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International Journal of Pharmaceutics 168 (1998) 9–15

international journal of pharmaceutics

Alternative approach to the preparation of chitosan beads

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Received 12 August 1997; received in revised form 22 January 1998; accepted 2 February 1998

Abstract

A controlled-release protein delivery system was investigated by using bovine serum albumin (BSA) as a model drug. Chitosan was reacted with sodium alginate in the presence of tripolyphosphate for bead formation. Spherical beads were produced with diameter in the range 0.78–0.92 mm and 13–90% encapsulation efficiency. It appeared that encapsulation of BSA was affected by the initial protein and sodium alginate concentrations and the presence of pectin (1%) in the external phase. Bead sizes changed with alginate concentration and pectin addition. Release properties of the beads were affected by their BSA content. Addition of pectin to the external phase decreased the percentage release of BSA from the beads. It can be concluded that alginate-reinforced chitosan beads might be a potential delivery system for protein encapsulation.

Keywords: Alginate; Beads; Bovine serum albumin; Chitosan; Controlled-release; Drug delivery systems; Pectin

1. Introduction

Biodegradable natural polymers are becoming increasingly important in the design of controlled-release drug delivery systems, particularly for the delivery of protein and peptide drugs (Polk et al., 1994, Sezer and Akbuğa, 1995a, Aydin and Akbuğa, 1996, Okhamafe et al., 1996). Among them, sodium alginate, chitosan and chitosan–alginate microcapsules and beads have been evaluated for proteins (Polk et al., 1994). Chitosan, the polycationic polysaccharide, forms gel beads with multivalent counterions (Shiraishi et al., 1993, Sezer and Akbuğa, 1995b). However, the membrane formed may have a limited strength. Furthermore, in order to obtain more stable alginate beads, complex coacervation between two oppositely charged polysaccharides has been studied (Murata et al., 1993, Okhamafe et al., 1996). In the preparation of chitosan beads, post-treatment of the beads with glutaraldehyde is necessary and this may be toxic in many cases.

Therefore, in the preparation of chitosan beads, sodium alginate could be used instead of glutaraldehyde for better cross-linking but there is no information about the addition of sodium alginate

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0378-5173/98/$19.00
PII S0378-5173(98)00072-6

Table 1
Composition, loading efficiency and diameter of BSA loaded chitosan beads

Formulation	BSA (%)	Na-alginate (%)	Pectin (%)	Glutaraldehyde (%)	Loading efficiency (%)	Diameter (μm $\pm$ S.D.)
A0	1.64	0.5	–	–	77.43	788.1 $\pm$ 6.4
A1	3.23	0.5	–	–	58.82	847.1 $\pm$ 3.6
A2	6.25	0.5	–	–	28.64	843.3 $\pm$ 5.4
A3	11.76	0.5	–	–	13.60	856.4 $\pm$ 1.8
B1	3.23	1.0	–	–	53.25	880.7 $\pm$ 8.5
B2	6.25	1.0	–	–	30.40	889.4 $\pm$ 4.9
C1	1.64	0.5	–	2.5	89.02	853.4 $\pm$ 3.2
C2	3.23	0.5	–	2.5	52.63	869.6 $\pm$ 5.0
D1	1.64	0.5	1	–	91.46	910.7 $\pm$ 4.9
D2	3.23	0.5	1	–	55.73	922.4 $\pm$ 5.3
D3	3.23	0.5	2	–	56.65	892.2 $\pm$ 7.2
E	3.23	–	–	10	39.44	898.6 $\pm$ 5.7

to the external phase for the preparation of chitosan beads. This paper describes a new approach for preparing more stable membranes, which are made of covalently bound biopolymers around chitosan beads. Alginate-reinforced chitosan gel beads were prepared using bovine serum albumin (BSA) for protein release as a model protein. The effects of different factors, such as initial BSA amount, sodium alginate concentration and addition of pectin and glutaraldehyde to the external phase, on bead properties were also studied.

2. Materials and methods

2.1. Materials

Chitosan (Sigma, USA), sodium alginate LF 20/50 (Pronova, Norway), tripolyphosphate (TPP; Sigma, USA), bovine serum albumin (BSA; Sigma, USA), pectin (from apple; BDH, UK), glutaraldehyde and glacial acetic acid (E. Merck, Germany) were used.

2.2. Preparation of beads

Alginate-treated chitosan beads containing BSA were prepared according to the method of Bodmeier et al. (1989) as follows. A weighed amount of BSA was dissolved in bidistilled water and added to an aqueous solution of chitosan (1.5%, w/v) in acetic acid. This mixture was dropped through a syringe into gently agitated TPP solution (1%, w/v) containing sodium alginate to form alginate-reinforced chitosan beads. The beads were separated and washed with bidistilled water at least three times. Finally, the beads were filtered and dried at room temperature. A number of variables such as BSA and sodium alginate concentrations, addition of pectin or glutaraldehyde into the external phase were investigated for optimization of bead properties (Table 1) ($n = 3$).

2.3. Physical characterization of beads

A photomicroscope (Olympus BH-2, Japan) was used to evaluate the shape and surface of the beads.

The size of the beads was determined using a standard light microscope with micrometer (Olympus BH, Japan).

2.4. Determination of BSA content of beads

The protein content of beads was determined using a digestion method. Briefly, digestion of beads was achieved by treating 25 mg of beads with 3 ml of phosphate-buffered saline (PBS) solution for at least 18 h at 25 $\pm$ 0.5°C. This procedure was repeated three times. BSA content was spectrophotometrically assayed at 595 nm according to Bradford's method (Bradford, 1976).

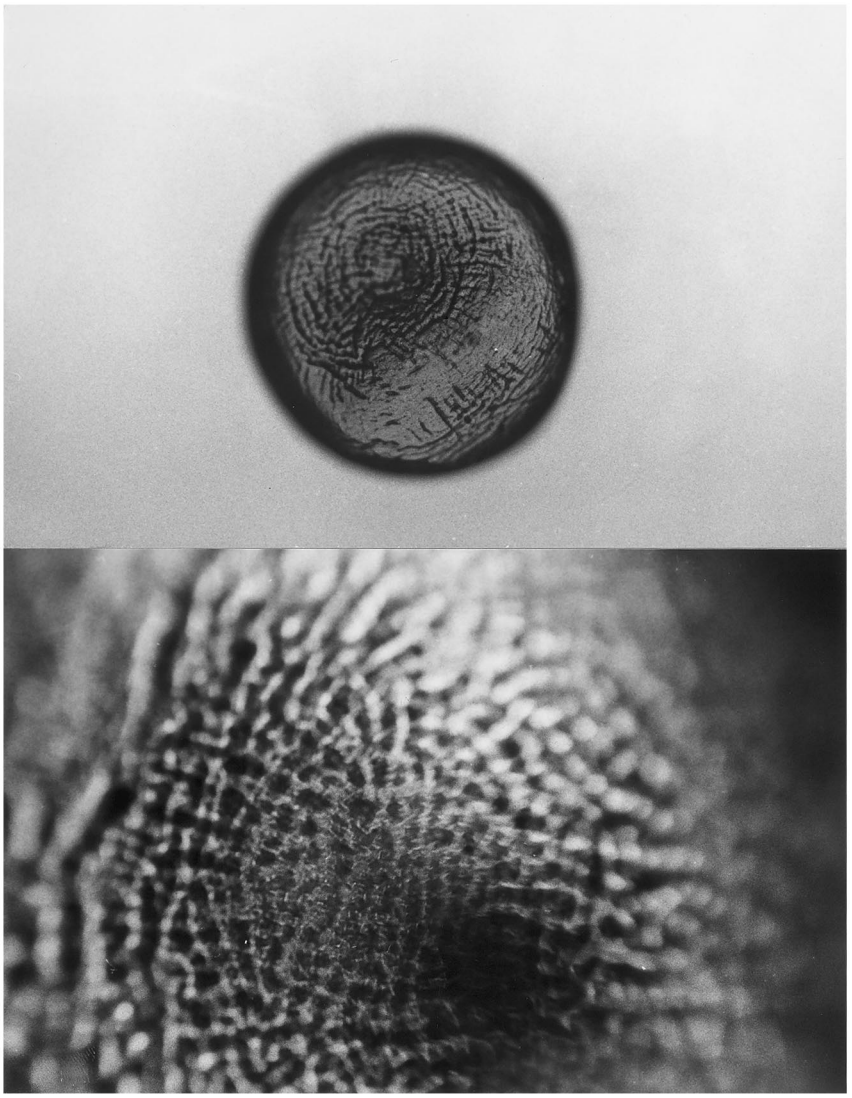

Fig. 1. Photographs of alginate-treated chitosan beads. Magnifications: top, $\times 4$; bottom, $\times 20$.

The efficiency of encapsulation was calculated by expressing the actual entrapment level divided by the theoretical entrapment level, as a percentage.

2.5. In vitro release studies

A weighed quantity of beads was suspended in PBS (pH 7.4) contained in a glass bottle. This medium was stirred at 100 rpm in a laboratory shaker and maintained at 37 ± 0.1°C in a water bath. Samples were periodically removed and BSA amount was analyzed spectrophotometrically (Shimadzu UV-2100S, Japan) at 595 nm according to Bradford's method. The means of three determinations are given.

2.6. Statistical analysis

Student's t-test was used to compare the results. They were considered statistically significant if $p < 0.05$.

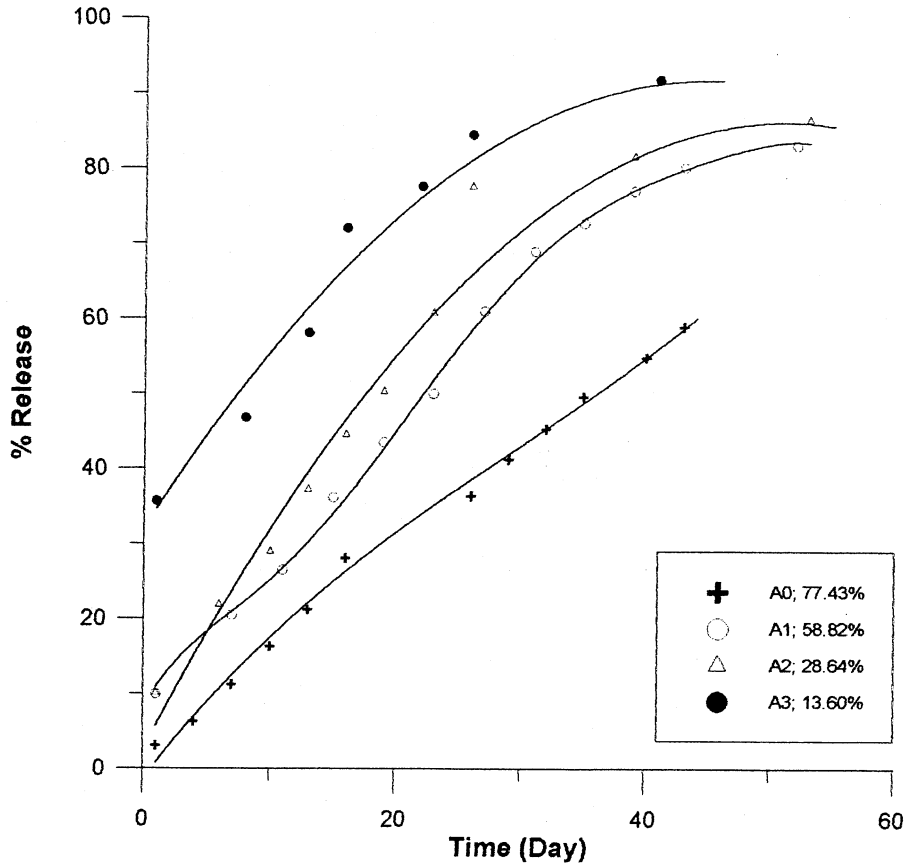

Fig. 2. Effect of initial BSA amount on protein release from beads.

3. Results and discussion

Gel beads composed of different negatively and positively charged polymers represent a type of drug delivery system that can be prepared without a tedious process.

In chitosan beads, the polyelectrolyte complex occurs not only between chitosan and TPP but also between chitosan and alginate, and it protects the gel matrix from environmental conditions. By using this ionotropic gelation technique, roughly spherical and regular shaped chitosan beads were obtained (Fig. 1). The beads were about 0.78–0.92 mm in diameter (Table 1). The weight distributions of beads were found to change within comparatively narrow ranges. Smaller beads were obtained by the addition of sodium alginate to TPP solution ($p < 0.05$). As seen in Table 1, bead sizes increased with pectin incorporation to the TPP phase (Formulation D1) and size also changed with alginate concentration (Formulations A1 and B1). It was observed that, with a sodium alginate concentration above 1% during the preparation of beads, the viscosity of the external phase was so high that the formation of drops was strongly hindered.

The encapsulation efficiencies of the beads varied from 13% to 90%. BSA content and encapsulation efficiencies are summarized in Table 1. It appears that encapsulation of BSA into alginate-treated chitosan beads was significantly affected by the initial protein concentration and the amount of sodium alginate added ($p < 0.05$); an increase in initial BSA amount led to a dramatic decrease in BSA encapsulation efficiency (Formulation A3). On the other hand, protein encapsula-

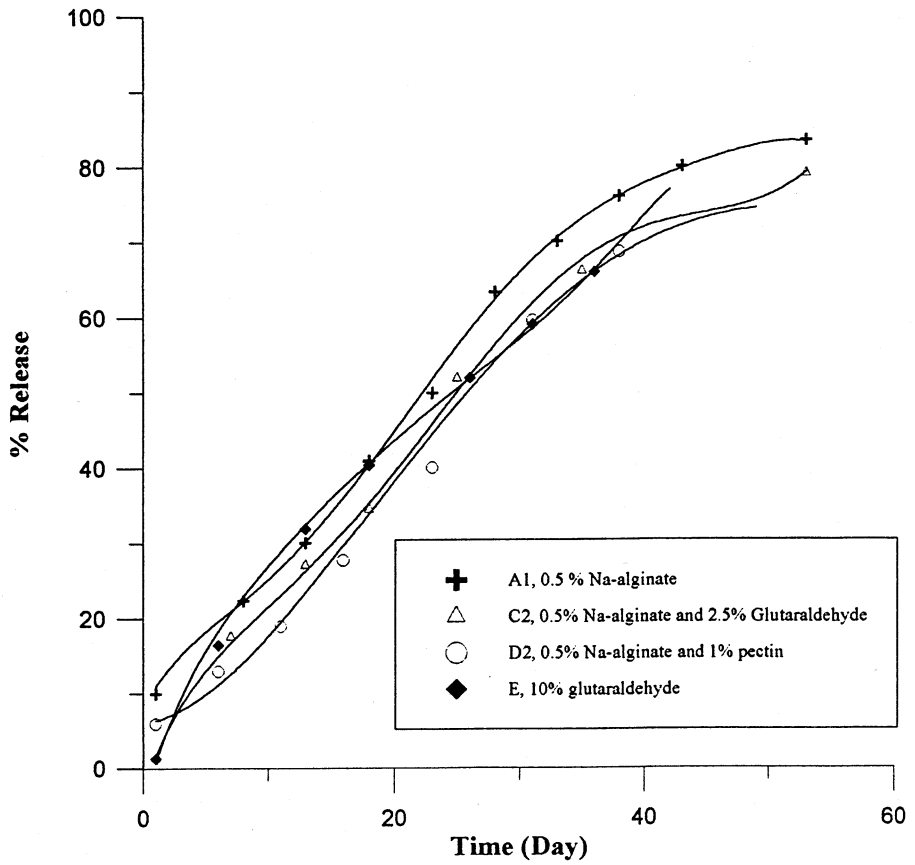

Fig. 3. Effect of formulation variables (pectin or glutaraldehyde addition to the structure of alginate-treated chitosan beads) on protein release from beads.

tion of alginate-treated chitosan beads changed with the addition of pectin in the TPP phase ($p < 0.05$). As seen in Table 1, in the case of pectin addition (1%) to the structure of alginate-treated chitosan beads, the highest encapsulation efficiency (91.46%) was obtained (Formulation D1). Moreover, no significant difference was found between the BSA encapsulation efficiencies of beads prepared with sodium alginate or glutaraldehyde (Formulations A1 and C2, respectively) ($p > 0.05$). Despite the high solubility of BSA in the aqueous phase, an encapsulation efficiency $> 50\%$ was generally achieved for many formulations.

Release profiles of BSA are given in Figs. 2–4. BSA release from alginate-treated chitosan beads was affected by the amount of protein in the beads. An increase in BSA in caused a decrease in its release from the beads (Fig. 2). In general, release profiles were characterized by a rapid initial drug release phase followed a second release phase (Formulation A). As seen in Fig. 2, except Formulation A_0, more than 10% of encapsulated BSA was released within the first day. However, by using the combination of glutaraldehyde and alginate in the external phase (Formulations C1 and C2) this burst effect disappeared (Fig. 3). On the other hand, as seen in Fig. 3, chitosan beads prepared with a sodium alginate–pectin (1%) combination, the burst effect was also reduced (Formulation D2). The best result was obtained with addition of a 1% concentration of pectin. Compared to the release profiles of chitosan beads prepared with sodium alginate (A1) and glu-

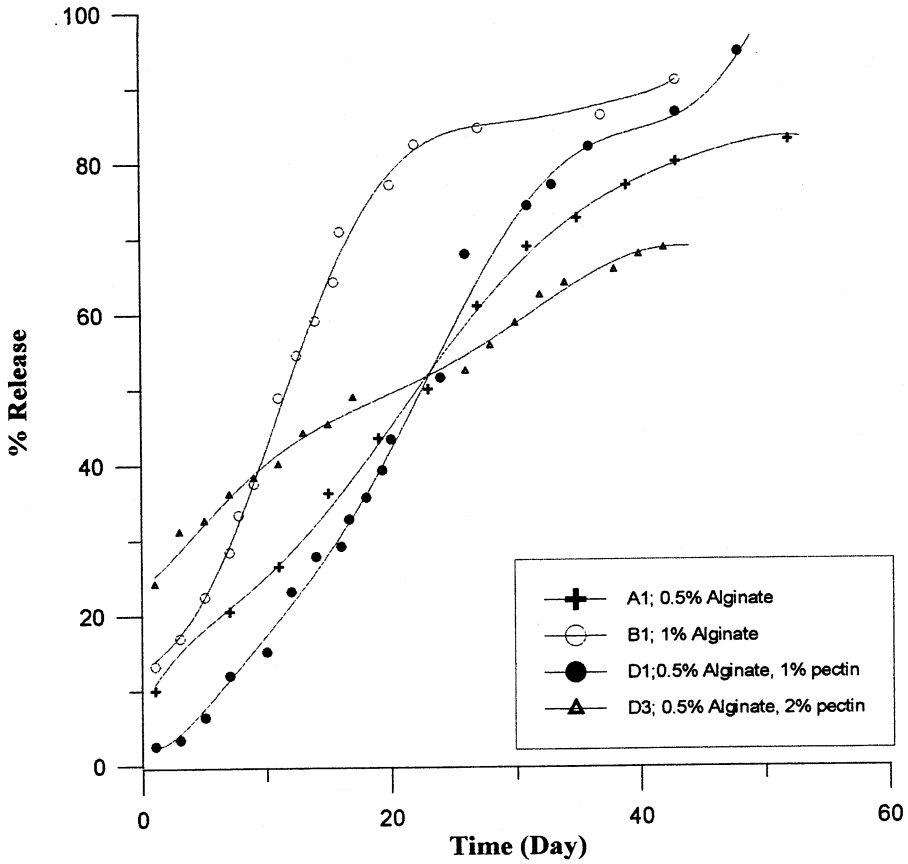

Fig. 4. Effect of alginate or pectin concentrations on release properties of alginate-treated chitosan beads.

taraldehyde (E), a significant difference was found between the release characteristics of beads ($p < 0.05$). The release of BSA was incomplete over the duration of the experiments.

Contrary to expectations, as the sodium alginate and pectin concentrations increased, BSA release did not significantly reduce (Fig. 4). The same result was also reported for chitosan-treated alginate beads by Polk et al. (1994). They explained this effect by the unchanged membrane permeability of chitosan beads.

However, better cross-linking was obtained in chitosan beads prepared with both alginate and pectin in a 0.5:1 ratio. By using this novel combination, a more stable chitosan membrane is obtained. Furthermore, this approach may be useful to protect protein and peptide drugs from toxic effect of glutaraldehyde.

In conclusion, the data presented here shows that alginate or alginate–pectin-reinforced chitosan beads might be a potential delivery system for the encapsulation of proteins.

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