International Journal of Pharmaceutics, 79 (1992) 11–19

IJP 02591

The controlled release of blue dextran from alginate beads

Chong-Kook Kim and Eun-Jin Lee

College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742 (Korea)

(Received 21 June 1991)
(Accepted 18 July 1991)

Key words: Alginate bead; Calcium-induced gelation; Blue dextran; Controlled release; Oral delivery system

Summary

The purpose of this paper is to explore the possible applicability of alginate gel beads as an oral controlled release system of macromolecular drugs. Blue dextran (M.W. approx. 2 000 000) was used as the model of macromolecular drugs. The release of blue dextran from alginate beads was strongly affected by drying time and blue dextran/sodium alginate ratio. However, the release was not particularly affected by the other factors such as sodium alginate concentration, calcium chloride concentration, curing time, and drop size. The drug release from alginate beads at pH 6.8 showed nearly zero-order release rate, which was more rapid than that at pH 1.2. Since the release of blue dextran as the model of macromolecular drugs could be controlled by the regulation of the preparation conditions of alginate beads, the alginate beads may be used for a potential oral controlled release system of such macromolecular drugs as vaccines and polypeptide drugs.

Introduction

Alginic acid is a hydrophilic, colloidal polysaccharide obtained from brown algae; it is a linear polymer of β-(1 → 4)-D-mannosyluronic acid (M) and α-(1 → 4)-L-gulosyluronic acid (G) residues and a macromolecular electrolyte that has one carboxyl group per constituent unit (Haug and Larsen, 1962; Haug et al., 1967a).

Alginate is easily gelled by the addition of Ca^{2+} to form an aqueous solution of sodium alginate, since insoluble calcium alginate is formed by cation exchange between Na^+ and Ca^{2+} (Haug and Smidsrod, 1965). The mechanism of gelation has been intensively investigated by circular dichroism (CD) and nuclear magnetic resonance studies. The gelation and crosslinking are due to the stacking of the guluronic acid (G) blocks of alginate chains with the formation of the egg-box junction (Katchalsky et al., 1961; Bryce et al., 1974).

Therefore, alginic acid or alginate is used as an immobilization matrix for cells and enzymes as well as pharmaceutical and food adjuvants (Awad and Awad, 1980; Kierstan et al., 1982; Connick, 1983; Badwan et al., 1985; Yang and Sharma, 1986; Yotsuyanagi et al., 1987; Segi et al., 1989). When an aqueous solution of sodium alginate was added dropwise to an aqueous solution of

Correspondence: C.-K. Kim, College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul, Korea.

calcium chloride, a spherical gel with regular shape and size was obtained. The spherical gel is termed 'alginate bead'.

Alginate beads have the following advantages: (1) alginate is known to be nontoxic when taken orally, and also to have a protective effect on the mucous membranes of the upper gastrointestinal tract (Koji et al., 1981a–c, 1982); (2) since dried alginate beads have the property of reswelling, they can act as a controlled-release system; (3) since their property of reswelling is susceptible to environmental pH, acid-sensitive drugs incorporated into the beads would be protected from gastric juice (Haug and Larsen, 1963; Haug et al., 1963, 1967b; Yotsuyanagi et al., 1987).

However, porosity gives alginate beads not only a fast release pattern of incorporated drugs but also a very low efficiency of incorporation of low molecular weight drugs, except for sparingly soluble drugs (Pfister et al., 1986). Therefore, it appears that alginate beads can be used for a controlled release system of macromolecular drugs or low molecular weight drugs bound to macromolecules through covalent or noncovalent bonds.

The purpose of this paper is to explore the possible applicability of alginate beads as an oral controlled release system of such macromolecular drugs as vaccines and polypeptide drugs. As the model of macromolecular drugs, blue dextran (M.W. approx. 2000000) was used, since it is chemically stable and readily identified. Furthermore, it can be used as a marker in estimation of the molecular weights of macromolecules by gel chromatography as its molecular size is reliably known (Mayes, 1984). In this experiment, blue dextran was incorporated into alginate beads which we investigated with regard to their physico-pharmaceutical characteristics.

Materials and Methods

Materials

Blue dextran (average M.W. approx. 2000000, Sigma Chemical Co., St. Louis, U.S.A.) and sodium alginate (Extra Pure, Junsei Chemical Co., Tokyo, Japan) were used. All other chemicals used were of reagent grade.

Preparation of alginate beads

Blue dextran (90 mg) was added to 4.5 g of 2.0% sodium alginate solution in a 10 ml beaker and dissolved completely. Using a 5 ml syringe (18 and 26 gauge needles), this solution was transferred dropwise to about 30 ml of 0.1 M calcium chloride solution in a 50 ml beaker with mild agitation within a period of 3 min. The mixture was then stirred slowly for 6 min to cure the alginate gel beads. The alginate beads were separated from the solution and dried at 80 °C for 12 h using an incubator (Model D7-2945, Dong-Yang Instruments, Inc., Seoul, Korea).

Assessment of drug incorporation into alginate beads

Alginate beads (20 mg) were added to 40 ml of pH 7.2 phosphate buffer in a 50 ml volumetric flask and dissolved completely. Subsequently, the solution was filled with pH 7.2 phosphate buffer to 50 ml and allowed to stand overnight. The solution was filtered and the absorbance of blue dextran was measured using a UV spectrophotometer (UV 2100, Shimadzu, Kyoto, Japan) at 260 nm.

Calcium content in alginate beads

Dried alginate beads prepared using different curing times were washed once with triple-distilled water and then dried at 80 °C for 1 h.

10 mg of dried alginate beads was added to 2 ml of 60% nitric acid and 1 ml of 60% perchloric acid in a 50 ml Erlenmeyer flask, and then incinerated by heating gradually until free of carbon. The carbon-free ash obtained was rinsed with triple-distilled water and poured into a 250 ml volumetric flask. The calcium concentration was determined spectrophotometrically by induced coupled-plasma atomic emission spectroscopy (ICP/5500B, Perkin-Elmer, Norwalk, CT, U.S.A.) at 393.37 nm.

Release test of blue dextran

The U.S.P. XXII rotating paddle method was used to determine the amount of blue dextran released from alginate beads. The vessels and paddles of the dissolution tester (DST-200, Fine Scientific Instruments, Seoul, Korea) were modi-

fied for use on a small scale. 125 ml of dissolution medium (pH 1.2 HCl solution or pH 6.8 phosphate buffer solution) was introduced into a vessel and stirred at 100 rpm. An accurately weighed amount (i.e. 32.5 mg as blue dextran) of alginate beads was added to the dissolution medium at 37 ° C.

At scheduled intervals, 2-ml samples were taken. The solution was filtered through a 0.45 μm membrane filter to remove any solid particles, and the filtrate was analyzed. An equivalent volume (2 ml) of fresh dissolution medium was added to keep the volume of dissolution medium in the beaker constant at 125 ml.

The amount of blue dextran released was determined spectrophotometrically at 260 nm.

Results and Discussion

Curing of alginate beads

The series of steps leading to the formation of alginate beads can be described as follows: the droplets of sodium alginate solution containing blue dextran came into contact with the $CaCl_2$ solution. The Na^+ in the droplets was substituted by Ca^{2+}, thus yielding the alginate gel. At this point, on inspection of an alginate bead against the light, a circular boundary was observed within the bead, constituting the border of the ion exchange between Na^+ and Ca^{2+}. This circular boundary gradually shrank with progression of the curing process and finally disappeared.

On preparing alginate beads with a drop size of approx. 2 mm in diameter, it was noted that the time required for this circular border to disappear was 6 min. Hence, the change in calcium content of alginate beads as a function of curing time was observed to determine the period required for full curing (Fig. 1). The curing of alginate beads appeared to reach near completion after 6 min. The weight of each alginate bead was also found to depend on the curing time, therefore, the calcium content was calculated per unit mass (mg) and per bead. However, the patterns of the curves were similar to each other.

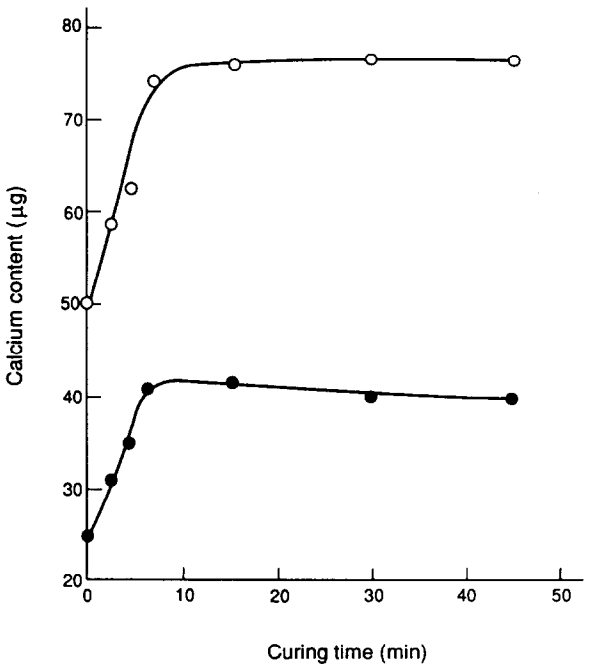

Fig. 1. Effect of curing time on calcium content per unit mass (mg) of alginate beads (○) and per bead (●). Alginate beads were prepared under the following conditions: 2.0% sodium alginate; 0.1 M $CaCl_2$; drying at 80 ° C for 60 min; drop size, diameter 2 mm; blue dextran/sodium alginate ratio, 1 : 1.

Shape of alginate beads

The alginate beads were very spherical (Fig. 2A) and, subsequent to drying, their sizes decreased to about 1/3 of the radius (Fig. 2B). The dried alginate beads, after release for 3 h, became slightly swollen at pH 1.2 (Fig. 2C). However, at pH 6.8, they became appreciably more swollen, dissolving gradually (Fig. 2D), and had mostly dissolved after 8 h. It appeared that they underwent a β-elimination reaction at pH 6.8, which is susceptible to the environmental pH (Haug and Larsen, 1963; Haug et al., 1963, 1967b).

Weight distribution of alginate beads

The alginate beads were prepared using 26 and 18 gauge needles (drop size of approx. 2 and approx. 4 mm, respectively). 50 beads were taken from each batch separately and the individual weight of the alginate beads was determined on an M5 Micro-Balance (E. Mettler, Zurich, Switzerland). The number distributions according

to the alginate bead weight were evaluated (Fig. 3). The weight distributions of alginate beads were found to fall within comparatively narrow ranges and were log-normally distributed.

Incorporation of drug into alginate beads

The efficiency of incorporation of blue dextran into alginate beads was not significantly affected by curing time. In the case of 2.0% sodium alginate, the fully cured alginate beads (after 6 min curing) had incorporation efficiencies particularly close to each other, namely, more than 80%.

Release of blue dextran

Release patterns according to the pH of the release medium The release of blue dextran from

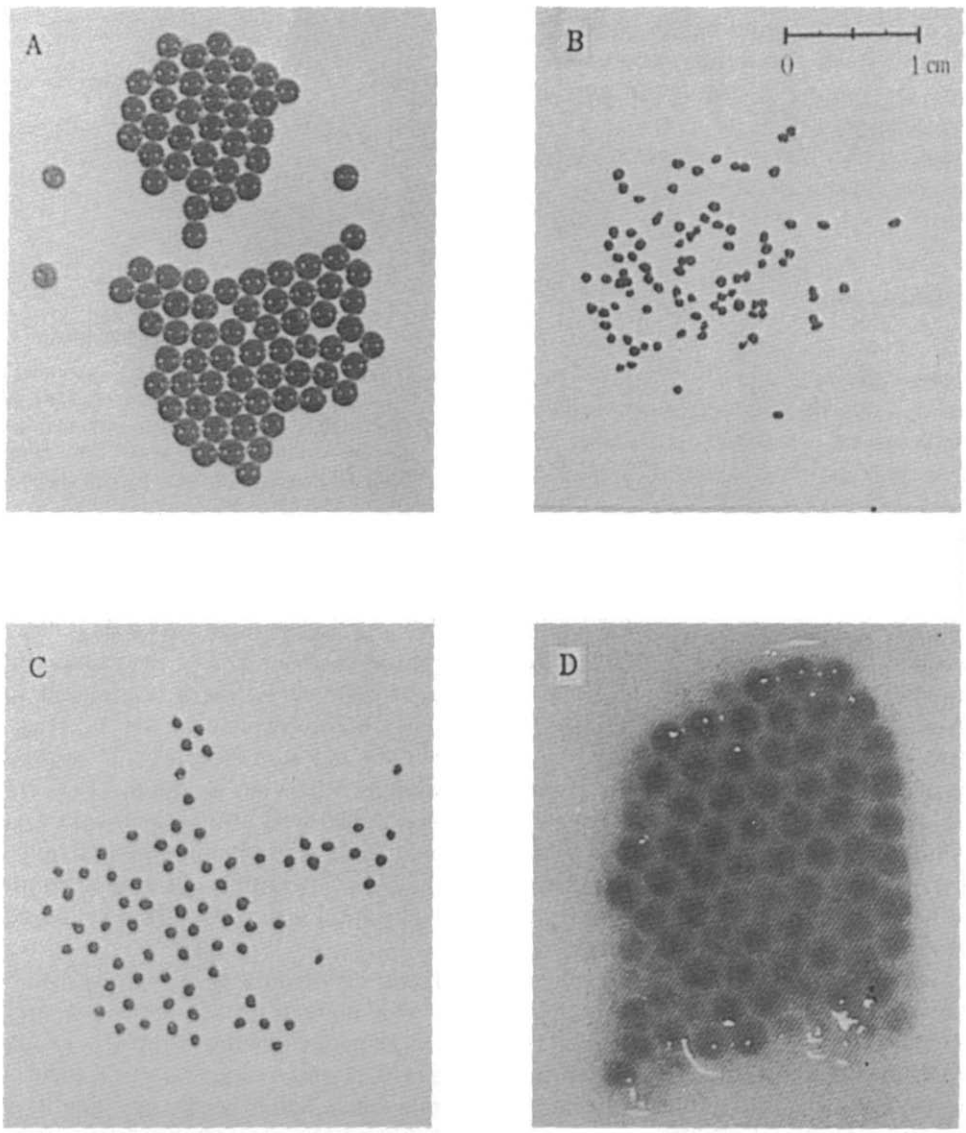

Fig. 2. Photographs of alginate beads before drying (A), after drying (B), and after release of blue dextran for 3 h at pH 1.2 (C) and pH 6.8 (D). Alginate beads were prepared under the following conditions: 2.0% sodium alginate; 0.1 M $CaCl_2$; curing time, 6 min; drying at 80 ° C for 12 h; drop size, diameter 2 mm; blue dextran/sodium alginate ratio, 1 : 1.

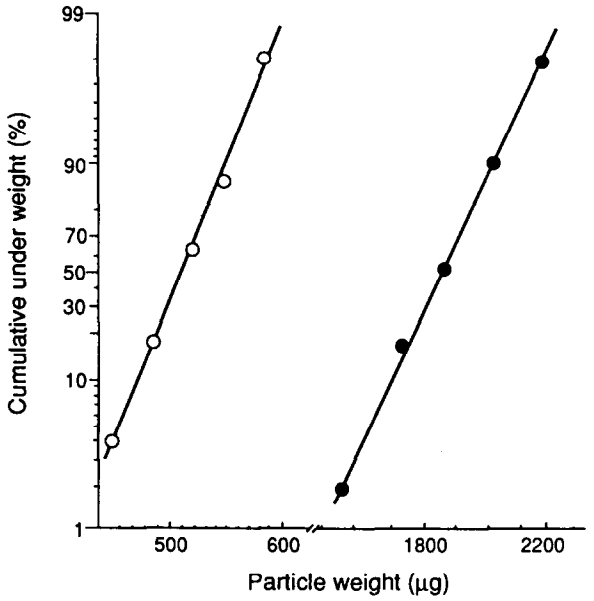

Fig. 3. Weight distribution of alginate beads according to drop sizes of diameter 2 mm (○) and 4 mm (●). Alginate beads were prepared under the following conditions: 2.0% sodium alginate; 0.1 M $CaCl_2$; curing time, 6 min; drying at 80 °C for 12 h; blue dextran/sodium alginate ratio, 1:1.

alginate beads was monitored at pH 1.2 and 6.8 and the results obtained were used for comparison (Figs 4–6). The release of blue dextran from alginate beads at pH 6.8 was more rapid than that at pH 1.2 and showed nearly zero-order kinetics. 10–30% of blue dextran was released during a period of 8 h at pH 1.2 as compared to almost 100% at pH 6.8.

In contrast to low molecular weight drugs, blue dextran is so large that it cannot diffuse through the pores of the alginate gel matrix which has not swollen. Therefore, no more than 30% of blue dextran was released over an 8 h period at pH 1.2, at which pH alginate beads scarcely swell. However, at pH 6.8, the release of blue dextran from alginate beads is due both to its diffusion through the swollen matrix of alginate beads and to escape from the beads' surface which underwent erosion after swelling. If this were due only to the diffusion of blue dextran, its rate of release would be first order at pH 6.8, since the path length of diffusion increases with time. However, its rate of release was found to be zero order, since it is also the result of erosion of alginate beads and the escape of blue dextran from the matrix.

Effect of sodium alginate concentration In the investigation of the effect of sodium alginate concentration in droplets on the process of gelation, sodium alginate concentrations of 1.8, 2.0 and 2.2% were employed in the preparation of alginate beads. The blue dextran/sodium alginate ratio was held constant at 1:1. The release of blue dextran into dissolution media (pH 1.2 and 6.8) was monitored by measuring the UV absorbance at 260 nm.

It was observed that, for sodium alginate concentrations above 2.2% during the preparation of the beads the viscosity of the sodium alginate solution was so high that the formation of drops was strongly hindered. Moreover, at sodium alginate concentrations below 1.8%, the alginate beads were unable to retain their spherical form during the drying process. Nevertheless, once prepared within the appropriate range of sodium alginate concentrations, alginate beads displayed similar patterns of release.

The alginate gels appeared to possess a constant composition of alginate beads, the release of blue dextran not noticeably being affected by the concentration of sodium alginate solution in droplets during gelation.

Effect of calcium chloride concentration A mixture of 2.0% sodium alginate solution (4.5 g) and blue dextran (90 mg) was extruded into 30 ml of 0.05 M $CaCl_2$ solution to form alginate beads of approx. 2 mm diameter. Release of blue dextran into dissolution media (pH 1.2 and 6.8) was monitored by measuring the absorbance at 260 nm. Alginate beads were also prepared according to the same procedure, but at concentrations of 0.1 and 0.2 M $CaCl_2$ solution. Alginate beads prepared within the above range of $CaCl_2$ concentrations showed comparable patterns of release.

Similar results have previously been reported by Kierstan et al. (1982). The latter workers observed that the diffusional behavior of hemoglobin (M.W. about 64500) from calcium alginate gels was unaffected by variations in $CaCl_2$ concentration from 0.125 to 0.5 M.

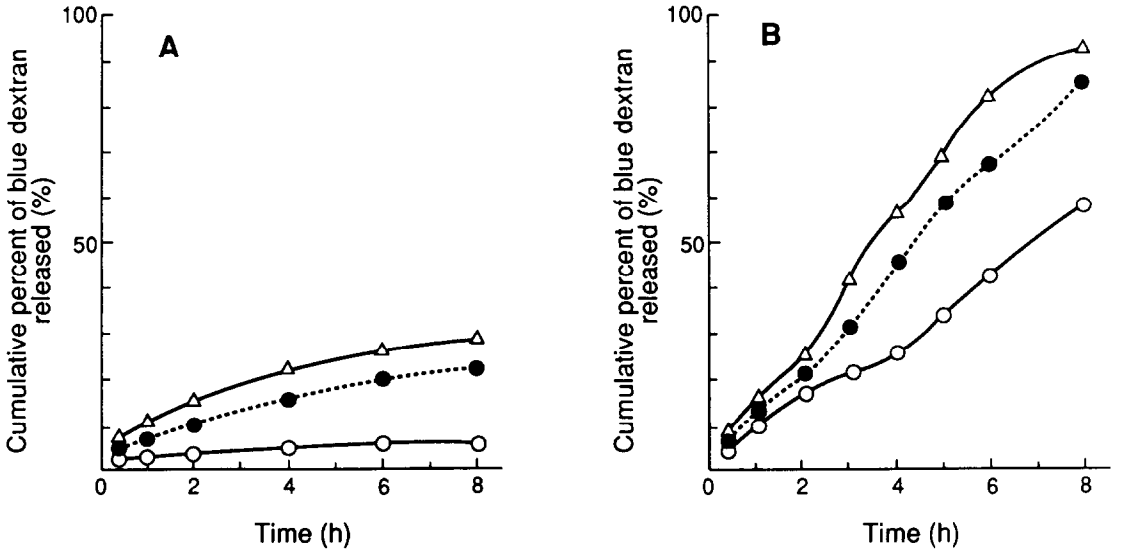

Fig. 4. Effect of drying time on in vitro release of blue dextran from alginate beads at pH 1.2 (A) and pH 6.8 (B). Alginate beads were prepared under the following conditions: 2.0% sodium alginate; 0.1 M $CaCl_2$; curing time, 6 min; drying at 80 °C for 0 (○), 6 (●), and 12 (△) h; drop size, diameter 2 mm; blue dextran/sodium alginate ratio, 1 : 1.

Effect of curing time The effect of curing time was examined: this corresponds to the period of immersion of the alginate droplets in $CaCl_2$ solution that is required for curing of the gel structure subsequent to droplet formation. Alginate beads of a particular batch were removed at appropriate intervals from the $CaCl_2$ solution for sample analysis.

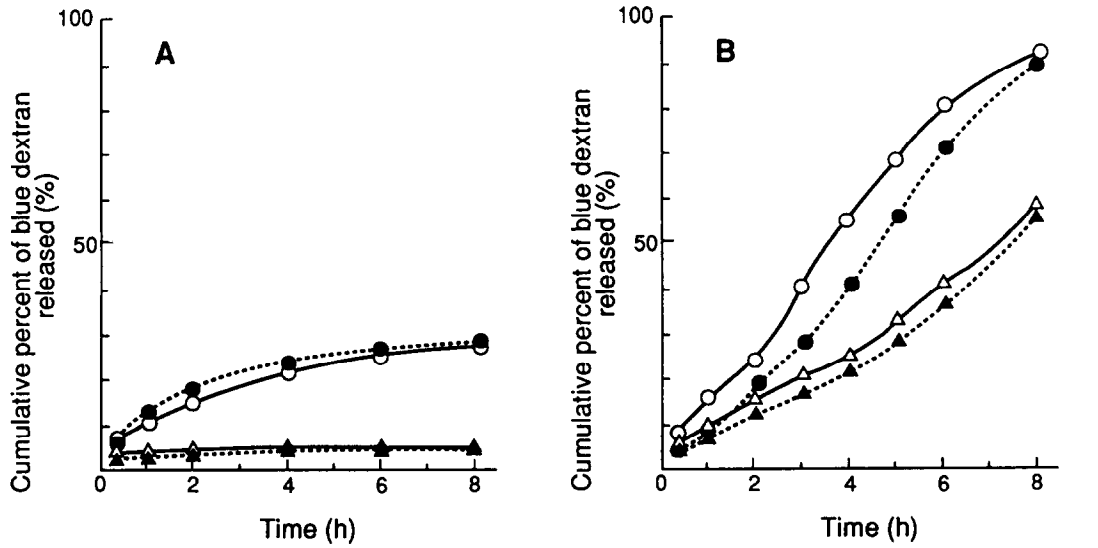

Fig. 5. Effect of drop size on in vitro release of blue dextran from alginate beads at pH 1.2 (A) and pH 6.8 (B). Alginate beads were prepared under the following conditions: 2.0% sodium alginate; 0.1 M $CaCl_2$; curing time, 6 min; drying at 80 °C for 12 h; before drying (triangle) and after drying (circle); drop size, diameter 2 mm (unfilled symbols) and 4 mm (filled symbols); blue dextran/sodium alginate ratio, 1 : 1.

Release of blue dextran from alginate beads cured for 60 min was somewhat slower than that from beads that had been cured for 6 min. However, the patterns of release showed very little variation between alginate bead samples cured for longer than 6 min. Therefore, it appears that the effect of curing time on the release of fully cured alginate beads is very minor.

Effect of drying time The influence of the degree of dehydration on the release of blue dextran was investigated by drying alginate bead samples at 80 °C for 6 and 12 h, whilst others were left undried. The alginate beads that had been heated at 80 °C for 12 h appeared to have become fully dehydrated.

Release of blue dextran from alginate beads dried to constant weight by heating at 80 °C for 12 h was observed to take place at a faster rate as compared to the beads subjected to a different experimental regime (Fig. 4). Dried alginate beads appeared to have developed a small degree of surface cracking during release. Such cracks facilitated surface erosion of the alginate beads and, consequently, blue dextran escaped more rapidly from the beads as compared to the undried samples.

Effect of drop size Dried alginate bead preparations of approx. 2 and 4 mm diameter in drop size exhibited somewhat different patterns of release. The plots corresponding to alginate beads before dehydration also showed a definite dependence on drop size, although rather minor in extent (Fig. 5). As compared to other factors in the present study which exert an influence on the process of release of blue dextran from alginate beads, the drop size had very little effect, at variance with the results reported by Kierstan et al. (1982).

Effect of blue dextran / sodium alginate ratio Different alginate bead samples were prepared such that the blue dextran/sodium alginate ratio was 1:1, 1:3 and 1:6. Release of blue dextran from the alginate beads was then monitored at pH 1.2 and 6.8.

It was observed that the greater the content of alginate, the slower was the rate of release of blue dextran from the alginate beads (Fig. 6). The alginate beads constitute a matrix of enterically dissolving material, therefore, the latter phenomenon was clearly in evidence and the beads additionally showed a sustained release pattern as compared to an enteric coating system. Blue

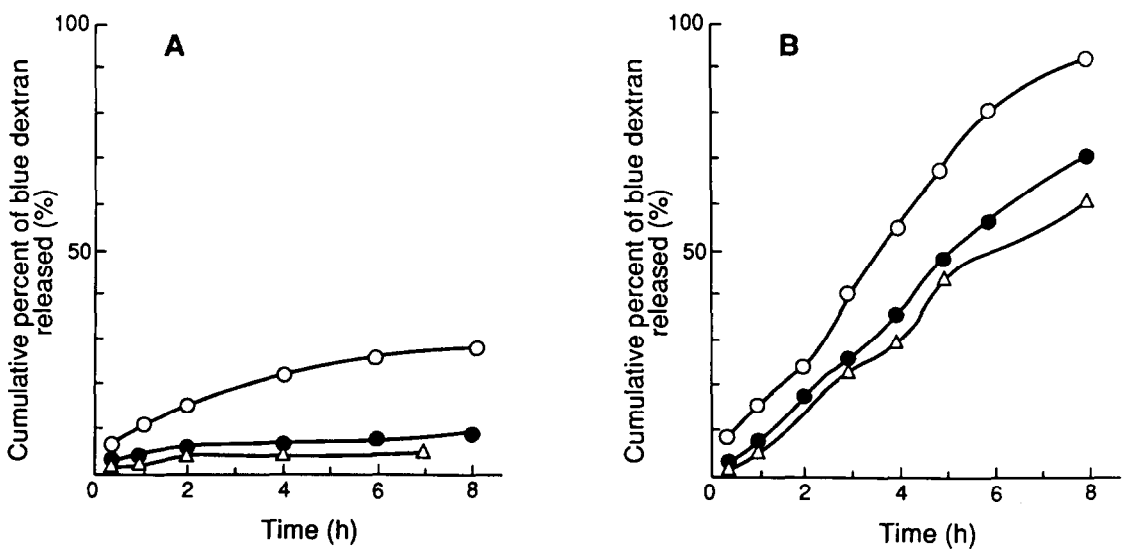

Fig. 6. Effect of blue dextran/sodium alginate ratio on in vitro release of blue dextran from alginate beads at pH 1.2 (A) and pH 6.8 (B). Alginate beads were prepared under the following conditions: 2.0% sodium alginate; 0.1 M $CaCl_2$; curing time, 6 min; drying at 80 °C for 12 h; drop size, diameter 2 mm; blue dextran/sodium alginate ratio, 1:1 (○), 1:3 (●) and 1:6 (△).

dextran is a high molecular weight substance and its release is strongly influenced by the swelling and erosion of the alginate gel matrix, with the result that the release of blue dextran has a significant dependence on the blue dextran/sodium alginate ratio. Similar results were reported by Kierstan et al. (1982).

Conclusion

The release of blue dextran from alginate beads was considerably affected by drying time and the blue dextran/sodium alginate ratio. Moreover, under preparative conditions that were maintained constant, the release of blue dextran displayed no significant dependence on the other experimental factors such as sodium alginate concentration, $CaCl_2$ concentration, curing time and drop size. Therefore, alginate beads can be prepared that show reproducible release behavior which indicates that the development of alginate beads hold promise for manufacturing purposes in the future.

The release of blue dextran as a model of macromolecular drugs was regulated by the appropriate choice of experimental conditions for the preparation of alginate beads, and hence, alginate beads may be used in oral controlled release systems for such macromolecular drugs as vaccines and polypeptide drugs.

Acknowledgement

This study was partly supported by a research grant from the Research Center for New Drug Development.

References

Awad, A. and Awad, O., Alginate matrix-supported acetylcholinesterase. *J. Chem. Tech. Biotechnol.*, 30 (1980) 583–586.

Badwan, A.A., Abumalooh, A., Sallam, E., Abuhalaf, A. and Jawan, O., A sustained release drug delivery system using calcium alginate beads. *Drug Dev. Ind. Pharm.*, 11 (1985) 239–256.

Bryce, T.A., McKinnon, A.A., Morris, E.R., Rees, D.A. and Thom, D., Chain conformations in the sol-gel transitions for polysaccharide systems, and their characterization by spectroscopic methods. *Faraday Disc. Chem. Soc.*, 57 (1974) 221–229.

Connick, W.J., Jr., Controlled release of bioactive materials using alginate gel beads, *US Patent* 4,401,456 (1983).

Haug, A. and Larsen, B., Quantitative determination of the uronic acid composition of alginates. *Acta Chem. Scand.*, 16 (1962) 1908–1918.

Haug, A. and Larsen, B., The solubility of alginate at low pH. *Acta Chem. Scand.*, 17 (1963) 1653–1662.

Haug, A., Larsen, B. and Smidsrod, O., The degradation of alginate at different pH values. *Acta Chem. Scand.*, 17 (1963) 1466–1469.

Haug, A. and Smidsrod, O., The effect of divalent metals on the properties of alginate solutions. *Acta Chem. Scand.*, 19 (1965) 341–351.

Haug, A., Larsen, B. and Smidsrod, O., Studies on the sequence of uronic acid residues in alginic acid. *Acta Chem. Scand.*, 21 (1967a) 691–704.

Haug, A., Larsen, B. and Smidsrod, O., Alkaline degradation of alginate. *Acta Chem. Scand.*, 21 (1967b) 2859–2870.

Katchalsky, A., Cooper, R.E., Upadhyay, J. and Wassermann, A., Counter-ion fixation in alginates. *J. Chem. Soc.*, 1961 (1961) 5198–5204.

Kierstan, M., Darcy, G. and Reilly, J., Studies on the characteristics of alginate gels in relation to their use in separation and immobilization applications. *Biotechnol. Bioeng.*, 24 (1982) 1507–1517.

Koji, D., Yutaka, W., Chiaki, Y., Mamabu, Y., Seiji, O., Masayuki, O. and Takashi, M., Pharmacological studies of sodium alginate. I. Protective effect of sodium alginate on mucous membranes of upper-gastrointestinal tract. *Yakugaku Zasshi*, 101 (1981a) 452–457.

Koji, D., Chiaki, Y., Yutaka, W., Mamabu, Y., Seiji, O., Masayuki, O. and Takashi, M., Pharmacological studies of sodium alginate. II. Hemostatic effect of sodium alginate on gastrointestinal bleeding. *Yakugaku Zasshi*, 101 (1981b) 458–463.

Koji, D., Mamabu, Y., Chiaki, Y., Yutaka, W., Seiji, O., Masayuki, O. and Takashi, M., Pharmacological studies of sodium alginate. III. Acceleration of fibrin formation by sodium alginate. *Yakugaku Zasshi*, 101 (1981c) 464–469.

Koji, D., Chiaki, Y., Mamabu, Y., Masayuki, O., Takashi, M. and Hisanao, K., Pharmacological studies of sodium alginate. IV. Erythrocyte aggregation by sodium alginate. *Yakugaku Zasshi*, 102 (1982) 573–578.

Mayes, E.L.V., Determination of protein molecular weights by gel permeation high pressure liquid chromatography. In Walker, J.M. (Ed.), *Methods in Molecular Biology*, Vol. 1, Humana, NJ, 1984, pp. 5–12.

Pfister, G., Bahadir, M. and Korte, F., Release characteristics of herbicides from calcium alginate gel formulations. *J. Controlled Release*, 3 (1986) 229–233.

Segi, N., Yotsuyanagi, T. and Ikeda, K., Interaction of calcium-induced gelation of alginic acid and pH-sensitive reswelling of dried gels. *Chem. Pharm. Bull.*, 37 (1989) 3092–3095.

Yang, R.K. and Sharma, S.C., Delivery system for an active ingredient and a process for preparation thereof, *European Patent Application*, 202,819 (1986).

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.