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Cyclodextrin–insulin complex encapsulated polymethacrylic acid based nanoparticles for oral insulin delivery

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Abstract

Present investigation was aimed at developing an oral insulin delivery system based on hydroxypropyl β cyclodextrin–insulin (HPβCD–I) complex encapsulated polymethacrylic acid-chitosan-polyether (polyethylene glycol-polypropylene glycol copolymer) (PMCP) nanoparticles. Nanoparticles were prepared by the free radical polymerization of methacrylic acid in presence of chitosan and polyether in a solvent/surfactant free medium. Dynamic light scattering (DLS) experiment was conducted with particles dispersed in phosphate buffer (pH 7.4) and size distribution curve was observed in the range of 500–800 nm. HPβCD was used to prepare non-covalent inclusion complex with insulin and complex was analyzed by Fourier transform infrared (FTIR) and fluorescence spectroscopic studies. HPβCD complexed insulin was encapsulated into PMCP nanoparticles by diffusion filling method and their *in vitro* release profile was evaluated at acidic/alkaline pH. PMCP nanoparticles displayed good insulin encapsulation efficiency and release profile was largely dependent on the pH of the medium. Enzyme linked immuno sorbent assay (ELISA) study demonstrated that insulin encapsulated inside the particles was biologically active. Trypsin inhibitory effect of PMCP nanoparticles was evaluated using *N*-α-benzoyl-L-arginine ethyl ester (BAEE) and casein as substrates. Mucoadhesive studies of PMCP nanoparticles were conducted using freshly excised rat intestinal mucosa and the particles were found fairly adhesive. From the preliminary studies, cyclodextrin complexed insulin encapsulated mucoadhesive nanoparticles appear to be a good candidate for oral insulin delivery.

Keywords: Insulin; Polymethacrylic acid; Chitosan; Nanoparticles; Cyclodextrin; Mucoadhesion

1. Introduction

Insulin is the most effective and durable drug in the treatment of advanced-stage diabetes. Despite of significant advancement in the field of pharmaceutical research, development of a proper non-invasive insulin delivery system remains a major challenge (Cefalu, 2004; Lin et al., 2004). Oral route represents the most convenient way of drug administration possibly due high patient compliance and comfort. Moreover an oral insulin delivery system is expected to follow the physiological route of insulin secretion (Lewis et al., 1996). Orally administered insulin undergoes a first hepatic pass, and will produce a similar effect as pancreas-secreted insulin by inhibiting the hepatic gluconeogenesis and suppressing the hepatic glucose production. Oral delivery of peptides/proteins is restricted mainly due to their susceptibility to proteolysis and inability to traverse across biological barriers (Woodley, 1994; Lee and Yamamoto, 1990). Various approaches have been adopted to overcome the inherent barriers for oral insulin, including chemical modification of insulin and co-administration of adjuvants either in the form of absorption enhancers or protease inhibitors (Hinds and Kim, 2002; Myers et al., 1997; Anderberg et al., 1992; Bernkop-Schnurch, 1998).

Among the promising approaches towards developing oral insulin delivery systems, use of polymeric nano/microparticles seems to be an effective strategy. Polyacrylic acid (PAA), chitosan based hydrogels are promising excipients for oral peptide delivery, as they are capable of opening epithelial tight junctions which are mainly responsible for limited paracellular uptake of hydrophilic macromolecules (Luessen et al., 1997). Moreover PAA based systems can protect peptides from proteolytic degradation occurring in the GI tract (Luessen et al., 1995;

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Madsen and Peppas, 1999). Binding of divalent cations such as zinc, calcium is reported to be major reason for inhibitory and permeation enhancing properties of PAA based systems. Further these materials can adhere onto the intestinal membrane and helps in enhancing the residence time of dosage forms. Hydrogel particles composed of PAA based systems have the ability to protect peptides from gastric degradation by virtue of their pH dependent release mechanism (Lowman and Peppas, 1999).

The tendency for insulin molecules to aggregate and form fibrils remains a fundamental obstacle in the long-term therapeutic systems. Protein aggregation happens mainly by the interaction of hydrophobic residues in the protein molecules and is often accompanied by drastic reduction of biological potency creating serious problems in formulating drug delivery systems (Sluzky et al., 1991). Cyclodextrin (CD) complexation represents a unique and effective strategy for improving the protein therapy by stabilizing them against aggregation, thermal denaturation and degradation. β cyclodextrin (βCD) forms of non-covalent inclusion complex with a wide variety of drugs/proteins and complexation often alters the physicochemical and biological properties of guest molecules (Stella and Rajewski, 1997). Hydrophilic βCD inhibits the adsorption of insulin to hydrophobic surfaces and prevents self-aggregating nature of insulin at neutral pH (Lovatt et al., 1996). Proteins are mostly hydrophilic and too bulky to be wholly included into a βCD cavity. Hydrophobic side chains in the peptides penetrate into the βCD cavity leading to the formation non-covalent inclusion complexes and cyclodextrins' ability to sequester hydrophobic moieties helps in improving the stability of proteins (Irie and Uekama, 1999). CD complexation perturbs the membrane fluidity to lower the barrier function and this may enhance the absorption of drugs across the biological barriers (Uekama, 2004). However clinical exploitation of cyclodextrins based systems has been restricted mainly due to safety concerns. Oral administration of βCD based systems raises minimal safety concerns since they are poorly absorbed from GI tract. βCD complexed insulin encapsulated mucoadhesive microparticles seems to be a promising system for improving oral insulin delivery (Victor and Sharma, 2004; Moses et al., 2000). We have demonstrated insulin absorption from GI tract by radioimmunoassay (RIA) following an oral administration of βCD–insulin complex encapsulated alginate/chitosan matrix (Jerry et al., 2001).

Presently HPβCD complexed insulin encapsulated mucoadhesive nanoparticles were developed for oral insulin delivery. Particles were prepared by an inter-ionic gelation method in aqueous medium by free radical polymerization of methacrylic acid in presence of chitosan. Average size of particles dispersed in phosphate buffer (pH 7.4) was determined by DLS study. HPβCD–insulin complex prepared was analyzed by FTIR and fluorescence emission studies. Drug loading onto these nanoparticles was achieved by diffusion filling method and loaded particles were subjected to *in vitro* release study at pH 1.2 and 7.4. Biological activity of entrapped insulin was measured using ELISA technique. Further mucoadhesivity and trypsin inhibitory effect of the particles were evaluated in order to establish their efficiency towards oral protein delivery.

2. Materials and methods

2.1. Materials

Methacrylic acid (MAA) was obtained from Merck-Schuchardt (Germany) and was purified by passing through inhibitor-removing column supplied by Aldrich (Milwaukee, USA). Ethylene glycol dimethacrylate (EDMA) and polyethylene glycol–polypropylene glycol–polyethylene glycol copolymer (polyether) were obtained from Aldrich (Milwaukee, USA). Chitosan with approximate molecular weight 270,000 and 85% deacetylated was graciously provided by Central Institute of Fisheries Technology (CIFT, India). 2-Hydroxypropyl β cyclodextrin (average degree of substitution: 0.67 hydroxypropyl groups per glucose unit, average molecular weight: 1396), Trypsin type III from bovine pancreas (11,100 U/mg), *N*-α-benzoyl-L-arginine ethyl ester (BAEE), casein sodium salt, 2-[*N*-morpholino] ethane sulfonic acid (MES) were from Sigma (St. Louis, MO, USA). Human insulin (recombinant DNA origin) 100 IU/ml was purchased from Elli Lilly (India) and human insulin ELISA kit was from Mercodia (Sweden). All other chemicals were extrapure reagent grade chemicals and were used as received.

2.2. Preparation of PMCP nanoparticles

PMCP nanoparticles were prepared by modified inter-ionic gelation process reported previously (Sajeesh and Sharma, 2006a). MAA (inhibitor free) was mixed with EDMA under room temperature, chitosan and polyether were introduced separately into this reaction medium. Double distilled water was used for dilution and the solution was stirred with a magnetic stirrer for 15 min in a round bottom flask at 60 °C. Polymerization was initiated by the addition of potassium persulfate. The reaction medium was maintained at 50–60 °C for 6 h and resulting suspension was allowed to settle overnight. Supernatant liquid was removed carefully and particles obtained were washed with double distilled water to remove the unreacted monomers.

In a typical experiment, 3 g MAA was mixed with 0.6 g EDMA, 0.1 g chitosan and 0.2 g polyether in a round bottom flask. Medium was diluted by the addition of 300 ml double distilled water and potassium persulfate was added to this reaction mixture to initialize polymerization. Particles formed were allowed to settle overnight and recovered particles were dried.

2.3. Particle size measurement by dynamic light scattering (DLS)

The mean size and distribution of nanoparticles were measured with a Malvern series dynamic light scattering instrument (Zetasize HAS, Malvern Instruments, UK). Dried particles were dispersed in phosphate buffer (pH 7.4) and were subjected to particle size analysis.

2.4. Preparation and characterization of HPβCD–insulin (HPβCD–I) complex

2.4.1. Preparation of HPβCD–I complex

HPβCD–I complex was prepared by mixing 100 mg of HPβCD with 200 IU insulin solution. Complex was stirred for 30 min at room temperature and was allowed to remain for another hour. Resultant complex formed was lyophilized (FreezeZone 4.5, Labconco) to obtain solid HPβCD–insulin complex.

2.4.2. FTIR studies

Infrared absorption spectra of HPβCD and HPβCD–I were obtained using a NICOLET 5700 FTIR (Thermo Electron Corporation) spectrophotometer. The samples were pressed with KBr into a pellet before measuring the infrared absorption spectra.

2.4.3. Fluorescence spectroscopic investigation of inclusion complexation

Fluorescence spectroscopic analysis of HPβCD–I inclusion complexes was done using Varian Cary Eclipse spectrophotometer. Insulin solution for the study (1 IU/ml) was prepared by diluting insulin solution (100 IU/ml) with phosphate buffer (pH 7.0) and 3 ml of this solution was used for the assay. Fluorescence emission spectra were recorded (290–350 nm) at an excitation wavelength of 280 nm. Fluorescence emission intensity of insulin solution was measured and increasing concentration of solid HPβCD (1, 3, 5, 10 mg) were added to the same insulin solution. Relative fluorescence emission intensity of insulin and complexed insulin was measured under similar conditions.

2.5. Insulin loading and release studies

2.5.1. Insulin loading by diffusion filling method

Insulin solution (100 IU/ml) was used for loading and release studies. A known amount of dried nanoparticles was kept in remote loading medium consisting of HPβCD complexed insulin (F1) and uncomplexed insulin (F2). After 6 h, nanoparticles were taken out and excess insulin solution was gently wiped off. Loaded nanoparticles were kept for drying at low temperature (2–4 °C).

2.5.2. Insulin encapsulation efficiency of PMCP particles

Insulin content inside the PMCP nanoparticles was determined by suspending 100 mg of sample in 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's method (Lowry et al., 1951). Absorbance was measured using Shimadzu (UV-160 A) UV/visible spectrophotometer at 750 nm. All experiments were carried out at room temperature (30 ± 3 °C) in triplicate.

Encapsulation efficiency of PMCP nanoparticles was calculated as per equation

$$\mathrm{EE} = \frac{\text{total amount of insulin} - \text{free amount of insulin}}{\text{total amount of insulin}} \times 100$$

2.5.3. In vitro insulin release study

Nanoparticles loaded with insulin were dried and weighed. Known amount of drug-loaded nanoparticles was suspended in 20 ml of buffer solutions of pH 1.2 and 7.4. At specified interval of time 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.

2.5.4. Biological activity evaluation of encapsulated insulin by ELISA

Biological activity of encapsulated insulin (F1 and F2) was assed using ELISA technique. Insulin loaded nanoparticles (100 mg) was suspended in phosphate buffer (pH 7.4) for 12 h. An aliquot of sample was withdrawn from the solution and insulin content was analyzed by ELISA as per standard protocol. Results were obtained by reading the optical density at 450 nm using micro plate reader (Finstruments Microplate Reader).

2.6. Trypsin inhibitory effect of PMCP nanoparticles

2.6.1. Using low molecular weight substrate (BAEE)

Trypsin inhibitory assay of PMCP particles with BAEE as substrate was performed using a biochemically optimized procedure reported elsewhere (Ameye et al., 2000). Nanoparticles were dispersed in 50 mM MES/KOH buffer (pH 6.8) to give a final concentration of 0.25%, 0.5%, 1% (w/v) solutions and 1 ml of the dispersion was used for the assay. BAEE (20 mM) was dissolved in 0.067 M phosphate buffer (pH 7.6) and 3 ml of the solution was used for study. BAEE and the polymer dispersion were mixed well and 1 ml 30 U/ml trypsin solution was added to this solution. The resultant mixture was incubated at 37 °C for 10 min. After stopping the enzymatic reaction by the addition of 1 ml 5% tricholoro acetic acid solution, 0.2 ml of sample was withdrawn and metabolite benzoyl arginine (BA) formed was analyzed by UV absorbance at 253 nm.

2.6.2. Using high molecular weight substrate (casein)

Trypsin inhibitory of PMCP particles with casein substrate was performed as follows. Trypsin solution with 500 μg/ml concentration was prepared and 0.2 ml of this solution was used for the assay. Enzyme solution was incubated with 1 ml polymer dispersion (0.25%, 0.5%, and 1%) at room temperature for 10 min and 2 ml casein solution (2%) was added. Medium was incubated for 30 min at 37 °C. The enzymatic reaction was stopped by addition of 4 ml trichloro acetic acid solution (10%). The resultant suspension was centrifuged at 10,000 rpm for 10 min and 0.2 ml of clear aliquot was taken out and protein content was analyzed by Lowry method.

Control experiments in both studies were performed in an exactly similar method avoiding use of polymer solutions. The activity of non-inhibited trypsin was taken as 100% and the activity of partially incubated trypsin was expressed relative to 100% activity calculated comparing the absorption of reaction mixtures.

2.7. Mucoadhesion studies

Mucoadhesion studies were performed on freshly excised rat intestinal mucosa according to a method described previously (Rao and Buri, 1989). Male Wistar rat (200–250 g) was sacrified by overdose anesthesia and small intestine was removed from the animal. Intestine was flushed with saline to remove lumenal contents and was carefully cut opened. Tissue was then placed in a polyethylene support with help of cyanoacrylate adhesive and nanoparticles were uniformly spread on the mucosal surface. Nanoparticles were allowed to interact with mucus for 10 min and then were mounted on a platform at an angle 45° under a constant flow rate (20 ml/min) of phosphate buffer (pH 7.4). Percentage of nanoparticles adhered on the intestine was calculated by comparing weight of particles adhered to weight of particles applied.

In another set of experiment PMCP nanoparticles were given orally to rats using oral feeding tube. After specified intervals (2 and 3 h) rats were sacrificed and small intestine was removed from the body. Intestinal portion was carefully opened and presence of particles was examined visually.

3. Results and discussion

In present study nanoparticles were prepared by novel inter-ionic gelation method in aqueous medium and particles were obtained spontaneously during the process. Recently polymethacrylic acid–chitosan microparticles were obtained by this technique, avoiding use of any organic solvents, surfactants and steric stabilizers (Sajeesh and Sharma, 2006a,b). Ionic interaction between amino groups in chitosan and acid groups of polyacrylic acid/polymethacrylic acid can lead to the formation of inter-ionic polymer complexes (Ahn et al., 2001). Polyionic hydrogels prepared by ionic gelation process have the definite advantage of creating an ionic environment that can favour the stabilization of bioactive agents. However most of the polyionic hydrogels lacks stability especially at extreme pH conditions (Hu et al., 2002). CS may take a randomly coiled conformation at alkaline/neutral pH, because of their unionized amino groups (Berger et al., 2004). At lower pH region CS acquires a net positive due to the protonation of amino groups and swelling can occur as a result of electrostatic repulsion of charged ionic groups. Such hydrogels displays extremely poor protein retention at gastric pH. Intermixing of cationic–anionic polymers leads to strong interpolymer complex formation and this can restrict the intrinsic mobility of individual polymer chains. Also acidic/alkaline functional groups may get involved in the formation of strong inter-ionic complexes and this may mask their efficiency towards mucoadhesive drug delivery system (Bernkop-Schnurch, 2002). So in present investigation role of CS in the matrix was restricted to serve as counter ion to facilitate ionic interaction between anionic–cationic polymers and the concentration of component polymers were optimized to make the material appropriate for oral protein delivery.

Block copolymers with basic poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) structure seems to be a promising material for biomedical application. Repeating hydrophilic-hydrophobic segments renders the material a surfactant property and their thermo-responsive behavior attracted wide interest in drug delivery. Recent reports suggest that these non-toxic materials can improve the permeability of epithelial tissues and can promote transport of hydrophilic materials across the biological membranes (Batrakova et al., 1998). Polyethers form inter-polymer complex with polyacids such as polyacrylic acid through cooperative hydrogen bonding between ether group and carboxylic acids (Lowman et al., 2000). Carboxylic acid group in polyacrylic acid can act as good proton donor, while ether groups may serve the purpose of a proton acceptor. A combination of polymethacrylic acid and polyether could be beneficial in generating mucoadhesive nanoparticles with better physico-chemical properties (Bromberg et al., 2004).

Typical dynamic light scattering data of PMCP particles dispersed in phosphate buffer is presented in Fig. 1. Particle size distribution was unimodal and mean diameter was found in the range of 500–800 nm. Size of drug carriers is considered to be a major parameter in determining the efficiency of particulate drug delivery systems. Nanoparticles are preferred over microparticles because smaller particle size may allow a better retention of drug delivery system in the GI tract and might provide higher local drug concentration. As compared micron sized particles nanoparticles have higher surface area that can lead to higher drug loading. They can maintain a more intimate contact with the biological tissues (Peppas and Huang, 2004). PMCP particles displayed good dispersion capacity in phosphate buffer pH 7.4 and observed size of the particles seems to be most appropriate for a hydrophilic oral insulin delivery system.

Ability cyclodextrin (CD) derivatives to form inclusion complexes with variety of peptides/drugs are well studied. Complexation enhances the stability of peptide formulation and improves their shelf-time. Unlike chemical modifications, no covalent bond is formed or broken during the CD complexation process (Uekama, 2004; Irie and Uekama, 1999). CD complexation happens spontaneously and the main driving force is the release of enthalpy rich water molecules from the CD cavity. CD complexation favors the stabilization of proteins without tampering the biological activity of native proteins and this method seems to

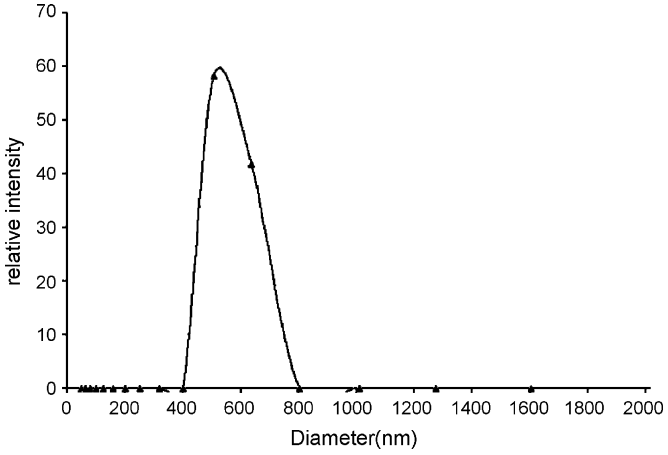

Fig. 1. Size distribution curve of PMCP particles at pH 7.4.

be a promising approach in the development of advanced drug delivery systems.

Numerous spectral techniques have been employed to analyze HPβCD-drug complexes. FTIR spectrum of HPβCD and HPβCD–I are given in Fig. 2 a and b. FTIR is a useful technique to confirm the formation of cyclodextrin-drug inclusion complexes. Insulin showed typical absorption peak at 1654 and 1541 cm^{-1} corresponding to amides I and II (spectra not shown). In case of pure HPβCD wide absorption band was observed in the range of 3349 cm^{-1} due to presence of OH group. Typical absorption peak was observed at 1643 cm^{-1} possibly due to the presence of hydroxyl groups part of an aromatic system. In case of freeze dried HPβCD–I complex this was observed in range of 1650 cm^{-1} and an additional peak was observed at 1537 cm^{-1} which could be due to the complex formation.

Further conclusive evidence of complex formation was observed during fluorescence studies. Relative fluorescence emission intensity of insulin with and without HPβCD is given in Fig. 3. Results from fluorescence spectroscopy studies clearly suggest that addition of HPβCD to insulin has enhanced the fluorescence emission intensity of insulin, suggesting an interaction between these compounds. As the concentration of HPβCD was increased, fluorescence intensity of insulin solution was enhanced significantly. A likely model for the interaction between HPβCD and insulin involves encapsulation of surface positional tyrosine with the cyclodextrin. The resulting exclusion of water from the immediate tyrosine neighborhood leads to the observed increase in fluorescence yield (Aachmann et al., 2003). These studies confirm the complex formation of HPβCD with insulin molecules.

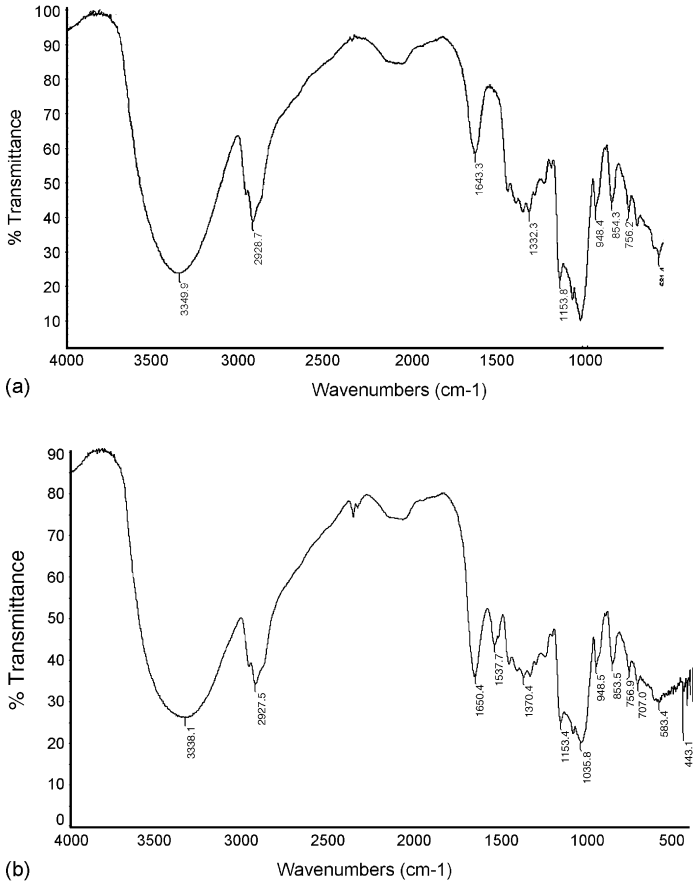

Fig. 2. FTIR spectra of HPβCD (a) and HPβCD–I complex (b).

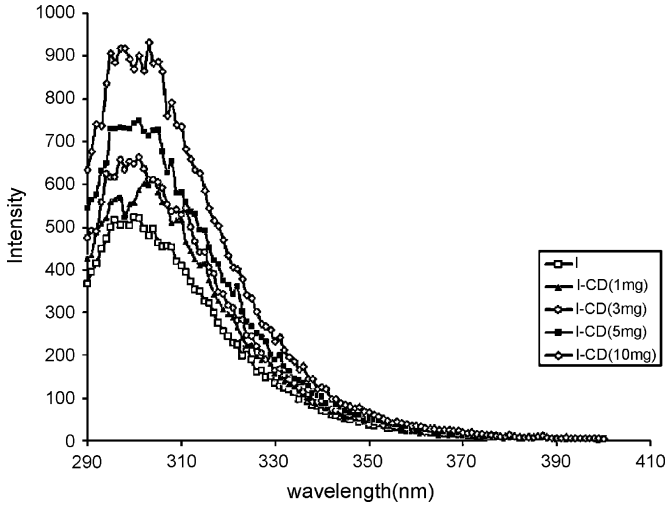

Fig. 3. Fluorescence emission spectra of I and HPβCD complexed I.

Drug loading onto these nanoparticles was done by diffusion filling method (remote loading) in which crosslinked particles were exposed to protein solutions, and drug diffuses in mainly by concentration gradient. As well established hydrogel with carboxylic acid group swells in weakly alkaline/neutral pH and by virtue of their pH sensitivity, drug diffuses into the matrix quite rapidly. A major advantage of this encapsulation process is that drug loading can be achieved without the aid of organic solvents or other harmful treatment. By this technique around 80% encapsulation efficiency was achieved with complexed and uncomplexed insulin formulations (Table 1). Cyclodextrins and their hydrophilic derivatives are known to enhance the loading efficiency of liposomes and polymeric nano/microparticles (Duchêne et al., 1999). However in present study significant variation was not observed in encapsulation efficiency with complexed and uncomplexed insulin formulations.

In vitro release profile of insulin from complexed and uncomplexed insulin (F1 and F2) encapsulated nanoparticles are given in Fig. 4. As expected pH dependent release kinetics was observed from these nanoparticles, possibly due to the ionic nature of materials used. Polymethacrylic acid being the major component in the system, rapid release is expected at alkaline/neutral conditions and more controlled release is expected at acidic pH. At low pH swelling of matrix is prevented possibly due to the existence of non-ionized carboxylic acid groups in the network and this protects encapsulated drugs from gastric conditions. On the other hand, drastic swelling occurs at high pH by the ionization of acid groups and this causes encapsulated

Table 1
Insulin encapsulation efficiency of PMCP nanoparticles

Formulations	Loading time (h)	Encapsulation efficiency
Fl	6	86 ± 2
F2	6	85 ± 2

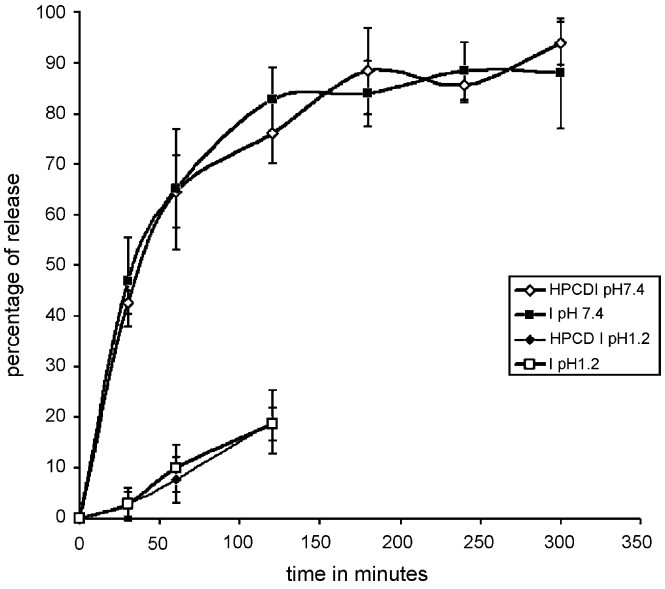

Fig. 4. *In vitro* release study of insulin from HPβCD–I (F1) and I (F2) encapsulated PMCP nanoparticles ($n = 3$).

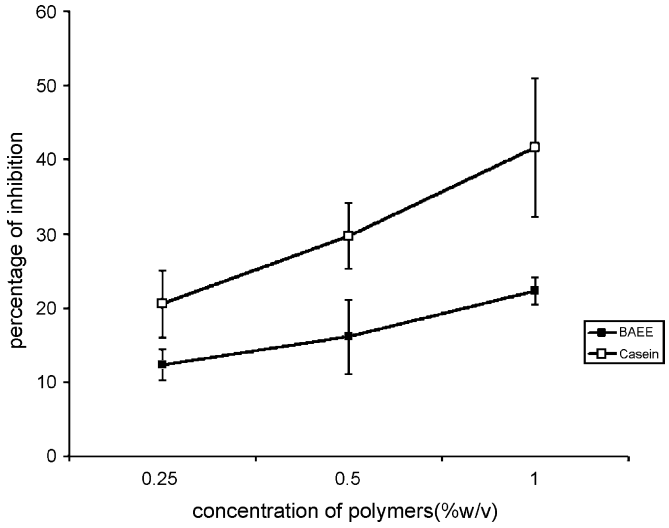

Fig. 5. Trypsin inhibitory effect of PMCP nanoparticles with BAEE and casein substrates ($n = 3$).

drug to leach out quickly from the matrix. In the process these hydrogels protects peptides from harmful gastric environment and releases in the small intestinal region where they have a chance to act. Release of insulin was completed in almost 3 h at neutral pH and around 20% of total insulin loaded was released at pH 1.2 in the first 2 h of study.

As observed from the release kinetics change in release properties was not observed with complexed and uncomplexed insulin under *in vitro* conditions. Release mechanism of guest molecules from cyclodextrin complexes is not fully understood yet. When placed in water the complex dissolves and this causes dissociation of cyclodextrin–drug complex. However release of drug from cyclodextrin based system under *in vivo* conditions may vary significantly, competitive displacement of drugs from their cyclodextrin complexes probably plays a vital role under *in vivo* (Stella et al., 1999). Cyclodextrin preferably binds relatively hydrophobic moieties rather hydrophilic and this may lead to the displacement of relatively hydrophilic guest molecules from cyclodextrin cavity. Competitive binding of hydrophobic residues under *in vivo* conditions is believed to play a vital role in altering absorption of drugs through mucosal membranes.

Biological activity of encapsulated insulin was evaluated using ELISA studies. ELISA studies proved that encapsulated insulin retained biological activity. 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 insulin assay measures biologically active insulin with a high degree of specificity, using a pair of mouse monoclonal antibodies. The full biological activity of proteins is dependent on preserving the integrity of its three dimensional structure. ELISA results suggest that these nanoparticles are capable of preserving biological activity of encapsulated insulin.

Trypsin inhibitory effect of PMCP nanoparticles is given in Fig. 5. We have attempted to evaluate the inhibitory effect of PMCP particles using BAEE as a low molecular substrate and casein as a high molecular weight substrate. As evident from these studies particles displayed a weak inhibitory effect against trypsin at neutral pH. Inhibitory effect was more pronounced with casein substrate rather with BAEE and concentration of polymer dispersion used also affected the inhibition process. Binding of divalent cations such as calcium, zinc, etc. is proposed to major reason for inhibitory effect of these polymers. Carboxylic acid groups can bind calcium from enzyme structure and this may cause thermodynamic instability to the enzymes. Calcium binding ability of PEG grafted PMAA hydrogels was directly co-related with the trypsin inhibition capability (Madsen and Peppas, 1999). Non-specific interactions such as Vanderwaals and electrostatic interaction of polymer with enzymes are also reported to be a cause for inhibition process (Luessen et al., 1995). Steric factors may also influence the inhibition process to a certain extend. Mucoadhesive polymeric particles with weak inhibitory effect may reduce the proteolytic attack in the small intestine and can localize the delivery systems onto the intestinal wall without exposing the active ingredient directly to the intestinal fluids.

Photographs demonstrating mucoadhesivity of PMCP particles on rat intestine are given in Figs. 6–8. Mucoadhesive studies conducted with isolated rat intestine clearly indicated the adhesive nature of these nanoparticles. Several theories are proposed to explain the phenomenon of mucoadhesion and theories varies with change in polymer structure. Non-covalent interactions (hydrogen bonding, vanderwaals forces, etc.) of carboxylic acid groups with hydroxyl groups of mucus membrane is proposed to be the major reason for mucoadhesion of PAA based systems (Dodou et al., 2005). However a proper *in vitro* model for evaluating the mucoadhesive properties of polymeric nanoparticles (with size in lower micrometer/nanometer range) is still lacking. By present method we observed that morethan $84 \pm 3\%$ ($n = 3$) of applied nanoparticles was retained in the intestine. Visually it was observed that PMCP nanoparticles were retained in the intestine even after continuous washing with buffer (Fig. 6). In

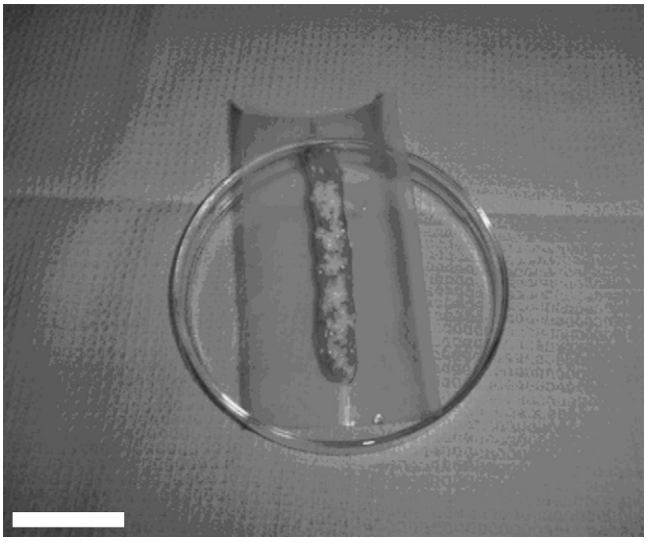

Fig. 6. Photograph demonstrating mucoadhesive property of PMCP nanoparticles in rat intestine at pH 7.4 (picture was taken after 20 min washing with phosphate buffer).

Fig. 7. Photograph demonstrating the adherence of PMCP nanoparticles onto rat small intestine following oral administration (picture was taken after 2 h).

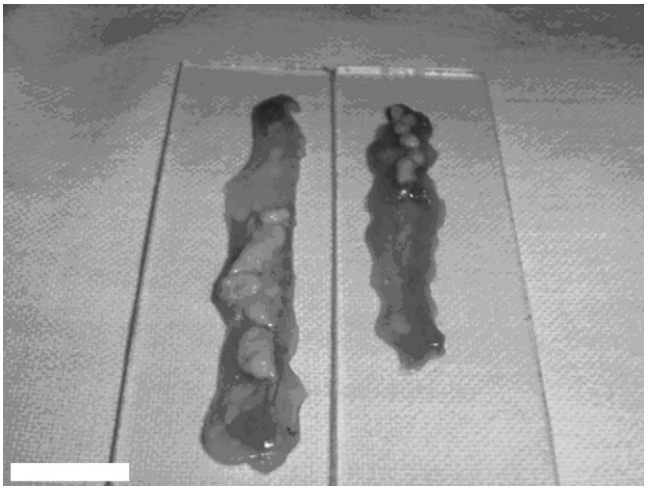

Fig. 8. Photograph demonstrating the adherence of PMCP nanoparticles onto rat small intestine following oral administration (picture was taken after 3 h).

other set of experiments presence of orally delivered nanoparticles inside the intestine was examined. It was observed that orally administrated nanoparticles were retained inside the intestine after 2–3 h (Figs. 7 and 8). These particles were found aggregated inside and appeared largely on ileum portion of small intestine. Preliminary mucoadhesive studies suggest that these nanoparticles may be useful in oral drug delivery.

4. Conclusion

Present investigation explored the possibility of formulating an oral insulin delivery system by combining the advantages of βCD complexation and nanoparticulate mucoadhesive delivery systems. HPβCD complexation was expected to improve the stability and absorption of insulin formulations, while mucoadhesive nanoparticles based on polymethacrylic acid was intended for achieving higher residence time and to reduce proteolytic degradation. Polymethacrylic acid based nanoparticles prepared by novel inter-ionic gelation technique displayed good stability and dispersion in phosphate buffer. Particles dispersed in phosphate buffer displayed a size range of 500–800 nm. FTIR and fluorescence spectroscopic investigations clearly indicated HPβCD–insulin complex formation. Nanoparticles displayed good insulin encapsulation efficiency and pH dependent release profile was observed at acidic/alkaline conditions. ELISA study confirmed that encapsulated insulin maintained the biological activity. PMCP nanoparticles were found fairly adhesive on isolated rat intestine at neutral pH and displayed weak inhibitory effect against trypsin.

From the preliminary studies, hydroxypropyl β cyclodextrin complexed insulin encapsulated mucoadhesive polymethacrylic acid based nanoparticles seems to be a useful candidate for oral insulin delivery.

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