Bioadhesive alginate copolymers as platforms for oral delivery of insulin

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Abstract: The objective of this study is to utilize the pH sensitivity of modified alginate for oral delivery of insulin. The chemical modification of natural polymers by grafting has received considerable attention in recent years because of the wide variety of monomers available. Acrylic-type polymeric prodrugs were synthesized by free radical copolymerization of acrylic acid, poly (ethyleneglycol monomethyl ether methacrylate) (PEGMA) and alginate in the presence of bis-acrylamide as a cross-linking agent and persulfate as an initiator. The composition of the cross-linked three-dimensional polymers was determined by FTIR spectroscopy. Equilibrium swelling studies were carried out in enzyme-free simulated gastric and intestinal fluids (SGF and SIF, respectively). Insulin was entrapped in these gels and the in vitro release profiles were established separately in both (SGF, pH 1) and (SIF, pH 7.4). Drug releases, in both SGF and SIF media from polymer bonded drugs containing alginate with or without calcium ions were significantly different from each other. The drug release rates from polymer bonded drugs prepared without ions and in the presence of sodium ions were faster. Incorporation of calcium ions into the graft copolymers led to a significant decrease in swelling as well as a substantial retardation of drug release. [Nature and Science. 2009;7(6):61-69]. (ISSN: 1545-0740).

Key words: Alginate, poly (ethylene glycol), pH-sensitive hydrogel, oral insulin delivery.

Introduction

The development of bioadhesive controlled-release systems has been the subject of many studies in recent years (1-9). The ideal drug delivery system should be inert, biocompatible, bioadhesive, comfortable for the patient and capable of achieving high drug loading.

Algicnic acid (Alg), a natural anionic polysaccharide, has been used as a medicine for stomach ulcers as well as a food additive because of the protective effect for the gastric mucosa on per oral administration. The solution of sodium alginate immediately forms a cured gel matrix in the presence of a divalent cation and this characteristic has been utilized practically as a bioreactor. Alginate is a linear copolymer composed of 2 monomeric units, D-mannuronic acid (M blocks) and L-guluronic acid (G blocks). Because of the particular shapes of the monomers and their modes of linkage in the polymer, the geometries of the G-block regions, M-block regions, and alternating regions are substantially different, as shown in Figure 1. The G blocks are buckled while the M blocks have a shape referred to as an extended ribbon. If 2 G-block regions are aligned side by side, a diamond-shaped hole results. This hole has dimensions that are ideal for the cooperative binding of calcium ions. When calcium ions are added to a sodium alginate solution, such an alignment of the G blocks occurs, and the calcium ions are bound between the 2 chains like eggs in an egg box. Thus, the calcium reactivity of alginate is the result of a calcium induced dimeric association of the G-block regions. Depending on the amount of calcium present in the system, these interchain associations can be either temporary or permanent. With low levels of calcium, temporary associations are obtained, giving rise to highly viscous, thixotropic solutions. At higher calcium levels, precipitation or gelation results from permanent associations of the chains. The pH sensitive nature and its ability to control gel permeability means; alginate based polymers have significant potential for drug delivery applications. However the bioadhesive potential of alginate is not sufficient to make suitable for prolonged contact with the intestinal mucosal surface in case of oral drug delivery of poorly absorbable agents. Because, it may be need to be further modified for some special applications. Among diverse approaches that are possible for modifying polysaccharides, grafting of synthetic polymer is a convenient method for adding new properties to a polysaccharide with minimum loss of its initial properties.

Polyacrylic acid (PAA) polymers with pH-sensitive properties have been shown to have good mucoadhesive properties, but their tendency to cause irritation has limited their broad application as buccal bioadhesives. Polysaccharides, such as starch, alginate, cellulose and cellulose derivatives, have been used
in buccal drug delivery systems due to their high biocompatibility and hydrophilicity (10-15). However, their application is limited by their low bioadhesive properties.

Grafting of PAA into alginate has been considered as an alternative procedure to produce nonirritant delivery systems in tablet form with good bioadhesion and controlled-release properties for buccal application. The usual procedure for preparation of alginate graft copolymers is to initiate a free radical on the alginate backbone and then allow the radical to initiate acrylate polymerization.

Existence of polar functionality groups as carboxylic acid need not only for bioadhesive properties but also for pH-sensitive properties of polymer (16-18). Then the incorporation of polyacrylates into biopolymers and, specifically, the grafting of acrylic monomers onto alginate could result in combined properties such as biocompatibility, nontoxicity, and higher bioadhesion, which would confer attractive characteristics on the newly prepared composite materials (19).

Poly(ethylene glycol) (PEG) is widely used in the drug delivery system (DDS) for many reasons, with one being their low toxicity to cells (20). Another reason is that PEGs bind relatively little with proteins (21), thereby enabling the long chain of the PEGs to protect the proteins and peptides in the DDS that are used to target the cells from reacting with other sites in the body (22). PEGs may also increase the chances of the DDS carriers to reach the desired cells (3), as the large molecular weight of the PEGs have been reported to increase the circulation time of the DDS carriers in the bloodstream (23).

In this study our aim was to utilize the pH sensitivity of alginate, (Alginate is stable in acidic pH of stomach, but it swells and starts dissolving slowly in the intestinal alkaline pH) which can be used for protecting insulin in stomach and the bioadhesivity of PAA to make prolonged contact with the intestinal mucosae, so as to increase the absorption of insulin. The free radical graft copolymerization poly acrylic acid (PAA) and poly (ethylene glycol monomethyl ether methacrylate) (PEGMA) onto alginate was carried out at 70 °C, bis-acrylamide as a cross-linking agent and persulfate as an initiator. Insulin was entrapped in these gels and the in vitro release profiles and stability of insulin in contact with these hydrogels during the release were studied. Influences of different factors, such as polymer composition, cross-linking, swelling, effect of the amount of calcium ions on drug released and bioadhesion properties were studied.

Experimental

Materials

The insulin used was recombinant human insulin (AK2U Nobel France; lot # 821156, Batch L-00023822). Poly(ethylene glycol) monomethyl ether methacrylate (PEGMA) was prepared by the method described in the literature (24). Poly (ethylene glycol) monomethyl ether (PEGME) was purchased from Aldrich (France) (Mn = 1000, 2000). Dicyclohexylcarbodiimide (DCC) were purchased from Merck Co. 4-dimethylaminopyridine (DMAP) and reagents were obtained from Fluka Co. Sodium alginate of medium viscosity (3500cps for a 2% solution at 250 °C) was obtained from Sigma chemicals Co. Acrylic acid (AA) and bis-acrylamide were purchased from Merck Co. All the other chemicals used were of analytical reagent grade.

The IR spectra were recorded on a Shimadzu FT-IR-408 spectrophotometer. The amount of released drug was analyzed using a high-performance liquid chromatography-ultraviolet (HPLC-UV) Waters bus SAT/IN Module at 210 nm. Isocratic elution was performed using 30% acetonitrile and 70% buffer containing 0.1M KH2PO4 and 1% triethylamine adjusted to pH 3.0 with phosphoric acid. The column used was Nucleosil-C185-m PHASE SEPARATIONS 4.6-250 mm Analytical Cartridge (part no. psl841020) equipped with a precolumn.

Methods

Preparation of graft copolymers of alginate with acrylic acid: General Procedure

Polymer bonded drugs (PBDs) were synthesized by graft copolymerization of alginate, PAA, PEGMA (variable feed ratio as shown in Table 1) and bis-acrylamide as a cross-linking agent in water as the solvent (50 mL). Copolymerization was carried out in the presence of persulfate as an initiator ([I] = 0.02 M) at 60-70 °C in a thermostatic water bath. All experiments were carried out in Pyrex glass ampoules. After the desired time (48 h) the precipitated hydrogels was collected, washed with deionized water for 1 week and the water was changed every 12 hours in order to remove any unreacted monomers. After washing, the samples were dried in air and stored in desiccators until use. The values are given in Table1. IR (KBr): 3450-2500 (broadened, -COOH group), 1730, 1650, 1220, 1210 cm⁻¹.
Buffer Solutions

Enzyme-free SGF (pH 1) or SIF (pH 7.4) were prepared according to the method described in the US Pharmacopeia (25). For the study of the influence of calcium ion on the properties of the graft copolymers, the buffers SGF (pH 1) or SIF (pH 7.4) were modified by adding calcium oxide. The pH change due to the addition of calcium oxide was corrected with either 1.0 N HCl or 1.0 N NaOH and then used for the drug release experiments.

Swelling ratio

Grafting of acrylic acid monomer onto biopolymers is usually performed to prepare materials with high absorbency for water. To measure the swelling, preweighed dry drug-free hydrogels were immersed in various buffer solutions (pH 7.4 and pH 1) at 37 ºC. After excess water on the surface was removed with the filter paper, the weight of the swollen samples was measured at various time intervals. The procedure was repeated until there was no further weight increase. The degree of swelling was calculated according the relation:

\[ \text{SW} (\%) = \left( \frac{W_s - W_d}{W_d} \right) \times 100 \]

Where, \( W_s \) and \( W_d \) represent the weight of swollen and dry samples, respectively. The study of swelling shows that swelling of hydrogels increases with time, first rapidly and then slowly, reaching maximum constant swelling (mass equilibrium swelling, MES). The swelling value of cross-linked polymers in pH 1 and pH 7.4 at 37 ºC are given in Table 2.

Insulin stability during release studies from hydrogels

In order to study the stability of insulin in contact with hydrogels, two different conditions were chosen: 37 ºC and darkness, 37 ºC and light. Insulin was loaded in hydrogels as described and then the peptide stability was investigated during release under the above mentioned conditions at two different pH values of 1 and 7.4. Samples were analyzed under each condition after 24 and 48 h. In this condition insulin remained fairly stable at both pH values during the course of experiments, indicating that adsorption of the peptide to the hydrogels and their release afterwards did not substantially influence the stability of this peptide drug.

To investigate the protective ability of the hydrogel for insulin in the harsh environment of the stomach, insulin and insulin-incorporated were treated with a simulated gastric solution that contained endopesidase pepsin. After the treatment in gastric solution, the biological activity of insulin was determined with HPLC. These results indicated that all insulin was degraded immediately after insulin was in contact with gastric fluid and the main cause of degradation was the proteolytic enzyme, pepsin. After being treated with gastric fluid, all of hydrogels demonstrated a protective effect on insulin and the biological activity remained after the treatment with gastric fluid of hydrogels. Studies of hydrogel showed that when the PAA content increased, degradation of insulin decreased.

Insulin release from hydrogels

Insulin release from the delivery systems was tested in the pyrex glasses. The powdered hydrogel (10 mg) was poured in 5ml of aqueous buffer solution (pH=7.4 & pH=1) at 37 ºC. The rotation speed was adjusted with stirrer. Samples were measured using HPLC-UV at 210 nm. The flow-rate and injection volume were 1 ml/min and 60 µL, respectively. Insulin was detected at a retention time of 5.5 min and the detection limit was 0.3 µg/mL. Triplicate samples were used. The amounts of insulin released from hydrogels was collected by taking 60-µL samples at predetermined time intervals and analyzed by HPLC.

Quantitative analysis of insulin

Three milligrams of polymer-drug adduct was dispersed in 3 mL of mobile phase solution. The reaction mixture was maintained at 37 ºC. After 4 h the hydrolysis solution filtered and analyzed by HPLC for the determination of total insulin in hydrogels. The results obtained are presented in Table 2.

In situ Bioadhesivity Studies

Bioadhesivity testing was done by a novel in situ method as described by Ranga Rao and Buri (26). A freshly cut 5-6cm long piece of small intestine of rat was obtained and cleaned by washing with
isotonic saline. The piece was cut open and the mucosal surface was exposed. Known weights of hydrogels were added evenly on the mucosal surface. The intestinal piece was maintained at 80% relative humidity for 30 mts in a desiccator. The piece was taken out and phosphate buffer pH 6 was allowed to flow over the intestinal piece for about 2 mts at a rate of 20 ml/min. The perfusate was collected and dried to get the particles not adhered. The percent of bioadhesion was estimated by the ratio of amount applied to adhere hydrogels. The values are given in Table 3.

Results and Discussion

To achieve successful colonic delivery, a drug needs to be protected from absorption of the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs. These requirements have prompted the development of polymeric systems that swell minimally under acidic conditions but extensively in basic intestinal medium.

When an aqueous solution of sodium alginate is added to an aqueous solution of calcium ions, spherical alginate beads with regular shape and size are produced, since an insoluble calcium alginate matrix is formed by the cation exchange between Na+ and Ca2+ (27). Alginate beads have the following advantages: 1) Alginate is known to be nontoxic as taken orally and to protect the mucous membrane of the upper gastrointestinal tract from the irritation of chemicals (28). 2) Since dried alginate beads have the property of reswelling, they can act as a controlled-release system. 3) Since the property of reswelling is susceptible to environmental pH, acid-sensitive drugs incorporated into beads would be protected from gastric juice (29). In addition, the presence of divalent calcium ions was found to affect the drug release and mucoadhesion properties of PAA polymers (8, 9).

In general, PEG has good biocompatibility and has been approved for a wide range of biomedical applications (30). Especially, the presence of grafted PEG chains in these hydrogels plays an important role. At low pH, the oxygen groups in the grafted PEG chain form hydrogen-bonded complexes by interacting with carboxylic groups of PAA. These hydrogen bonds lead to more collapsed polymer networks resulting in protection of drug incorporated in the hydrogels. Moreover, several studies have shown that these grafted PEG chains promote mucoadhesion by chain interpenetration leading to increased drug absorption through the intestinal wall.

All the matrices with the presence of PEG and increase in the content of AA had shown increased bioadhesivity (Table 3). However, in the graft copolymers, especially those with incorporated calcium ions, had better bioadhesive properties than without calcium ions and were shown to be promising buccal drug carriers for systemic delivery. The binding of those with sialic acid residues make prolonged contact of the drug with the epithelium, also it was assumed that opening of the intercellular junctions by PEG could lead to the enhancement of insulin absorption across the mucosa.

The swelling value shows that, an increase in the content of AA in the feed monomer mixtures resulted in less swelling in SGF but greater swelling in SIF. The loading numbers in Table 2 shows existence of polar functionality groups as carboxylic acid need not only for loading insulin on the polymer but also for pH-sensitive properties of polymer. Insulin molecules have a tendency to attach to polar groups due to hydrogen-bonding. Hydrogen bonding is a key contributor to the specificity of intramolecular and intermolecular interactions in biological systems. Because the increase of AA content in the hydrogels provides more hydrogen bonds at low pH and more electrostatic repulsion at high pH.

In vitro release of insulin

Drug releases, in both SGF and SIF media with or without calcium ions were significantly different from each other. For these hydrogels, the released insulin in the acid media increased with the molecular weight of the grafted PEG in the network. At the incorporating pH of 7.0, the carboxylic acid groups in the networks, as well as the insulin, (pI of 5), were negatively charged resulting in repulsion. Thus, the negatively charged insulin was mainly distributed in the neutral PEG chain domains. A researcher shows that insulin appeared to partition into the PEG phase in hydrogels containing PEG and a negatively charged component (31). When insulin-incorporated polymer bonded drugs were placed in acidic media, particles with longer PEG chains, where more insulin was distributed, had more chance to contact the outer aqueous environment, and as a result insulin was released by a concentration gradient at low pH. However,
there was no significant difference of insulin release at high pHs from systems with different PEG molecular weights.

The degree of hydrolysis of the hydrogels containing insulin as a function of time is shown in figure 2. It appears that the degree of hydrolysis network polymers depends on their degree of swelling and reticulated degree. With increased cross-linking and an increase in the reticulated degree of the polymer, diffusion of the hydrolyzing agents in the networks polymer is reduced and the hydrolysis rate is slower. A high different hydrolysis rate for polymers at pH 1 and pH 7.4 can be related to the number of carboxylic acid groups units along the polymer chain. Existence of hydrogen-bonding interactions between –COOH groups in the polymer matrix results in a complex structure within the network, and so the movement of polymeric segments is restricted. This also accounts for minimum hydrolyzing of the gel in a medium of pH 1. However, when the sample is placed in a medium of pH 7.4, the almost complete ionization of –COOH groups present within the polymer network not only increases the ion osmotic swelling pressure to a great extent but also enhances the relaxation of macromolecular chains because of repulsion among similarly charged –COO groups. These two factors ultimately result in a greater increase in the water uptake. In pH 7.4 with completed ionization and an increase in the hydrophilicity of the polymer, diffusion of the hydrolyzing agents on polymer is increased and the hydrolysis rate increased (32).

The calcium cation can act as chelating agents and cross-linkers. Incorporation of the (Ca\(^{2+}\)) resulted in a remarkable retardation of drug release. Evidently, the divalent ions may have acted as simple cross-linking agents by interacting with carboxyl groups and forming bridges between polymer chains. This interaction may in turn reduce swelling and drug release. The effect of the amount of calcium added on drug release in SGF and SIF is shown in Table 4. At pH 7.4, the drug release decreased apparently when 3 mg of calcium ions was added into the 300 mg of Polymer Bonded Drugs (PBDs). The calcium ions may have acted as simple cross-linking agents by interacting with carboxyl groups and forming bridges between polymer chains. Furthermore, the dissolved calcium ions interact with alginate, thus forming a calcium alginate gel matrix. Therefore, the slower drug release from PBDs with calcium ions was due to the retention of the drug in the calcium alginate gel matrix. However, a large amount of added calcium 30 mg produced a faster drug release (Table 4). This can be explained by the influence of calcium on the gel formation. The gel strength increases with the addition of calcium up to a critical concentration. Above this concentration, the gel strength weakens. This weakening is due to excessive cross-linking by the calcium and hence formation of a nonhomogeneous gel matrix. The PBDs disintegrated partially, and the larger surface area created resulted in the faster drug release.

This result was consistent with findings from previous studies (33, 34). In pH 1, the drug release was insignificantly decreased in the alginate-based matrix containing a small amount of calcium ions 3 mg as shown in Table 4, probably because the added calcium ions were replaced by protons in the medium. Increasing the calcium amount in the formulations to 30 mg clearly increased the release rate. The complete disintegration of matrices would cause the faster drug release in an acidic medium.

**Table 1. Composition of alginate based copolymers**

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Molar composition of monomers in the feed</th>
<th>Alginate</th>
<th>PEGMA(^{1000})</th>
<th>PEGMA(^{2000})</th>
<th>MAA</th>
<th>CA(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-2</td>
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<td>3</td>
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<td>P-3</td>
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<td>5</td>
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<td>1</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-5</td>
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<td>1</td>
<td>3</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-6</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-7</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-8</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>10</td>
<td></td>
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</table>
**Figure 1.** A schematic illustration of the principal structure of alginate: (a) the alginate chain (b) calcium alginate matrix.

**Table 2. Percent of swelling and drug loading numbers**

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Maximum constant swelling (‰)</th>
<th>Percent of Insulin loading (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>pH 1</td>
<td>pH 7.4</td>
</tr>
<tr>
<td>P-1</td>
<td>200</td>
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</tr>
<tr>
<td>P-2</td>
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<td>600</td>
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<tr>
<td>P-3</td>
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<td>1050</td>
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<td>P-4</td>
<td>100</td>
<td>950</td>
</tr>
<tr>
<td>P-5</td>
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</tr>
<tr>
<td>P-6</td>
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<td>750</td>
</tr>
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<td>P-7</td>
<td>240</td>
<td>1200</td>
</tr>
<tr>
<td>P-8</td>
<td>160</td>
<td>1100</td>
</tr>
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</table>

**Table 3. Percentage of particles adhered onto rat intestine**

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Percentage adherence</th>
<th>Percentage adherence containing Ca(^{2+}) (3 mg)</th>
<th>Percentage adherence containing Ca(^{2+}) (30 mg)</th>
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<tbody>
<tr>
<td>P-1</td>
<td>55</td>
<td>58</td>
<td>63</td>
</tr>
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</tr>
<tr>
<td>P-3</td>
<td>59</td>
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<tr>
<td>P-4</td>
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<td>60</td>
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<td>P-7</td>
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<td>65</td>
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<tr>
<td>P-8</td>
<td>60</td>
<td>63</td>
<td>68</td>
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</table>
Figure 2. Release of insulin from polymeric carriers as a function of time at 37°C.
Table 4. Effect of amount of calcium added on insulin released from polymeric carriers at 37 °C.

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Maximum constant of release (%) at 7 hour pH 1</th>
<th>Maximum constant of release (%) containing Ca²⁺ (3 mg) at 7 hour pH 1</th>
<th>Maximum constant of release (%) containing Ca²⁺ (30 mg) at 7 hour pH 1</th>
<th>Maximum constant of release (%) containing Ca²⁺ (3 mg) at 7 hour pH 7.4</th>
<th>Maximum constant of release (%) containing Ca²⁺ (30 mg) at 7 hour pH 7.4</th>
</tr>
</thead>
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<td>P-2</td>
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<td>25</td>
<td>8</td>
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<td>23</td>
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</table>

Conclusion

A new nonirritating buccal adhesive system for the controlled-release of insulin was developed using graft copolymers of alginate and acrylic acid in various compositions. Novel bioadhesive and pH-responsive hydrogels containing pendant alginate (Poly(alginate-co-AA-co-MEG)) were synthesized by free-radical crosslinked copolymerization. By regulating the crosslinking percentage of the AA copolymers, pH-sensitive hydrogels with improved optimal hydrolysis rates were obtained. The hydrolysis of the drug-polymer conjugates were performed at pH 1 and 7.4 at 37 °C. The drug-release profiles of PBDs indicated that the amount of drug released depended on the amount of calcium ions.

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References


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