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Chitosan–alginate multilayer beads for controlled release of ampicillin

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Abstract

The aim of this study is to develop multilayer beads with improved properties for controlled delivery of the antibiotic ampicillin. Ionotropic gelation was applied to prepare single and multilayer beads using various combinations of chitosan and Ca^{2+} as cationic components and alginate and polyphosphate as anions. Beads prepared with higher concentrations of chitosan entrapped more ampicillin. During incubation in simulated gastric fluid, the beads swelled and started to float but did not show any sign of erosion. Single layer chitosan–alginate beads released 70% of the drug within 4 h. Multilayer beads released only 20–30% in the same period of time. During subsequent incubation in simulated intestinal fluid, both single and multilayer beads continued to release drug. At least part of this release is due to disintegration of the beads. The rate of release both in gastric and intestinal fluid and the kinetics of disintegration in intestinal fluid can be controlled by changing the chitosan concentration in the coagulation fluid. The release of the drug can also be controlled by the degree of cross-linking using polyphosphate. Cross-linked multilayer beads were prepared that released only 40% of the entrapped drug during 24 h. It is concluded that chitosan–alginate multilayer beads, cross-linked with polyphosphate offer an opportunity for controlled gastrointestinal passage of compounds with low molecular weight like ampicillin.

Keywords: Chitosan; Alginate; Multilayer beads; Ampicillin; Controlled release

1. Introduction

Ideally, a drug delivery system releases the drug in the right body compartment at the rate required for a specific treatment. Most available drug delivery systems use biodegradable, biocompatible and natural biopolymers and are capable of rate and/or time controlled drug release. Considerable research efforts are being spent on oral sustained drug delivery systems, with the majority of these systems being solid dosage forms. They distribute their drug load more uniformly in the gastrointestinal tract with the aim to reduce local irritation (Lauwo et al., 1990; Bodmeier et al., 1991).

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Beads loaded with antibiotics would be useful for oral delivery to treat gastric diseases such as peptic ulcer (Shu and Zhu, 2000; Remuñañ-Lǒpez et al., 2000; Orienti et al., 2002) and for ulcerative colitis, carcinomas and infections in the intestine (Shah et al., 1999; Tozaki et al., 2002). In addition, sustained systemic absorption specifically in the intestinal region offers interesting possibilities for the treatment of diseases susceptible to the diurnal rhythm, such as asthma, arthritis or inflammation (Yeh et al., 1995; Tozer et al., 1995; Lorenzo-Lamosa et al., 1998). Ampicillin, being a broad spectrum antibiotic, is commonly used for systemic therapy as well as locally for gastric or intestinal infections. It is acid resistant and therefore can be given orally. It has a short biological half-life of 0.75–1.5 h. In order to make the application of ampicillin more effective, research has been directed to design formulations for its sustained and controlled release.

The biopolymer, chitosan, the N-deacetylated product of the polysaccharide chitin, is gaining importance in the pharmaceutical field owing to its unique polymeric cationic character, good biocompatibility, non-toxicity and biodegradability. Chitosan has been proposed as a useful excipient for either sustained release of water-soluble drugs and for enhancing the bioavailability of poorly water-soluble compounds. Large chitosan microspheres and beads (with diameter up to a few millimeters) have been proposed for the controlled release of drugs (Sezer and Akbûga, 1995; Remuñan-Lǒpez et al., 1998; Mi et al., 1999a,b). In order to achieve sufficient stability, chitosan gel beads and microspheres are often chemically cross-linked with glutaraldehyde (Jameela and Jayakrishnan, 1995; Berthold et al., 1996; Genta et al., 1997) and ethylene glycol diglycidyl ether (Mi et al., 1999a,b). However, residues of these compounds in the chitosan beads can cause damage or irritation to mucosal membranes and may induce undesirable side effects (Bodmeier et al., 1991; Jameela and Jayakrishnan, 1995; He et al., 1999). Recently, polyelectrolyte complexes have been proposed for the design of drug delivery systems. Cationic chitosan can form gels with non-toxic multivalent anionic counterions such as polyphosphate (Bodmeier et al., 1989; Lin and Lin, 1992; Mi et al., 1999a,b) and sodium alginate (Aral and Akbûga, 1998; Anal et al., 2003) by ionic cross-linking.

In this study, we prepared chitosan–alginate multilayer beads cross-linked with polyphosphate to develop a stable, non-toxic, interpolymer complex of ionic-cross-linked chitosan–alginate–tripolyphosphate (TPP) beads with improved drug release properties. TPP is a non-toxic and multivalent anionic compound. It can form a gel by ionic interaction with the positively charged amino groups of chitosan. These reinforced beads have been investigated for sustained release under conditions representative for the gastro-intestinal system. This report shows the drug entrapment and drug release behavior in the case of model drug, ampicillin for various single and multilayer chitosan–alginate beads. Drug release was studied in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF).

2. Materials and methods

2.1. Materials

Alginic acid (sodium salt) extracted from brown algae (molecular weight 200 kD) with a glucuronic:manuronic acid (G:M) ratio of 2:1, and calcium chloride dihydrate were obtained from Fluka Chemika, ampicillin and tripolyphosphate (TPP) from Sigma Chemicals, USA. The 2% (w/v) aqueous solution of sodium alginate exhibits a viscosity of 220 cp at 25 °C. Chitosan (85% degree of deacetylation, molecular weight 10^3 kD) was prepared in author's laboratory from shrimp shell (*Penaeus monodon*) after deproteinization and decalcification followed by deacetylation (Stevens, 2002). Pepsin and pancreatin powder were supplied by Acros Organics and Carlo Erba Reagent, respectively. SGF (pH 1.2) and SIF (pH 7.5) were prepared as prescribed in US Pharmacopoeia (USP XII 1995). All other reagents were of analytical grade and used without further purification.

2.2. Preparation of chitosan–alginate beads

Various types of beads were prepared and named after their multivalent components: chitosan, alginate and polyphosphate. Chitosan–alginate beads were produced by the gelation method (Anal et al., 2003). A homogenous mixture of 2% (w/v) sodium alginate and 20% (w/w) ampicillin in distilled water was used as dope. The pH was adjusted to 5.5 ± 0.1. Homogenous aqueous solutions of chitosan (CTS) and calcium chloride ($CaCl_2$) in various ratios were used as coagulation

fluid. Chitosan (1%, w/v) was dissolved in 1% (v/v) acetic acid at room temperature. The coagulation fluids were prepared by diluting the chitosan stock solution to the desired chitosan concentration with $CaCl_2$ solutions of sufficient concentration. The composition of the coagulation fluids is listed in Table 1. The solutions were mixed for 2 h before use. The pH of the coagulation fluids was adjusted to 4.5 ± 0.1. Dope (20 ml) was dropped through a 27 gauge blunt ended needle into 200 ml of coagulation fluid under mechanical stirring at 200 rpm. The flow rate of the dope was maintained at 10 ml/h using compressed nitrogen. The smooth, spherical and homogenous beads obtained were kept for an hour in the coagulation fluid under stirring. Thereafter beads were collected, washed with distilled water and air-dried. Their properties are listed in Table 1 (formulations A0–A3). Three batches of beads were prepared for further study.

2.3. Preparation of multilayer chitosan–alginate beads

Multilayer chitosan–alginate beads were prepared as described in Table 1 (A4–A6). In case of formulation A4 and A5, the beads were firstly prepared as described for A0 and A3. The resultant beads were washed once with distilled water and transferred into 100 ml of 0.08% chitosan solution for 30 min. In formulation A6, beads obtained as A3 were subsequently incubated in 0.08% CTS, 0.08% sodium alginate solution (30 min) and 0.5% $CaCl_2$ (10 min). The chitosan–alginate multilayer beads were rinsed with distilled water and subsequently air-dried. Three batches of beads were produced for further characterization.

2.4. Preparation of multilayer chitosan–alginate beads cross-linked with tripolyphosphate

Single-layer beads were produced by dropping chitosan–ampicillin as dope into sodium alginate and TPP (Table 2, D0–D1) while multilayer chitosan–alginate–TPP beads were produced as described for formulations in Table 2 (E0–E3). Multilayer beads E0 were made by transferring D0 beads into 0.08% aqueous sodium alginate solution and stirred for 1 h. Formulations (E1–E3) were prepared from D1 beads by an hour post-coagulation treatments subsequentially in alginate and $CaCl_2$ (E1), CTS and TPP (E2) and alginate, CTS, TPP and $CaCl_2$ (E3).

Table 1
Properties of various chitosan–alginate single and multilayer beads

Formulation	Coagulation fluid		Post coagulation treatment subsequently in the following solutions			Characteristics of the beads				
	CTS (%)	$CaCl_2$ (%)	CTS (%)	SA (%)	$CaCl_2$ (%)	Mean size (μm) (±S.D.)	Entrapment efficiency (%) (±S.D.)	Swelling index (%) (±S.D.)	DT_1 (h)	DT_2 (h)
A0	–	3.0	–	–	–	450 ± 15	15.5 ± 2.5	68 ± 11	3–4	3–4
A1	0.2	3.0	–	–	–	551 ± 12	46.7 ± 4.6	132 ±	7–9	7–9
A2	0.4	3.0	–	–	–	601 ± 22	59.8 ± 3.5	178 ±	10–12	10–12
A3	0.8	3.0	–	–	–	675 ± 18	75.7 ± 4.2	222 ± 18	12–14	12–14
A4	–	3.0	0.08	–	–	610 ± 15	12.3 ± 3.5	105 ± 2	12–16	12–16
A5	0.8	3.0	0.08	–	–	810 ± 20	65.4 ± 5.2	201 ± 8	>24	>24
A6	0.8	3.0	0.08	0.08	0.5	885 ± 30	60.1 ± 3.8	85 ± 11	>24	>24

The dope: 2% (w/v) aqueous sodium alginate (SA) containing 20% (w/w) ampicillin was dropped into 200 ml of coagulation fluid containing chitosan (CTS) and calcium chloride ($CaCl_2$). In post-coagulation treatment, beads were washed and treated in 100 ml solution as specified. In A6, post-coagulation was done subsequently in SA (30 min), CTS (30 min) and $CaCl_2$ (10 min). Finally dried beads were assayed for mean size (μm), entrapment efficiency, swelling index, disintegration time (h) in simulated intestinal fluid (SIF) without pancreatin, prior incubated in simulated gastric fluid (SGF) for 4 h (DT_1) disintegration time (h) in SIF with pancreatin, prior incubated in SGF for 4 h (DT_2).

Table 2
Properties of various chitosan–alginate–TPP single and multilayer beads

Formulation	Coagulation fluid		Post coagulation fluid				Characteristics				
	SA (%)	TPP (%)	SA (%)	$CaCl_2$ (%)	CTS (%)	TPP (%)	Mean size (μm) (±S.D.)	Entrapment efficiency (%) (±S.D.)	Swelling index (%) (±S.D.)	DT_1 (h)	DT_2 (h)
D0	–	8.0	–	–	–	–	544 ± 52	61.9 ± 4.4	98 ± 10	12–14	10–14
D1	0.8	8.0	–	–	–	–	548 ± 65	77.9 ± 5.4	75 ± 4	22–24	22–24
E0	–	8.0	0.08	–	–	–	602 ± 25	25.5 ± 5.7	102 ± 3.6	12–14	12–14
E1	0.8	8.0	0.08	2.0	–	–	615 ± 22	65.1 ± 2.9	82 ± 6.3	>24	>24
E2	0.8	8.0	–	–	0.08	2.0	756 ± 20	69.2 ± 3.6	169 ± 5.1	>24	>24
E3	0.8	8.0	0.08	2.0	0.08	2.0	706 ± 11	57.1 ± 2	135 ± 3.2	>24	>24

The dope: 2% (w/v) chitosan in 1% (v/v) acetic acid containing 20% (w/w) ampicillin. Coagulation fluid contained alginate (SA) and tripolyphosphate (TPP). Post-coagulation was carried out for each fluid separately and sequentially; each treatment had duration of 1 h. Finally dried beads were assayed for mean size (μm), entrapment efficiency, swelling index, disintegration time (h) in simulated intestinal fluid (SIF) without pancreatin, prior incubated in simulated gastric fluid (SGF) for 4 h (DT_1) disintegration time (h) in SIF with pancreatin, prior incubated in SGF for 4 h (DT_2).

2.5. Particle size determination

The particle size of the beads in a sample was measured with a micrometer (Mittotuyo micrometer, NSK Co., Japan) and calculated as the average value of the size of 100 beads.

2.6. Determination of encapsulation efficiency

The ampicillin content in the beads was determined by a digestion method. The ampicillin-loaded beads (10 mg) were pulverized and incubated in 10 ml 0.02 M phosphate buffer (pH = 7.4) at room temperature for 24 h. The suspension was then centrifuged at 6000 rpm for 30 min. The supernatant was assayed spectrophotometrically for ampicillin content at the wavelength of 203 nm. Supernatant from the empty beads (without ampicillin) was taken as blank. All samples were analyzed in triplicate.

2.7. Swelling studies

The swelling properties of the chitosan–alginate beads were determined in SGF (pH 1.2) with and without pepsin. Samples of beads of known weight (10 mg) were placed in a glass vial containing 10 ml of swelling solution and allowed to swell at 37 °C. The swollen beads were periodically removed and weighed. The wet weight of the swollen beads was determined by blotting them with filter paper to remove moisture adhering to the surface, immediately followed by weighing on an electronic balance. All experiments were done in triplicate. The percentage of swelling of the beads was calculated from the formula:

$$\left[\frac{\text{final weight of beads } (W_t) - \text{initial weight of beads } (W_0)}{\text{initial weight of beads } (W_0)}\right] \times 100$$

2.8. Disintegration of beads

Disintegration of the beads in SIF (pH 7.5) was studied at different intervals of time. Beads (10 mg) were preincubated with 10 ml of SGF with and without pepsin for 4 h at 37 °C in an incubator with 100 rpm shaking. After filtering, the swollen beads were trans-

ferred to another vial, containing 10 ml of SIF with and without pancreatin. The samples were incubated at 37 °C at 100 rpm in a shaking incubator. The physical appearance of the beads and their fragments during incubation was observed through a microscope. The time of complete disintegration was registered. All experiments were done in quadruplet.

2.9. In vitro drug release studies

The ampicillin release from beads was studied by incubating 50 mg of beads in 50 ml of SGF without pepsin (pH 1.2) in a 125 ml conical flask kept in a shaking water bath at 37 °C at 100 strokes per min. At time = 4 h, the beads were filtered and transferred to 50 ml of SIF without pancreatin (pH 7.5), incubated at 37° C in a water bath at 100 strokes/min. Starting from time = 0 h and at desired intervals of time, 1.5 ml sample was withdrawn and replaced with same amount of fresh medium. Ampicillin in the release medium was measured directly spectrophotometrically after removing debris by centrifugation at 6000 rpm for 30 min. The release studies were also carried out in SIF only, without prior incubation in SGF. The corresponding empty chitosan–alginate beads (without ampicillin) were taken as reference. Each experiment was repeated at least three times.

2.10. Microbiological assay for extracted ampicillin from beads

The assay of extracted ampicillin from beads, based on its action as bactericidal agent, was performed by a test tube serial dilution method (Bailey and Scott, 1970). The test bacterium was *Staphylococcus aureus* obtained from the Thailand Institute of Science and Technology Research (TISTR, Bangkok, Thailand). The extracted ampicillin was diluted in a phosphate buffer saline solution and serial two-fold dilutions were made in liquid Mueller-Hinton broth. Duplicate tubes containing 1 ml of each dilution were inoculated with 1×10^5 bacterial cells. The tubes were then incubated at 37 °C for 24 h. Antibiotic activity was expressed as the minimum inhibitory concentration (MIC), recorded as the highest dilution the antibiotic solution that was effective to inhibit bacterial growth. Antibiotic not subjected to the microencapsulation procedure and blank chitosan microspheres were used as control. All the experiments were done in triplicate.

2.11. Statistical analysis

Results were analyzed and expressed as mean ± S.D. Statistical analysis was done by using factorial design (Randomized Complete Block Design, RCBD) for characterization of chitosan–alginate beads. Effects of various post-treatments on multilayer chitosan–alginate beads were statistically analyzed by one-way ANOVA. The differences were considered significant at the level of $p < 0.05$. SPSS 7.5 for Windows was used for all statistical analysis.

3. Results and discussion

Single chitosan–alginate beads (Table 1, A0–A3) were obtained by dropping aqueous solution of sodium alginate into the coagulation fluid containing (0–0.8%, w/v) chitosan and 3% $CaCl_2$ (w/v). Beads were also obtained by post-coagulation treatment of calcium-alginate beads with chitosan (A4). Multilayer beads (A5 and A6) were obtained by treating the beads more times in a coagulation fluid. The beads after additional treatment with chitosan were intact and compact. The beads, additionally treated with sodium alginate became like clumps after washing. In this case, an extra treatment with 0.5% $CaCl_2$ solution was given to obtain smooth and spherical beads (A6). Single chitosan–alginate–TPP beads (Table 2, D0 and D1) were obtained by dropping the chitosan solution as the dope in an aqueous coagulation solution of TPP/alginate. The multilayer beads (E0–E3) were obtained after an extra treatment either with sodium alginate, Ca^{2+} and/or chitosan solution. The multilayer beads E2 and E3 were further cross-linked with TPP.

3.1. Particle size

3.1.1. Chitosan–alginate beads

The shape of the chitosan–alginate single layer beads was spherical. The weight of the beads was found to increase with the increase in concentration of chitosan in the coagulation fluid suggesting formation of a thicker chitosan layer. Mean particle size of chitosan–alginate single layer beads (formulations

A0–A3) was between 450 and 675 μm (Table 1). Earlier reports also suggested that the size of the beads increases with the use of chitosan in the coagulation fluid (Sezer and Akbûga, 1995; Anal et al., 2003; Lim et al., 1997). The multilayer beads showed an increase in particle size, probably due to extra coating (Table 1, A4–A6). There was a significant difference ($p < 0.05$) in size between single layer and multilayer chitosan–alginate beads (Table 1).

3.1.2. Chitosan–alginate–TPP beads

The weight of the chitosan–alginate–TPP beads did not increase when alginate was added to the coagulation fluid. The single layer beads (Table 2, D0 and D1) were in the 544–548 μm range. With the extra treatment by either alginate or chitosan (E0–E3), bead size increased. It was observed that, with a chitosan or alginate concentrations above 0.8% (w/v), the viscosity of coagulation fluid became so high that the formation of drops was strongly impaired.

3.2. Entrapment efficiency of ampicillin in beads

The variation in the concentrations of chitosan had a significant effect on the entrapment of ampicillin in chitosan–alginate beads. Entrapment is expressed as the percentage of total available ampicillin in the dope that actually becomes entrapped in the beads. In the absence of chitosan, entrapment of ampicillin was very low (15%, Table 1, A0). This may be due to insufficient cross-linking and large pore size permitting the ampicillin to diffuse out during and after gelation. Addition of 0.4–0.8% of chitosan to the coagulation fluid (A2, A3) resulted in a large increase of the entrapment. This is probably due to more firmness in the alginate–chitosan complex during gelation caused by increased ionic interactions at pH 4.5 between the carboxylate groups in the alginate and the protonated amine groups in the chitosan. As a result less ampicillin is lost during gelation. In the presence of more chitosan, this process will also go faster. Moreover, a denser membrane will be formed because of the greater number of alginate–chitosan ionic linkages. The entrapment efficiency for ampicillin in alginate–chitosan multilayer beads shows the same trends as in the single layer beads. Entrapment in chitosan–alginate–TPP beads is listed in Table 2.

3.3. Swelling index of dried beads

In order to obtain data on the behavior of chitosan–alginate beads during gastro-intestinal passage, the swelling, the stability in SGF and the release of ampicillin in SIF were studied. In SGF, the beads showed swelling and floating without any sign of disintegration during 4 h.

The swelling index of chitosan–alginate beads increased up to 222% with the addition of chitosan in the coagulation fluid (Table 1, A0–A3). Swelling was found similar in SGF with or without pepsin (data not shown). Beads with an extra treatment with sodium alginate (A6) showed low swelling.

3.4. Behavior of beads in SIF

In the next series of experiments, swollen beads in the gastric fluid were subsequently incubated in SIF with and without pancreatin and observed by light microscopy. Beads prepared without chitosan (Table 1, formulation A0) and swollen in SGF appeared not to be stable in SIF. Complete disintegration was observed within 3–4 h. Addition of chitosan in the coagulation fluid resulted in stronger beads with delayed disintegration (A1–A3). Multilayer beads showed slower disintegration. Beads A5 and A6 remained intact for more than 24 h. Post-coagulation coating of the alginate complex with chitosan retarded the erosion process. Pancreatin did not affect the erosion or disintegration of the beads (data not shown). The multilayer chitosan–alginate–TPP beads (Table 2) were more stable and remained intact more than 24 h in the disintegration solution. After transfer to neutral pH in SIF, the hydroxyl ions will tend to displace the anionic alginate in the calcium-alginate–chitosan complex. Even more important, the chitosan will loose most of its positive charge. As a result, the complex will dissociate and the matrix will erode. The stronger the binding forces in the matrix, the longer this process will take.

3.5. Release of ampicillin from chitosan–calcium-alginate single and multilayer beads

Ampicillin loaded beads prepared as described in Tables 1 and 2 have been investigated for the release of drug in SGF and SIF. The chitosan–alginate single

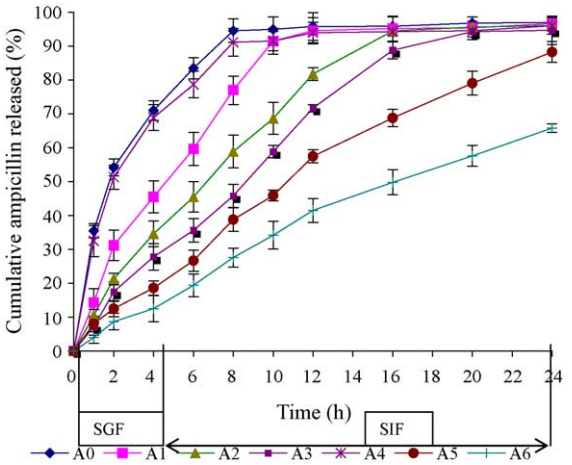

Fig. 1. Release from chitosan–alginate beads and multilayer beads in SGF and SIF. The beads were first incubated in SGF for 4 h and then transferred to SIF. Notations are described in Table 1 (formulations A0–A3: single layer beads; A4–A6: multilayer beads, post-coagulation treatment with chitosan or alginate or with both).

and multilayer beads were incubated in SGF (pH 1.2) during 4 h and then in SIF (pH 7.5) for the next 20 h. The release of ampicillin from alginate beads (A0) was about 70% in SGF within 4 h (Fig. 1). Ampicillin, being a small molecule and hydrophilic in nature, has the tendency to diffuse out easily. As mentioned above, alginate beads, not reinforced by chitosan, had probably insufficient cross-linking density to prevent drug molecules to diffuse out. Likewise, the alginate beads coated with chitosan only in post-coagulation treatment (A4), released more than 60% of the entrapped drug in SGF within 4 h. In these formulations, the entrapment was very low as well. With the addition of chitosan in the coagulation fluid (A1–A3), the release of entrapped ampicillin during the first 4 h in SGF was significantly reduced. The release was very low (<15%) of entrapped drug from multilayer beads (A6).

After 4 h, the beads were transferred to SIF. The alginate beads (A0) disintegrated and lost all remaining drug molecules within 4 h in SIF. Dainty et al. (1986) reported that the disruption of calcium-alginate gel matrix occurs faster in phosphate buffer above a pH 5.5 due to the chelating action of the phosphate ions. At these neutral pH values, the affinity of phosphate for calcium is higher than that of alginate and the solubility of the calcium-phosphate complex, once formed, is high.

Sustained release of ampicillin was observed in case of the multilayer beads, A5–A6. The sustained release was observed in beads prepared by treatment with chitosan, both during coagulation and during post-coagulation. The lower rate of release coincides with the delay in the erosion of the multilayer beads. The delayed erosion and concomitantly the more sustained release of ampicillin from chitosan-reinforced alginate beads probably reflect again the strengthening of the beads by ionic interaction of chitosan (NH_3^+) with alginate (COO^-) ions.

3.6. Release of ampicillin from chitosan–alginate–TPP beads

The release behavior of ampicillin-loaded beads formulated with TPP to replace Ca^{2+} ions is presented in Fig. 2. Chitosan–TPP beads (E0) remained intact for about 10–12 h. Release of ampicillin coincided with the disintegration of chitosan–TPP beads. Sustained release of ampicillin during more than 24 h was observed for chitosan–TPP–alginate beads that received post-coagulation treatment. The delay in the release by chitosan containing beads coincides with the delay in the erosion of beads.

The effect of prior incubation in SGF on the release of ampicillin was investigated during incubation by a parallel study with incubation in SIF only (Fig. 3).

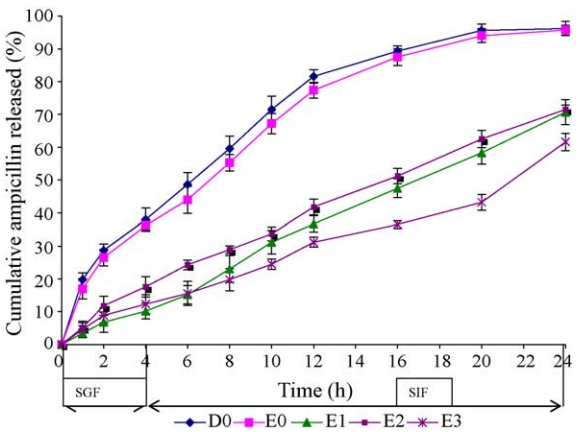

Fig. 2. In vitro release in SGF and SIF from chitosan–alginate multilayer beads cross-linked with tripolyphosphate. The beads were incubated first in SGF for 4 h and then for a period of 20 h in SIF. The notations are given in Table 2 (formulation D0: chitosan–TPP beads; E0–E3: chitosan–alginate–TPP multilayer beads).

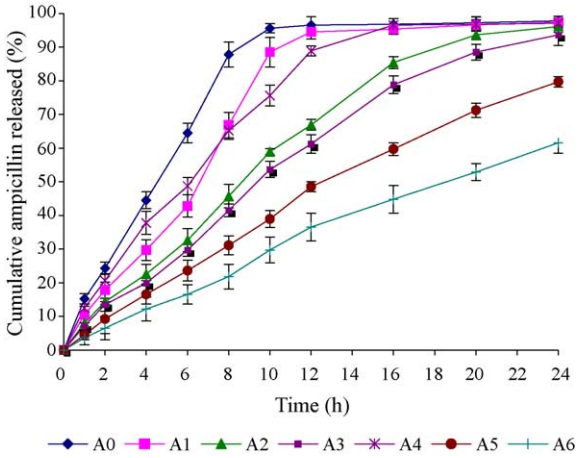

Fig. 3. In vitro release of ampicillin from chitosan–alginate multilayer beads in SIF only. The notations are given in Table 1 (formulations A0–A3: chitosan–alginate single layer beads; A4–A6: multilayer beads, given post-coagulation treatment either with chitosan or alginate or both).

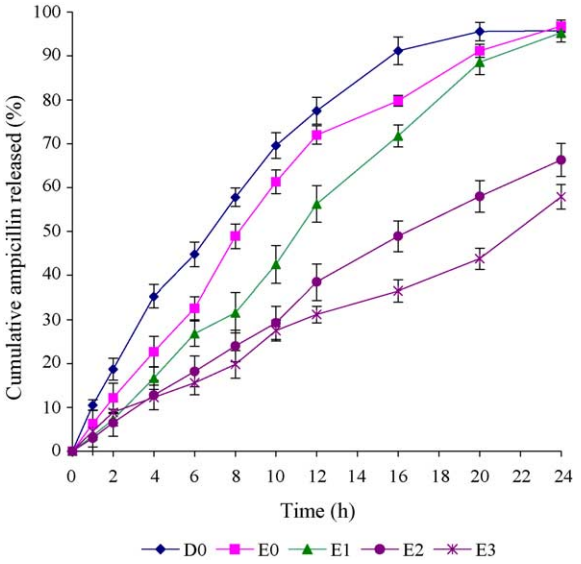

Fig. 4. In vitro release of ampicillin from chitosan–alginate multilayer beads cross-linked with TPP in SIF only. The notations are given in Table 2 (formulation D0: chitosan–TPP beads; E0–E3: chitosan–alginate–TPP multilayer beads).

The chitosan–alginate beads were initially more stable showing a delayed release of ampicillin. Ampicillin release was extended over a longer period of time especially for beads with post-coagulation treatment by chitosan and alginate (Fig. 3, A5 and A6); the multilayer beads showed significant less release of the entrapped ampicillin during 12 h in SIF.

The release of ampicillin from chitosan–TPP (D0) and chitosan–alginate–TPP multilayer beads (E0–E3) in SIF only is shown in Fig. 4. About 70% of the entrapped ampicillin was released about in 12 h from chitosan–TPP beads but TPP multilayer beads release only 25–30%. The release of ampicillin from TPP multilayer beads is similar in SIF (Fig. 4) and in SIF preceded by 4 h incubation in SGF (Fig. 2).

3.7. Microbiological assay for extracted ampicillin

For the assessment of a possible medical application, it is important to confirm the nature of the compound released from the beads as ampicillin. The release of functionally active ampicillin, by various bead formulations was confirmed by its effectiveness to inhibit the microbial growth of *S. aureus*. This was tested in test tube serial dilution method, in order to establish the minimum inhibitory concentration (MIC) at which drug could prevent microbial growth. The MIC value for most samples of extracted ampicillin was found 12.5 μg/ml and few values of 15.5 μg/ml. The values are in good agreement with the MIC value of ampicillin against *S. aureus* of ≥10 μg/ml.

4. Conclusions

Experiments were done to establish the optimum conditions for the preparation of homogenous and spherical chitosan–alginate single and multilayer beads with a smooth surface. The beads were prepared by dropping an alginate–ampicillin mixture in calcium-chitosan and also by dropping of a chitosan–ampicillin mixture in a mixture of TPP and alginate. Chitosan beads are more efficient in the entrapment of the drug. Chitosan drops loose less of their drug content during ionotropic gelation. Chitosan reinforces the bead structure and its impermeability, preventing the drug and ions to leak out. Simultaneously, chitosan makes the bead more flexible and allows the bead wall to enlarge its surface and to swell to compensate for osmotic differences between interior and exterior of the beads.

The release of ampicillin seems to occur both by diffusion and by erosion of the beads. The disintegration of the beads was pH-dependent. The multilayer beads showed more delay in the release of ampicillin, more than chitosan–alginate or chitosan–alginate–TPP beads prepared in a single step. The release in multiple chitosan–alginate beads was more sustained in intestinal fluid without prior incubating in gastric fluid. The classical alginate and chitosan–TPP beads eroded completely within 20 h while the beads reinforced by chitosan and cross-linked with TPP remained intact and released only 30% of the low molecular weight drug in 24 h. Based on these findings, it has been concluded that the multilayer beads, especially those cross-linked with TPP are suitable for the oral sustained release of low molecular and highly hydrophilic compounds. These biocompatible bead systems can bypass the acidity of gastric fluid without liberating substantial amounts of the loaded compound.

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