Synthesis and Characterization of Poly(HEMA-MAA) Hydrogel Carrier for Oral Delivery of Insulin

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ABSTRACT: Poly(HEMA-MAA) hydrogel particles were synthesized by redox free-radical polymerization using 2-hydroxyethylmethacrylate, different concentration of methacrylic acid as monomer, ethyleneglycol dimethacrylate as crosslinking agent, and APS/TEMED as free-radical initiator. Fourier transform infrared spectrum of poly(HEMA-MAA) hydrogels showed intense absorption peak of carbonyl group at $\sim$ 1700 cm^{-1} due to carboxylic acid groups of MAA, peak at $\sim$ 2960 cm^{-1} due to CH stretching and vinylic peak at 1700 cm^{-1} independent of MAA concentration. Highest swelling percentage 587% was observed in case of poly(HEMA-MAA) hydrogel synthesized using 30% of MAA while lowest swelling percentage 413% was observed in hydrogel synthesized 10% of MAA at basic pH (8.0). Scanning electron micrograph of copolymeric particles showed the irregular shape of poly(HEMA-MAA) particles with conglomeration with each due to ionization of carboxylic groups. Insulin was radiolabeled using *technetium-99m* radionuclide and the radiolabeling efficiency was found to be 99%. Poly(HEMA-MAA) hydrogel having 60% of MAA showed the highest insulin loading efficiency of 68% while lowest 37% was observed in case of 10% MAA hydrogel. Insulin release studies showed only 35–65% of insulin was released into the medium from particles at pH 2.5 in 60 min, while insulin release was significantly higher at pH 7.4. Hypoglycemic effect of the 60 and 80 I.U./kg insulin dose loaded in poly(HEMA-MAA) copolymeric particles were carried out in fasted diabetic rats and highest decrease in blood glucose level from 506 mg/dL to 170 mg/dL was observed within first 3 h. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 2004–2012, 2011

Key words: HEMA; drug delivery; insulin; diabetes; hydrogel

INTRODUCTION

Peptide drugs are usually indicated for chronic conditions, and the use of injections on a daily basis during long-term treatment has obvious drawbacks.[1] The gastrointestinal tract (GIT) is the route of choice for the administration of most drugs, regardless of their molecular structure or weight. It would be highly advantageous if insulin could be administered orally, because the oral delivery of insulin can mimic the physiological fate of insulin and may provide better glucose homeostasis. This would also lessen the incidence of peripheral hyperinsulinemia, which is associated with neuropathy, retinoendopathy, and so forth.[2,3] Almost since the initial discovery of insulin alternative effective routes other than subcutaneous injection have been an elusive goal for many investigators. The oral route is considered to be the most convenient and comfortable means for administration of insulin for less invasive and painless diabetes management, leading to a higher patient compliance.[4–7] Oral administration of insulin has some limitations, including low oral bioavailability due to degradation in the stomach, inactivation, and digestion by proteolytic enzymes in the luminal cavity, poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity.[5] Consequently, various approaches have been examined to overcome the delivery problems of these peptides when orally administered.[8] Polymeric carrier systems, such as liposomes,[9] microemulsions,[10] nanoparticles,[11] and microspheres,[12] have shown to improve the gastrointestinal absorption of peptide drugs. Also, hydrogels,[13] azopolymer coating,[14] site-specific drug delivery systems,[15] absorption enhancers,[16] enzyme inhibitors,[17] and modification of chemical structure have shown similar effects. Nevertheless, the intestinal epithelium is a major barrier to the absorption of hydrophilic drugs, as they cannot diffuse across epithelial cells through lipid-bilayer cell membranes to the bloodstream.[18] The transport of hydrophilic macromolecules via the paracellular pathway is, however, severely restricted by the presence of tight junctions, which are located at the luminal aspect of adjacent epithelial cells.[19,20] The crosslinked

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polymeric hydrogels based drug delivery carrier for the oral delivery of insulin showed the promising results because of their pH sensitive nature, high-loading efficiency, and biocompatiblity.[21]

In view of the above, this work is devoted toward the development of a pH sensitive copolymeric hydrogel formulation with high-insulin loading efficiency, sustained and efficient delivery of insulin in gastrointestinal tract. To achieve the desired properties, various poly(HEMA-MAA) copolymeric hydrogel carrier were synthesized by free-radical polymerization using different concentration of methacrylic acid (MAA) in the reaction mixture. Scanning electron microscope (SEM), Fourier transform infrared (FTIR), swelling studies, insulin loading, and release studies were used to characterize these poly(HEMA-MAA) hydrogel carrier. Insulin loading and *in vitro* release studies were carried out using radiolabeled (technittium-99m) at pH 2.5 and 7.4, and the samples were analyzed using dose calibrator. Insulin-loaded particles were also evaluated for hypoglycemic effect on diabetic rats.

EXPERIMENTAL

Materials

Hydrochloric acid, alloxan, ammonium persulfate (APS), and sodium hydroxide pellets were obtained from Central Drug House (Delhi, India). MAA, 2-hydroxyethyl methacrylate (HEMA), and ethylene glycol dimethacrylate (EGDMA) monomers were obtained from Merck Germany. *N*,*N*,*N*′,*N*′-Tetramethylethylene diamine (TEMED) was supplied by Ranbaxy Chemicals (Delhi, India). Monocomponent human insulin (*r-DNA* origin) of 100 I.U./mL concentration from Eli Lilly and company (USA) was used. All the chemicals were used as received.

Synthesis of poly(HEMA-MAA) polymeric hydrogel

Poly(HEMA-MAA) hydrogels were synthesized by redox free-radical polymerization using 0.6% APS, 0.8% of *N*,*N*,*N*′,*N*′-TEMED of monomer concentration as free-radical initiator. Different concentration of MAA (10–60% w/w) and 1% EGDMA as cross-linking agent were added before polymerization in the reaction mixture and poured in the thin-walled glass test tube and kept separately at 60°C for 2 h. After 2 h of reaction, hydrogels were taken out from the test tube (by breaking the test tube) and post-cured at 80°C for 2.5 h and washed repeatedly with deionized water to remove the unreacted monomers.

Poly(HEMA-MAA) copolymeric hydrogel thus synthesized were crushed thoroughly using Phillips grinder, Mumbai, India for 15 min at medium speed. The copolymeric hydrogel material thus obtained was further poured through the muslin cloth to get the uniform size hydrogel particles. The hydrogel particles thus obtained were used in further studies.

Fourier-transform infrared spectroscopy

Attenuated total reflectance-Fourier transform infrared (FTIR) spectroscopy of poly(HEMA-MAA) hydrogel particles synthesized using different percentage of MAA was recorded on a Perkin–Elmer one spectrometer. FTIR of all the samples were recorded in the transmittance mode.

Swelling studies of poly(HEMA-MAA) copolymeric hydrogel

The swelling characteristics of poly(HEMA-MAA) hydrogel synthesized using various amount of MAA were determined by immersing dried test samples in different pH (at pH 8.0 and 1.2) solution at 37°C separately. At specific time intervals, samples were removed from the swelling medium and blotted with a piece of paper for 5 s to absorb excess water on surface. The swelling percentage (Q_s) of the test samples were calculated from the following equation:

$$Q_s = \frac{W_s - W_d}{W_d} \times 100$$

where W_s is the weight of the swollen test sample, and W_d is the weight of the dried test sample.

Scanning electron microscopy

Surface morphological studies of various copolymeric hydrogel particles of poly(HEMA-MAA) were carried out at pH 7.4 using SEM (Leo, VP-435, UK). Small volumes (50–100 mg) of copolymeric particles were suspended separately in buffer solutions of pH 7.4 for 6 h and then dried on a double-sided adhesive tape. The dried samples were sputter coated with gold particles under reduced pressure conditions and observed under SEM at constant 15 kV accelerating voltage.

Optical microscopy

Surface morphological studies of poly(HEMA-MAA) polymeric microparticles synthesized using different concentration (10, 30, and 50%) of MAA were carried out using optical microscope (Leica DC-350F Microsystems, Germany). Small amount (50–100 mg) of copolymeric particles were suspended separately in buffer solutions of pH 7.4 for 6 h and then

placed on the cover slip and observed under the microscope.

Radiolabeling of insulin by ^{99m}Tc-pertechnetate as radionuclide

Concentrated solution of insulin (100I.U./mL) was radiolabeled with *technetium-99m* (^{99m}Tc), which is a suitable radiotracer for analytical works because of its favorable physical properties. *Technetium-99m* has a short half-life of 6.02 h, easily eluted from the generator and cost effective. Insulin was radiolabeled with *technetium-99m* by the standard procedure developed by INMAS, Delhi. Briefly, 10 mL of insulin (100 I.U./mL) was mixed with 1 mL of 5 mg/mL concentration solution of stannous chloride in 0.1*N* HCl (pH of the solution was kept between 5 and 6). Fifty mille baqurel (MBq) of $Na^{99m}TcO_4$ was added to it, and the mixture was incubated for 15 min. Radiochemical purity of the radiocomplex was determined using instant thin layer chromatography (ITLC).

The pH of the radiolabeled insulin solution (concentration 100 I.U./mL) was adjusted to 7.4 by adding 1*N* NaOH solution using micropipette and 0.02% (v/v) of Tween 20 was added to ensure that insulin did not adsorb on the glass surface.[22] One gram each of copolymeric poly(HEMA-MAA) hydrogel particles was placed separately in 5 mL of insulin solution at 37°C for 5 h at pH 7.4 to allow maximum loading and then pH of the insulin solution was lowered gradually to 2.5 by adding 1*N* HCl, to trap the insulin inside the hydrogel particles.[23] Insulin-loaded copolymeric hydrogel particles was filtered and stored at 4°C for further studies. Loading efficiency of insulin-loaded copolymeric hydrogel particles were measured by the activity measurement using Dose calibrator (CAPINTEC CRC® 15®, USA). The insulin loading efficiency percentage was calculated by the following equation:

$$\text{Loading efficiency percentage} = \frac{M_{\text{bound}}}{M_{\text{theoretical}}} \times 100$$

where M_{bound} is the amount of insulin (mg) eluted from the particles (bound insulin) and $M_{\text{theoretical}}$ is the theoretical loading amount of insulin (mg) originally added in the reaction mixture. Conversion of I.U. of insulin into milligrams was carried out by using the International standard (1 I.U. = 45.5 μg).

In vitro release studies of insulin from poly(HEMA-MAA) hydrogel particles

In vitro insulin release studies were performed by placing 1 g of various poly(HEMA-MAA) insulin-loaded copolymeric hydrogel particles (synthesized using 10–60% MAA in reaction mixture) separately in 10 mL of pH 2.5 citrate-phosphate buffer solutions at 37°C and the mixture was stirred at 50 rpm using magnetic stirrer. At every 15-min time interval, insulin-loaded copolymeric hydrogel particles were filtered using Whattman filter paper No1 and re-suspended in fresh 10 mL of pH 2.5 buffer and release studies were continued till 90 min. After 90 min, insulin release from the hydrogel particles were carried out with fresh 7.4 pH phosphate buffer solution and samples were collected again separately at 15-min time interval in the same manner till 165 min. *In vitro* release studies from different poly (HEMA-MAA) insulin-loaded copolymeric hydrogel (synthesized using different concentration of MAA) particles were carried out separately at different time and day. Each filtered sample was analyzed by radioactive Dose Calibrator (CAPINTEC CRC® 15®, USA) and the amount of insulin released was calculated. The studies were performed to mimic the GIT conditions. Cumulative release of insulin was calculated by adding the release of insulin at pH 2.5 and 7.4.

In vivo insulin release from poly(HEMA-MAA) hydrogel particles

Sprague-Dawley rats weighing 280–330 g (15–16 weeks old) were provided by experimental animal facility of INMAS, Delhi. The study protocol was reviewed and approved by Institutional Animal Ethics Committee.

In vivo insulin release studies on rats

Diabetes was induced in the experimental animals by injecting single dose of alloxan (140 mg/kg body weight) dissolved in saline water (0.9% NaCl solution in distilled water) intraperitonially and their blood glucose levels were checked for diabetic conditions after 48 h. Animals with blood glucose level $\geq$ 300 mg/dL were only used for further studies.[24] Twenty-four diabetic rats were selected randomly and divided into four groups and each with six rats was housed in one cage. Insulin-loaded poly (HEMA-MAA) hydrogel particles synthesized using 30 and 40% of MAA were used for *in vivo* efficacy evaluation. Insulin-loaded copolymeric hydrogel particles were filled in gelatin capsules and administered orally down to the esophagus using forcep. Animal studies were carried out in two phases.

Evaluation of insulin-loaded poly(HEMA-MAA) hydrogel particles on fasted-diabetic rats

In first phase, diabetic animals were fasted overnight prior to oral administration of insulin-loaded hydrogel particles. Animals of first group were taken as

TABLE I
Synthesis of Polymeric Hydrogel Particles Using APS (0.8% of Monomer Conc.)/TEMED (0.6% of Monomer Conc.), 1% of EGDMA as Crosslinker and Variable Amount of MAA (10–60% of Monomer Concentration) at 60°C

HEMA (g)	MAA (% age)	Physical properties of the hydrogel
8.9	10 (1 mL)	Bubble free, with good mechanical properties
7.9	20 (2 mL)	Light yellow, bubble free, optimal strength
6.9	30 (3 mL)	Yellow, large number of bubble, brittle in nature
5.9	40 (4 mL)	Yellow, large number of bubble, brittle in nature
4.9	50 (5 mL)	Yellow, large number of bubble, brittle in nature
3.9	60 (6 mL)	Dark yellow, large size bubble, very hard in nature

control and fed with poly(HEMA-MAA) hydrogel particles (synthesized using 30% of MAA) without insulin loading. In second group animals were fed with insulin-loaded (60 I.U./kg dose) poly(HEMA-MAA) hydrogel particles (synthesized using 30% of MAA). Third group of animals were fed with insulin loaded (60 I.U./kg dose) with poly(HEMA-MAA) hydrogel particles (synthesized using 40% of MAA). While fourth group of animals were fed with insulin loaded (80 I.U./kg dose) with poly(HEMA-MAA) hydrogel particles (synthesized using 30% of MAA). The copolymeric hydrogel particles loaded with insulin dose given to second, third, and fourth group of rats were 60 and 80 I.U./kg animal body weight and their blood glucose level was checked at regular time interval up to 8 h.

Evaluation of insulin-loaded poly(HEMA-MAA) hydrogel particles on fed-diabetic rats

In second phase of experiments, animals were allowed to move freely and fed properly to simulate the natural conditions. Animals of first group were taken as control and fed with poly(HEMA-MAA) hydrogel particles (synthesized using 30% of MAA) without insulin loading. In second group, animals were fed with insulin loaded with poly(HEMA-MAA) hydrogel particles (synthesized using 30% of MAA). The copolymeric hydrogel particles loaded with insulin dose given to second group of rats was 60 I.U./kg animal body weight and their blood glucose level was checked at regular time interval up to 8 h.

Blood samples were collected from the tail vein of rats at specific time interval after administration of insulin-loaded hydrogel particles and blood glucose level was measured using Accutrend® blood glucometer (Roche, Germany).

RESULTS AND DISCUSSION

Synthesis of poly(HEMA-MAA) polymeric hydrogel

In view of synthesizing pH sensitive copolymeric hydrogel carrier MAA was added during polymerization reaction. Variable amount of MAA (10–60%) was added in reaction mixture during polymerization in addition to 2-hydroxyethylmethacrylate (HEMA). One percent of EGDMA was also added as crosslinking monomer as given in Table I. Hydrogel particles synthesized using 10% of MAA were found to be bubble free, with good mechanical properties. Hydrogel particles containing 20% of MAA content were yellowish in color, bubble free, with optimal mechanical strength. Poly(HEMA-MAA) hydrogel particles having 30, 40, and 50% of MAA content were yellow, small size bubble appear on the surface and brittle in nature. Poly(HEMA) hydrogel particles having 60% of MAA content were dark yellow, large size and number of bubble, brittle in nature and difficult to remove from the glass test tube. The copolymeric hydrogel were taken out from the thin glass test tube by using mechanical pressure. The hydrogel thus obtained were postcured at 80°C for 1 h in deionized water repeatedly to remove the unreacted monomers of HEMA, MAA, and EGDMA, if any.

FTIR studies of poly(HEMA-MAA)

FTIR spectrum of poly(HEMA-MAA) copolymeric particles containing different concentration of MAA are given in Figure 1. FTIR spectrum showed of poly(HEMA-MAA) hydrogel, showed intense absorption peak of carbonyl group at $\sim$ 1700 cm^{-1} due to carboxylic acid groups of MAA irrespective of MAA concentration as shown in Figure 1. Copolymeric poly(HEMA-MAA) hydrogel particles showed the intense absorption peak at $\sim$ 1700 cm^{-1} due to merging of carbonyl and vinyl peaks of monomers and peak at $\sim$ 2940 cm^{-1} due to CH stretching confirms the polymerization of the monomers. FTIR analysis of copolymeric hydrogel was carried out to confirm the copolymerization of HEMA and MAA to poly(HEMA-MAA) copolymer. FTIR spectrum of MAA monomer showed characteristic absorption peaks at $\sim$ 1635 cm^{-1} for vinylic groups and $\sim$ 1690 cm^{-1} for carbonyl group and a band from 3000 to 3450 cm^{-1} for OH group of carboxylic group. FTIR spectrum of HEMA monomer showed a band at 3428 cm^{-1} due to OH group, peak at 2957 cm^{-1} due to CH stretching, carbonyl peak at 1717 cm^{-1} and C—O—C stretching at 1146 cm^{-1}. FTIR spectrum of

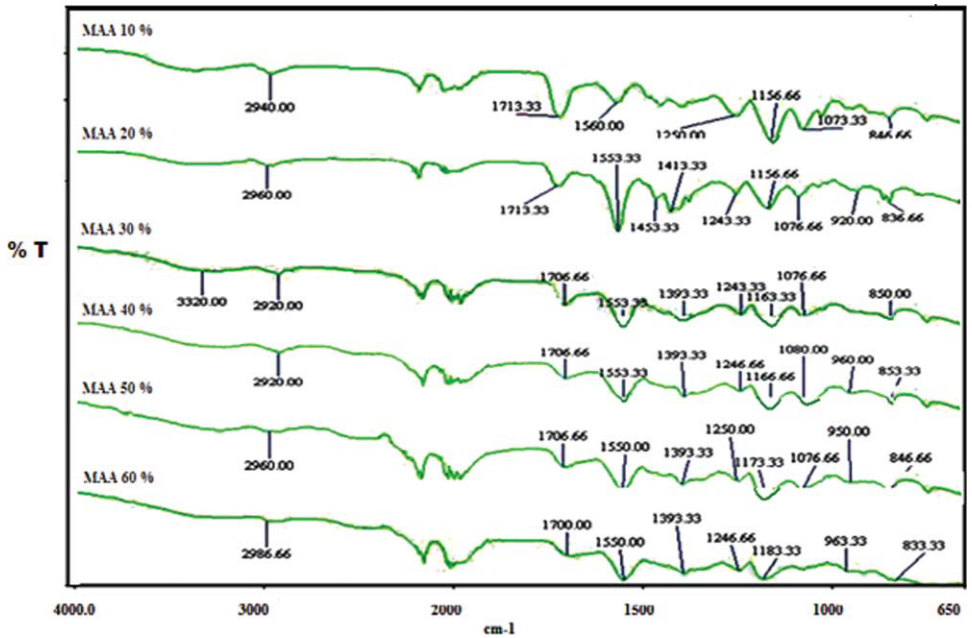

Figure 1 FTIR spectra of poly(HEMA-MAA) hydrogel synthesized using 10–60% of MAA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

EGDMA showed peaks at 1637 cm^{-1} for vinylic groups and 1717 cm^{-1} for carbonyl group.

Swelling studies of poly(HEMA-MAA) hydrogel

Swelling studies of poly(HEMA-MAA) copolymeric hydrogel particles synthesized using various concentration of MAA showed in Figure 2. Swelling studies were carried out in two phases (at pH 8.0 and 1.2) in view of mimicking GIT condition. In the first phase, swelling studies were carried out at basic pH (pH 8.0) for 24 h at 37°C. At basic pH, highest swelling 587% was observed in case of poly(HEMA-MAA) hydrogel synthesized using 30% of MAA while lowest swelling percentage 413% was observed in hydrogel synthesized 10% of MAA. Poly(HEMA-MAA) hydrogel synthesized using 20, 40, 50, and 60% of MAA showed 458, 462, 454, and 436, respectively. Decrease in swelling percentage in case of 40, 50, and 60% MAA based hydrogel is probably due to homo polymerization of MAA monomer, which also revealed by the brittle nature of the polymeric hydrogel with increase in MAA concentration in the hydrogel backbone. In the second phase, swelling studies were carried out at acidic pH (pH 1.2) for 24 h. At acidic pH, highest swelling 48% was observed in case of poly(HEMA-MAA) hydrogel synthesized using 0% of MAA while lowest swelling percentage 34% was observed in hydrogel synthesized 50% of MAA. Poly(HEMA-MAA) hydrogel synthesized using 10, 20, 30, 40, and 60% of MAA showed 45, 42, 41, 35, and 36 respectively. Swelling percentage at acidic pH of poly(HEMA-MAA) does not shows significant change in swelling percentage with increase in the concentration of MAA, probably due to the presence of carboxylic backbone into the copolymer which is inert to acidic environment. At acidic pH inter-/intraparticle hydrogen bonding takes place in hydrogel particles, which acts as a secondary crosslinker and resulted in low degree of swelling. While at basic pH, disruption of hydrogen bonding and ionization of carboxylic acid moieties resulted in high degree of swelling.

Scanning electron microscopy

SEM micrograph of poly(HEMA-MAA) dried hydrogel particles synthesized using 10 and 20% of MAA concentration are given in Figure 3. The copolymeric particles were found to be irregular in shape.

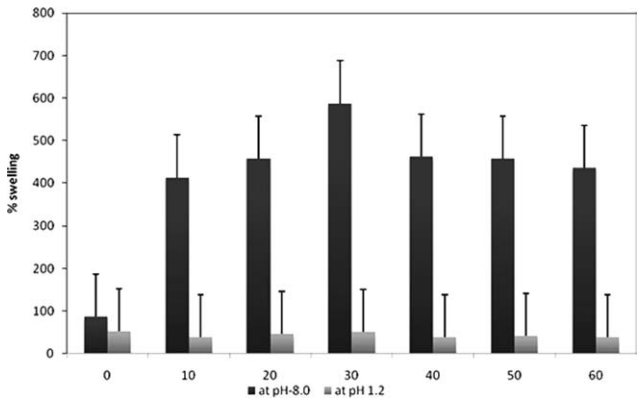

Figure 2 Swelling percentage of various poly(HEMA-MAA) hydrogel at acidic pH (1.2) and basic pH (8.0).

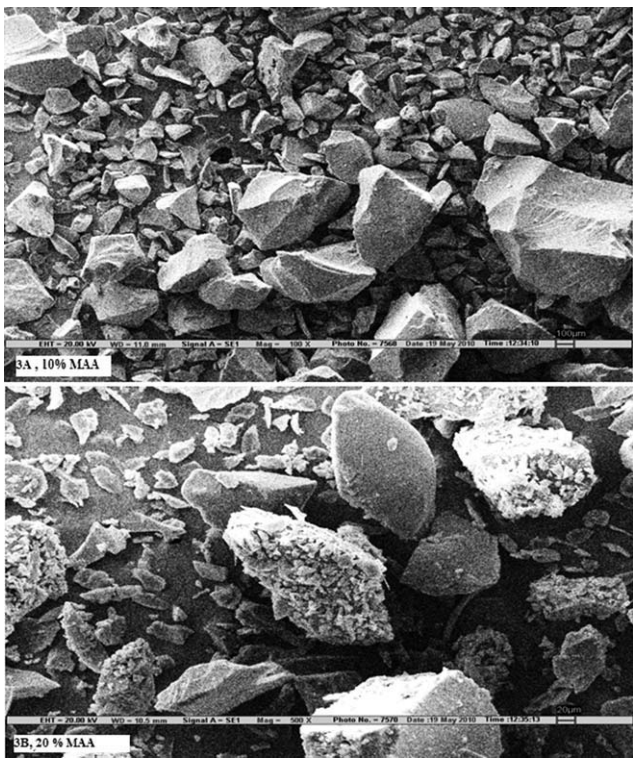

Figure 3 SEM micrograph of poly(HEMA-MAA) copolymeric hydrogel particles synthesized using 10% (3A) and 20% (3B) of MAA concentration.

Poly(HEMA-MAA) copolymeric hydrogel particles were conglomerating with each other independent of MAA concentration. It was probably due to small mesh size, inter-/intraparticle hydrogen bonding and strong interaction between carboxylic acid groups of MAA with the HEMA (hydrogen bonding). It was also observed that the aggregation of the copolymeric particles increased with increasing MAA concentration, probably due to increase in the interparticle hydrogen bonding because of increase in acidic moieties over the surface of the hydrogel particles. SEM micrographs also showed the aggregation of particles in various poly(HEMA-MAA) copolymeric particles independent of MAA concentration. Foss and Peppas also found[25] in the copolymers of p(AA-*g*-PEG) and p(MAA-*g*-PEG) the carboxylic acid groups present in the networks complex with the etheric groups of PEG at low pH environment.

Optical microscopy

Optical microscopic studies of poly(HEMA-MAA) copolymeric hydrogel synthesized using different concentration (10, 30, and 50%) of MAA showed the highly irregular surface independent of the MAA concentration, while maximum roughness was observed in case of poly(HEMA-MAA) hydrogel synthesized using 50% MAA. Minimum roughness was observed in case of poly(HEMA-MAA) hydrogel synthesized using 10% MAA concentration. It was probably due to uneven swelling in the aqueous media. Poly(HEMA-MAA) hydrogels also showed the irregular cracks over the surface independent of MAA concentration. Poly(HEMA-MAA) hydrogel synthesized using 30% of MAA showed the irregular pores over the surface. The uneven swelling of the copolymeric hydrogel was also supported by the swelling studies of the carrier. Uneven swelling in the copolymeric hydrogel synthesized using higher concentration (40–60%) of MAA was probably due to the homo-polymerization of MAA monomer due to the higher availability during polymerization reaction.

Radiolabeling of insulin by ^{99m}Tc-pertechnetate as radionuclide

Insulin was radiolabeled with *technetium-99m* and the radiolabeling efficiency was found to be 99%, which was calculated on the basis of ITLC-SA. ITLC-SA as stationary phase and tetrahydrofuran as mobile phase was used to resolve TcO^{4-} from Tc-99m-Insulin. Free pertechnetate and reduced hydrolyzed technetium always <1% in all the experiments. When the preparation was incubated at 37°C there was no increase in the free pertechnetate in the complex till 6 h and only 1% free pertechnetate was measured at 24 h. The data suggest the high *in vitro* stability, reproducibility with high labeling efficiency of the radiocomplex (Tc-99m-Insulin).

Insulin loading in copolymeric poly(HEMA-MAA) hydrogel particles

Insulin loading into poly(HEMA-MAA) particles was carried out at pH 7.4, where the mesh size of the gel carrier was large enough due to ionization of carboxylic groups of MAA and insulin could diffuse easily into the network within 5 h of insulin loading. Insulin loading efficiency of poly(HEMA-MAA) particles is given in Figure 4. Insulin was trapped inside the network by lowering the pH to 2.5. Insulin loading into the particles depends on various factors like mesh size of the network, molecular composition of the particles, and the pH of the surrounding media. Poly(HEMA-MAA) hydrogel synthesized using 60% of MAA showed the highest insulin loading efficiency of 68% (15.47 mg or 340 I.U. of insulin), while the lowest loading efficiency 37% (8.42 mg or 185 I.U. of insulin) was observed in case of 10% MAA hydrogel. Poly(HEMA-MAA) hydrogel synthesized using 30 and 40% of MAA showed 56% (12.74 mg or 280 I.U. of insulin) and 63% (14.33 mg or 315 I.U. of insulin) of insulin loading efficiency, respectively.

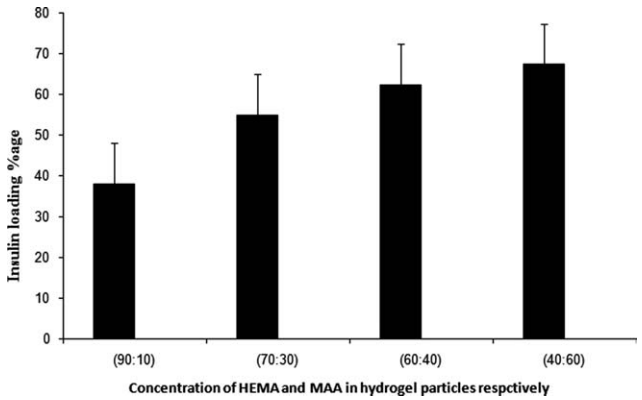

Figure 4 Insulin loading percentage of poly(HEMA-MAA) hydrogel synthesized using 10, 30, 40, and 60% of MAA.

In vitro cumulative release studies of insulin from poly(HEMA-MAA) hydrogel

Insulin release from insulin-loaded poly(HEMA-MAA) particles synthesized using various concentration of MAA (10, 30, 50, and 60% MAA) were carried out at 37°C as a function of pH and duration of exposure is shown in Figure 5. Minimum insulin release was observed from the particles at pH 2.5, probably due to presence of carboxylic groups present in the network of poly(HEMA:MAA) particles complexed with the each other with hydrogen bonding at low pH and thus the mesh size became small as the network was in its complexed state while at high pH the disruption of the hydrogen bonding and ionization of carboxylic groups led to an increase in the mesh size of the copolymeric particles. Only 35–65% of insulin was released into the medium from particles at pH 2.5 in 60 min, while insulin release was significantly higher at pH 7.4. It was observed that approximately 20–30% of left insulin released from the particles within first 15 min while the rest released within next 75 min at pH 7.4. Poly(HEMA-MAA) particles suspended in pH 7.4 showed the burst release independent of MAA concentration as shown in Figure 5. Poly (HEMA-MAA) particles having 60% of MAA showed the highest release in pH 2.5 and 7.4, due to the highest loading efficiency. Increase in the hydrophilicity with increase in the concentration of MAA used for synthesis of copolymer particles is responsible for more release of insulin from poly(HEMA: MAA) having 60% of in comparison with any other particles. Insulin release from poly(HEMA:MAA) having 60% MAA showed very high release at acidic media in comparison to 30% MAA hydrogel, which is probably due to large mesh size of the hydrogel having 60% MAA in comparison to the 30% poly (HEMA-MAA) hydrogel at basic pH. In view of the aforementioned results poly(HEMA-MAA) hydrogel having 30% MAA showed the most sustained and controlled release of insulin followed by the hydrogel having 40% MAA and thus used for further studies.

In vivo insulin release studies in rats

Eighteen diabetic rats were selected randomly and divided into three groups and each with six rats was housed in one cage. Insulin-loaded poly(HEMA-MAA) copolymeric particles were filled in gelatin capsules and administered orally down to the esophagus using forcep. The *in vivo* insulin release studies were carried out in two phases. In first phase, all the diabetic experimental animals were fasted overnight prior to oral administration of insulin-loaded copolymeric hydrogel carrier. In second phase, all the diabetic experimental animals were fed properly with the standard feed and left freely to mimic the natural condition. The experimental animals did not show any significant change in body weight and showed the normal behavior as the control group animals during the experiment.

Evaluation of insulin-loaded poly(HEMA-MAA) hydrogel particles on fasted diabetic rats

In the first phase, efficacy of insulin-loaded poly (HEMA-MAA) hydrogel carrier was tested on overnight fasted diabetic rats. Hypoglycemic effect of the 60 and 80 I.U./kg insulin dose loaded in poly (HEMA-MAA) copolymeric particles were carried out in diabetic rats and highest decrease in blood glucose level from 506 mg/dL to 170 mg/dL was observed within first 3 h. After 3 h blood glucose level started rising slowly and approach to the control value within next 3 h as shown in Figure 6. Oral administration of 60 I.U./kg insulin dose loaded in poly(HEMA-MAA) particles having 30 and 40% of MAA showed almost similar effect and reduced the

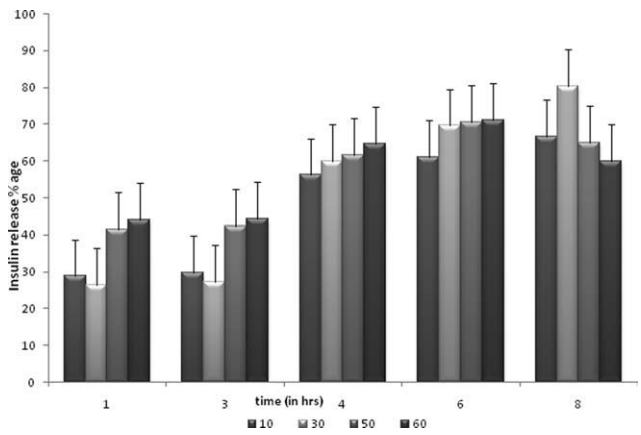

Figure 5 *In vitro* cumulative release of insulin from poly(-HEMA-MAA) hydrogel.

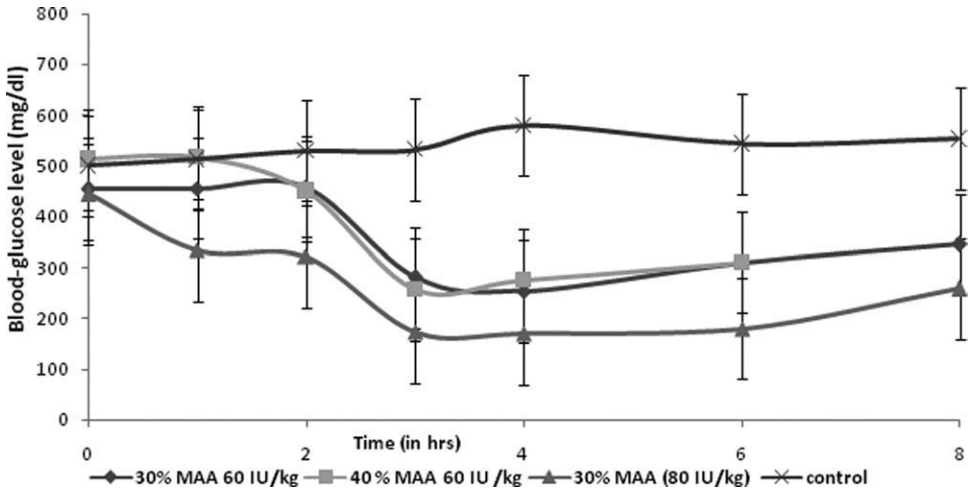

Figure 6 Hypoglycemic effect of orally administered poly(HEMA-MAA) particles to overnight-fasted diabetic rats.

fasted blood glucose level by 50% within first 3 h of the treatment and maintain the same for next 3 h and then slowly rose and approached the control value within next 2 h. Control animals fed with poly(HEMA-MAA) particles without insulin showed almost no change in the blood glucose level during experiments. Hypoglycemic effect of 80 I.U./kg dose of insulin loaded in poly(HEMA-MAA) hydrogel particles having 30% of MAA showed the 65% reduction in blood glucose level.

Evaluation of insulin-loaded poly(HEMA-MAA) hydrogel particles on fed diabetic rats

In the second phase, efficacy of insulin-loaded poly (HEMA-MAA) hydrogel carrier was tested on fed diabetic rats. Oral administration of 60 I.U./kg insulin dose loaded in poly(HEMA-MAA) having 30% MAA to fed diabetic rats reduced the initial blood glucose level by 25% within 3 h in comparison with 50% reduction in case of fasted animals. The highest reduction of 35% in blood glucose level was observed after 4 h as shown in Figure 7. Less reduction of blood glucose level in fed diabetic animals is due to the continuous absorption of glucose from the GIT.

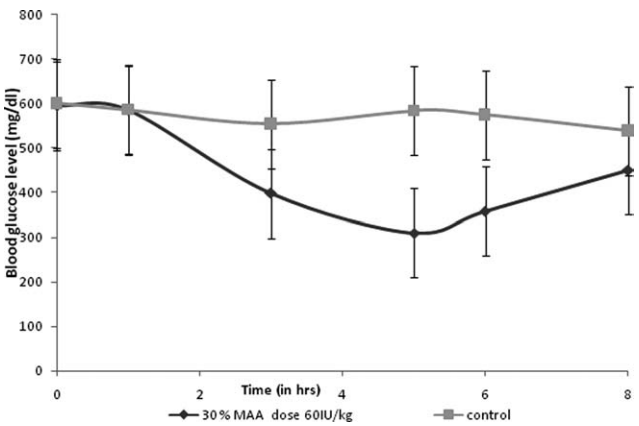

Figure 7 Hypoglycemic effect of orally administered poly(HEMA-MAA) particles to fed diabetic rats.

CONCLUSIONS

Various poly(HEMA-MAA) copolymeric hydrogel particles were synthesized successfully as confirmed by FTIR. Copolymeric particles synthesized using various concentration of MAA were found to be hydrophilic and pH sensitive. Poly(HEMA-MAA) particles synthesized using 30% of MAA showed the 58% loading efficiency and minimum insulin release in acidic medium while sustained and sufficient release was observed in basic medium as required in GIT. Poly(HEMA-MAA) showed the efficiency to reduce the blood glucose level in diabetic rats and the effect was lasted for 8 h. Insulin-loaded cross-linked poly(HEMA-MAA) hydrogel particles have potential to be used as a convenient, safe, and effective oral delivery system for diabetic patients.

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