ELSEVIER

International Journal of Pharmaceutics 201 (2000) 51–58

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

A novel approach to prepare tripolyphosphate/chitosan complex beads for controlled release drug delivery

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

Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, People's Republic of China

Received 27 October 1999; received in revised form 1 March 2000; accepted 6 March 2000

Abstract

A novel approach was developed to improve the mechanical strength of tripolyphosphate (TPP)/chitosan beads prepared under coagulation condition at 4°C in the presence of gelatin. Cross-sectional analysis indicated that the beads had a homogeneous crosslinked structure, as a result the beads were strengthened greatly (the mechanical strength increased more than ten times). Furthermore sodium alginate (a polyanion) can interact with cationic chitosan on the surface of these TPP/chitosan beads to form polyelectrolyte complex film for the improvement of the drug sustained release performances. The loading efficiency of model drugs (brilliant blue and FITC-dextran) in these beads was very high (more than 90%). Crosslinking time, TPP solution pH and other preparation factors had an effect on the drug release performance of beads. The release period of brilliant blue (a poor water soluble dye) was more than 2-months at a fairly constant rate in 0.9% NaCl, 10 mM PBS pH 7.4. However, for FITC-dextran (a water soluble polysaccharide) only 1–2 days in the same conditions. It seems that TPP/chitosan bead prepared by the novel method is a promising formulation for drug delivery.

Keywords: Chitosan; Tripolyphosphate; Polyelectrolyte complex beads; Drug controlled release

1. Introduction

Chitosan with excellent biodegradable and biocompatible characteristics, is a naturally occurring polysaccharide. Due to its unique polymeric cationic character and its gel and film forming properties, chitosan has been examined extensively in the pharmaceutical industry for its potential in the development of drug delivery systems (Yao et al., 1995; Illum, 1998).

Recently, the use of complexation between oppositely charged macromolecules to prepare chitosan beads (or microspheres) as a drug controlled release formulation, especially for peptide and protein drug delivery, has attracted much attention, because this process is very simple and mild (Polk et al., 1994; Liu et al., 1997). In addition, reversible physical crosslinking by electrostatic interaction, instead of chemical crosslinking, is applied to avoid possible toxicity of reagents and other undesirable effects.

Tripolyphosphate (TPP) is a polyanion, and can interact with cationic chitosan by electrostatic

* Corresponding author. Tel.: +86-0571-795-2046.
E-mail address: kjzhu@ipsm.zju.edu.cn (K.J. Zhu)

0378-5173/00/$ - see front matter
PII: S0378-5173(00)00403-8

forces (Kawashima et al., 1985a,b). Since Bodmeier et al. (1989) reported a TPP/chitosan complex can be prepared by dropping chitosan droplets into a TPP solution, many workers have explored the potential pharmaceutical usage (Shirashi et al., 1993; Sezer and Akbuga, 1995; Aydin and Akbuga, 1996; Calvo et al., 1997a,b; Shu and Zhu, 1999). However, the mechanical strength of these chitosan beads is very poor, so its usage in the pharmaceutical industry is still limited.

Aral and Akbuga (1998) have strengthened TPP/chitosan beads by coating sodium alginate on the bead surface to form a polyelectrolyte complex film. But the poor mechanical strength of TPP/chitosan beads still needs to be improved. Herein we report a new method to prepare TPP/chitosan beads with a more homogeneous structure. The controlled release behavior of model drugs from these beads was also investigated.

2. Materials and methods

2.1. Materials

Chitosan was obtained from Tianbao Chitosan Co. Ltd (China), and refined twice by dissolving in dilute HAc solution and precipitating from dilute ammonia, the degree of deacetylation was 86%, Mv was 460 000. Fluorescein isothiocyanate dextran (FITC-dextran, Mw 71 200), Gelatin (type B, approx. 225 Bloom) and Sodium alginate (low viscosity) were all obtained from Sigma (USA). Coomassie brilliant blue R250 (BB, Mw 825) was purchased from Fluka A.G. (Switzerland) and used after sieving (less than 50 μm). Tripolyphosphate (TPP) and other reagents were all commercially available and used as received.

2.2. Preparation of chitosan beads

The beads were prepared by two methods (conventional method and novel method), and described as follows.

2.2.1. Mixture solution preparation

A model drug (FITC-dextran or brilliant blue) was dissolved or dispersed in double-distilled water and added to an aqueous solution of chitosan, containing gelatin dissolved in acetic acid, at 37°C under agitation. The component concentration in the solution was (w/v): chitosan 4%, gelatin 4%, and model drug 1%.

2.2.2. Novel method

A total of 2 ml of the above mixture/solution (37°C) was dropped through a syringe needle (0.45 mm in diameter) into 250 ml cold sesame-seed oil (4°C) to induce the coagulation of gelatin. After 30 min, the oil was discarded, and 100 ml cold crosslinking solution was added under gentle agitation at 4°C. The crosslinking solution contained 1.0% TPP with or without 0.5% sodium alginate, and solution pH varied from 4.0–8.0. After a certain time, the beads were separated and washed with double distilled-water, then used in the following experiments or dried under vacuum at room temperature.

2.2.3. Conventional method

This process is similar to that reported by Bodmeier et al. (1989). Briefly, the above mixture/solution was directly dropped through a syringe needle (0.45 mm in diameter) into the same crosskinking solution used in the novel method at 30°C (slightly above the gelation point of gelatin to avoid the coagulation of chitosan and gelatin droplet). After a certain time, the beads were separated and washed with double distilled-water, then used in the following experiments or dried under vacuum at room temperature. In some conditions, beads only with chitosan (not containing gelatin) were also prepared by this method as a reference.

For simplification, in the following part, the novel method (the solution containing 4% chitosan and 4% gelatin) was named as method 1, the conventional method using the solution containing 4% chitosan and 4% gelation as method 2(a), also the conventional method using the solution only containing 4% chitosan as method 2(b). Furthermore without further specification, the beads used in the following experiments were all prepared with 1.0% TPP, in crosslinking time 30 min at pH 6.0 of crosslinking solution.

2.3. Model drug loading efficiency determination

During the bead preparation process, the aqueous phase was collected, and the drug content in the aqueous phase was determined. For FITC-dextran by fluorescence measurements (Fluorescence Spectrophotometer, model F-4000, HITACHI), and for BB by UV-visible spectrophotometric measurements at 590 nm (Murata et al., 1993).

Loading efficiency =

(the drug given − the drug loss)/the drug given $\times 100\%$

2.4. Bead mechanical strength determination

The mechanical strength of the beads was evaluated using a similar method described by Daly and Knorr (1988). It was expressed as the value that the maximum force used when the bead was broken under uniaxial compression at a crosshead speed of 0.5 cm/min (Model TM, Instron Corp., Canton, MA).

2.5. Model drug release studies

An amount of beads containing ca. 10 mg of model drug was suspended in a glass bottle containing 100 ml solution (0.9% NaCl, 10 mM PBS pH 7.4), and incubated on a shaking water-bath at 37°C, 50 rpm. A total of 4 ml samples were withdrawn, at appropriate intervals and FITC-dextran content determined by fluorescence measurements and BB by UV-visible spectrophotometric measurements at 590 nm. An equal volume of the same dissolution medium was added to maintain a constant volume.

2.6. Morphology observation

The surface and cross-sectional morphologies of the dried beads were examined using scanning electron microscopy (S.E.M., S-500, HITACHI). Cross-sectional samples were prepared by fracturing beads in liquid nitrogen. Prior to observation, samples were mounted on metal grids, using double-sided adhesive tape, and coated by gold under vacuum before observation.

To investigate the crosslinking mechanism, the wet beads in certain crosslinking time were collected during preparation, cut into two halves with blades and the uncrosslinked part was washed out with 30°C distilled-water. Then the cross-sectional morphologies in wet condition were examined using an optical microscope (XJZ-6, Jiangnan Optical Instrument Plant, China).

3. Results and discussion

3.1. Morphology observation

Scanning electron micrographs of TPP/chitosan beads and their surface morphology are shown in Fig. 1. TPP/chitosan beads prepared by method 1 were not very spherical in shape (about 1.2–1.5 mm in size) (Fig. 1(A)), and had a rough surface with large wrinkles (Fig. 1(B)). Coating sodium alginate on these bead surfaces had dramatically improved the surface morphology (Fig. 1(C)), because sodium alginate can form a polyelectrolyte complex film on the bead surface with cationic chitosan. Cross-sectional analysis of TPP/chitosan beads by S.E.M. indicated that the sodium alginate coating beads prepared by method 1 had a roughly circular cross-section with a distinctive complex film layer. A distinctive boundary between the film layer and the core layer of the bead was observed (Fig. 1(D)). The thickness of the dried film layer appeared to vary from 15 to 25 μm on average.

In contrast, TPP/chitosan beads prepared by method 2(a) or (b) were very brittle and the shape was irregular. Even after the surface was coated by sodium alginate, the beads were still easily destroyed when dried (Fig. 1(E)).

3.2. Bead mechanical strength

The mechanical strength of TPP/chitosan beads prepared under different conditions is shown in Fig. 2. When the beads were prepared by method 1, the mechanical strength improved greatly, with

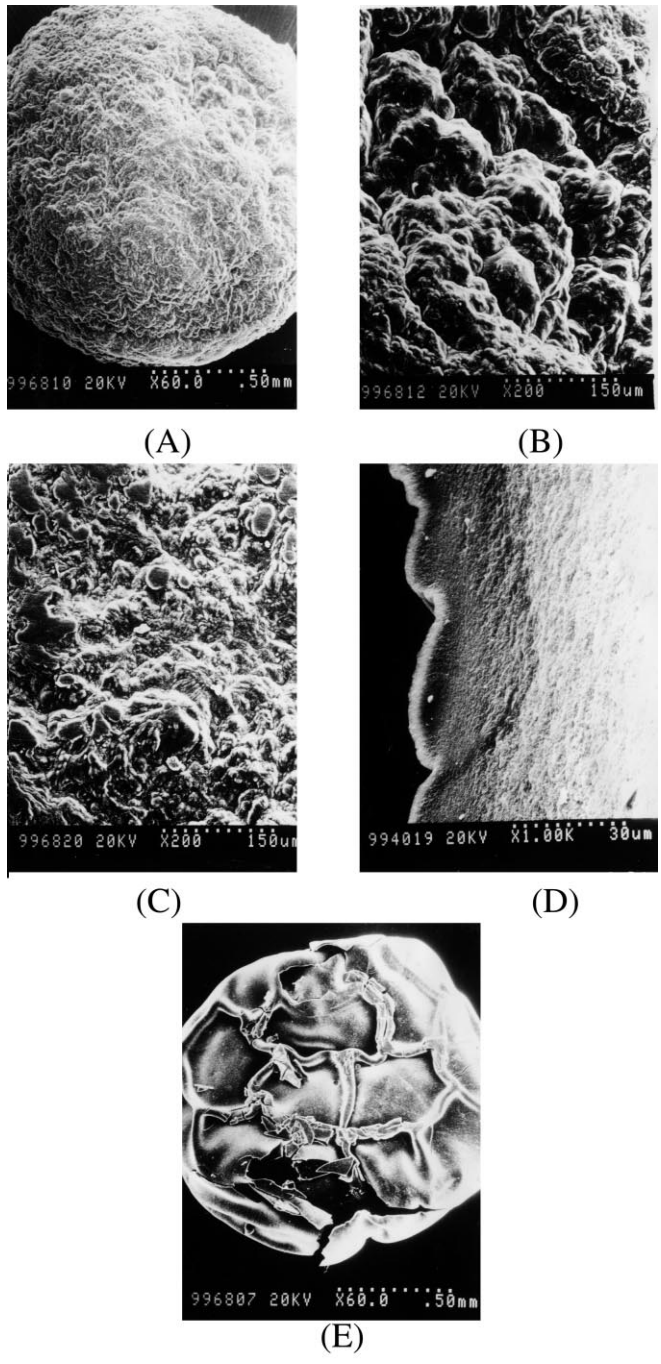

Fig. 1. S.E.M. micrographs (A) TPP/chitosan beads prepared by method 1; (B) surface morphology of TPP/chitosan beads prepared by method 1; (C) surface morphology of sodium alginate coating TPP/chitosan beads prepared by method 1; (D) the cross-sectional morphology of sodium alginate coating TPP/chitosan beads prepared by method 1; and (E) sodium alginate coating TPP/chitosan beads prepared by method 2(b).

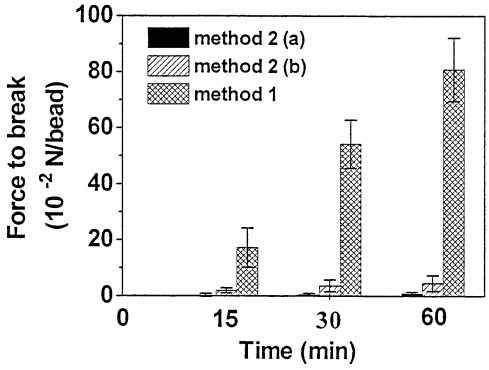

Fig. 2. The relationship between crosslinking time and mechanical strength of TPP/chitosan beads prepared by different method ($n > 10$).

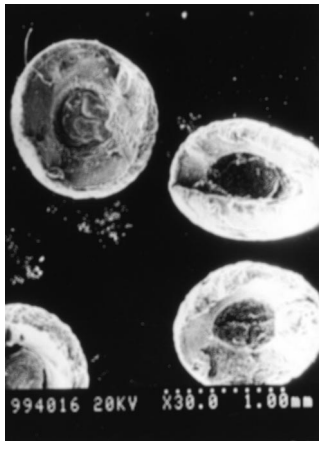

Fig. 3. Cross-sectional S.E.M. micrographs of TPP/chitosan beads prepared by method 1 in crosslinking time 30 min (the beads were cut into two halves under wet condition, then dried at room temperature for S.E.M. observation).

Table 1
The influence of sodium alginate (SA) coating on the loading efficiency of FITC-dextran in TPP/chitosan beads prepared by different methods ($n = 3$)

	SA concentration (% w/v)	
	0	0.5
Method 2(a)	47.2 ± 5.2	75.6 ± 4.7
Method 2(b)	71.5 ± 4.6	89.7 ± 4.2
Method 1	94.7 ± 4.3	98.7 ± 1.5

increased crosslinking time for a crosslinking time of 30 min, the force required to break the beads that were prepared using method 1 increased to more than 50 times than that required to break the beads prepared using method 2(a) and more than ten times than that of the beads in method 2(b).

In the case of method 2(a) or 2(b), as TPP diffused into the droplet core, chitosan molecules also diffuse freely from the droplet core towards an inward moving TPP-induced crosslinking zone during the crosslinking process, a gradient zone of TPP crosslinked chitosan beads was formed. The polymer density in the interface between the chitosan droplet and TPP solution was greatest and it decreased towards the bead center, resulting in poor mechanical strength and the poor shape of the beads (Fig. 1(E)). However when method 1 was employed, the homogeneous chitosan/gelatin droplet was coagulated at 4°C and the diffusion of chitosan molecules was retarded. As a result, only TPP diffused from surface into the inner core of the droplet to interact with chitosan, therefore a homogeneous crosslinking structure was formed.

Cross-sectional observation indicated that there was a very loose core of the TPP/chitosan wet beads prepared by methods 2(a) or 2(b). In contrast, the structure of beads prepared by method 1 was more homogeneous. With TPP diffusing from outside into the core of the coagulated droplet gradually, the droplet was crosslinked from the surface to the center step by step. In a crosslinking time of 30 min, a distinctive boundary between the crosslinked surface layer and the uncrosslinked core was observed (Fig. 3). In a crosslinking time of 60 min, homogeneous and completely crosslinked beads were formed (the picture is not shown).

3.3. Model drug loading efficiency

Table 1 shows the influence of sodium alginate coating on FITC-dextran loading efficiency in beads prepared under different conditions. Generally, sodium alginate coating facilitates increased drug loading efficiency in all three cases. It also can be seen that the loading efficiency of FITC-dextran is very high ($> 90\%$), even without

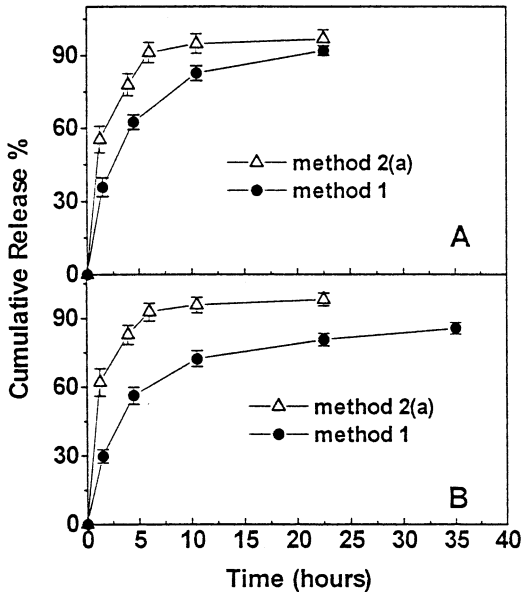

Fig. 4. The release curves of FITC-dextran from wet TPP/chitosan beads prepared by different methods (A) without coated by sodium alginate; and (B) coated with sodium alginate ($n = 4$).

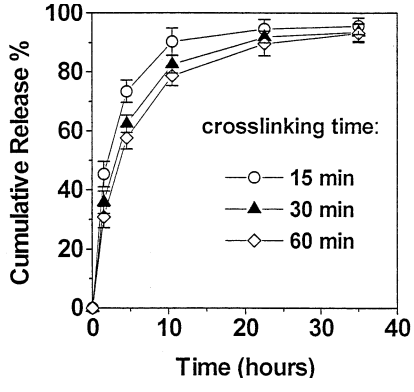

Fig. 5. The influence of crosslinking time of wet TPP/chitosan beads prepared by method 1 on the FITC-dextran release behavior ($n = 3$).

sodium alginate being coated in the case of method 1, which should be attributed to the fact that the drug loss decreased greatly under coagulation conditions in the preparation process.

The pH values of the crosslinking solution had little effect on the loading efficiency in method 1, and in all cases when pH is in the range 4.0–8.0, high loading efficiency (more than 90%) of FITC-dextran in beads can be obtained. This result is also in accordance with the above discussion that the determining factor resulting in high loading efficiency is that the coagulation condition.

The loading efficiency of brilliant blue (a small molecular dye with poor water solubility) was much higher than that of FITC-dextran, and almost 100% of the drug remained in the beads.

3.4. Model drug release studies

The release curves of FITC-dextran from TPP/chitosan beads are shown in Fig. 4(A). It was obvious that the dextran release can be extended using method 1. In 10 h, about 81% of the drug released from beads prepared by method 1, whereas about 93% released from that by method 2(a). For beads prepared by method 1 with sodium alginate on the surface, 80% drug release required ca. 24 h (Fig. 4(B)). However, in method 2(a), the sodium alginate coating did not extend the drug release. When using other polyacids [such as poly(methylacrylic acid), poly(aspartic acid), carboxylmethyl cellulose, etc.] as the coating material the same results were obtained. Aral and Akbuga (1998) and Polk et al. (1994) also reported similar results, but the reason still need to be understood.

The effect of extending the crosslinking time from 15 to 60 min on the drug release period from TPP/chitosan beads prepared by the novel method was examined and shown in Fig. 5. In 10 h, the cumulative drug release from beads at crosslinking time of 15, 30, and 60 min was 91, 83, and 76%, respectively. This result is in accordance with the bead cross-sectional observation that a more crosslinked structure was formed with crosslinking time extended from 15 to 60 min.

Due to the ionic interaction nature of crosslinking, the charge density of chitosan and TPP under preparation conditions may affect the bead formation and drug release performance. Fig. 6 shows the effect of TPP solution pH on the FITC-dextran release behavior from TPP/chitosan beads prepared by method 1. Increasing TPP solution pH led to quicker drug release. This result is complicated and may be explained as

follows. Chitosan is a weak polybase, and the pK_a was reported to be 6.3 (Yalpani and Hall, 1984). With the pH increasing, the ionization of amine groups decreased. As a result, the crosslinking density of beads formed at pH 7.0 TPP solution is lower than that at pH 5.0, so the drug release from beads prepared at pH 7.0 TPP solution is quicker.

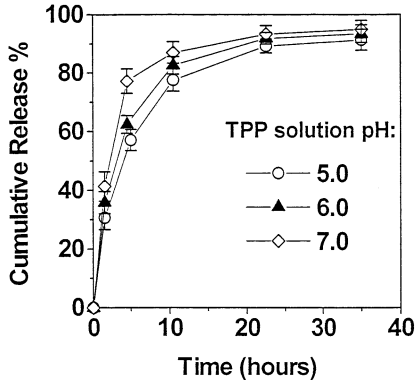

Fig. 6. The influence of TPP solution pH on the FITC-dextran release behavior from wet TPP/chitosan beads prepared by method 1 ($n = 3$).

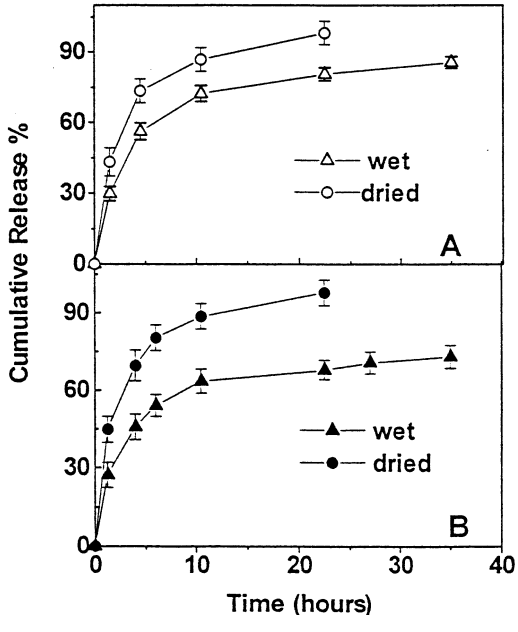

Fig. 7. The influence of dry process on the FITC-dextran release behavior from sodium alginate coating TPP/chitosan beads prepared by different method: (A) method 1; and (B) method 2(b) ($n = 3$).

Fig. 7 shows the influence of dry processing on the drug release from sodium alginate coating TPP/chitosan beads prepared by different methods. Generally, the drying process will destroy the structured integrity of the beads, thus accelerating drug release. For example, in the case of method 1 within 10 h, 72% FITC-dextran was released from wet beads, but 84% from dried beads (Fig. 7(A)). Moreover, when method 2(b) was employed, the acceleration effect of the drying process on drug release was more serious, 63% FITC-dextran was released from wet beads and 87% from dried (Fig. 7(B)) in 10 h, because the heterogeneous structure of the beads made them very easy to destroy when dried (Fig. 1(E)). Another phenomenon which should be noticed in Fig. 7 is that the release of FITC-dextran from wet sodium alginate coating beads prepared by method 2(b) is much slower than from that prepared by the novel method. We thought that in the case of method 2(b), these beads were prepared with chitosan only, sodium alginate can form dense and integral polyelectrolyte complex film in a wet state, which retarded drug release greatly if the beads were not dried. However, when method 1 was employed, the presence of gelatin may disturb the formation of polyelectrolyte complex film, therefore, less dense film was formed.

The release of BB from TPP/chitosan beads prepared by method 1 was sustained slowly as a result of its poor aqueous solubility, and the release time was more than 2 months at a fairly constant rate (data not shown).

4. Conclusions

In comparison with the conventional method (method 2(a) or 2(b)), TPP/chitosan beads prepared by the novel method (method 1) had a more homogeneous structure as a result of the more homogeneous crosslinking process, therefore the beads were strengthened greatly. The drug loading efficiency was very high (more than 90%) because during the preparation process the chi-

tosan droplet was under coagulation conditions. Polyacids (such as sodium alginate) also can be coated on the surface of TPP/chitosan beads prepared by the novel method to form a polyelectrolyte complex film, which increased the drug loading efficiency as well as prolonging the drug release period. The crosslinking time and TPP solution pH had an effect on the drug release performances from beads. The dry process destroyed the integrity of the beads structure and accelerated drug release, which was more serious in the case of beads prepared using the conventional method as a result of the heterogeneous bead structure.

Acknowledgements

The authors wish to express their thanks to National Natural Science Foundation of China for financial support.

References

Aral, C., Akbuga, J., 1998. Alternative approach to the preparation of chitosan beads. Int. J. Pharm. 168, 9–15.

Aydin, Z., Akbuga, J., 1996. Chitosan beads for the delivery of salmon calcitonin: preparation and characteristics. Int. J. Pharm. 131, 101–103.

Bodmeier, R., Oh, K.H., Pramar, Y., 1989. Preparation and evaluation of drug-containing chitosan beads. Drug Dev. Ind. Pharm. 15, 1475–1494.

Calvo, P., Remunan-Lopez, C., Vila-Jata, J.L., Alonso, M.J., 1997a. Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for protein and vaccines. Pharm. Res. 14, 1431–1436.

Calvo, P., Remunan-Lopez, C., Vila-Jata, J.L., Alonso, M.J., 1997b. Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. J. Appl. Polym. Sci. 63, 125–132.

Daly, M.M., Knorr, D., 1988. Chitosan-alginate complex coacervate capsules: effects of calcium chloride, plasticizer, and polyelectrolyte on mechanical stability. Biotech. Prog. 4, 76–81.

Illum, L., 1998. Chitosan and its use as a pharmaceutical excipient. Pharm. Res. 15, 1326–1331.

Kawashima, Y., Handa, T., Takenaka, H., Lin, S.Y., Ando, Y., 1985a. Novel method for the preparation of controlled-release theophylline granules coated with a polyelectrolyte complex of sodium polyphosphate-chitosan. J. Pharm. Sci. 74, 264–268.

Kawashima, Y., Handa, T., Kasai, A., Takenaka, H., Lin, S.Y., 1985b. The effect of thickness and hardness of the coating film on the drug release of theophylline granules. Chem. Pharm. Bull. 33, 2469–2474.

Liu, L.S., Liu, S.Q., Ng, S.Y., Froix, M., Ohno, T., Heller, J., 1997. Controlled release of interleukin-2 for tumor immunotherapy using alginate/chitosan porous microspheres. J. Control. Rel. 43, 65–74.

Murata, Y., Maeda, T., Miyamato, E., Kawashima, S., 1993. Preparation of chitosan-reinforced alginate gel beads: effects of chitosan on gel matrix erosion. Int. J. Pharm. 96, 139–145.

Polk, A., Amsden, B., Yao, K.D, Peng, T., Goosen, M.F.A., 1994. Controlled release of albumin from chitosan-alginate microcapsules. J. Pharm. Sci. 83, 178–185.

Sezer, A.D., Akbuga, J., 1995. Controlled release of piroxicam from chitosan beads. Int. J. Pharm. 121, 113–116.

Shirashi, S., Imai, T., Otagiri, M., 1993. Controlled release of indomethacin by chitosan-polyelectrolyte complex: optimization and in vivo/in vitro evaluation. J. Control. Rel. 25, 217–225.

Shu, X.Z., Zhu, K.J., 1999. A novel approach to prepare polyanion/chitosan complex beads as drug delivery formulation. Preprints of 2nd International Symposium on High-tech Polymers and Polymeric Complexes (HPPC-II), Zhengzhou, China, in press.

Yalpani, M., Hall, L.D., 1984. Some chemical and analytical aspects of polysaccharide modification. 3. Formation of branched-chain, soluble chitosan derivatives. Macromolecules 17, 272–279.

Yao, K.D., Peng, T., Yin, Y.J., Xu, M.X., 1995. Microcapsules/microspheres related to chitosan. J.M.S.-REV., Macromol. Chem. Phys. C35, 155–180.