Evaluation of Alginate–Chitosan Bioadhesive Beads as a Drug Delivery System for the Controlled Release of Theophylline

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ABSTRACT: This study describes the preparation of mucoadhesive alginate–chitosan beads containing theophylline intended for colon-specific delivery. The calcium alginate beads were coated with chitosan by the ionotropic hydrogelation method with a polyelectrolyte complex reaction between two oppositely charged polyions. The release profiles of theophylline from the beads were determined by ultraviolet–visible absorption measurement at 272 nm. Scanning electron microscopy was used for morphology observation. The *in vitro* mucoadhesive tests for particles were carried out with the freshly excised jejunum of Sprague-Dawley rats. The bead particles, which ranged in size from 200 to 400 μm, exhibited excellent mucoadhesive properties. The results showed that the formulated coated beads succeeded in controlling the release of theophylline over a 24-h period. In conclusion, the release of theophylline was found to be dependent on the composition of the beads, the component polymer and its possible interactions, and the bioadhesiveness. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 2452–2459, 2009

Key words: biopolymers; drug delivery systems; hydrogels; microencapsulation; polysaccharides

INTRODUCTION

The use of new natural polymers as drug carriers has received considerable attention in the last few years. One of the goals of such systems is to prolong the residence time of a drug carrier in the gastrointestinal (GI) tract.[1,2] The bioadhesive bond can be of a covalent, electrostatic, hydrophobic, or hydrogen-bond nature.[3] Ionic polymers have been reported to be promising for bioadhesive medical applications, and increased charge density will also give better adhesion;[3] this suggests that electrostatic interactions are of great importance.

The entire GI tract, including the stomach, is covered with a continuous layer of an insoluble mucus hydrogel.[4] The mucus hydrogel mainly consists of glycoproteins, and because of their ester sulfate and sialic acid groups, the mucus layer has an overall strong net negative charge.[4] Mucoadhesive drug delivery systems work by increasing the drug residence time at the site of activity or resorption. The mucoadhesive feature of alginate and chitosan may aid in its utility as a potential delivery vehicle for drugs to mucosal tissues such as the GI tract. Studies have shown that polymers with charge density can serve as good mucoadhesive agents. Increased charge density will give better adhesion. It has also been reported that polyanion polymers are more effective as bioadhesives than polycation polymers or nonionic polymers. Alginate, being an anionic polymer with carboxyl end groups, is a good mucoadhesive agent. Also, the adhesive properties of chitosan in a swollen state have been shown to persist well during repeated contacts of chitosan and the substrate, and this implies that, in addition to adhesion by hydration, many other mechanisms, such as hydrogen bonding and ionic interactions, might also be involved.[5–7] An important mechanism of action has been suggested to be ionic interactions between positively charged amino groups in chitosan and the negatively charged mucus gel layer. Because of the adherence of alginate and chitosan particles to the mucosal tissues, the bioactive agent transit time is delayed, and the drug is localized to the absorptive surfaces. This improves drug bioavailability and effectiveness.[2,8–12]

The formation of a polyelectrolyte complex has been demonstrated to occur when a cationic polymer and an anionic polymer are present simultaneously in an aqueous solution.[13,14] Polyelectrolyte complexes have numerous applications, such as membranes,

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antistatic coatings, environmental sensors, chemical detectors, and medical prosthetic materials.[15,16] Alginate is a polyanionic copolymer of mannuronic and guluronic acid residues. Alginate–chitosan complexes can be important in oral peptide delivery systems. Alginate has the property of shrinking at a low pH and dissolving at a higher pH, whereas chitosan dissolves at a low pH and is insoluble in higher pH ranges. In view of these limitations encountered in pure alginate and chitosan bead systems, the concept of alginate–chitosan polyelectrolyte complexes has gained acceptance.[8] Upon mixing, the carboxyl residues of alginate and the amino groups of chitosan ionically interact to form the polyelectrolyte complex. The complexation of chitosan with alginate reduces the porosity of alginate beads and decreases the leakage of the encapsulated drugs. A chitosan complex with alginate has been studied as a coating on alginate beads, alginate–chitosan coacervates, and so forth. The easy solubility of chitosan at a low pH is prevented by the alginate network because alginate is insoluble under low-pH conditions. The possible dissolution of alginate at a higher pH is prevented by chitosan, which is stable at higher pH ranges.[5,17–19]

Because theophylline (TPH) is an effective drug used for the treatment of asthma and pulmonary disease[20] and has been widely used as a model drug in various controlled-release studies, this study focuses on the development of beads with an alginate core and chitosan coatings containing TPH (as a model drug) to control the release of TPH over a 24-h period by investigating the influence of the chitosan coating on the drug release properties. Alginate–chitosan beads were prepared by the ionotropic hydrogelation method with a polyelectrolyte complex reaction between two oppositely charged polyions with sodium alginate as a gel core. The preparation procedure was a two-step method including the formation of alginate–TPH followed by a membrane-forming step in which the beads were suspended in a solution of chitosan. This work also focuses on the study of the morphology, swelling, and stability of the beads. Many authors[21–23] have succeeded in preparing a polymer delivery system for TPH able to provide 100–250 mg of accumulative release in a time period of 2–8 h. In this work, we tried to validate the concept of diffusion-controlled release because the diffusion of drug molecules within a hydrogel matrix is hindered by the insoluble hydrogel network in which drug molecules have to travel through tortuous pathways to exit the hydrogel matrix. Different factors affecting the release process, such as the alginate and chitosan concentration, chitosan molecular weight, and $CaCl_2$ solution pH, have been studied. The slow drug release after coating suggests a controlled prolonged pathway by which the drug can travel through the polymer network.

EXPERIMENTAL

Sodium alginate (low viscosity = 200 cP, medium viscosity = 3600 cP, and high viscosity = 14,000 cP for a 2.5% aqueous solution at 20°C) was obtained from Sigma–Aldrich Chemicals, Ltd. (CHEMIE GmbH, Steinheim, Germany). Chitosan from crab shells (low viscosity = 2–200 cP, medium viscosity = 200–800 cP, and high viscosity = 800–2000 cP; minimum deacetylation = 85%) was obtained from Aldrich Chemicals, Ltd. (Germany). Calcium chloride (anhydrous, fine, general reagent grade (GRG) 90%) was purchased from Fisher Scientific (Fairlawn, NJ). Anhydrous TPH powder (≥99%) was obtained from Sigma–Aldrich Chemicals.

Preparation of the chitosan–alginate beads

Chitosan–alginate hydrogel beads were prepared according to the following protocol:

- First, sodium alginate was dissolved in 50 mL of distilled water to obtain an alginate solution of a certain concentration [1.5, 2, 5, or 5% (w/v)]. TPH (50 mg) was then added to the alginate solution with continuous stirring to finally obtain a homogeneous alginate–TPH suspension.
- The obtained suspension was then added dropwise into a 2% (w/v) $CaCl_2$ solution with a peristaltic pump at a pumping rate of 1 mL/min and was left to cure for 10 min.
- The formed beads were then transferred to a chitosan solution dissolved in 1% (w/v) acetic acid and left for 15 min to allow the coating process; finally, the chitosan-coated alginate beads were loaded with TPH.
- The coated beads were then separated and washed with distilled water to be ready for further investigations.
- The dried beads were obtained through drying in air for 48 h.
- For studying the effect of the preparation pH, $CaCl_2$ solutions were prepared with different pHs.

Dissolution test

Drug dissolution studies were carried out in 0.1*M* phosphate-buffered saline (PBS) of pH 7.4 at 37 ± 0.5°C; dried beads were put into a flask containing 100 mL of the release medium. The flasks were put in a shaking incubator at the shaking rate of 150 ± 5 rpm. The samples were collected from the release medium at regular intervals. The amount of each

sample was 3 mL. After each sample collection, the same amount of fresh release medium at the same temperature was added to the release medium to maintain the sink condition. The drug concentration of each sample was determined spectrophotometrically at 274 nm. All measurements were carried out in triplicate, and values were plotted with standard deviation errors.

Morphology observation

The surface of the beads was examined with scanning electron microscopy. Before observation, samples were mounted on metal grids with double-sided adhesive tape and coated with gold *in vacuo* before observation

Bead stability: explosion assay[24]

The beads could burst when the mechanical strength of the beads could not withstand the osmotic swelling pressure inside the beads. One hundred beads were suspended in a 500-mL beaker with 0.1*M* PBS (pH 7.4) and then thermostated in a shaking water bath at 37°C and 50 rpm for 20 h with time intervals of 2.5 h. The percentage of burst beads was defined as the number of burst beads divided by the number of beads given times 100%.

Swelling degree determination

Calcium alginate hydrogel beads were sampled in a graduated test tube. The diameters of 50 beads were randomly measured under an optical microscope and averaged as the diameter of the beads before swelling (D_0). The beads were then immersed in 500 mL of 0.1*M* PBS (pH 7.4) at 37 ± 0.5°C with stirring at 50 rpm for 10, 20, 40, 60, 80, 100, or 120 min. The diameters of 50 beads were randomly measured under an optical microscope and averaged as the diameter of the beads after swelling (D_t). Thus, the degree of swelling (S_w) could be calculated as follows:[25]

$$S_w(\%) = 100\left[\left(\frac{D_t}{D_0}\right)^3 - 1\right]$$

The high S_w value suggests severe volume swelling of the beads.

Determination of the bioadhesive strength

Preparation of isolated rat jejunum

Unfastened male Sprague-Dawley rats (250–350 g) were sacrificed with an overdose of urethane. Before the test, the jejunum (5–7 cm) was cut open longitudinally, emptied of food, and washed with 0.1*M* HCl (20 mL/min) and a phosphate buffer (pH 6.0, 20 mL/min) until it was clean. The jejunum was cut and kept in a phosphate buffer solution (pH 6.0) and was used within 4 h of sacrifice. Pieces (5–7 cm) of the jejunum were placed on one half of a longitudinally cut rubber tube (2-cm diameter) with the help of pins. They were used as the biosurface for the *in situ* test.

In situ falling adhesion test

This procedure was adopted from Rao and Buri's method.[26] Isolated rat jejunum and stomach pieces were used as the biosurface to test the bioadhesive strength of the test beads. Silica-coated glass beads (700 or 100 μm) were used as the control samples. A humidity chamber (80% relative humidity) was prepared by saturation with a saturated solution of ammonium chloride at room temperature (25 ± 1°C). Approximately 50-mg test samples were placed on a piece of jejunum tissue and incubated for 20 min in a humidity chamber. This procedure allowed the beads to hydrate and interact with the mucosal surface of the gut. Then, the tissue–particulate assembly was placed on the plastic support and fixed at an angle of 45°C. A rubber tube connected to a peristaltic pump was placed about 1 cm above the tissue sample to obtain an even flow of liquid. The beads were washed by the pumping of the phosphate buffer solution at 30 mL/min. The percentage of beads retained on the test tissue was used as an index of the bioadhesive property of the beads. It was determined by the collection and counting of the washed beads.

RESULTS AND DISCUSSION

The important features of a good drug delivery system include versatility to carry drugs with different physicochemical properties, simplicity of the method of preparation, and feasibility for mass production. We have attempted to bear these factors in mind while formulating the polymer-based delivery system. Our method for preparing drug-loaded beads involves only aqueous solvents. Although at this initial stage of development we have used conventional drug molecules as mode1 compounds, the technique is also expected to be applicable to peptide and protein drugs.

This work focused on the preparation of alginate–chitosan beads with an inner alginate core and an outer chitosan–alginate complex membrane (Fig. 1) and its effect on the release behavior of encapsulated TPH in both wet and dry beads.

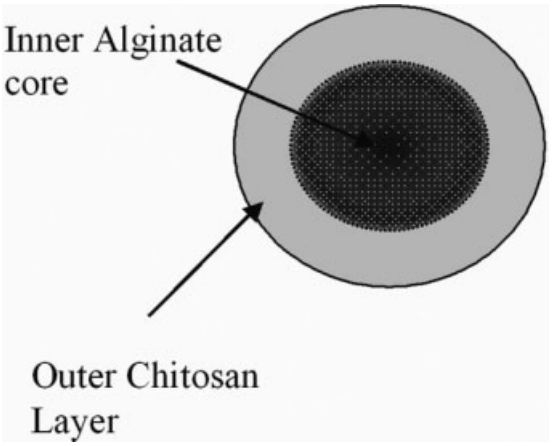

Figure 1 Crosslinking-reinforced chitosan–alginate complex bead.

Release studies

The water content of the beads was found to have a determining effect on the drug release rate, so the release of TPH from both wet and dried beads was studied, and the results were compared.

Undried beads

Figure 2 shows the effect of the chitosan concentration in the coating solution on the drug release from 2.50% (w/v) alginate beads in 0.1*M* PBS (pH 7.4). It was observed that the drug release percentage from coated beads, in general, was less than that from uncoated ones. A further increase in the chitosan concentration beyond 0.1% (w/v) accelerated the drug release. This result could be explained by the explosion assay of the beads in the release medium (Fig. 3), which showed that the higher the chitosan concentration was, the higher the percentage of burst beads was. In comparison with a chitosan concentration of 0.3 or 0.5% (w/v), the lower burst of beads at a chitosan concentration of 0.1% (w/v) resulted in the slowest release of the drug (Fig. 2). This could be explained as follows: when the beads were incubated in PBS, the mechanical strength of the beads decreased because of the displacement of crosslinking calcium by sodium ions,[27] but the osmotic activity of the ions increased. When the mechanical strength of the beads could not bear the osmotic pressure, the beads probably burst. This effect seriously affected the loaded drug release, especially when a high concentration of chitosan (0.3 or 0.5% w/v) was used for the coating, which formed a denser chitosan–alginate membrane and greatly limited drug release.

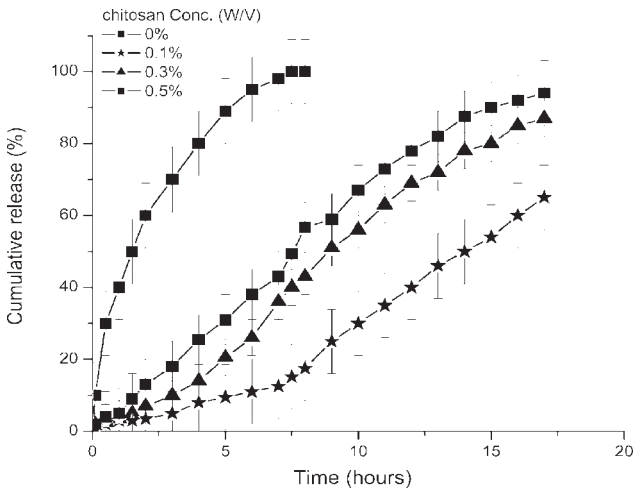

Figure 2 Effect of the chitosan concentration in the coating solution on the drug release from 2.50% (w/v) alginate beads in 0.1*M* PBS (pH 7.4). There was a further increase in the chitosan concentration over 0.1% (w/v).

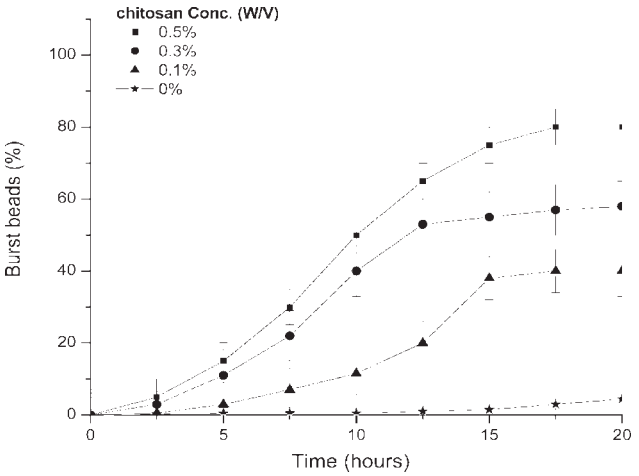

Figure 3 Effect of the chitosan concentration (increasing from 0 to 0.5) on the percentage of beads burst in 0.1*M* PBS (pH 7.4).

Dried beads

The drying process of coated beads is known by its effect on the release behavior as a result of destroying the formed alginate–chitosan films.[28] Figure 4 shows the drug release curves from dried beads in 0.1*M* PBS. In comparison with the wet beads; drying prolonged the release more effectively, especially at a higher concentration of alginate. This result is in agreement with other obtained results.[28] This result could be explained in the light of the scanning electron micrographs of the surfaces of the dried beads (Fig. 5). Figure 5 shows that the surfaces of beads prepared with 1.5% alginate had very clear cracks as result of the drying process. Such cracks accelerated the release of TPH and in turn reduced the prolongation effect of the coating process with chitosan. These cracks disappeared with a higher concentration of alginate, and the surfaces of both 2.5 and 5%

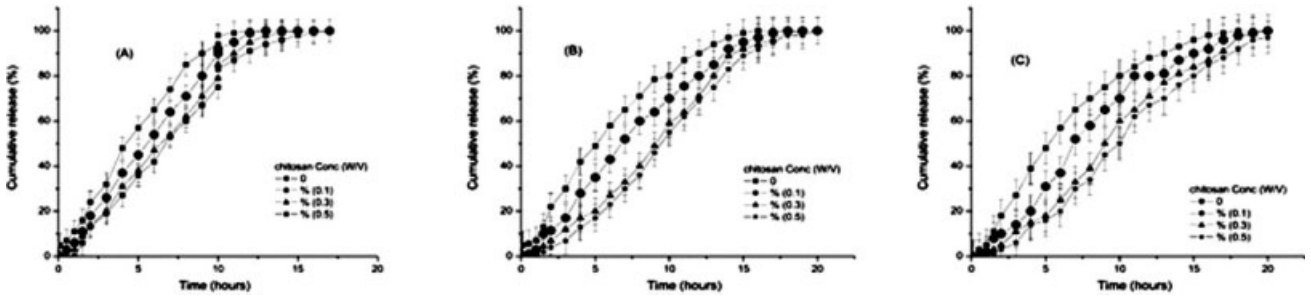

Figure 4 Influence of the chitosan coating on the release of TPH from dried coated beads: (A) 1.5, (B) 2.5, and (C) 5% sodium alginate concentration (w/v). The release medium was 0.1*M* PBS (pH 7.4).

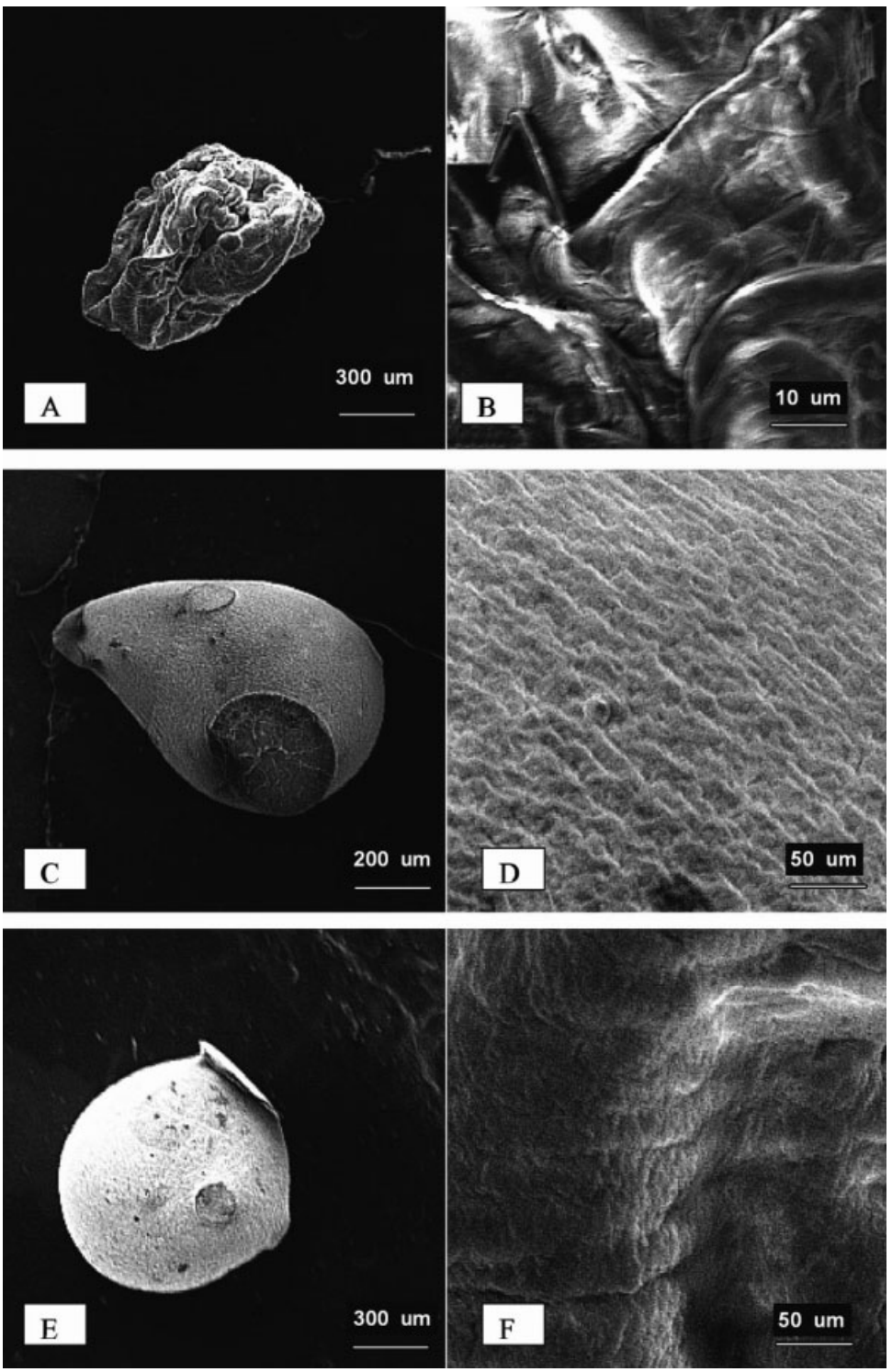

Figure 5 Scanning electron micrographs showing the typical surface morphology of dried beads prepared with 0.3% (w/v) chitosan and 1.0, 2.5, or 5.0% (w/v) alginate.

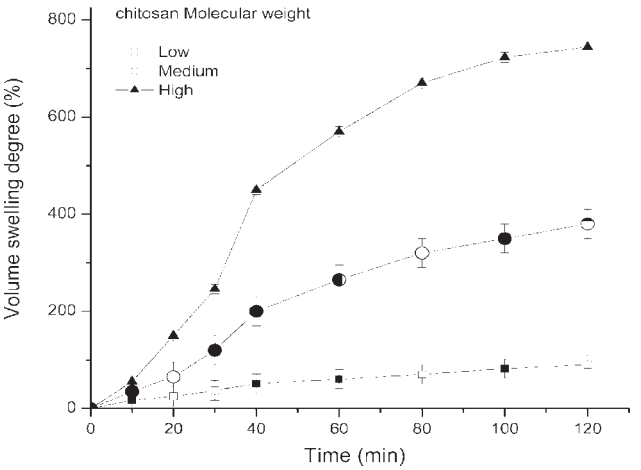

Figure 6 Influence of the chitosan molecular weight on the volume swelling degree of wet coated beads with a 2.5% (w/v) sodium alginate concentration and 0.3% (w/v) chitosan. The release medium was 0.1*M* PBS (pH 7.4).

alginate coated beads were very smooth; this led to clear prolongation of the release time.

Effect of the chitosan molecular weight on the drug release and volume swelling degree

Because the core of the beads is a calcium alginate hydrogel, when they encounter an electrolyte solution such as sodium chloride, sodium citrate, or even PBS, ionotropy will occur between Ca^{2+} and Na^{+}, resulting in the conversion of the beads to a liquid accompanied by volume expansion, which is called liquefaction. When drug-loaded alginate–chitosan beads are administered, volume swelling usually occurs in the environment *in vivo*, and the rupture of beads even takes place under some conditions; this results in the burst release of entrapped drugs. On the one hand, a series of pharmacological side effects, including drug intoxication, will be caused, threatening the lives of patients; on the other hand, the burst release will lead to the instant exposure of drugs to the severe *in vivo* environment, including a low pH and more enzymes, which will increase the chance of drug inactivation, especially for proteins, so the expected therapeutic effect cannot be realized. Therefore, it is a prerequisite to have knowledge of the swelling behavior for beads applied in drug delivery systems.

The changes in the swelling volume for beads coated with chitosans of different molecular weights with time are shown in Figure 6. The obtained results can be interpreted according to the rate of diffusion of chitosan molecules into the three-dimensional network of calcium alginate and the extent of the reaction between chitosan and sodium alginate molecules.

In general, the larger the weight-average molecular weight (M_w) of chitosan is, the more positively charged the amino of the chitosan chain is, and this means more binding sites with alginate. However, the large spatial size of chains for large chitosan molecules results in high diffusion resistance and causes a low diffusion rate and lower diffusion extent. The reaction occurs mainly at the surface of calcium alginate beads to form a thin membrane, which has weak antiswelling ability while being liquefied. On the contrary, when chitosan with a low M_w value is used, the small steric hindrance results in thick membrane formation with strong antiswelling ability. Therefore, a low M_w value of chitosan results in beads with a thick and strong membrane. In conclusion, the drug release rate changes gradually with changes in the molecular weight of chitosan as follows:

$$\text{High } M_w > \text{Medium } M_w > \text{Low } M_w$$

This is shown in Fig. 7. The obtained results agree with results obtained by other authors.[29]

Effect of the preparation pH

Because most drug systems such as proteins and polypeptides are usually sensitive to pH variations of the environment, pH control during the preparation process is very important to maintain their bioactivity. Also, the rate of drug release from alginate–chitosan beads changes according to the pH change of the dissolution medium. When an alginate bead falls into a chitosan solution, an interphasic membrane is formed by complexation between two

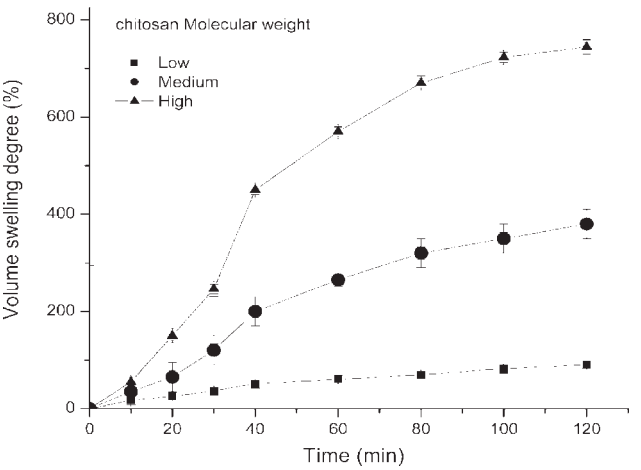

Figure 7 Influence of the chitosan molecular weight on the release of TPH from wet coated beads with a 2.5% (w/v) sodium alginate concentration and 0.3% (w/v) chitosan. The release medium was 0.1*M* PBS (pH 7.4).

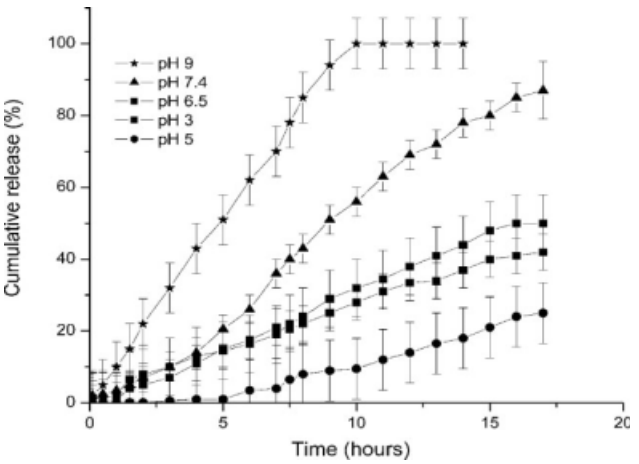

Figure 8 Influence of the preparation pH on the release of TPH from wet coated beads prepared with a 2.5% (w/v) sodium alginate concentration and 0.3% (w/v) chitosan.

polyelectrolytes of opposite charges through electrostatic interactions. The formed polyelectrolyte complex can protect the hydrogel matrix from environmental conditions and provide an excellent GI delivery system for the drug.

Figure 8 shows the changes in the release rate of beads affected by the variation of the preparation pH: beads prepared at pH 5 showed a minimum release rate, and the release rate increased as the pH increased. Because both amine and carboxylic groups in both polyelectrolytes had an approximately 70–80% degree of dissociation near pH 5.0, each polysaccharide could sustain the rigid, linear conformation to result in dense membrane formation [Fig. 9(A)]. It has been reported that the pK values of mannuronic acid (pK_M) and guluronic acid (pK_G) of alginate chains are 3.38 and 3.65, respectively.[30] The pK value of chitosan (pK_χ) is 6.3.[31] It has also been found that the carboxyl of the mannuronic acid unit reacts with the amino base during membrane formation between alginate and polylysine.[32] Thus, in the pH range of 3.5–5.5, pK_M < pH < pK_χ. According to the Lewis law of acid–base equilibrium, with the pH increasing from 3.5 to 5.5, COO^- of the mannuronic acid unit of alginate and NH_3^+ of chitosan increase so that there are more reaction sites to take part in membrane formation.

Above pH 5.0, the degree of dissociation of chitosan is suppressed, and the chitosan may form some kind of loop. This loop formation makes chitosan–alginate membranes less dense and increases the rate of release. A schematic representation is shown in Figure 9(C,D). Because at pH 6.5, pK_M < pK_χ < pH, NH_3^+ of chitosan is less than that at pH 5. Therefore, the reaction extent is reduced, and the release rate is higher than that at pH 5. In the case of pH 3, the release rate is similar to that at pH 6.5, and this also can be explained by the schematic representation in Figure 9(B). Because pH < pK_M < pK_χ at pH 3, COO^- of alginate is less at pH 3; therefore, the release rate is higher than that at pH 5.

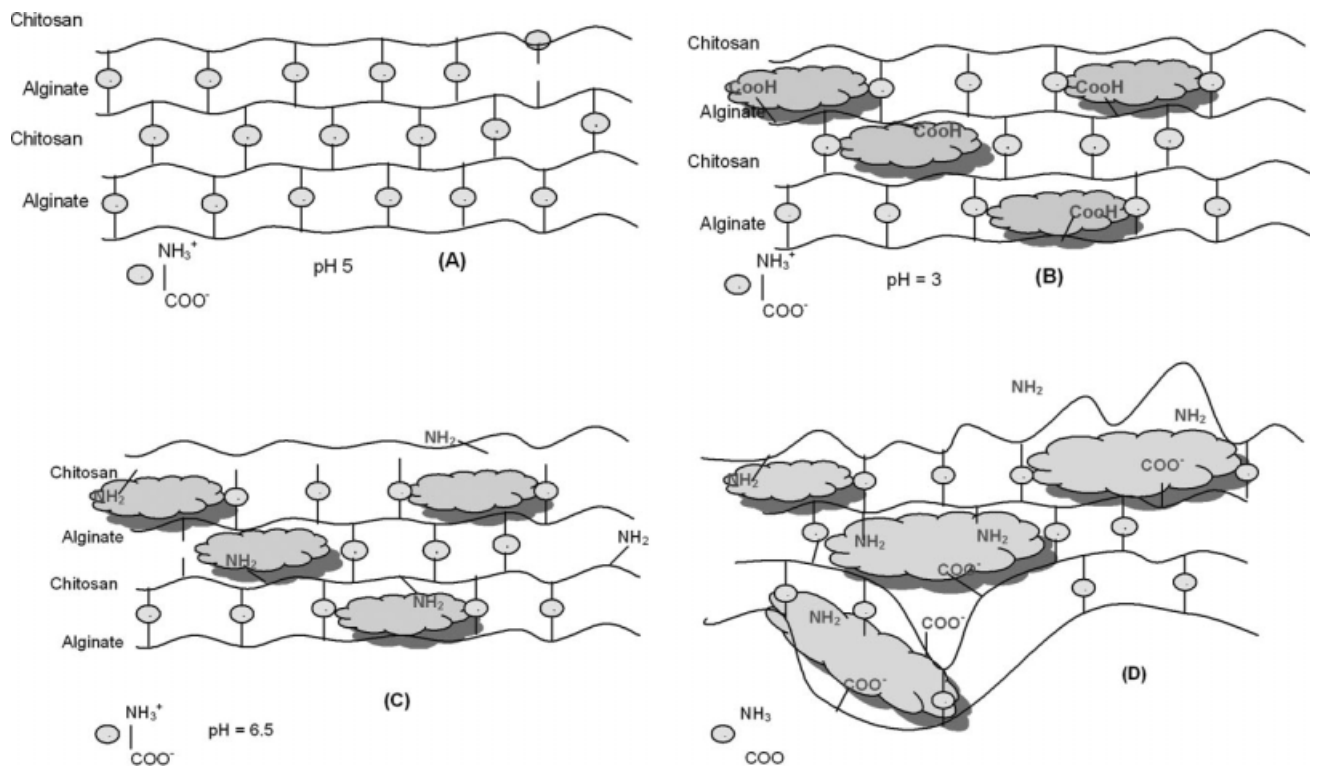

Figure 9 Schematic representation of a polyelectrolyte complex between chitosan and alginate at different pHs.

TABLE I
Percentage of Bioadhesive Beads Adhering to the Intestine Segment

Bead type	Particulate adhering to the intestine (%; n = 20)	
	200–400 μm	304 mm
Alginate	100	30
Chitosan	100	50
Alginate–chitosan	100	55

Bioadhesion test

For testing the bioadhesive properties of beads, Rao and Buri's[26] method has been widely accepted. We adapted this method to test our beads, and the percentage of beads adhering to the intestine was considered a primary index of the bioadhesive property of the beads (Table I). The results indicated that this method was more qualitative rather than quantitative in nature. Silicon-coated glass beads (0.7–1.1 mm in diameter), used as the control, had no bioadhesive property at all. These beads had a surface area comparable to that of the test polymeric beads and hence were considered adequate as a control. In contrast, the test beads of alginate, chitosan, and alginate–chitosan, which had sizes ranging from 200 to 400 μm and from 3 to 4 mm, adhered to intestinal mucosae. The adhesion was affected by the surface area and/or its particle size. The 200–400-μm beads were 100% adhered, regardless of the type of polymer used to prepare the beads. The type of polymer showed a clear effect on the adhesion percentage for 3–4-mm beads. Thus, from these results, it is reasonable to conclude that the investigated beads could have good bioadhesive properties.

CONCLUSIONS

This work demonstrates the effects of formulation and process variables on the particle size, drug content, drug release, and especially mucoadhesiveness of beads made from alginate as the core and chitosan as the outer coating. The developed beads consisted of the drug TPH entrapped within sodium alginate and coated with chitosan as an outer layer to control the release of TPH over a 24-h period. Alginate–chitosan beads were prepared by the ionotropic hydrogelation method with a polyelectrolyte complex reaction between two oppositely charged polyions with sodium alginate as the gel core. The preparation procedure was a two-step method including the formation of alginate–TPH followed by a membrane-forming step in which the beads were suspended in a solution of chitosan. The results from the physical characterization of the prepared beads were in favor of their localization and prolonged presence at the release site. The main contribution of the results of this study was the successful prolongation of the release time and/or reduction of the rate of TPH release to provide the required dose through one administration per day for the drug. This success was mainly due to the multiple effects of the chitosan coating, ranging from controlling the release to increasing the adhesion time to increasing the mechanical strength of the formulated beads.

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