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Development of PEGDMA: MAA based hydrogel microparticles for oral insulin delivery

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Abstract

An oral insulin delivery system based on copolymers of poly(ethylene glycol) dimethacrylate and methacrylic acid was developed and its functional activity was tested in non-obese diabetic rats. Poly(ethylene glycol) dimethacrylates (PEGDMA) were synthesized by esterification reaction of different molecular weight poly(ethylene glycol) with methacrylic acid (MAA) in presence of acid catalyst. PEG dimethacrylates of molecular weight ranging from 400 to 4000 and methacrylic acid were further copolymerized by suspension polymerization to obtain pH sensitive hydrogel microparticles. The diameter of poly(PEGDMA:MAA) microparticles increased with increasing the molecular weight of the poly(ethylene glycol) dimethacrylate used for respective microparticle synthesis. Insulin was loaded into the hydrogel microparticles by partitioning from concentrated insulin solution. In vitro release studies of insulin loaded microparticles were performed by simulating the condition of gastrointestinal tract, which showed the minimal insulin leakage (18–25%) at acidic pH (2.5) and significantly higher release at basic pH (7.4). Animal studies were carried out to investigate the abilities of the insulin loaded hydrogel microparticles to influence the blood glucose levels of the diabetic rats. In studies with diabetic rats, the blood glucose level reduced for animals that received the insulin loaded hydrogel microparticles and the effect lasted for 8–10 h. It was also observed, two capsules per day of poly(PEGDMA4000:MAA) hydrogel microparticles containing 80 I.U./kg of insulin dose were sufficient to control the blood glucose level of fed diabetic rats between 100 and 300 mg/dl.

Keywords: Oral drug delivery; Insulin; Diabetes; Hydrogel microparticles

1. Introduction

Oral delivery of insulin is limited due to barriers such as loss of activity during formulation and storage conditions, acidic environment of stomach, enzymatic degradation and low epithelial permeability in gastrointestinal tract (Gerardo et al., 1999; Lee et al., 2000). In case of insulin, less than 0.1% of orally dosed drug reaches the blood stream intact (Lowman et al., 1999; Foss et al., 2004). Currently, insulin injections are the only option available for diabetic patients. Unfortunately, injections are often painful, have low patient compliance, are required two to three times a day and have high chances of infection. Several other routes of insulin administration for treatment of diabetes mellitus have been investigated that include oral, nasal (Kublik and Vidgren, 1998; Surendrakumar et al., 2003), rectal (Onuli et al., 2000) and transdermal (Brand et al., 1997; Kanikkannan et al., 1999; Boucaud et al., 2002).

In order to benefit from the advantages of oral delivery, a number of studies have been carried out to develop oral insulin formulations. Actually, orally administered insulin is delivered first to the liver through portal circulation, similar to the physiological route of insulin secretion in non-diabetic individuals. Furthermore, potential benefits from this route include improved disease management, enhanced patient compliance and reduction of long term complications of diabetes (Sastry et al., 2000; Lee, 2002; Shein, 2003; Calceti et al., 2004). To improve the oral delivery of insulin, different strategies have been pursued such as the use of sustained release polymeric systems with enzyme inhibitors and permeation enhancers. Polymeric systems attempted for oral insulin delivery include enteric coated dosage forms and microencapsulations (Nakamura et al., 2004; Peppas, 2004). Microparticulates/microspheres of insulin have

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been prepared with microcrystalline cellulose (Trenktrog et al., 1996), polyvinyl alcohol (Kimura et al., 1996) and polymethacrylates (Morishita et al., 1992; Agarwal et al., 2001). Various copolymers, comprised either of methacrylic acid or acrylic acid for their pH sensitive nature (Lowman et al., 1999; Torres-lugo et al., 2002; Peppas et al., 1999; Kleir and Peppas, 1989) and ability to bind calcium (Madsen and Peppas, 1999) and poly(ethylene glycol) for its ability to stabilize and protect insulin have also been used for oral insulin delivery (Peppas et al., 2000). However, disadvantages with these approaches include exposure of the proteins to the harsh possessing conditions, long processing times to prepare the microparticles, rapid release (2 h) and the lower encapsulation efficiencies (as low as 40%). Some investigators have also used absorption enhancers to increase the intestinal permeability (Aungst, 1994; Coudhari et al., 1994; Senel et al., 1997).

The goal of the present research work is to develop a formulation with high insulin loading efficiency, sustained and efficient delivery of insulin in gastrointestinal tract. To achieve the desired properties, copolymers based on various molecular weight (400–4000 g/mol) poly(ethylene glycol) dimethacrylates and methacrylic acid were synthesized, loaded with insulin and evaluated for hypoglycemic effect of the developed formulations on diabetic rats.

2. Materials and methods

2.1. Materials

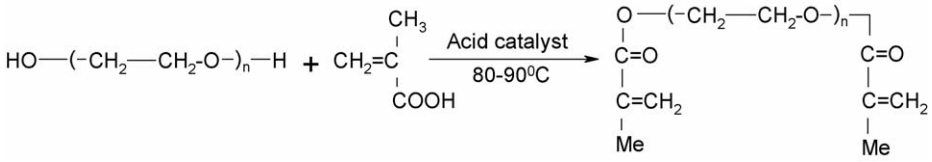
Poly(ethylene glycol) with molecular weight 400, 600, 1000, 2000 and 4000 were purchased from Loba Chemie (Mumbai, India). Azobisisobutyronitrile (AIBN) obtained from Acros Organics (NJ, USA). Methacrylic acid, hydrochloric acid, sodium sulphate, sodium hydroxide pellets and alloxan were obtained from Central Drug House (Delhi, India). Acetonitrile (HPLC grade), water (HPLC grade) and hexane was purchased from Ranbaxy Chemicals (Delhi, India). Monocomponent human insulin (r-DNA origin) of 100 I.U./ml concentration from Eli Lilly and Company (USA) was used as received.

2.2. Animals

Sprague–Dawley rats weighing 280–330 g (15–16 weeks old) were provided by Experimental Animal Facility of Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi. The study protocol was reviewed and approved by Institutional Animal Ethics Committee.

2.3. Synthesis and characterization of PEG dimethacrylate

Two hundred grams of α,w-dihydroxy PEG with molecular weight of 400, 600, 1000, 2000 and 4000 g/mol were taken separately with methacrylic acid in two-fold molar excess based on PEG diol in the presence of methane sulphonic acid as catalyst (1.5% of monomer concentration) and hydroquinone as free radical inhibitor (0.01% of methacrylic acid). Toluene was used as azotropic solvent to remove water formed during esterification reaction. The reaction was carried out in 500 ml three-neck flask from 80 to 90 °C at 40 rpm for 7 h.



Poly(ethylene glycol) dimethacrylate (PEGDMA) thus formed was neutralized with 5% of aqueous sodium bicarbonate ($NaHCO_3$). PEGDMA was precipitated from the solution by adding ice-cold hexane and dried under vacuum at 60 °C for 24 h (Pathak et al., 1992; Cruise et al., 1998). Various PEGDMA macromers thus synthesized are referred as PEGDMA400, PEGDMA600, PEGDMA1000, PEGDMA2000 and PEGDMA4000.

The degree of substitution of the PEG terminal alcohol with acrylate was determined using proton NMR spectrum of the respective PEGDMA. The method compares the ratio of the integral value of PEG backbone (~4.2 ppm) and the acrylate (~5.8–6.4 ppm) taken into consideration to calculate the percentage of acrylation. The extent of acrylation was calculated using the following formula:

$$\text{Degree of acrylation} = \left[\frac{\text{vinylic integral}/4}{(\text{vinylic integral}/4) + (\text{oxyethylene integral}/4)/(44/\text{PEG molecular weight})} \right] \times 100$$

2.4. Synthesis of poly(PEGDMA–MAA) particles

The pH sensitive copolymeric hydrogel microparticles were synthesized by free radical suspension polymerization. Various molecular weights PEGDMA (ranging from 400 to 4000 g/mol) and methacrylic acid were taken separately in the molar feed ratio of 1:2, respectively. Azobisisobutyronitrile (0.6% of monomer concentration) was used as free radical initiator. Polymerization reaction was carried out in 500 ml flask at 75 °C, using water as continuous medium, with a stirring speed of 300 rpm for 4 h. A typical copolymerization recipe consisted of 1 g of PEGDMA, 2 g of methacrylic acid, 97 g of water and 0.018 g of AIBN. The resulting cross-linked copolymeric particles were washed repeatedly with deionized water to remove unreacted monomers. The wet copolymeric particles were freeze-dried and stored for further studies (Morishita et al., 2004).

2.4.1. Particle size analysis

A submicron particle size analyzer (90 plus particle size analyzer, Brookhaven Instruments, NY, USA) was used to determine the diameter of various copolymeric particles. Polymeric particles were suspended in buffer solution of pH 2.5 and 7.4 for 2 h at 37 °C and light scattering measurement was performed for 200 s per sample and data were analyzed.

2.4.2. FTIR analysis

ATR–FTIR spectrum (attenuated total reflectance–Fourier transform infrared spectroscopy) of vacuum-dried samples of MAA, PEG4000, PEGDMA4000 and poly(PEGDMA4000: MAA) microparticles were recorded on a Perkin-Elmer spectrum one spectrometer.

2.4.3. Scanning electron microscopy (SEM)

The poly(PEGDMA400:MAA) and poly(PEGDMA4000: MAA) copolymeric hydrogel particles were suspended separately in buffer solutions of pH 2.5 and 7.4 for 6 h and then small volumes (50–100 μl) of mixture were poured and dried on a double-sided adhesive tape at 60 °C overnight. The dried samples were sputter coated with gold particles under reduced pressure conditions and observed under scanning electron microscope (Leo, VP-435, UK) at constant 15 kV accelerating voltage for surface morphological studies.

2.4.4. Swelling studies

The swelling characteristics of copolymeric poly(PEGDMA: MAA) hydrogel microparticles synthesized using various molecular weights PEGDMA were determined by immersing dried test samples in 10 ml phosphate buffer solution of pH 1.5 and 7.4 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 ratios (Q_s) of the test samples were calculated from the following equation:

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

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

2.4.5. Insulin loading of microparticles

The pH of the insulin solution (concentration 100 I.U./ml) was adjusted to 7.4 by adding 1N NaOH solution and 0.02 mg of Tween 80 was added to ensure that insulin did not adsorb on the glass surface (Foss and Peppas, 2004). One gram each of copolymeric microparticles was placed separately in 5 ml of insulin solution at 37 °C for 6 h at pH 7.4 to allow maximum loading and then pH of the insulin solution was lowered gradually to 2.5 by adding 1N HCl, to trap the insulin inside the microparticles (Joshi and Misra, 2001; Zimmermann and Muller, 2001). Insulin loaded copolymeric microparticles was filtered, freeze-dried and stored at 4 °C for further studies. The reverse phase high performance liquid chromatography (RP-HPLC) was used to determine the insulin concentration eluted from the samples at pH 7.4 for calculating insulin loading efficiency and percentage. Briefly, Kromasil C18 column was employed and the wavelength of instrument detector was set at 214 nm (Oliva et al., 1996; Yomota et al., 1996). The mobile phase was a mixture of acetonitrile and the sodium sulphate buffer of pH 2.3 in the ratio 24:76 with a flow rate of 1.0 ml/min. Insulin loading capacity and the loading efficiency was calculated from the following equations (Morcöl et al., 2004):

$$\text{Insulin loading percentage (w/w)} = \frac{M_{\text{bound}}}{M_{\text{particle}}} \times 100$$

$$\text{Insulin efficiency (\%)} = \frac{M_{\text{bound}}}{M_{\text{theoretical}}} \times 100$$

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

2.4.6. In vitro insulin release studies

In vitro insulin release studies were performed by placing 1 g of various molecular weight insulin loaded poly(PEGDMA: MAA) copolymeric microparticles separately in 10 ml of pH 2.5 citrate–phosphate buffer solutions at 37 °C and the mixture was stirred at 100 rpm (Trotta et al., 2005). At every 15 min time interval insulin loaded copolymeric microparticles were filtered using Whattman filter paper no. 1 and re-suspended in fresh 10 ml of pH 2.5 buffer till 90 min. After 90 min, insulin release from the microparticles were carried out with fresh 7.4 pH phosphate buffers and samples were collected again at 15 min time interval in the same manner till 240 min (4 h). Each filtered sample was analyzed by RP-HPLC and the amount of insulin released was calculated by means of a standard calibration curve. These studies were performed to mimic the gastrointestinal tract conditions.

2.5. Animal studies

Insulin loaded poly(PEGDMA2000:MAA) and poly-(PEGDMA4000:MAA) microparticles were used for in vivo oral release studies. 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 ≥300 mg/dl were only used for further studies (Kisel et al., 2001). Eighteen diabetic rats were selected randomly and divided into three groups and each with six rats was housed in one cage. Insulin loaded copolymeric microparticles were filled in gelatin capsules and administered orally down to the oesophagus using forcep. Animal studies were carried out in two phases.

2.5.1. Effect of oral microparticles on fasted diabetic rats

In first phase, diabetic animals were fasted overnight prior to oral administrations. Animals of first group were taken as

control and fed with poly(PEGDMA4000:MAA) microparticles without insulin loading. In second group animals were fed with insulin loaded poly(PEGDMA2000:MAA) copolymeric microparticles while in the third group all the six animals were fed with insulin loaded poly(PEGDMA4000:MAA) copolymeric microparticles. The copolymeric microparticles loaded with insulin dose given to second and third group of rats was 60 I.U./kg animal body weight and there blood glucose level was checked at regular time interval up to 8 h.

2.5.2. Effect of oral microparticles on fed diabetic rats

In second phase of experiments, animals were allowed to move freely and fed properly to simulate the natural conditions and blood glucose level was attempted to control between 100 and 300 mg/dl using an appropriate dose of insulin loaded poly(PEGDMA4000:MAA) polymeric particles up to 8 days. Blood samples were collected from the tail vein of rats at specific time interval after administration of insulin loaded microparticles and blood glucose level was measured using Accutrend® blood glucometer (Roche, Germany). The reduction in blood glucose concentration (C_{max}) was obtained from the blood glucose concentration–time curves (%change of initial) of each rat using equation:

$$\%\text{Change} = \left[\frac{F - P_t}{F}\right] \times 100$$

where F is the fasting glucose level and P_t is the plasma glucose level at time (t) after oral administration of the insulin loaded microparticles.

3. Results and discussion

3.1. Synthesis and characterization of PEG dimethacrylate

PEG dimethacrylates were synthesized by esterification reaction of different molecular weight poly(ethylene glycol) with methacrylic acid in presence of acid catalyst. Degree of acrylation was found to be in the range of 93–95% in all the PEG dimethacrylates, based on proton NMR spectroscopy.

3.2. Particle size analysis

The mean diameter of the hydrogel microparticles at pH 2.5 and 7.0 are given in Table 1. It was observed that the size of the hydrogel microparticles increased with increasing molecular weight of the PEG dimethacrylates used for polymerization. Poly(PEGDMA4000:MAA) microparticles showed the largest mean diameter of 25 μm while the lowest mean diameter of 5.0 μm was observed for poly(PEGDMA400:MAA) microparticles at pH 2.5. Basically, the carboxylic groups present in the network of all the microparticles complex with the etheric groups of PEG due to hydrogen bonding at pH 2.5 and thus the mesh size should be small (Foss et al., 2004), but particle size analyzer showed the opposite results as given in Table 1. The reason for this anomalous behavior is due to the aggregation of microparticles with each other due to interparticle hydrogen bonding at the surface and subsequently adhered microparticles showed the larger mean size with wide distribution at pH 2.5. While at pH 7.4 the disruption of the hydrogen bonding and ionization of carboxylic groups leads to larger mesh size but at the same time, generates the repulsive force between the particles as a result of which, particles separate from each other in aqueous basic media and showed smaller mean size with narrow size distribution. Aggregation of microparticles with each other at pH 2.5 also supported by high polydispersity index of microparticles while at pH 7.4 low polydispersity index was observed which confirmed the aggregation of microparticles at acidic pH and separation of microparticles at basic pH.

3.3. FTIR analysis

FTIR spectra of MAA, PEG4000, PEGDMA4000 and the copolymeric microparticles of poly(PEGDMA4000:MAA) are given in Fig. 1. MAA had characteristic absorption peaks at 1635 cm^{-1} for carbonyl group and 1697 cm^{-1} for vinyl groups and a wide band from 3000 to 3450 cm^{-1} for −OH of carboxylic group as given in Fig. 1a, while PEG4000 showed the weak band at 3450 cm^{-1} for hydroxyl group, 2882 cm^{-1} for CH stretching and 1466 cm^{-1} for CH banding as given in Fig. 1b. In case of PEGDMA4000 a broader peak at 1639 cm^{-1} was observed due to merging of carbonyl and vinyl peaks as given in Fig. 1c. In case of poly(PEGDMA4000:MAA) microparticles, IR absorption peak of carbonyl group at 1695 cm^{-1} was further intensified due to carboxylic acid groups of PEGDMA4000 and MAA, which confirms the hydrophilic character and pH sensitive nature of the microparticles as shown in Fig. 1d. Almost similar peaks were observed in all other molecular weight PEGDMA and their microparticles.

Table 1
Particle size distribution of poly(PEGDMA:MAA) hydrogel microparticles synthesized using various molecular weights PEGDMA

Poly(PEGDMA–MAA) microparticles based on various molecular weight PEGDMA	Mean diameter of microparticles (μm) at pH 2.5	Polydispersity of microparticles (μm) at pH 2.5	Mean diameter of microparticles (μm) at pH 7.4	Polydispersity of microparticles (μm) at pH 7.4
PEGDMA400	5.22 ± 1.021	0.362	0.405 ± 0.003	0.021
PEGDMA600	8.25 ± 1.476	0.471	0.864 ± 0.021	0.005
PEGDMA1000	10.59 ± 1.362	0.406	1.65 ± 0.054	0.004
PEGDMA2000	18.80 ± 2.58	0.358	1.84 ± 0.146	0.042
PEGDMA4000	25.29 ± 2.971	0.453	2.694 ± 0.213	0.005

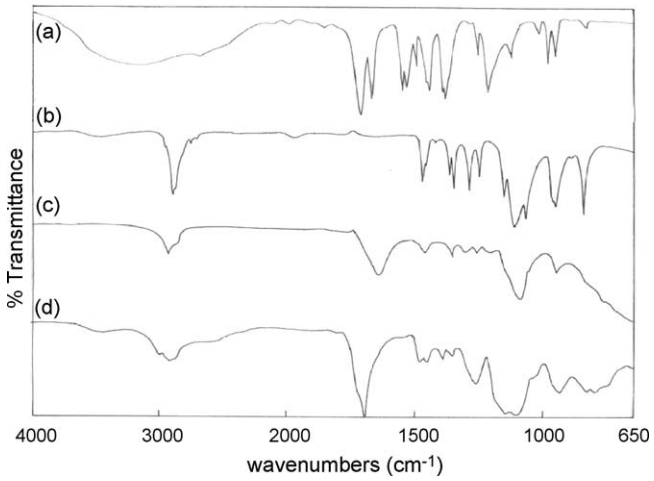


Fig. 1. FTIR spectra of MAA (a), PEG4000 (b), PEGDMA4000 (c) and poly(PEGDMA4000:MAA) microparticles (d).

3.4. Scanning electron microscopy

SEM micrographs of dried poly(PEGDMA400:MAA) and poly(PEGDMA4000:MAA) hydrogel microparticles are given in Figs. 2 and 3, respectively. The poly(PEGDMA400:MAA) and poly(PEGDMA4000:MAA) microparticles were found to be spherical in shape at pH 2.5 because of small mesh size due to hydrogen bonding between carboxylic acid groups of MAA with the etheric groups of PEG as shown in Figs. 2A and 3A, respectively, while at pH 7.4 poly(PEGDMA400:MAA) and poly(PEGDMA4000:MAA) microparticles, irrespective of their PEGDMA molecular weights (used for synthesis of microparticles) swells enormously due to ionization of carboxylic groups and consequently coalesce with each other and appeared like a continuous film as given in Figs. 2B and 3B. SEM studies also confirms the hydrophilic and pH sensitive nature of the microparticles as all the microparticles swell enormously at pH 7.4 while maintain their spherical appearance at pH 2.5.

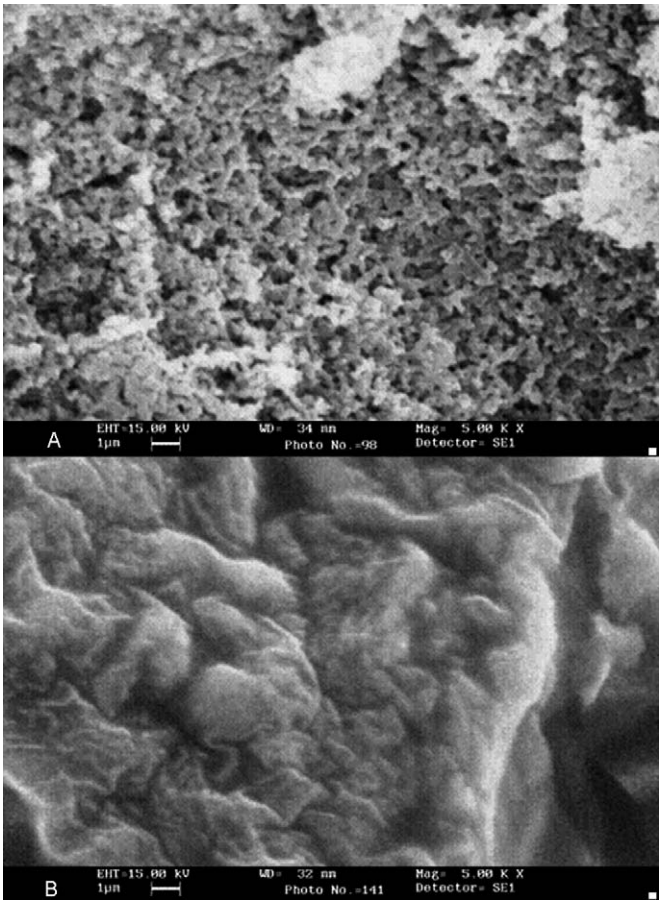


Fig. 2. Scanning electron microphotograph of poly(PEGDMA400:MAA) microparticles at pH 2.5 (A) and pH 7.4 (B).

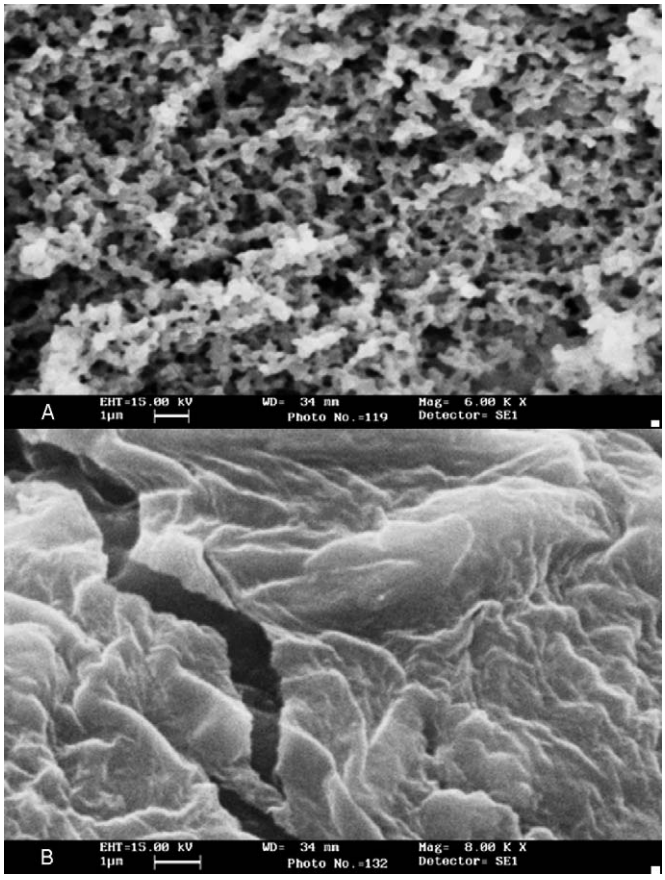


Fig. 3. Scanning electron microphotograph of poly(PEGDMA4000:MAA) microparticles at pH 2.5 (A) and pH 7.4 (B).

3.5. Swelling studies

The swelling characteristics of poly(PEGDMA400:MAA), poly(PEGDMA1000:MAA) and poly(PEGDMA4000:MAA) microparticles are shown in Fig. 4. The degree of swelling of hydrogel microparticles was found to be in the range of 2–6 at pH 1.2 and 11–15 at pH 7.4. It was also observed that the degree of swelling of microparticles increased with the increase in molecular weight of PEGDMA used for synthesis and pH of the surrounding medium. Poly(PEGDMA4000:MAA) microparticles showed the highest degree of swelling 6 and 15 at pH 1.2 and 7.4, respectively. At acidic pH inter/intraparticle hydrogen bonding takes place in microparticles which acts as

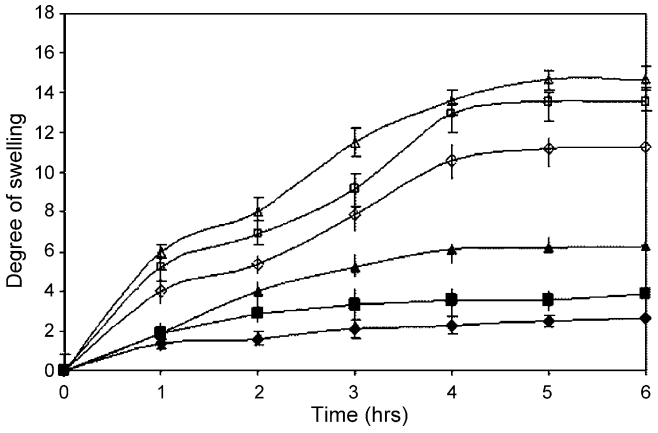


Fig. 4. Swelling characteristics of poly(PEGDMA–MAA) microparticles synthesized using various molecular weight PEGDMA and MAA: (♦) poly(PEGDMA400:MAA), (■) poly(PEGDMA1000:MAA) and (▲) poly(PEGDMA4000:MAA) microparticles swelling at pH 1.2. (◊) Poly(PEGDMA400:MAA), (□) poly(PEGDMA1000:MAA) and (Δ) poly(PEGDMA4000:MAA) microparticles swelling at pH 7.4.

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. On the other hand, decrease in cross-linking density with increase in the molecular weight of PEGDMA used for synthesis of copolymer microparticles is responsible for higher swelling of poly(PEGDMA4000:MAA) in comparison to poly(PEGDMA400:MAA) microparticles.

3.6. Insulin loading of microparticles

Insulin loading into microparticles 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 6 h of insulin loading. On lowering the pH to 2.5, insulin was trapped inside the network. Insulin loading into the microparticles depends on various factors like mesh size of the network, molecular composition of the microparticles and the pH of the surrounding media. Basically, the carboxylic groups present in the network of poly(PEGDMA:MAA) microparticles complexed with the etheric groups of PEG due to 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 microparticles. Moreover, the long neutral PEG chains could interact with the negatively charged carboxylic groups to shield the insulin molecule from charged forces (Foss and Peppas, 2004). Insulin has high affinity for PEG-rich environment and the favorable interaction between PEG and insulin allowed the protein to diffuse and stabilize inside the copolymeric particles (Iwanaga et al., 1997; Preswich et al., 2000). Previously, Lowman and Peppas (2000) have also observed that copolymeric nanoparticles composed purely of MAA exhibit low loading as compared to copolymeric particles based on MAA and monomethoxy terminated poly(ethylene glycol) acrylates.

Table 2
Insulin loading efficiency/loading percentage of poly(PEGDMA:MAA) microparticles synthesized using various molecular weight PEGDMA

Various molecular weight PEGDMA based microparticles	Loading efficiency	Insulin loading percentage
400	43.95 ± 5	0.99
600	49.61 ± 7	1.13
1000	58.88 ± 5.2	1.34
2000	69 ± 7.32	1.57
4000 (2:1)	82 ± 6.34	1.87

Loading efficiency and percentage of insulin loading of various hydrogel microparticles (Table 2) increases with increasing the molecular weight of the PEG dimethacrylate used for microparticles synthesis. It was found that poly(PEGDMA4000:MAA) microparticles showed the maximum loading efficiency (82%) while the lowest (43%) loading efficiency was observed in case of poly(PEGDMA400:MAA) copolymeric microparticles. Decrease in cross-linking density and increase in the hydrophilicity with increase in the molecular weight of PEGDMA used for synthesis of copolymer microparticles is responsible for high loading of insulin in poly(PEGDMA4000:MAA) in comparison to poly(PEGDMA400:MAA) microparticles.

3.7. In vitro insulin release

Cumulative insulin release from insulin loaded poly(PEGDMA:MAA) microparticles based on various molecular weight PEG dimethacrylates and MAA with molar ratio 1:2, respectively, at 37 °C as a function of pH and duration of exposure is shown in Fig. 5. Minimum insulin release was observed from the microparticles at pH 2.5, most likely due to shrinkage of network taking place at acidic pH as mentioned above. Only 18–25% of insulin was released into the medium from microparticles at pH 2.5 in 90 min, while insulin release was significantly higher at pH 7.4. It was observed that approximately 35% of left insulin released from the microparticles

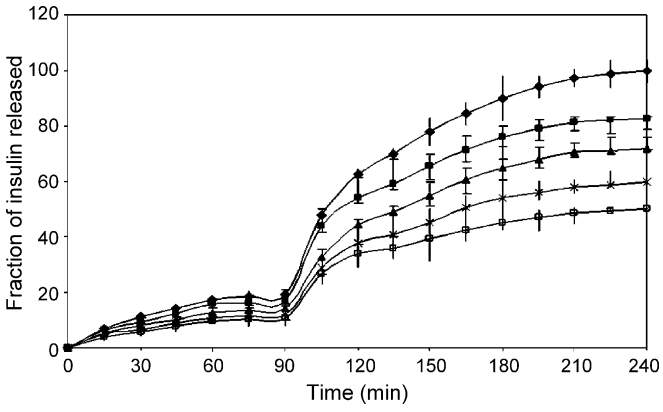


Fig. 5. In vitro cumulative release of insulin from poly(PEGDMA400–MAA) microparticles (□), poly(PEGDMA600–MAA) microparticles (×), poly(PEGDMA1000–MAA) microparticles (▲), poly(PEGDMA2000–MAA) microparticles (■) and poly(PEGDMA4000:MAA) microparticles (♦) at pH 2.5 (from 0 to 90 min) and pH 7.4 at 37 °C (from 90 to 240 min).

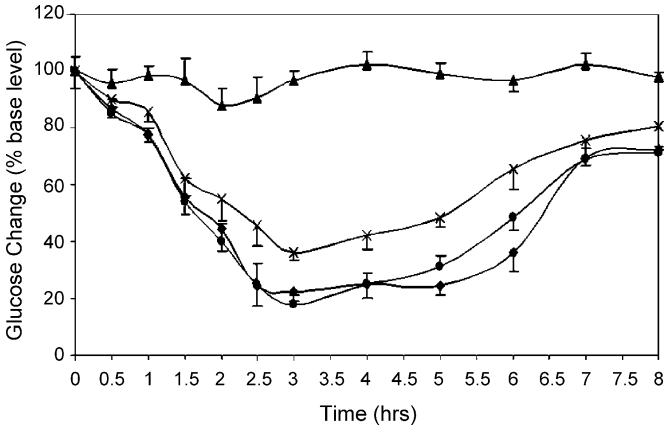


Fig. 6. Hypoglycemic effect of orally administered microparticles to over night fasted diabetic rats: (▲) poly(PEGDMA4000:MAA) microparticles without insulin to control animals, (×) 40 I.U./kg insulin dose loaded in poly(PEGDMA4000:MAA) microparticles, (⧫) 60 I.U./kg of insulin dose loaded in poly(PEGDMA4000:MAA) microparticles and (●) 60 I.U./kg of insulin dose loaded in poly(PEGDMA2000:MAA) microparticles.

within first 15 min while the rest released within next 150 min (2.5 h) at pH 7.4. Poly(PEGDMA4000:MAA) microparticles showed the highest release in pH 2.5 and 7.4, due to the highest loading efficiency as shown in Table 2. Again, decrease in cross-linking density and increase in the hydrophilicity with increase in the molecular weight of PEGDMA used for synthesis of copolymer microparticles is responsible for more release of insulin of poly(PEGDMA4000:MAA) in comparison to poly(PEGDMA400:MAA) microparticles while, the rate of insulin release increases with increase in molecular weight but pattern of release remains same in all the poly(PEGDMA:MAA) microparticles.

3.8. Animal studies

3.8.1. Effect of oral microparticles on fasted diabetic rats

Hypoglycemic effect of the 40 I.U./kg insulin dose loaded in poly(PEGDMA4000:MAA) copolymeric microparticles were carried out in diabetic rats and it was observed that blood glucose level reduced by 65% within 3 h but started rising slowly and approach to the control value within next 5 h as shown in Fig. 6. Oral administration of 60 I.U./kg insulin dose loaded in poly(PEGDMA4000:MAA) microparticles and poly(PEGDMA2000:MAA) microparticles showed almost similar effect (Fig. 6) and reduced the fasted blood glucose level by 78% within first 2.5 h of the treatment and maintain the same for next 3.5 h and then slowly rose and approached the control value within next 2 h. Control animals fed with poly(PEGDMA4000:MAA) microparticles without insulin showed almost no change in the blood glucose level during experiments. It was also observed from the experiment (Table 1) that lower amount poly(PEGDMA4000:MAA) microparticles was required to deliver the same dose of insulin in comparison to poly(PEGDMA2000:MAA) microparticles due to the less cross-linking density higher hydrophilicity and thus high loading of insulin in copolymeric microparticles synthesized using PEGDMA4000.

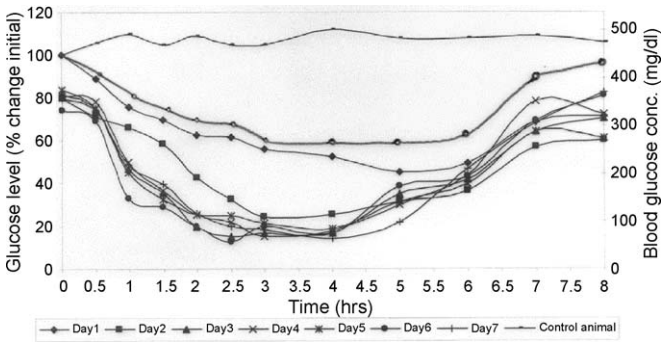


Fig. 7. Hypoglycemic effect of orally administered microparticles to fed diabetic rats: (—) poly(PEGDMA4000:MAA) microparticles without insulin loaded to the control animals and (◯) single dose of 60 I.U./kg of insulin loaded in poly(PEGDMA4000:MAA) microparticles, 80 I.U./kg of insulin dose loaded in poly(PEGDMA4000:MAA) microparticles administered twice a day at 12 h time intervals studies were continued for 7 days.

3.8.2. Effect of oral microparticles on fed diabetic rats

Oral administration of 60 I.U./kg insulin dose loaded in poly(PEGDMA4000:MAA) to fed diabetic rats reduced the initial blood glucose level by 30% within 2.5 h in comparison to 78% in case of fasted animals, while the highest reduction of 45% in blood glucose level was observed after 4 h in comparison to 76% in case of fasted animals as shown in Fig. 7. Lower reduction of blood glucose level in fed diabetic animals is due to the continuous absorption of glucose from the gastrointestinal tract. It was also observed that 80 I.U./kg of insulin loaded microparticles two times a day were sufficient to maintain the blood glucose level between 100 and 300 mg/dl. Studies were continued till 7 days and almost similar trend were observed in all days as shown in Fig. 7. These studies shows, the effect of oral administration of insulin loaded copolymeric microparticles reduce the blood glucose level and the effect was lasted for at least 8–10 h which confirms the sustained release of active insulin from the copolymeric microparticles.

4. Conclusion

Copolymeric particles synthesized using various molecular weights PEGDMA and MAA were found to be biostable, hydrophilic and pH sensitive. Poly(PEGDMA:MAA) microparticles with molar ratio 1:2 were found to have the high loading efficiency and showed the minimum insulin release in acidic medium while significant but sustained release in basic medium as required in gastrointestinal tract.

Poly(PEGDMA4000:MAA) copolymeric microparticles had the highest efficiency to reduce the blood glucose level in diabetic rats and it was observed that two capsule loaded with 80 I.U./kg insulin dose were sufficient to control the blood glucose level between 100 and 300 mg/dl. Detailed studies of sustained release mechanism of insulin loaded microparticles using radiolabelled insulin in higher animals are under progress. Poly(PEGDMA4000:MAA) hydrogel microparticles have strong potential to be used as an oral insulin delivery system for diabetic patients.

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