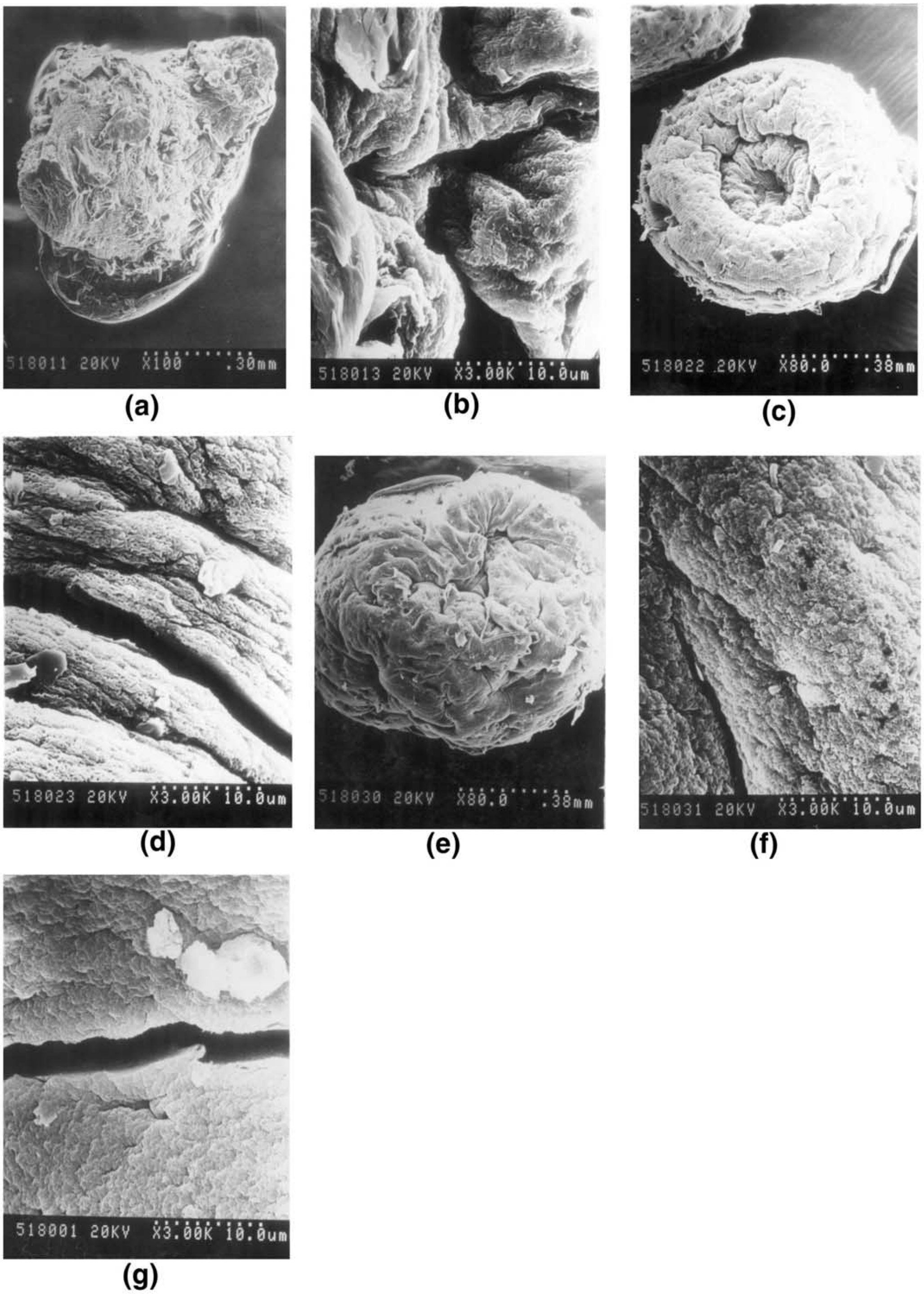

Fig. 1. SEM micrographs of 0.3% (w/v) CCAGB prepared by method 1 with different concentrations of alginate – 1.5% (w/v) alginate: dried beads (a) and surface morphology (b); 3.0% (w/v) alginate: dried beads (c) and surface morphology (d); 5.0% alginate: dried beads (e) and surface morphology (f); and the surface morphology of CAGB with 3.0% (w/v) alginate without chitosan (g).

was determined by UV–Visible spectrophotometric measurements at 590 nm [13,23]. In some cases, the erosion of alginate was monitored by the phenol-sulfuric acid color reaction [24,25]. An equal volume of the same dissolution medium was added back to maintain a constant volume.

3. Results and discussion

3.1. Morphology observation

SEM micrographs and typical surface morphology of dried CCAGB prepared with 0.3% (w/v) chitosan and 1.5, 3.0, or 5.0% (w/v) alginate by method 1(a) are shown in Fig. 1. The spherical shape of beads in wet state was usually lost after drying especially those beads prepared with low alginate concentration. In 1.5% (w/v) alginate, the dried beads was very irregular (Fig. 1a), and had a rather rough surface with large gaps (Fig. 1b). With the increasing of alginate concentration (3.0% w/v), the shape of beads changed to a spherical disc with a collapsed center (Fig. 1c); the surface morphology was also improved, but still showed some roughness and large gaps (ca. 2 μm in width) (Fig. 1d). Normally the spherical shape was retained when the alginate concentration was as high as 5.0% (w/v) (Fig. 1e), and the surface was relatively smooth (Fig. 1f), but gaps (ca. 1 μm) were still observed. These results indicated that the integrity of alginate–chitosan membrane was seriously destroyed in the drying process, and the spherical shape of dried beads improved with the increase of alginate concentration.

In method 1(b), CCAGB can be obtained with chitosan concentration higher than 0.3% (w/v). The diameter of these beads in the wet state was very large, and their mechanical strength was relatively poor. With 3.0% (w/v) alginate and 0.5% (w/v) chitosan, the diameter of wet beads in method 1(b) was ca. 4.3 mm, while it was ca. 2.9 mm in both methods 1(a) and 2. After drying, the spherical shape of the beads in method 1(b) was lost completely (picture not shown).

On the other hand, CAGB maintained some spherical shape after drying even in 1.5% (w/v) alginate, and the surface was relatively smoother (Fig. 1g) though with large gaps (ca. 3 μm). It was reported by Skjåk-Bræk et al. [22] that CAGB usually had a heterogeneous structure with dense surface layer and loose core due to the heterogeneous gelation mechanism, which resulted in the collapse of beads during the drying process [9]. Compared to CAGB, the poorer shape of CCAGB after drying was possibly attributed to their more heterogeneous structure. The surface layer of CCAGB was denser and the core was looser, since in the preparation process alginate diffused from the droplet core toward the interface between droplet and gelling solution, to form membrane with calcium ions and chitosan which resulted in the more heterogeneous structure of CCAGB [22].

The heterogeneous structure of CCAGB was also related to the preparation method. In method 1(b), CCAGB had the most heterogeneous structure because the diffusion of alginate from the droplet core toward the surface to react with chitosan was relatively free, while in method 1(a) it was limited due to the gradual gelling of alginate with calcium. In method 2 CCAGB had the least heterogeneous structure because the alginate was gelled at first and then chitosan was coated on the surface. In accordance with that, with the same concentration of alginate and chitosan the shape of dried CCAGB in method 2 (Fig. 2a) was more spherical than in method 1(a) (Fig. 1c), and the shape in method 1(b) was the poorest (picture not shown).

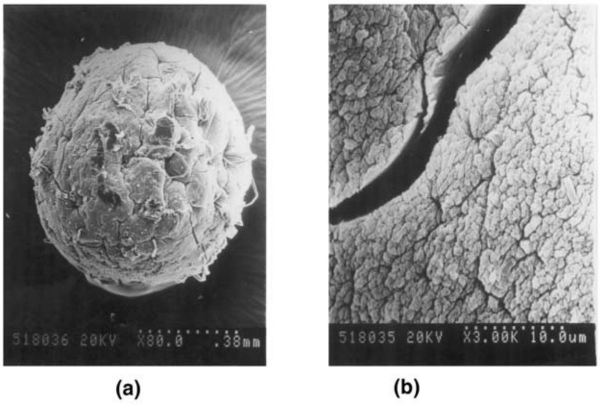

Fig. 2. SEM micrograph (a) and surface morphology (b) of 0.3% (w/v) CCAGB prepared by method 2.

3.2. Chitosan density

Elemental analysis indicated that the chitosan content coated on the bead surface increased with the increasing of the chitosan concentration (result not given). However, the increase in alginate concentration did not always result in the increase of bound chitosan content on the bead surface (Table 1). For instance, in method 1(a) with the increase of alginate concentration from 1.5 to 3.0% (w/v), the chitosan density increased from 0.247 to 0.352 μg/mm^2. However, further increase of alginate concentration to 5.0% (w/v) resulted in the slight decrease of chitosan density (0.345 μg/mm^2). It was reported that with the increase of alginate concentration, more alginate with negative charges existed on the bead surface. Therefore more chitosan with positive charges were bound on the bead surface. However, the increase of alginate concentration also led to the decrease of bead porosity and limited the diffusion of chitosan into the inside of beads, hence less chitosan was bound [7]. Our results may be attributed to the above two factors.

From Table 1, it can be also seen that under the same condition the chitosan density on the bead surface in method 2 (0.379 μg/mm^2) was higher than the value in method 1(a) (0.352 μg/mm^2), because in method 2 chitosan can diffuse into gel beads more rapidly [7]. In method 1(b), when alginate solution was dropped into chitosan solution, an integral membrane of alginate–chitosan formed immediately; chit-

Table 1
The chitosan density on CCAGB surface (0.5% w/v chitosan, pH 5.0) ($n = 3$)[a]

Alginate concentration (w/v%)	Chitosan density (μg/mm^2)
$1.5^{1(a)}$	0.247 ± 0.012
$3.0^{1(a)}$	0.352 ± 0.013
$5.0^{1(a)}$	0.345 ± 0.023
$3.0^{1(b)}$	0.155 ± 0.008
3.0^{2}	0.379 ± 0.011

[a] 1(a), 1(b) and 2 refers to methods 1(a), 1(b) and 2, respectively.

osan bound only on the alginate droplet surface and could not diffuse into gel beads further, so the chitosan density was the lowest (0.155 μg/mm^2).

3.3. Release studies

3.3.1. In un-dried bead state

In the un-dried beads prepared by method 1(a) it was observed that BB release was prolonged, and the release rates were proportional to the decrease in chitosan concentration. For example, with 3.0% (w/v) alginate, the release percent of BB was 100, 86, 55 and 44% in 18 h with chitosan concentrations 0, 0.1, 0.3 and 0.5% (w/v), respectively (Fig. 3a, b).

However, the increase of alginate concentration did not always prolong BB release. Fig. 3 shows the BB release with chitosan coating. When the concentration of chitosan was low (0 and 0.1% w/v), slower BB release from CCAGB was observed (Fig. 3a). For example, with chitosan concentration 0.1% (w/v), it needed ca. 13 h for 80% BB to release from beads with alginate 1.5%, while it took ca. 15 h in alginate concentration of 3.0% (w/v). However, in chitosan concentrations of 0.3 and 0.5% (w/v), the BB release behavior changed greatly (Fig. 3b), though the initial diameters of beads with 3.0 and 1.5% (w/v) alginate was changed slightly (Table 2). In the first several hours, the release rates were still ranked in the decrease of alginate concentration; but after ca.15 h the BB release from beads (3.0% w/v alginate) was much quicker. In 24 h, 86% BB were released from beads with 3.0% w/v alginate while the value was 49% for 1.5% w/v alginate (0.3% w/v chitosan used). In the chitosan concentration of 0.5% (w/v), 72% BB were released from beads with 3.0% w/v alginate while the value was 25% for 1.5% w/v alginate. This phenomenon may be related to the burst of beads in release media. When the CAGB or CCAGB were incubated in 0.9% NaCl, the mechanical strength of beads decreased due to the displacement of cross-linking calcium by sodium ions [25], but the osmotic activity of ions increased. When the mechanical strength of beads cannot bear the osmotic pressure, the beads probably burst. This effect had seriously affected BB release, especially when high concentration of chitosan (0.3 and 0.5% w/v) was used for coating which formed denser chitosan–alginate membrane and greatly limited BB release.

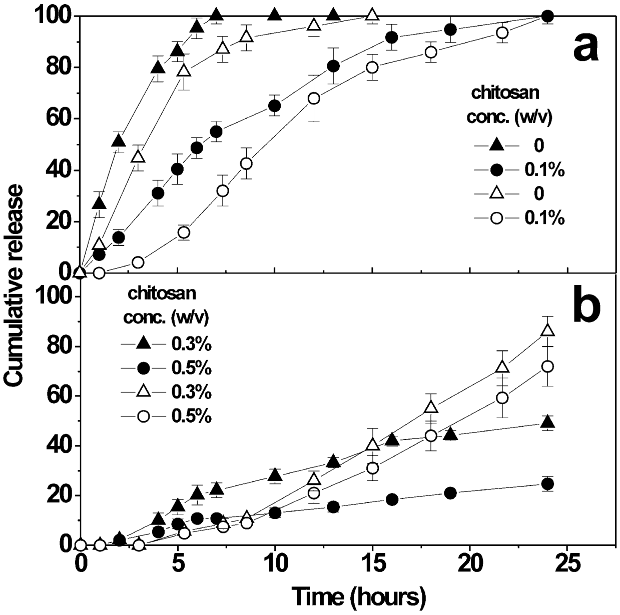

Fig. 3. The release curves of BB from CCAGB prepared by method 1(a) in wet bead state. (a) Chitosan concentration was 0 and 0.1% (w/v), (b) chitosan concentration was 0.3 and 0.5% (w/v). Sodium alginate concentration: solid symbol curves 1.5% (w/v); open symbol curves 3.0% (w/v). Release media: 0.9% (w/v) NaCl ($n = 3$).

Table 2
The diameter (mm) of CCAGB prepared by method 1(a) or 2 ($n \geq 20$)[a]

Alginate concentration (w/v%)	Chitosan concentration (w/v%)			
	0	0.1	0.3	0.5
1.5$^{1(a)}$	2.36 ± 0.04	2.55 ± 0.04	2.42 ± 0.05	2.39 ± 0.04
3.0$^{1(a)}$	2.90 ± 0.06	2.96 ± 0.05	2.93 ± 0.07	2.88 ± 0.08
3.0$^{(2)}$	2.90 ± 0.06	2.86 ± 0.02	2.82 ± 0.06	2.80 ± 0.05

[a] 1(a) and 2 refers to methods 1(a) and 2, respectively.

Fig. 4 shows the influence of chitosan concentration on the burst percent of beads prepared with 1.5, 3.0 and 5.0% (w/v) alginate by method 1(a) in 24 h. Serious burst of CCAGB occurred in alginate concentration of 3.0 and 5.0% (w/v), while no burst of beads was observed with 1.5% (w/v) alginate. It may be related to the higher osmotic pressure inside beads in the case of alginate with higher concentration. Compared to 3.0% alginate, the less significant burst of beads in 5.0% (w/v) alginate was possibly caused by the compensation of the higher mechanical strength of beads.

In method 2, BB release profiles were similar to those in method 1(a), but with some changes. Fig. 5a shows the BB

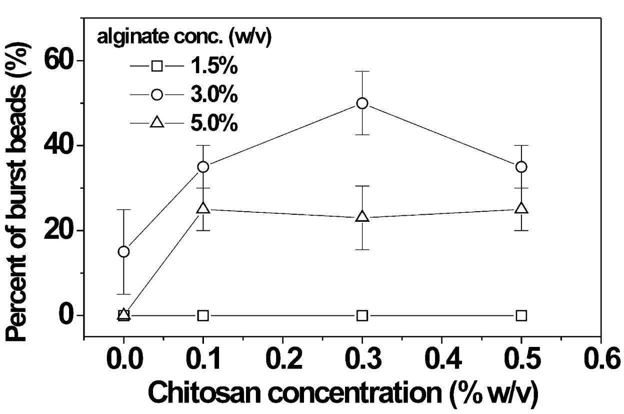

Fig. 4. The effect of chitosan concentration on the percent of burst beads in 0.9%(w/v) NaCl for 24 h. Beads were prepared by method 1 with 1.5, 3.0 or 5.0% (w/v) alginate and in wet beads state ($n = 3$).

release curves from CCAGB in method 2 with 3.0% (w/v) alginate in 0.9% (w/v) NaCl. The release rates did not proportionate to the decrease of chitosan concentration as in method 1(a) though the beads had similar diameters (Table 2). It was observed that the further increase of chitosan concentration over 0.1% (w/v) accelerated the BB release.

Fig. 5b shows the swelling curves of CCAGB in method 2 with 3.0% (w/v) alginate in 0.9% (w/v) NaCl. It was observed that chitosan coating resulted in the larger swelling ratio of beads, which was similar to those reported by Lee et al. [17]. In 6.5 h, the swelling ratio of CCAGB with 0.1, 0.3 and 0.5% (w/v) chitosan was 2.66, 2.63 and 2.60, respectively, while the value for CAGB was 2.40. After 6.5 h, serious burst of beads in 0.9% (w/v) NaCl occurred, which disturbed the measurement of the swelling ratio.

The swelling results (Fig. 5b) cannot explain the phenomenon that beads coated by 0.1% (w/v) chitosan prolonged BB release. Fig. 5c shows the result of ‘explosion assay’ in 0.9% (w/v) NaCl. Significant burst of CCAGB was observed; the higher the chitosan concentration, the higher the percent of burst beads. In 24 h with the chitosan concentration of 0, 0.1, 0.3 and 0.5% (w/v), about 15, 39, 55 and 90% beads were burst, respectively. Compared to chitosan concentration of 0.3 or 0.5% (w/v), the less burst of beads in chitosan concentration of 0.1% (w/v) resulted in the slowest release of BB (Fig. 5a).

In Figs. 4 and 5c, it can be seen that the burst of CCAGB was more significant than CAGB in 0.9% NaCl, which suggested that the bead stability decreased due to chitosan coating. In general the less stability of CCAGB should lead to a quicker BB release. However, in our experiments in the un-dried beads it was not observed. The possible reason is that the release of BB from CAGB in 0.9% (w/v) NaCl was too fast, and most of BB was released when significant burst

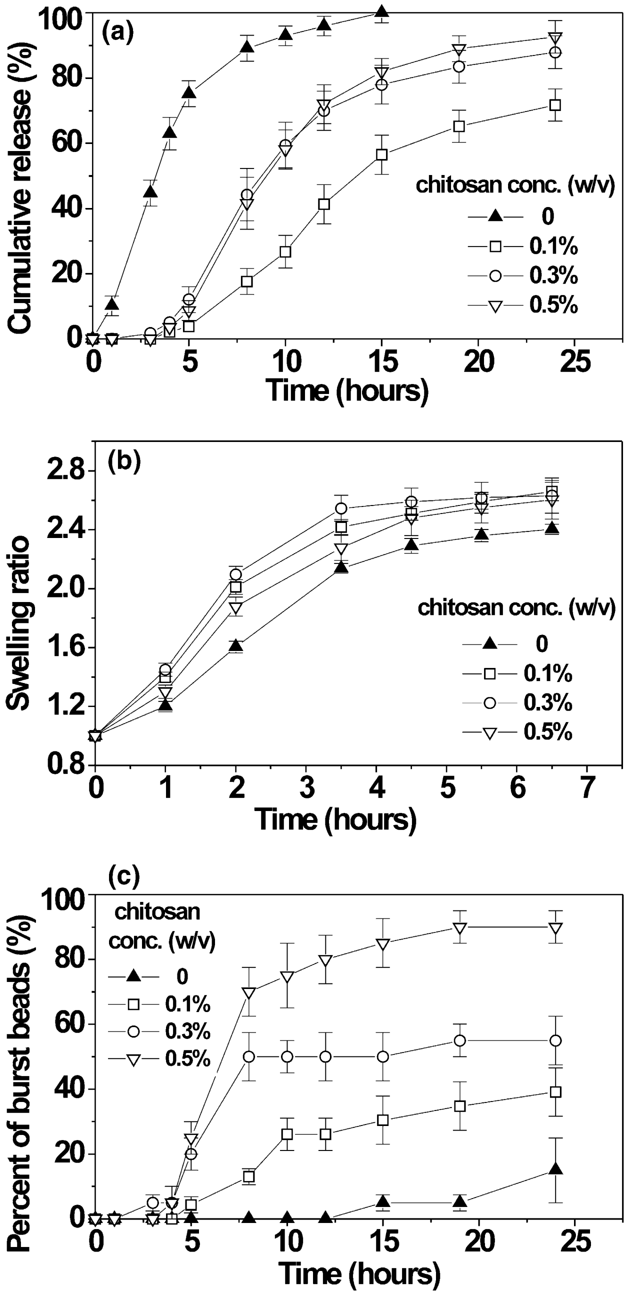

Fig. 5. The influence of chitosan coating (increasing concentration from 0 to 0.5% w/v) on the BB release from wet CAGB (a), swelling ratio (b) and percent of burst beads (c) in media of 0.9% (w/v) NaCl. The beads were prepared by method 2 with 3.0% (w/v) alginate ($n = 3$).

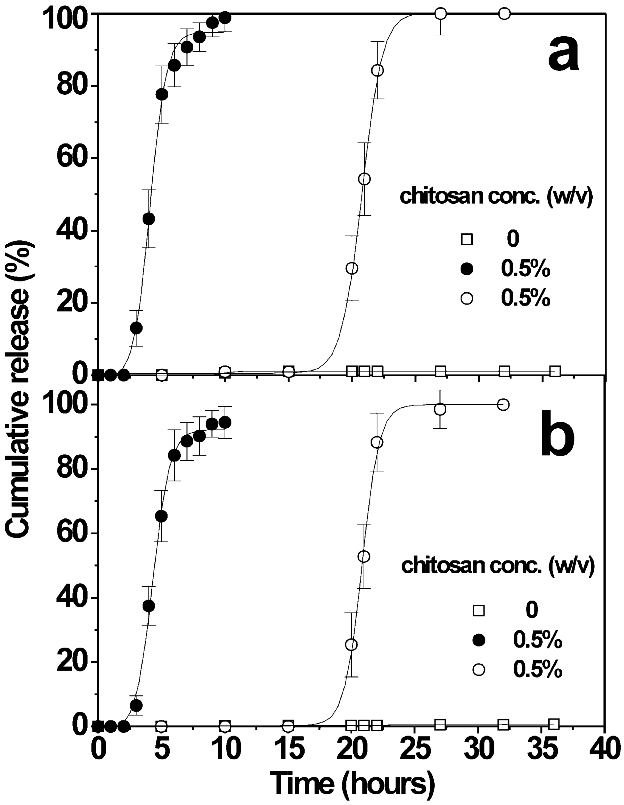

Fig. 6. The BB (a) and alginate (b) release from CAGB coated with 0.5% chitosan (w/v) or not. Solid circle curves: method 1(a); open circle curves: method 2. Release media: 0.1 M PBS ($n = 3$).

of CCAGB occurred. Therefore, we conducted some release studies in media of 0.1 M PBS.

Fig. 6 shows the behavior of BB release and alginate leaching in 0.10 M PBS from CAGB, and CCAGB with 0.5% (w/v) chitosan by method 1(a) or method 2. It was obvious that the BB and alginate leaching accelerated due to chitosan coating especially in method 1(a). In 36 h, little BB and alginate leaching (both less than 2%) were observed for CAGB, while in ca. 22 h for method 2 and ca. 10 h for method 1(a) the BB release and alginate leaching were completed. In Fig. 6a, b, it can also be seen that BB release and alginate leaching were both in a pulsed way (especially for method 2) and they were simultaneous, which meant BB release was controlled by the dissociation of beads in 0.10 M PBS.

According to our previous experiments, the little BB release and matrix erosion were attributed to the heterogeneous structure of CAGB, i.e. the dense surface layer of CAGB retarded BB release and matrix erosion. However, in the case of CCAGB, the BB release and alginate leaching were accelerated. It suggested that chitosan coating possibly destroyed the structure of CAGB, especially for method 1(a) according to the discussion of morphology observation, therefore the acceleration of BB release and matrix erosion were more significant in method 1(a) than in method 2.

Fig. 7a shows the BB release in 0.9% (w/v) NaCl from wet CCAGB prepared by different methods with 3.0% alginate (w/v) and 0.5% w/v chitosan. In method 2, the burst of beads was the most serious (Fig. 7b), resulting in the quickest BB release. Method 1(a) prolonged BB release most effectively though the initial diameter of beads (2.88 ± 0.08 mm) was slightly larger than that of method 2 (2.80 ± 0.03 mm), and the burst of beads was less significant. In method 1(b), no burst of beads occurred due to the lowest alginate concentration in beads, but with the largest diameter (4.3 ± 0.22 mm), the release rate of BB was quicker than method 1(a), but lower than method 2.

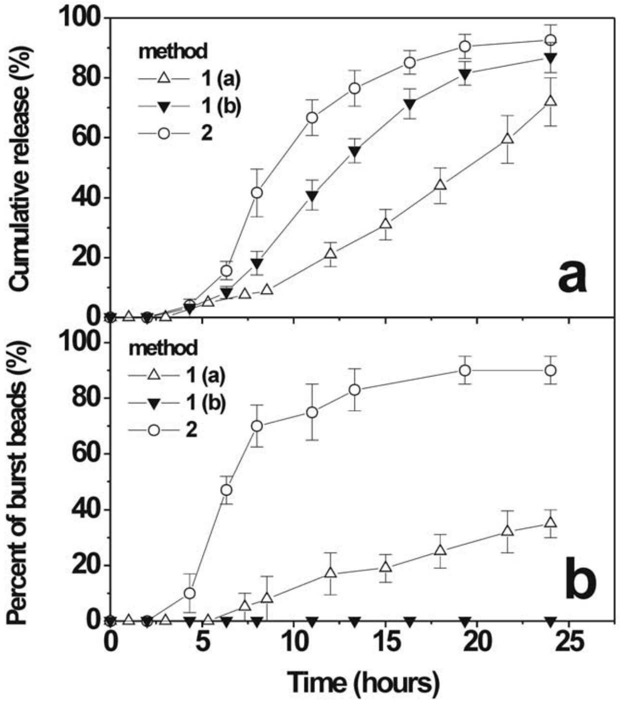

Fig. 7. The release curves of BB from CCAGB (a) and the curves of the percent of burst chitosan coating calcium–alginate beads (b). The beads were prepared by different methods and in wet beads state (3.0% sodium alginate, 0.5% chitosan). Release media: 0.9% (w/v) NaCl ($n = 3$).

3.3.2. In dried beads

When CCAGB were dried, the influence of chitosan coating on BB release became very complicated. Fig. 8 shows BB release curves from dried beads by method 1(a) in 0.9% (w/v) NaCl. It was found that in some cases chitosan coating accelerated BB release. For example, with 1.5% (w/v) alginate, 64, 70, 76 and 79% BB were released in 3 h from the beads coated by 0, 0.1, 0.3 and 0.5% (w/v) chitosan, respectively (Fig. 8a), while in the alginate concentration of 3.0% (w/v), BB release was prolonged slightly (Fig. 8b). It was also found that the BB release was slightly faster from 0.1%

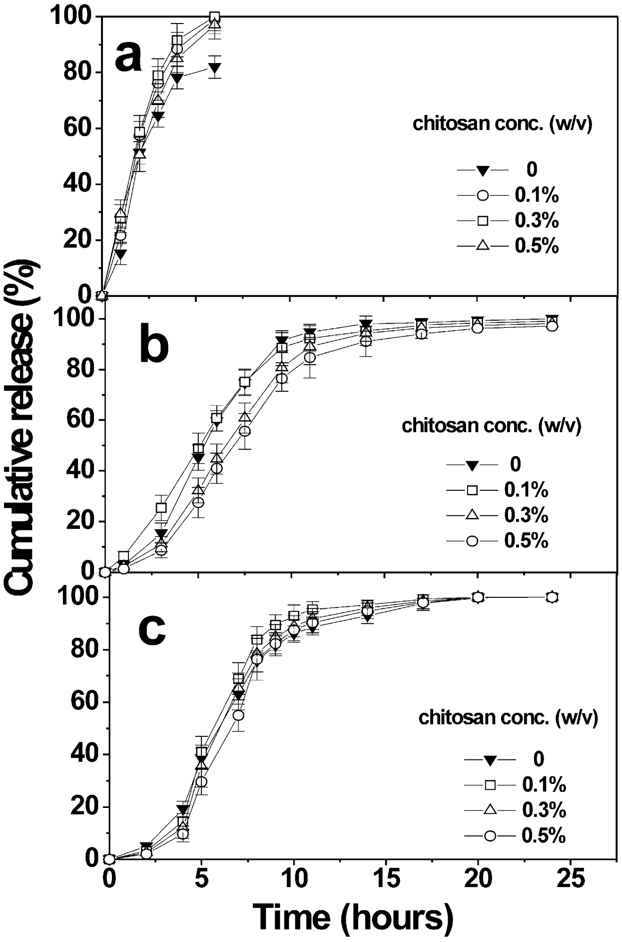

Fig. 8. The influence of chitosan coating on the BB release from dried CAGB prepared by method 1 with different sodium alginate concentration (w/v): (a) 1.5%; (b) 3.0%; (c) 5.0%. Release media: 0.9% (w/v) NaCl ($n = 3$).

(w/v) chitosan coated alginate than from those without chitosan in the first several hours. In 7.5 h, 74.8, 75.1, 60.9 and 55.6% BB were released in chitosan concentration of 0, 0.1, 0.3 and 0.5% (w/v), respectively. In 5.0% (w/v) alginate, the chitosan coating seldom prolonged BB release and chitosan concentration had little effect on BB release (Fig. 8c).

'Explosion assay' revealed that no bead burst occurred in 1.5% (w/v) alginate in method 1(a) in 24 h in 0.9% (w/v) NaCl no matter whether the beads were dried or not. So the above release results in Fig. 8a were possibly caused by the destroying of the integrity of chitosan–alginate complex membrane by the drying process. On the other hand, in alginate concentration of 3.0 and 5.0% (w/v), the burst of CCAGB in 0.9% (w/v) NaCl in the dried state was more serious than in the un-dried state, and usually 100% beads were burst in 24 h.

Fig. 9 shows BB release curves from dried beads prepared by method 2 in 0.9% (w/v) NaCl. In the alginate concentration of 1.5, 3.0 and 5.0% (w/v), chitosan coating prolonged BB release except in the later stage (after 5 h) in 1.5% (w/v) alginate (Fig. 9a). Compared to method 1(a), method 2 prolonged BB release more effectively in dried condition.

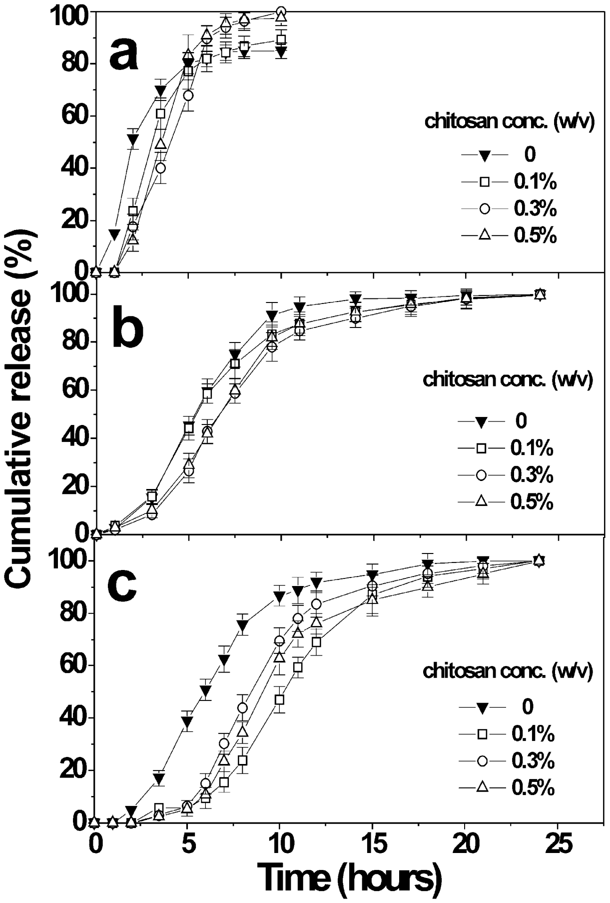

Fig. 9. The influence of chitosan coating on the BB release from dried CAGB prepared by method 2 with different sodium alginate concentrations (w/v): (a) 1.5%; (b) 3.0%; (c) 5.0%. Release media: 0.9% (w/v) NaCl ($n = 3$).

Table 3
The influence of chitosan coating on the time needed for 80% BB release in 0.9% (w/v) NaCl from CAGB with 3.0% (w/v) alginate

Chitosan concentration (w/v%)	Method 1(a)		Method 2	
	Wet	Dried	Wet	Wet
0	5.7	8.2	5.7	8.2
0.1	14.9	8.5	14.5	8.9
0.3	22.9	9.4	16.2	9.4
0.5	29.5	10.1	29.2	10.0

From Figs. 3, 4, 8 and 9, it can be seen that the drying process accelerated BB release from CCAGB in both methods 1(a) and 2 greatly, whereas for CAGB it was not the case (Table 3). For example, in method 1(a) with 3.0% (w/v) alginate, the time needed for 80% BB release was ca. 5.7, 14.9, 22.9 and 29.5 h in the un-dried state in chitosan concentrations of 0, 0.1, 0.3 and 0.5% (w/v), respectively; while in the dried state, under the same condition the value was ca. 8.2, 8.5, 9.4 and 10.1 h, respectively. This result was in contrast with that reported by Kim and Lee [26] that the drying process accelerated blue-dextran release from CAGB greatly, which was possibly attributed to the different model drugs used. In our experiment, the water solubility of BB was poor, and it was released from CAGB only when the beads swelled significantly, and it took more time for the dried CAGB to swell to the extent where BB began to release.

4. Conclusions

Chitosan coating on CAGB resulted in the poor shape and surface morphology of dried CCAGB especially in methods 1(b) and (a), and the instability of CCAGB in 0.9% NaCl. The influence of chitosan on the BB release from beads was complicated, which was not only related to the chitosan density on bead surface, but also other factors, such as preparation method, drying process etc. In the un-dried beads, when alginate concentration was low (such as 1.5% w/v) BB release was mainly related to the chitosan density on beads surface; while alginate concentration was higher (such as 3.0 or 5.0% w/v) where osmotic pressure inside beads was significant, the serious burst of CCAGB facilitated BB release. The drying process also influenced the BB release profile. The destroying of chitosan–alginate complex surface layer in the drying process accelerated BB release from CCAGB, but the retardation of BB release from CAGB was observed. Furthermore in the dried bead state the prolongation of BB release from GGAGB prepared in method 2 was more efficient than in method 1(a).

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