International Journal of Pharmaceutics, 96 (1993) 139–145

© 1993 Elsevier Science Publishers B.V. All rights reserved 0378-5173/93/$06.00

IJP 03192

Preparation of chitosan-reinforced alginate gel beads – effects of chitosan on gel matrix erosion

Y. Murata, T. Maeda, E. Miyamoto, and S. Kawashima

School of Pharmacy, Hokuriku University, Kanagawa-machi, Kanazawa 920-11 (Japan)

(Received 2 April 1992)
(Modified version received 22 August 1992)
(Accepted 22 December 1992)

Key words: Alginate gel beads; Chitosan; Complex formation; Sustained release; Coloring matter; Phenytoin

Summary

Chitosan-reinforced alginate gel beads were prepared and the release patterns of coloring matter (Brilliant Blue G, BB) held within them were investigated. The release rates of BB from the gel beads were much slower after an initial lag time when they were incubated with chitosan compared with the original intact gels prepared without chitosan. These differences were observed irrespective of the β-D-mannuronic acid/α-L-guluronic acid (M/G) ratio of the alginate used and an additive effect on the release rate was observed when two species of alginates were combined. The initial release rates were reduced gradually in proportion to the increases in the chitosan concentration and/or incubation times used when preparing the gel beads. Furthermore, erosion of the gel beads was suppressed by chitosan treatment. The release of phenytoin from alginate gel beads showed the same characteristics as that of BB.

Introduction

Calcium-induced alginate gels (Ca-alginate gels) have been studied to assess their usefulness for pharmaceutical preparations (Yotsuyanagi et al., 1991; Kim et al., 1992). Alginate is a polymer, which consists of α-L-guluronic acid (G) and β-D-mannuronic acid (M). Some properties of Ca-alginate gels are associated with the M/G ratio and/or sequence (Cesaro et al., 1988). Divalent cation-induced alginate gelation is known to arise mainly at junctions in the G-G-sequence rich chain region, which are called 'egg box junctions'. Cured gel beads form gradually following the permeation of cations into the gel matrix, although this reaction occurs immediately if adequate cations for gelation are present. For example, calcium-induced alginate gel beads have been prepared by incubating alginate gels in $CaCl_2$ solution for a few days (Yotsuyanagi et al., 1987). However, it is believed that some uronic acids and/or monomer unit sequences such as M-M or M-G blocks, do not participate in the gelation process. Moreover, formation of a polyelectrolyte complex has been demonstrated to occur when a cationic and an anionic polymer are present si-

Correspondence to: Y. Murata, School of Pharmacy, Hokuriku University, Kanagawa-machi, Kanazawa 920-11, Japan.

multaneously in aqueous solution. Such complex coacervation between two oppositely charged polysaccharides has been studied (Nakajima et al., 1976). Thus, it was recognized that the formation of complexes between alginate and chitosan, anionic and cationic polysaccharides respectively, could be ascribed to this phenomenon (Takahashi et al., 1990).

We have reported that gel erosion accelerates the release rate of coloring matter incorporated within them (Murata et al., 1993). If complex coacervation between alginates contained in gel beads and chitosan applied subsequently occurs, then suppression of gel erosion in aqueous media, like that shown in Fig. 1 (Nakajima et al., 1972; Takahashi, 1987), would be expected to occur. In this study, we prepared chitosan-reinforced calcium-induced alginate gel beads and investigated the release characteristics of compounds held within them.

Materials and Methods

Gel materials

Four alginates (500M, 500G, 150M and 150G) were purchased from Kibun Food Chemifa Co. (Tokyo, Japan), and chitosan (grade F; degree of deacetylation 75–85%) was obtained from Kimitsu Chemical Industries (Tokyo). The technical

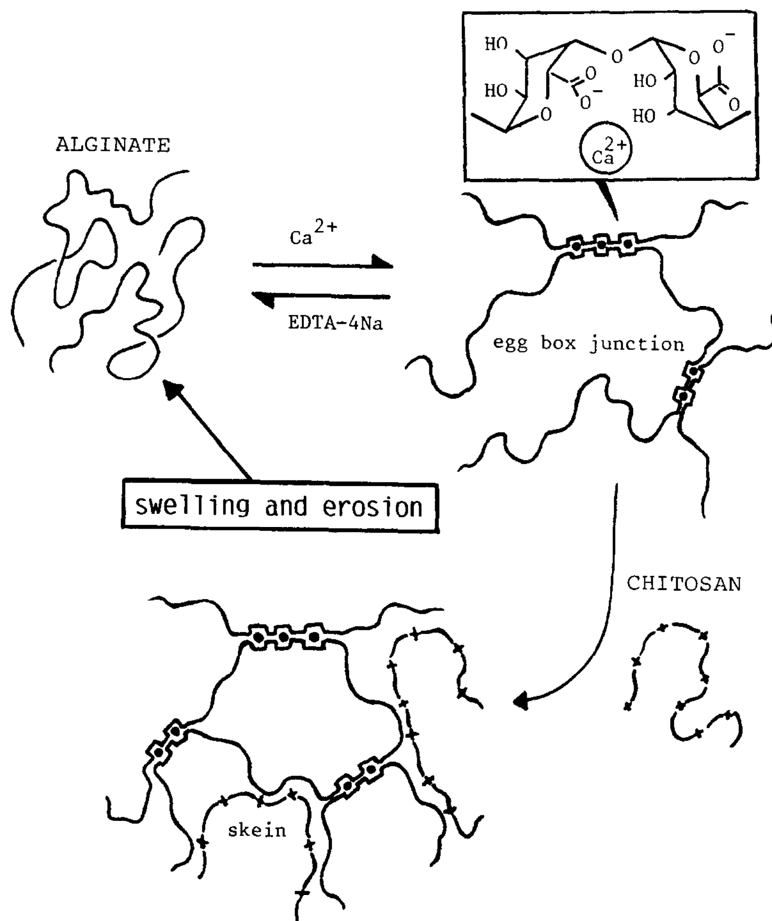

Fig. 1. Diagrammatic representation of the formation of calcium induced alginate gel in the presence of chitosan (Takahashi, 1987; Nakajima and Sato, 1972).

TABLE 1

Physical data for alginates

Species	MW [a] (dispersion)	M/G ratio
500M	2400000 (3.5)	0.89
500G	2100000 (7.8)	0.21
150M	1800000 (13.6)	1.13
150G	1300000 (10.7)	0.20

[a] Molecular weight was determined by gel-permeation chromatography.

and chemical data of these alginates are summerized in Table 1. The chitosan-reinforced gels prepared from each alginate, $CaCl_2$ and chitosan are described as 500M-CS, 500G-CS, 150M-CS, and 150G-CS respectively.

Reagents

Brilliant Blue G (BB) and phenytoin were purchased from Sigma Chemical Co. and Wako Pure Chemical Ind. (Osaka), respectively, and used after sieving (< 75 μm). The tetrasodium salt of EDTA was purchased from Nacalai Tesque (Kyoto), as was 2-nitrophenylhydrazine, which was converted to the hydrochloride and recrystallized from methanol-ether (ONPH).

Preparation of chitosan-reinforced Ca-alginate gel beads

Alginate gel beads were prepared using a modified method of that developed by Yotsuyanagi et al. (1991), as follows. Each alginate was dissolved in distilled, demineralized water with agitation, BB or phenytoin was added to the solution, which was agitated for 1 h, followed by standing in an ultrasonic cleaner (Branson, B-42H, U.S.A.) for 30 min to remove trapped air bubbles. The solution (5 ml) was dropped slowly into 50 ml 0.2 M $CaCl_2$ at 37°C using a peristaltic pump (ATTO, Tokyo) and left to stand at 37°C for 1 day, after which the gel beads were transferred to 50 ml 0.1 M pyridine-HCl buffer (pH 4.5), which contained 0.2 M $CaCl_2$ and chitosan (0, 0.02, 0.05 and 1.0%) and incubated at 37°C for 1, 3 or 5 days. Each type of alginate gel beads (90–100 drops of which were calculated to contain 10 mg BB) was immersed five times in 50 ml fresh distilled, demineralized water for 2 min each prior to carrying out the dissolution test described below.

Dissolution test

The release of BB and elution of alginate from each alginate gel in 500 ml physiological saline were measured quantitatively on a JP XII dissolution test apparatus using the paddle method rotating at 150 rpm with the temperature maintained at 37 ± 0.5°C. A 4 ml aliquot of each solution was removed periodically for analysis, and 4 ml saline (37°C) was added after each removal to maintain a constant volume. The absorbance of each solution collected was determined spectrophotometrically at 590 nm using a Hitachi Spectrophotometer Model 200-20. On completion of the release test (180–360 min) the gel beads were destroyed by adding EDTA (200 mg) and the total amount of BB retained by each alginate gel was determined spectrophotometrically, as described above.

2-ml aliquots of each sample solution were transferred into new glass tubes and the amount of alginate in each sample was determined by the colorimetric method using ONPH as the indicator (Murata et al., 1990). The dissolution tests on the gels containing phenytoin were performed using 500 ml 50 mM phosphate buffer (pH 6.8) according to a similar method, except the sample volume used was 0.5 ml and the amount released was determined using high-performance liquid chromatography (Farinotti et al., 1979), as follows. The system comprised an LC-6A pump (Shimadzu, Kyoto), a column (150 mm $\times$ 4 mm i.d.) packed with ODS-80 TM (Tosoh Manufacturing Co., Ltd), and a Uvidec-100-IV variable-wavelength UV detector (Japan Spectroscopic Co., Ltd). Chromatography was conducted at ambient temperature using an eluent comprising 55% (v/v) methanol and 45% (v/v) distilled water at a flow rate of 0.5 ml/min and the detector wavelength was set at 230 nm. All dissolution tests were performed in triplicate.

Results and Discussion

The effects of the incubation time on the release of BB from alginate gel beads in 0.1 M

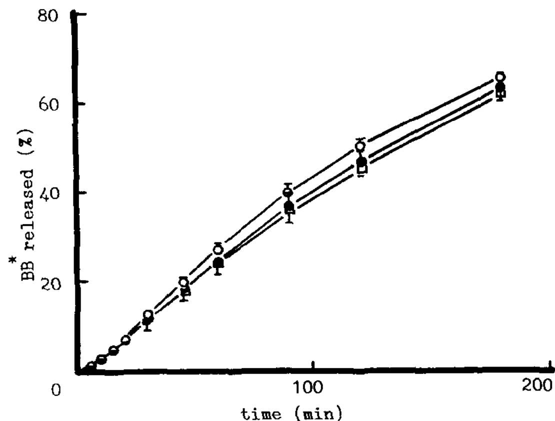

Fig. 2. Effect of incubation time for preparation of alginate (500G) gel beads in pyridine-HCl buffer (pH 4.5) without chitosan. (○) 1 day, (●) 3 days, (□) 5 days. (BB) Brilliant Blue.

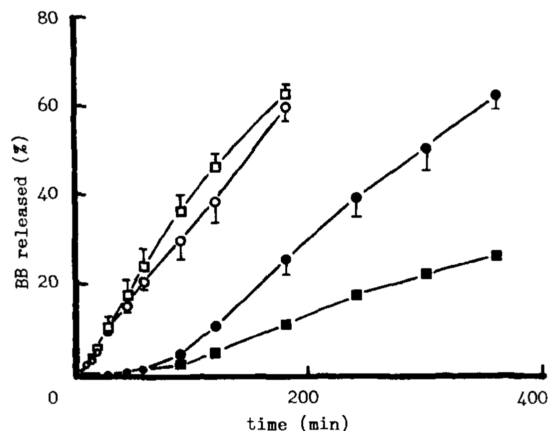

Fig. 3. Release curves of BB from alginate gel beads treated with 0.05% chitosan and 0.2 M $CaCl_2$ for 1 day. (●) 500M, (■) 500G, (○) 500M (without chitosan), (□) 500G (without chitosan). All beads were prepared with 1% alginate. Each gel bead contained 9.8 ± 0.3 mg of BB ($n = 12$).

pyridine buffer (pH 4.5) with 0.2 M $CaCl_2$ but without chitosan are shown in Fig. 2. The release of BB from the hydrogels appeared to follow zero-order kinetics and, even when the incubation time was prolonged, BB was released at a constant rate throughout; the release rates from the gels incubated for 5 days were similar to those incubated for 1 day. A marked change in the release of BB was observed when chitosan was included in the incubation media. The release curves of BB from some hydrogels prepared in the buffer solution containing 0.05% (w/v) chitosan and 0.2 M $CaCl_2$ are shown in Fig. 3. The release of BB from 500M-CS started after a 60 min delay and showed zero-order kinetics. The release rate was determined as 0.21%/min and the square of the correlation coefficient (r^2) = 0.984, which was calculated by subjecting all the BB dissolution data collected after incubation for 60–360 min from 500M-CS to least-squares linear fitting. The release rate (1–180 min) from the 500M gel prepared without chitosan was 0.33%/min ($r^2 = 0.989$) and there was no lag time before release. Similar results were obtained with 500G-CS, the release rate from which was 0.09%/min ($r^2 = 0.991$). The results obtained with 150M-CS and 150G-CS are depicted in Fig. 4. The release of BB from 150G-CS was delayed compared with that from the chitosan-free gel to the same extent as that from 500G-CS compared with chitosan-free 500G. However, no such delayed release was observed with 150M-CS. If the delayed release characteristics of the chitosan-reinforced gel were due only to alginate and chitosan complex formation, then the effects of chitosan on the gels prepared with M-rich and G-rich alginates would be expected to be the same. However, this was not the case and, therefore, not only complex formation but also the formation of new cross-linking induced by Ca^{2+} contributed to the characteristic release patterns de-

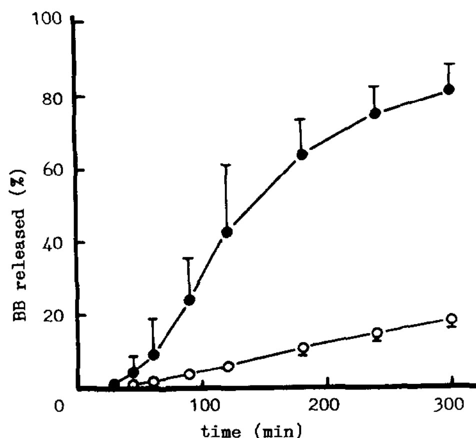

Fig. 4. Effect of the molecular weight of alginate. (●) 150M, (○) 150G.

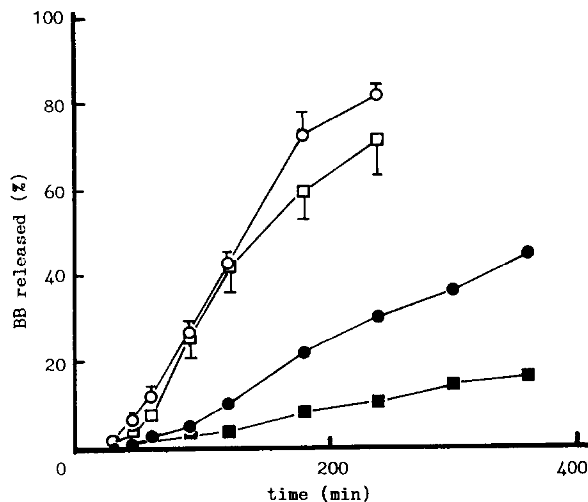

Fig. 5. Release curves of BB from alginate gel beads treated with 0.05% chitosan. (○) 500M (without $CaCl_2$, 1 day incubation); (□) 500G (without $CaCl_2$, 1 day incubation); (●) 500M (with 0.2 M $CaCl_2$, 3 days incubation); (■) 500G (with 0.2 M $CaCl_2$, 3 days incubation).

TABLE 3

Release of alginate from alginate gel beads

Chitosan (%)	Release (%)		
	90 min	180 min	300 min
0	5.5 ± 0.5	13.6 ± 0.9	–
0.02	2.2	4.2	11.4
0.05	1.7 ± 0.2	3.5 ± 0.2	8.2 ± 0.4
0.1	2.2	3.4	5.2

All tests were performed in physiological saline. Data represent means or means ± S.D. (n = 2–3).

scribed above. Furthermore, an additive effect of the different alginate species was observed, as the release rate was 0.13%/min ($r^2 = 0.973$) when the alginate gel comprised both 0.5% (w/w) 500M and 0.5% (w/w) 500G, which suggests that the release rate of a compound from the matrix can be controlled by utilizing two alginate species.

The release patterns from hydrogels prepared in the pyridine-HCl buffer solution containing chitosan but no $CaCl_2$ are shown in Fig. 5. No sustained release was observed with any gel, although there was a delay before release, which was attributable to complex formation that occurred on the alginate gel bead surfaces. This result suggests that capsule formation may occur with alginate and chitosan, as reported previously (Pondya et al., 1991). Therefore, we conclude that a change of gel matrix conformation occurred in the gel bead interiors in the presence of adequate $CaCl_2$ and chitosan. Complex coacervation has been demonstrated to proceed gradually in aqueous media. The release rates of BB from 500M-CS and 500G-CS decreased with increasing incubation time (shown in Fig. 5).

The effect of the chitosan concentration in the buffer solution on the release of BB is shown in Table 2. The initial release rate from both 500M-CS and 500G-CS decreased gradually in proportion to the increase in chitosan concentration. In particular, a marked change was observed with 500G-CS, from which only 13% of the BB was released after incubation for 8 h. The percentages of alginate released from 500G-CS are listed in Table 3. All the hydrogels prepared without chitosan decayed progressively during incubation

TABLE 2

Release of BB from alginate gel beads

Chitosan (%)	Release (%)					
	90 min		180 min		300 min	
	500M-CS	500G-CS	500M-CS	500G-CS	500M-CS	500G-CS
0	30.2 ± 4.2	36.7 ± 3.7	59.9 ± 3.4	62.8 ± 1.9	–	–
0.02	5.6 ± 1.8	4.3 ± 0.4	34.5 ± 10.5	19.2 ± 3.1	73.6 ± 4.1	40.3 ± 5.8
0.05	4.5 ± 0.2	2.3 ± 0.4	25.8 ± 3.2	11.2 ± 0.8	50.7 ± 5.1	22.6 ± 0.5
0.1	4.6 ± 0.3	2.1 ± 0.3	16.8 ± 1.5	5.9 ± 1.2	30.6 ± 3.3	9.7 ± 2.7

All tests were performed in physiological saline. Data represent means ± S.D. (n = 3).

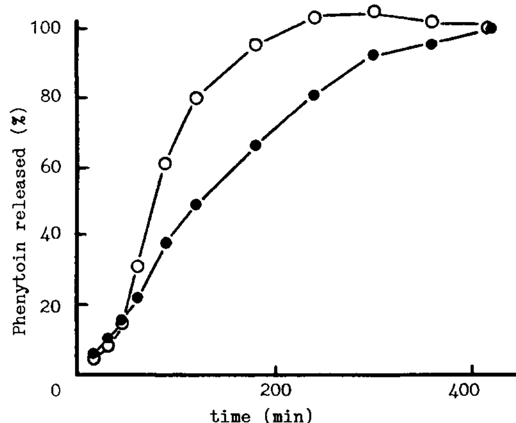

Fig. 6. Release curves of phenytoin from alginate (500G) gel beads. Treated for 1 day with (●) 0.2% chitosan and without (○) chitosan. All tests were carried out in 50 mM phosphate buffer (pH 6.8). Each gel bead contained 8.1 ± 0.6 mg of phenytoin ($n = 4$).

with physiological saline. However, this erosion was suppressed, in a concentration-dependent manner, by adding chitosan. Therefore, we concluded that erosion of the alginate gels in physiological saline affected the release of the solutes held within the beads.

As described above, the chitosan-treated alginate gels treated with chitosan resisted erosion in aqueous media which contained fully univalent cations, which would be expected to reflect the release of some drugs from the alginate gel matrix. The release of phenytoin held within 500G-CS incubated in phosphate buffer was delayed markedly compared with that from intact alginate gel prepared without chitosan (Fig. 6). We presume that the release rate was particularly affected by matrix erosion, because the phenytoin was dispersed in the gel matrix due to its poor solubility in aqueous media. In the Introduction, it was suggested that the reinforcement of calcium-induced alginate gel beads with chitosan may be attributable to complex coacervation between alginate and chitosan. We believe the process proceeds as follows: (a) rapid gelation is induced by Ca^{2+} followed by (b) gradual complex formation and gel matrix reconstruction. Thus, gel matrix erosion is suppressed and the compound held within it, such as coloring matter or phenytoin, is released slowly. We shall investigate further the effects of the characteristics of chitosan on this phenomenon (Imai et al., 1991).

Acknowledgements

The authors wish to thank Kibun Food Chemifa Co. for the use of the gel-permeation chromatography apparatus and Mr K. Saito (National Sanatorium, Iou Hospital) for valuable suggestions.

References

Cesaro, A., Delben, F. and Paoletti, S., Interaction of divalent cations with polyuronates. *J. Chem. Soc. Faraday Trans. 1*, 84 (1988) 2573–2584.

Farinotti, R. and Mahuzier, G., Simultaneous determination of six anticonvulsants in serum by high performance liquid chromatography. *J. Liq. Chromatogr.*, 2 (1979) 345–365.

Imai, T., Shiraishi, S., Saito, H. and Otagiri, M., Interaction of indomethacin with low molecular weight chitosan, and improvements of some pharmaceutical properties of indomethacin by low molecular weight chitosans. *Int. J. Pharm.*, 67 (1991) 11–20.

Kim, C.K. and Lee, E.J., The controlled release of blue dextran from alginate beads. *Int. J. Pharm.*, 79 (1992) 11–19.

Murata, Y., Kawashima, S., Miyamoto, E., Masauji, N. and Honda, A., Colorimetric determination of alginates and fragments thereof as the 2-nitrophenylhydrazides. *Carbohydr. Res.*, 208 (1990) 289–292.

Murata, Y., Nakada, K., Miyamoto, E. and Kawashima, S., Influence of erosion of calcium-induced alginate gel matrix on release of coloring matter. *J. Controlled Release*, 23 (1993) 21–26.

Nakajima, A. and Sato, H., Phase relation of an equivalent mixture of sulfated poly(vinyl alcohol) and aminoacetalyzed poly (vinyl alcohol) in microsalt aqueous solution. *Biopolymers*, 11 (1972) 1345–1355.

Nakajima, A. and Shinoda, K., Complex formation between oppositely charged polysaccharides. *J. Colloid Interface Sci.*, 55 (1976) 126–132.

Pondya, Y. and Knorr, D., Diffusion characteristics and properties of chitosan coacervate capsules. *Process Biochem.*, 26 (1991) 75–81.

Takahashi, A., Sol-gel transition of ionic polysaccharides. *Polym. J.*, 36 (1987) 786–789.

Takahashi, T., Takayama, K., Machida, Y. and Nagai, T., Characteristics of polyion complex of chitosan with sodium

alginate and sodium polyacrylate. *Int. J. Pharm.*, 61 (1990) 35–41.

Yotsuyanagi, T., Ohkubo, T., Ohhashi, T. and Ikeda, K., Calcium-induced gelation of alginic acid and pH-sensitive reswelling of dried gels. *Chem. Pharm. Bull.*, 35 (1987) 1555–1563.

Yotsuyanagi, T., Yoshioka, I., Segi, N. and Ikeda, K., Acid-induced and calcium-induced gelation of alginic acid: Bead formation and pH-dependent swelling. *Chem. Pharm. Bull.*, 39 (1991) 1072–1074.