Interpolymer Complex Microparticles Based on Polymethacrylic Acid-Chitosan for Oral Insulin Delivery

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ABSTRACT: In the present study, microparticles composed of polymethacrylic acid-chitosan (PMAA-CS) were prepared by a novel interionic gelation method. Free-radical polymerization of methacrylic acid was carried out in the presence of CS, using a water-soluble initiator, and application of these microparticles toward oral insulin delivery was evaluated. Microparticles obtained were characterized by scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) studies. From SEM studies, it was observed that microparticles had an aggregated morphology with size ~20 μm, while FTIR confirmed the presence of ionic interaction between PMAA and CS chains. Protein loading was done by diffusion filling method, and from in vitro release study, it was observed that insulin-loaded microparticles displayed a pH depended release profile at alkaline/acidic pH. Microparticles exhibited sustained release of insulin for 3–4 h at neutral pH, and enzyme linked immunosorbent assay (ELISA) proved that encapsulated protein maintained 100% biological activity at neutral pH. Preliminary study suggests that these microparticles can serve as good candidate for oral protein delivery. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 99: 506–512, 2006

Key words: microparticles; template polymerization; insulin; polymethacrylic acid; chitosan

INTRODUCTION

Oral administration of proteins/peptides still remains a challenge for scientific community, as they are inhibited by enzymatic and absorption barriers posed by gastrointestinal (GI) tract.[1,2] Hydrophilic carriers composed of polyacrylic acid/polymethacrylic acid (PAA/PMAA) and chitosan (CS) have been widely investigated for oral peptide delivery due to their ability to modulate tight epithelial junctions across GI tract.[3,4]Studies proved that polymers containing carboxylic acid groups have the ability to protect peptide drugs from protease enzymes like trypsin and chymotrypsin. Binding of divalent cations (calcium and zinc) is proposed to be the major reason for their enzyme inhibitory effect.[5,6] Further reduction of extracellular divalent ion concentration can result in opening of tight junctions and improves paracellular peptide drug transport across the intestinal epithelium.[7] Moreover, mucoadhesive properties exhibited by these polymers increases the residence time of oral dosage form at the epithelial surface and improves drug absorption.[8,9]

Compared with conventional delivery systems, hydrophilic nano/microparticles of alginate, CS, and PAA/PMAA have shown promise in developing efficient and effective oral insulin delivery systems.[10–12] It is proposed that microparticles may allow a better retention of drug delivery system in the GI tract, and reduction in particle size may improve the bioavailability of drugs.[13] Different polymerization techniques have been adopted for the preparation of PAA/PMAA nano/microparticles, which include suspension, inverse emulsion, reverse emulsion, and dispersion/precipitation polymerization processes.[14–17] However, most of such procedures use either surfactants or steric stabilizers for the stabilization of resultant particles.

Ionic interaction between anionic–cationic polymers could lead to the formation of polyelectrolyte complexes, and this method is often employed in the preparation of polyion nano/microparticles (complex coacervation). Polyion complexes of sodium alginate-CS, sodium carboxy methyl cellulose-CS, etc. have been widely investigated for different biomedical applications.[18–21] CS nano/microparticles can be prepared spontaneously through ionic crosslinking between positively charged amino group and low molecular weight counter ions like sodium tripolyphosphate, sodium sulfate, etc. (often referred as ionotropic gelation method).[22–25] In either case, ionic interaction between oppositely charged ions is the key factor leading to gelation. An attractive feature of these preparative techniques is that stable micropar-

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ticles can be obtained spontaneously without using surfactants or organic solvents, which makes them a suitable choice for drug delivery/tissue engineering applications.

In the present study, an attempt was made to utilize polycationic polymer (CS) as a counter ion for the generation of hydrophilic PMAA microparticles. MAA being anionic in nature, our studies were intended on inducing gelation by its interaction with cationic polymer CS. MAA was polymerized in the presence of CS under aqueous medium avoiding use of any organic solvents, surfactants, or steric stabilizers. SEM and FTIR were used to characterize these PMAA-CS microparticles. Swelling studies of microparticles were conducted in acidic and neutral pH. Insulin loading was done by diffusion filling method, and drug-loaded particles were subjected to in vitro release study at pH 1.2 and 7.4. Biological activity of loaded insulin at pH 7.4 was analyzed using ELISA technique.

EXPERIMENTAL

Materials and methods

Methacrylic acid (MAA) was purchased from Merck (Germany), and ethylene dimethacrylate (EDMA) was from Polysciences (Warrington, PA). Chitosan (CS) with approximate molecular weight 270,000 and 85% deacetylated was a gift from Central Institute of Fisheries Technology (Cochin, India). Human insulin100 IU/mL was purchased from Elli Lilly (India) while Human insulin ELISA Kit was from DAKO Cytomation (UK). Potassium persulfate was from BDH (India). All other chemicals were of reagent grade and used without further purification.

Insulin estimation was done by Lowry protein assay.[26] Absorbance was measured using SHIMADZU (UV-160 A) at 750 nm. All experiments were carried out at room temperature (30°C ± 2°C) in triplicate.

Preparation of microparticles

MAA was polymerized in the presence of CS to obtain PMAA-CS interpolymer complex microparticles. CS was purified by dissolving in acetic acid followed by precipitation in sodium hydroxide solution. Monomer (MAA) was mixed with comonomer EDMA and CS prior to the process. Reaction mixture was diluted with double-distilled water, and potassium persulfate was added to the solution as a free-radical initiator. Nitrogen was purged through the mixture for 15 min, and the whole content was stirred at high temperature (40–60°C) for 6 h. Resulting suspension was allowed to settle overnight, and supernatant solution was decanted carefully. Particles obtained were washed with distilled water and dried in vacuum.

TABLE I
Composition of MAA and CS Used for the Preparation of Microparticles

Sample code	MAA (g)	Chitosan (mg)	EDMA (g)
MCI	1.6	20	0.1
MCII	1.6	50	0.1
MCIII	1.6	100	0.1

Three different compositions of PMAA-CS microparticles were prepared by varying CS concentration and designated as MC I, MC II, and MC III. Composition of MAA and CS used for the preparation of microparticles is given in Table I.

Surface morphology

Microsphere surface was examined using scanning electron microscope (HITACHI S-2400). It was used to evaluate surface texture and shape of the microparticles. Samples were mounted on metal stubs using double-sided adhesive tape, coated with gold under vacuum, and then examined.

FTIR analysis

Dried samples were mixed with KBr and pressed into a disk. FTIR spectra were recorded using Nicolet Impact 410 spectrometer.

Swelling studies of microparticles

Swelling behavior of PMAA-CS microparticles was studied in buffer solutions of pH 1.2 and 7.4. Fifty milligrams of drug-free microparticles was suspended in 10-mL buffer solution. At each predetermined time interval, excess buffer solution was carefully removed, and weight of swollen particles was determined. The degree of swelling for each sample was calculated as per equation

$$\text{Degree of swelling} = (W_s - W_d)/W_d$$

where W_s and W_d are the weight of samples at the equilibrium and dry state, respectively.

Drug loading by diffusion filling method

A known amount of dried microparticles was kept in insulin solution (100 IU/mL) for remote loading. After specified time interval, microparticles were taken out and excess insulin solution was gently wiped off. Loaded microparticles were kept for drying at low temperature (2–4°C).

Insulin content inside the PMAA-CS microparticles was determined by suspending 100 mg of sample in

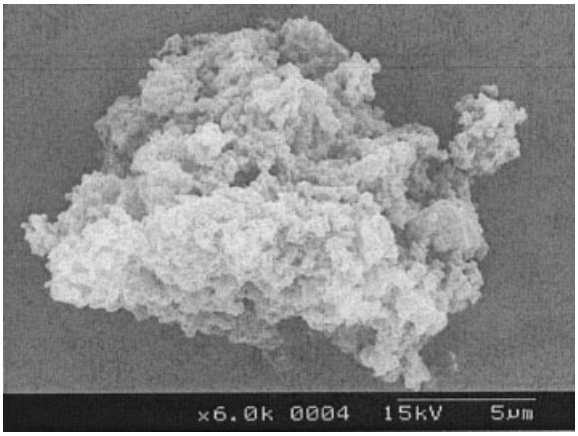

Figure 1 Scanning electron microphotograph of PMAA-CS microparticles (MC I).

20-mL phosphate buffer for 24 h with constant stirring. After specified time, aliquot of sample was withdrawn, and protein content was analyzed by Lowry protein assay.

Encapsulation efficiency (EE) of PMAA-CS microparticles was calculated as per equation

$$\text{EE} = \frac{\text{Total amount of insulin} - \text{Free amount of insulin}}{\text{Total amount of insulin}} \times 100$$

In vitro drug release study

Microparticles loaded with insulin were dried and weighed. Known amount of drug-loaded microparticles was suspended in 20 mL of buffer solutions (pH 1.2 and 7.4). At specified time interval, aliquot of sample was withdrawn, and protein content was estimated by Lowry protein assay. The dissolution medium was replaced with fresh buffer to maintain total volume after each withdrawal.

Biological activity of loaded insulin inside the microparticles was investigated using ELISA (DAKO Cytomation) techinique. Hundred milligrams of insulin-loaded microparticles was suspended in phosphate buffer (pH 7.4) for 24 h. After specified interval, an aliquot of sample was withdrawn, and ELISA was done as per standard procedure. Results were obtained by reading the optical density at 450 nm using micro plate reader (Finstruments Microplate Reader).

RESULTS AND DISCUSSION

PMAA-CS microparticles were prepared by polymerizing MAA in the presence of CS, using a water-soluble initiator under mild aqueous conditions. Macromolecular hydrogels based on PAA/PMAA-CS, prepared by template polymerization of acrylic monomers in the presence of CS, have been widely investigated for drug delivery applications.[27–31] However, objective of this study was to prepare PMAA microparticles by virtue of their ionic interaction with cationic polymer CS. Amino group of CS interacts with carboxylic acid of MAA, and microparticles were spontaneously generated during the polymerization. CS is a polycationic polymer widely investigated for biomedical applications, possibly due to their biodegradability, biocompatibility, and nontoxicity.[32,33] Further, CS posses good mucoadhesive properties and have the ability to improve the transport of hydrophilic drugs across intestinal epithelium by opening tight intestinal epithelium.[34] However, CS was selected for the present study mainly due to their ability to form gel upon contact with anionic polymers/low molecular weight counter ions. CS was utilized as a counter ion for the preparation of anionic PMAA microparticles, and intermolecular interactions between MAA-CS was responsible for the generation of microparticles during the process, since little success was obtained with the process tried in the absence of CS. EDMA crosslinking of MAA provides necessary stability to the system and renders a pH dependent swelling/deswelling capacity to the microparticles.

PMAA-CS microparticles were found to be aggregated and irregular as indicated by scanning electron microphotographs (Figs. 1–3). Size of these particles was found to be in the range of 10–20 μm. PMAA microparticles prepared in the absence of CS[35] was used for a comparison in this present study (Fig. 4). PMAA microparticles were found to be smaller in size and had totally different morphology as compared with those of PMAA-CS particles. It is reported that CS can act as an ionic template for free-radical polymerization of acrylic acid/methacrylic acid, and presence of template during the polymerization procedure

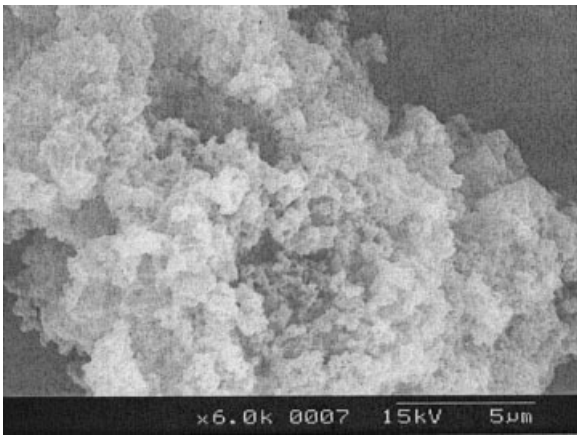

Figure 2 Scanning electron microphotograph of PMAA-CS microparticles (MC II).

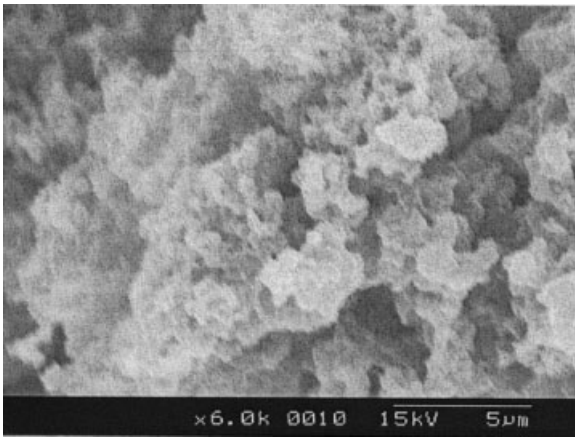

Figure 3 Scanning electron microphotograph of PMAA-CS microparticles (MC III).

has kinetic and structural effects on growing polymer chains.[36,37] SEM studies clearly indicated that morphological characteristics of the microparticles were affected by the inclusion of CS.

FTIR investigation of the microparticles was done to establish the ionic interaction of PMAA with CS in the matrix (Fig. 5). PMAA had characteristic IR absorption at 3529, 1722, and 1540 cm^{-1}(1 of Fig. 5). Typical IR absorption was reported for CS at 3450 cm^{-1} (for hydroxyl group), 1650 and 1550 cm^{-1} (for amide I and II groups, respectively).[28] In case of PMAA-CS microparticles (2–4 of Fig. 5), carbonyl group of PMAA appeared at 1718 cm^{-1} with a predominant shoulder at 1663 cm^{-1}.[27–30] This was expected due to the ionic interaction between PMAA-CS. Further, peak at 1540 cm^{-1}, which was assigned as symmetric deformation of ammonium ion, confirmed the presence of CS in the matrix.[30]

Swelling studies of drug-free microparticles were conducted in acidic and alkaline pH (Fig. 6). As expected PMAA-CS microparticles displayed higher degree of swelling at neutral pH and lower degree of swelling at acidic pH, possibly due to the higher PMAA concentration in the matrix. Hydrogels containing carboxylic acid groups collapses in strong acidic solution and swells in a weakly alkaline medium, due to the ionization/deionization behavior of acid groups. At low pH, acid group exist in protonated form and this causes network to shrink. However, at neutral/alkaline pH, these acid groups ionizes, and swelling occurs as a result of electrostatic repulsion between ionized acid groups.[38] From the swelling studies, it was obvious that the presence of CS also affected the swelling pattern of PMAA-CS particles. At neutral pH, swelling of PMAA-CS was reduced with higher CS concentration, while degree of swelling was increased with higher CS concentration, at acidic pH. Observed degree of swelling of PMAA-CS at pH 7.4 followed the order MC I > MC II > MC III, and swelling at pH 1.2 followed the order MC III >MC II >MC I.

Being polycationic in nature, CS exists in nonprotonated form at neutral pH. Higher CS concentration may retard the swelling of PMAA matrix possibly due to their hydrophobic nature at this pH. On the other hand, acid medium generates free positive charge on amino groups of CS, and swelling happens due to the mutual repulsion of NH_3^+ groups. Higher CS content may result in increased electrostatic repulsion between charged groups, and this can lead to a greater separation among polymer network at acidic conditions.

Insulin is a peptide hormone widely used in the treatment of diabetes mellitus and is susceptible to physical and chemical decomposition. Microencapsulation procedures involving use of either organic solvents and/or high temperature are not ideal for sensitive peptide drugs. In the present study, drug loading was carried out by diffusion filling method, in which crosslinked microparticles were exposed to remote loading medium. Major advantage of remote loading was that sensitive proteins were not exposed to harsh process involved in microparticle fabrication. By present technique, a high concentration of drug was achieved inside the microparticles, without affecting its biological activity.

EE of microparticles were found be 73 ± 3 (%), 73.5 ± 2 (%), and 65 ± 3 (%) for MC I, MC II, and MC III, respectively (n = 3). Drug-loading capacity of the microparticles was depended on composition of particles. MC I and MC II displayed somewhat similar drug-loading capacity, whereas MC III showed slightly lower drug EE. PMAA-CS forms interpolymer complexes, and this may affect the loading efficiency of microparticles. Close entanglement of CS chains with PMAA hinders the swelling capacity of the microparticles and can reduce the loading efficiency.

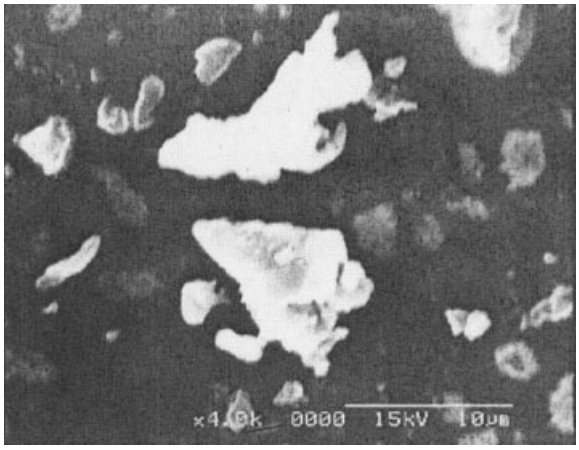

Figure 4 Scanning electron microphotograph of PMAA microparticles.

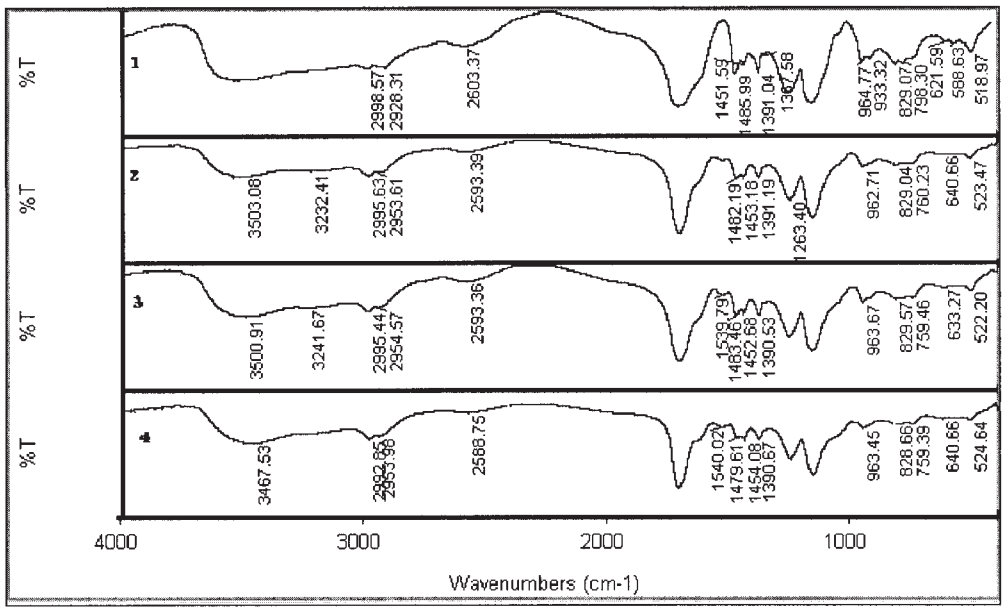

Figure 5 FTIR spectra of PMAA/PMAA-CS microparticles.

Hydrogels made of pH sensitive polymers (e.g., PMAA, PAA, etc.) are widely investigated as enteric coating material in pharmaceutical industry due to their ability to protect drugs from harmful gastric environment. At low pH (1.2), acid groups in these materials remain collapsed, and the drug encapsulated will be protected from the acidic environment. As pH increases, acid groups in the network ionizes, and hydrogels dissolves in intestinal fluids releasing the encapsulated material in its active form.[38,39] In vitro release profiles of PMAA-CS microparticles at pH 7.4 and 1.2 are given in Figure 7. At pH 7.4, the rapid release of insulin was observed possibly due to swelling ratio of the microparticles. In the first hour itself, about 50% of loaded insulin was released, and >90% of loaded insulin was released within 4 h of study. Several studies conducted proved that release of drug from hydrogel microparticles happens mainly

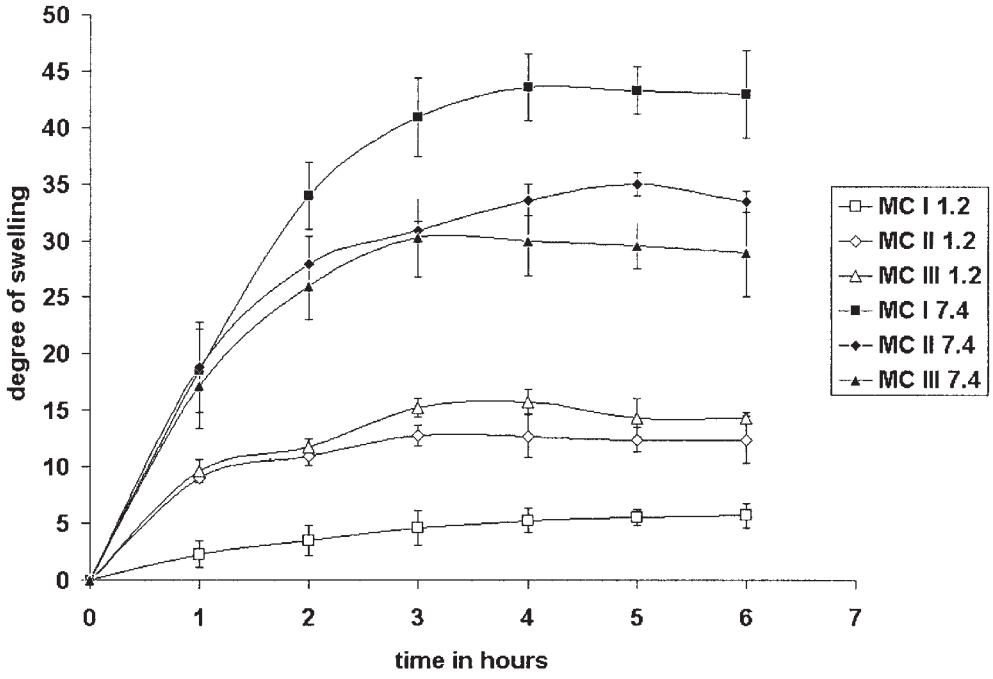

Figure 6 Swelling characteristics of PMAA-CS microparticles at pH 1.2 and 7.4.

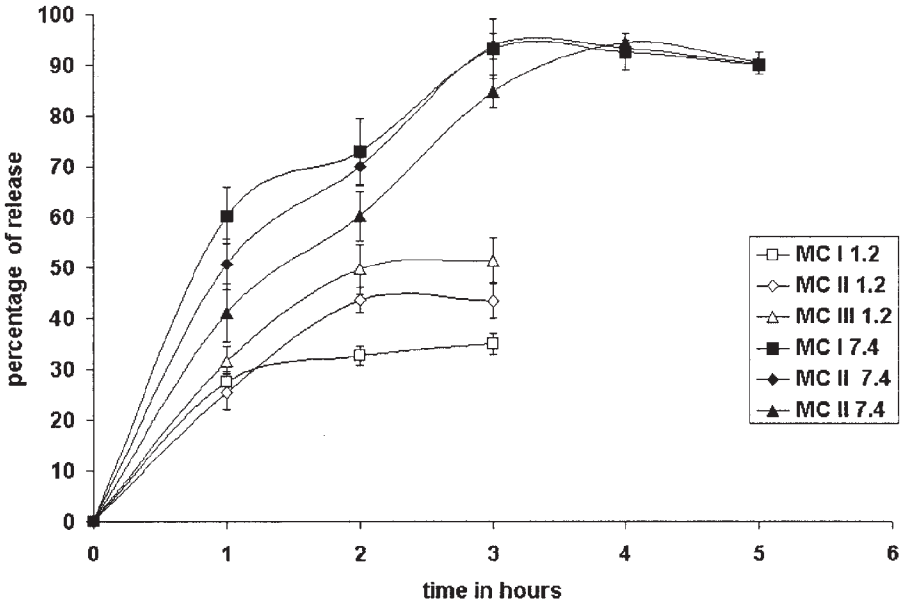

Figure 7 In vitro release profile of insulin from PMAA-CS microparticles at pH 1.2 and 7.4.

via diffusion through swollen matrix. For system containing acid groups, such as PAA/PMAA, pH plays a major role in controlling diffusion of loaded drug. Insulin is a relatively water-soluble peptide with approximate molecular weight of 5750, and these molecules diffuse out rapidly through the matrix. However, release of drug in actual in vivo conditions may vary due to the divalent ion binding of ionized carboxylic acid groups present in the matrix.

Release study of microparticles was carried at pH 1.2 for 3 h. Release of insulin was relatively low at pH 1.2, which can be related to the comparatively low degree of swelling at pH 1.2. However, some amount of encapsulated insulin was released at this pH, which could be possibly due to the diffusion of drug through the microparticles even in the collapsed state. At low pH, protonated amino group repel each other leading to separation among polymer chains and encapsulated-insulin easily diffuses out through these gaps. Matrix with higher CS content was unable to hold insulin at this pH, and >50% of insulin was lost from MC III at pH 1.2, within 2 h of the study. For microparticles having lower CS content (MC I and MC II), insulin release was slightly lower at acidic pH. So, it was obvious that microparticles having higher CS content may not be appropriate for present application because of its poor protein retention capacity at low pH.

Biological activity of loaded insulin was estimated using ELISA technique. Proteins are fragile molecules with labile bonds and reactive side-chains. Disruption of these complex structures or modification of side chains or use of extreme conditions can lead to loss of biological activity. ELISA measures biologically active insulin with a high degree of specificity, using a pair of mouse monoclonal antibodies.[40] Protein content inside the microparticles was analyzed by Lowry method, and biological activity of these proteins was further estimated using ELISA technique (Table II). Similar results were obtained from Lowry and ELISA studies, which suggest that 100% activity was maintained inside these microparticles. Full biological activity of proteins is dependent on preserving the integrity of its three-dimensional structure, and ELISA results suggest that PMAA-CS microparticles are capable of preserving biological activity of encapsulated-insulin.

CONCLUSIONS

Presently, a hydrophilic microparticle-based delivery system was utilized for oral delivery insulin. Microparticles were composed of PMAA and CS, which were developed by a novel ionic-gelation method. This process was successful in delivering stable micro-

TABLE II
Insulin Content Analysis by Lowry and ELISA Techniques (n = 3)

Sample code	Loading time (h)	Insulin content (IU/100 mg)	
		Lowry method	ELISA method
MCI	24	56 ± 2	57 ± 1.5
MCII	24	58 ± 1.5	58 ± 2
MCIII	24	47 ± 2	48 ± 1

particles under mild conditions without using organic solvents, steric stabilizer, or surfactants. SEM was illustrative of their surface morphology while FTIR showed the presence of ionic interaction between acid–amino groups in the PMAA-CS matrix. Insulin-loaded microparticles displayed pH dependent release properties, releasing >90% of loaded insulin in <4 h at neutral pH. Particles with relatively higher CS content displayed less than ideal property, releasing >50% of loaded drug within 2 h at acidic pH. However, particles with lower CS content displayed good swelling/release properties and can find application in oral peptide delivery applications. ELISA results showed that biological activity of loaded insulin was maintained at neutral pH inside the particles.

From preliminary studies, it seems PMAA-CS microparticles, prepared by CS-based "ionic gelation process," can find application in oral peptide delivery.

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