Synthesis and Characterization of pH Sensitive Poly(PEGDMA-MAA) Copolymeric Microparticles for Oral Insulin Delivery

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Received 13 March 2006; accepted 11 January 2007
DOI 10.1002/app.26181
Published online 27 September 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Poly(ethylene glycol) dimethacrylates (PEGDMA) were synthesized by esterification reaction of different molecular weight poly(ethylene glycol) with methacrylic acid in presence of acid catalyst. Their degrees of acrylation were found to be in the range of 93–95% using nuclear magnetic resonance (NMR) spectroscopy. PEG dimethacrylates of molecular weight ranging from 400 to 4000 and methacrylic acid were further copolymerized to obtain pH sensitive crosslinked hydrogel microparticles. The diameters of poly(PEGDMA-MAA) microparticles increased with increasing molecular weight of the poly(ethylene glycol) dimethacrylates and was found to be in the range of 0.4–2.7 μm at pH 7.4 and 5.2–25.3 μm at pH 2.5 in aqueous solution. Surface morphology of various polymeric samples were observed using SEM, which showed partial aggregation of particles at pH 2.5 but microparticles coalesce with each other and appeared like a continuous film at pH 7.4. *In vitro* insulin release studies were performed by simulating the condition of gastrointestinal tract, which showed only 18–25% insulin release into the aqueous medium at pH 2.5 in 90 min, while significantly higher release was observed at pH 7.4. In studies with diabetic rabbits, the blood glucose levels were lower for animals that received the insulin loaded hydrogel microparticles and the effect lasted for 8–10 h. J Appl Polym Sci 107: 863–871, 2008

Key words: oral drug delivery; insulin; diabetes; hydrogel microparticles; poly(ethylene glycol)

INTRODUCTION

Oral delivery of proteins/peptides still remains a challenge for scientific community, because of barriers such as loss of activity during formulation and storage conditions, acidic environment of stomach, enzymatic degradation, and low epithelial permeability in gastro intestinal tract.[1,2] 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 nondiabetic individuals. Furthermore, potential benefits from this route include improved disease management, enhanced patient compliance, and reduction of long term complications of diabetes.[3–6] Polymeric systems attempted for oral insulin delivery include enteric coated dosage forms and microencapsulations.[7,8] Various copolymers, comprised either of methacrylic acid or acrylic acid for their pH-sensitive nature and methoxytermenated poly(ethylene glycol) for its ability to stabilize and protect insulin, have also been used for oral insulin delivery.[9–13] Some of the disadvantages with this approach 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.[14–16] Studies have proved that polymers containing carboxylic acid groups have the ability to protect insulin from protease enzymes like trypsin and chymotrypsin. Binding of divalent cations (calcium and zinc) by these polymers was proposed to be the major reason for their enzyme inhibitory effect.[17,18] Furthermore, reduction of extracellular divalent ion concentration can result in opening of tight junctions and improves the paracellular peptide transport across the intestinal epithelium.[19] Moreover, mucoaddhesive properties exhibited by these polymers increases the residence time of oral doses forms at the epithelial surface and improves drug absorption.[20]

The present work is devoted towards the development of a pH sensitive polymeric formulation with high insulin loading efficiency, sustained, and

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efficient delivery of insulin in gastro intestinal tract. To achieve the desired properties, various molecular weight poly(ethylene glycol) dimethacrylates (PEGDMA) were synthesized and further copolymerized with methacrylic acid (MAA) under aqueous medium avoiding the use of any organic solvent and surfactants. SEM, FTIR, swelling studies, and particle size analyzer were used to characterize these poly (PEGDMA-MAA) microparticles. Insulin loading and *in vitro* release studies were carried out at pH 2.5 and 7.4 and the samples were analyzed using reverse phase high performance liquid chromatography (RP-HPLC). Insulin loaded microparticles were also evaluated for hypoglycemic effect on diabetic rabbits.

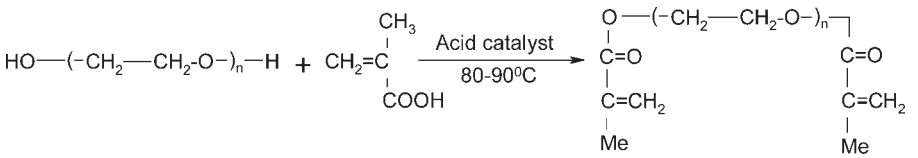
EXPERIMENTAL

Materials

Poly (ethylene glycol) with molecular weight 400, 600, 1000, 2000, and 4000 were purchased from Loba Chemie (Mumbai, India). Methacrylic acid, sodium sulfate, sodium hydroxide pellets and alloxan were obtained from Central Drug House (Delhi, India). Acetonitrile (HPLC grade), water (HPLC grade), hexane, Methane sulfonic acid, and ammonium persulfate were supplied by Ranbaxy Chemicals (Delhi, India). Monocomponent human insulin (r-DNA origin) with concentration of 100 IU/mL from Eli Lilly and company (USA) was used as received.

Synthesis of PEG dimethacrylate

Poly(ethylene glycol) dimethacrylates of different molecular weight were synthesized by esterification of 1 mol of diol with 2.5 mol of methacrylic acid in three neck round bottom flask, equipped with mechanical stirrer and dean-stark apparatus in heavy paraffin oil bath at 200 rpm. Methane sulfonic acid (1.4%) as catalyst, hydroquinone (0.15%) as inhibitor and toluene (22%) as azotropic solvent were used for esterification reaction. The amount of catalyst, inhibitor, and solvent were taken (w/w) percentage of total monomer. The temperature of reaction was maintained at (80 ± 2)°C for initial 1 h to avoid homopolymerization of highly reactive methacrylic acid and then slowly raised to (90 ± 2)°C in 2 h and continued at this temperature for a total time of 6.5 h ± 30 min. Reaction was stopped when the acid value was less than 20 mg of KOH/g of the sample.[21]



Poly(ethylene glycol) dimethacrylate(PEGDMA) thus formed was neutralized with 5% sodium bicarbonate ($NaHCO_3$), precipitated from the solution by adding ice cold hexane and dried under vacuum at 60°C for 24 h.[22] Various PEGDMA macromers thus synthesized are referred as PEGDMA400, PEGDMA600, PEGDMA1000, PEGDMA2000, and PEGDMA4000.

Characterization of PEG dimethacrylates

The degree of acrylation of various molecular weight PEGs were determined from their ^{1}H-NMR spectra recorded on a Bruker AC 300 spectrophotometer at a frequency of 300 MHz. The monomer was taken in $CDCl_3$ (5 wt/vol %) solvent using tetra methyl silane (TMS) as internal reference. The ratio of the integral value of PEG backbone (~ 4.2 and 3.6 ppm) and the acrylate (~ 5.8–6.4 ppm) were taken into consideration to calculate the degree of acrylation. The extent of acrylation was calculated using the following formula:[23]

$$\text{Degree of acrylation} = \left[\frac{(\text{Vinylic integral}/4)}{(\text{Vinylic integral}/4)+(\text{Oxyethylene integral}/4)(44/\text{PEGm.wt})}\right] \times 100 \quad (1)$$

Preparation of poly(PEGDMA-MAA) particles

Various copolymeric hydrogel microparticles were synthesized by free radical suspension polymerization. Methacrylic acid and PEG dimethacrylates with molecular weight ranging from 400 to 4000 g/mol were taken in the molar feed ratio of 2:1 respectively, and ammonium per sulfate (0.6% of monomer concentration) was used as free radical initiator. The polymerization reaction was carried out in 500 mL

flask at 75°C, using water (97% of monomer concentration) as continuous medium with a stirring speed of 300 rpm for 3–4 h. The resulting crosslinked copolymeric particles were repeatedly washed with deionized water to remove unreacted monomers. The wet copolymeric particles were freeze dried and stored for further insulin loading and release studies.[21]

Particle size analysis

A submicron particle size analyzer (90 plus particle size analyzer, Brookhaven Instruments, NewYork, 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/sample and data were analyzed.

Surface morphology

Surface morphological studies of polymeric microparticles poly(PEGDMA400-MAA), poly(PEGDMA1000-MAA), and poly(PEGDMA4000-MAA) were carried out at pH 2.5 and 7.4 using scanning electron microscope (SEM) (Leo, VP-435, UK). Small volumes (50–100 μL) of copolymeric particles were suspended separately in buffer solutions of pH 2.5 and 7.4 for 6 h and then dried overnight at 50°C on a double-sided adhesive tape. The dried samples were sputter coated with gold particles under reduced pressure conditions and observed under scanning electron microscope at constant 15 KV accelerating voltage.

FTIR analysis

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

Swelling studies

The swelling characteristics of copolymeric hydrogel microparticles based on 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 to absorb excess water on surface. The swelling ratios (Q_s) of the test samples were calculated from the following equation:

$$Q_s = (W_s - W_d)/W_d \quad (2)$$

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

Insulin loading of microparticles

One gram of each copolymeric microparticles was placed in 5 mL of insulin solution with concentration of 100 IU/mL at 37°C for 6 h separately at pH 7.4 to allow maximum loading and then pH of the insulin solution was gradually lowered to 2.5 by adding 1*N* HCL, to trap the insulin inside the microparticles. Insulin loaded copolymeric microparticles was freeze dried and stored at 4°C for further *in vitro* and *in vivo* studies.[24,25] The reverse phase high performance liquid chromatography (RP-HPLC) was used to determine the insulin concentration eluted from the samples for calculating insulin loading efficiency. Briefly, Kromasil C18 column was employed and the wavelength of instrument detector was set at 214 nm.[26] The mobile phase was mixture of acetonitrile and the sodium sulfate buffer of pH 2.3 in the ratio 24:76 with a flow rate of 1.0 mL/min. Conversion of IU of insulin into mg was carried out by using the international standard (1 IU = 45.5 μg).

$$\text{Insulin loading percentage(w/w)} = \frac{M_{\text{bound}}}{M_{\text{particle}}} \times 100 \quad (3)$$

where M_{bound} is the amount of insulin (mg) eluted from the particles (bound insulin), M_{particle} is the amount of particles (mg) utilized for insulin loading.

In vitro insulin release studies

Insulin release studies were performed by placing 1 g of various molecular weight insulin-loaded poly (PEGDMA-MAA) copolymeric particles separately in 10 mL of pH 2.5 citrate-phosphate buffer solution and the mixture was stirred at 100 rpm with a magnetic stirrer. The mixture was filtered at every 15 min time interval to measure the insulin release from the microparticles in the supernatant from loaded copolymeric particles and the solution was replaced with 10 mL of fresh buffer till 90 min. Poly (PEGDMA-MAA) microparticles were resuspended in 10 mL of 7.4 pH buffer solution after 90 min of release at 2.5 pH buffer solution. The supernatant samples were collected again at 15 min time interval in the same manner till 240 min (4 h). Each sample was analyzed by RP-HPLC and the amount of insu-

TABLE I
Particle Size Distribution of Hydrogel Microparticles Based on Various Molecular Weights PEG Dimethacrylates

Poly(PEGDMA-MAA) microparticles based on various mol wt 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
PEGDMA 400	5.22 ± 1.021	0.362	0.405 ± 0.003	0.021
PEGDMA 600	8.25 ± 1.476	0.471	0.864 ± 0.021	0.005
PEGDMA 1000	10.59 ± 1.362	0.406	1.65 ± 0.054	0.004
PEGDMA 2000	18.80 ± 2.58	0.358	1.84 ± 0.146	0.042
PEGDMA 4000	25.29 ± 2.971	0.453	2.694 ± 0.213	0.005

lin released was calculated by means of a standard calibration curve.

Animal studies

Male New Zealand rabbits weighing 2.5–3.0 kg were provided by experimental animal facility of Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi. Diabetes was induced in the experimental rabbits by injecting single dose of alloxan (150 mg/kg body weight) dissolved in sterilized water intravenously and their glucose levels were checked for diabetic conditions after 48 h. Animals with glucose levels above ≥300mg/dL were only used in subsequent studies.[27] Eight diabetic rabbits were selected randomly, fasted overnight prior to oral administration of the formulation and divided into two groups. 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(PEGDMA4000-MAA) microparticles with insulin dose of 50 IU/kg animal body weight. Blood samples were collected from the ear vein of rabbits at specific time intervals upto 8 h after oral 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 rabbit using equation:

$$\text{Change}(\%) = [(F - P_t)/F] \times 100$$

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

RESULTS AND DISCUSSION

Synthesis and characterization PEG dimethacrylate

PEG dimethacrylates were successfully synthesized by esterification reaction of different molecular weight poly(ethylene glycol) with methacrylic acid in presence of acid catalyst. NMR spectra of PEGDMA showed characteristic peaks of vinyl proton at ~ 5.8–6.4 ppm, oxyethylene ester proton and ether proton at ~ 4.2–3.6 ppm respectively. The degree of acrylation as calculated from Equation 1 was found to be in the range of 93–96%.

Particle size analysis

The mean diameter of the hydrogel microparticles at pH 2.5 and 7.4 are given in Table I. It was observed that the size of the hydrogel microparticles increases with increasing molecular weight of the PEG dimethacrylates used for synthesis. Poly(PEGDMA4000-MAA) hydrogel microparticles showed the largest

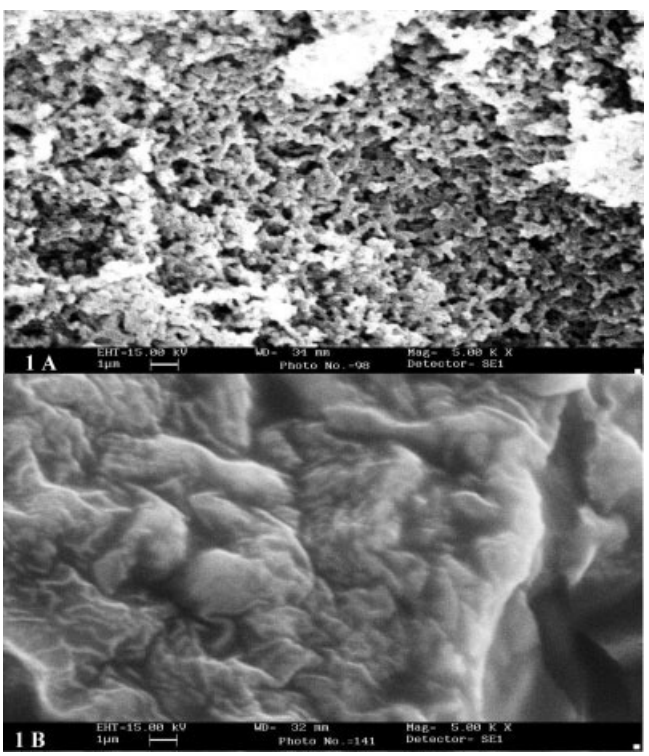


Figure 1 SEM analysis of microparticles based on PEGDMA400 at pH 2.5 (1A), and at pH 7.4 (1B).

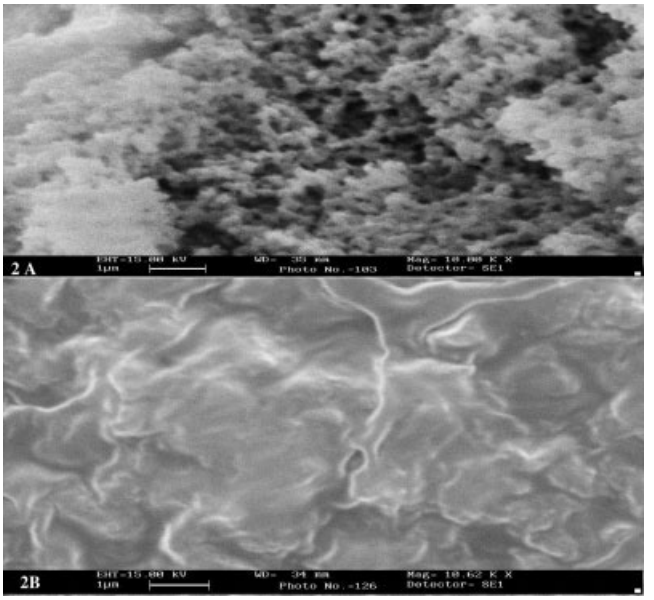


Figure 2 SEM analysis of microparticles based on PEGDMA600 at pH 2.5 (2A), and at pH 7.4 (2B).

mean diameter of 25 μm, while the poly (PEGDMA400-MAA) microparticles showed the lowest mean diameter of 5.0 μm at pH 2.5 (as given in Table I). It was also observed that the size of microparticles was higher at acidic pH (2.5) with wide size distribution, while at pH 7.4 size of microparticles decreases with narrow size distribution as given in Figures 6 and 7, respectively. Basically, the carboxylic groups present in the network of poly (PEGDMA-MAA) microparticles complex with the etheric groups of PEG because of hydrogen bonding at low pH and thus the mesh size becomes small. The microparticles also adhere to each other because of interparticle hydrogen bonding at the surface and subsequently adhered particles showed the larger size with wide size 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 show smaller size with narrow size distribution.

Surface morphology

SEM micrographs of dried hydrogel microparticles at pH 2.5 and 7.4 are given in Figures 1–4. The copolymeric microparticles were found to be aggregated particles at pH 2.5 as shown in Figures 1A, 2A, 3A, and 4A because of strong interaction

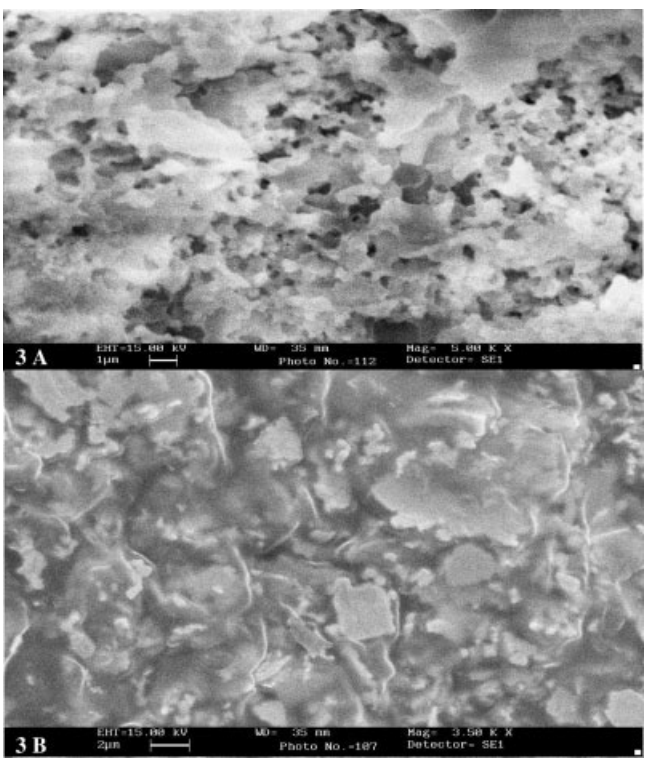


Figure 3 SEM analysis of microparticles based on PEGDMA1000 at pH 2.5 (3A), and at pH 7.4 (3B).

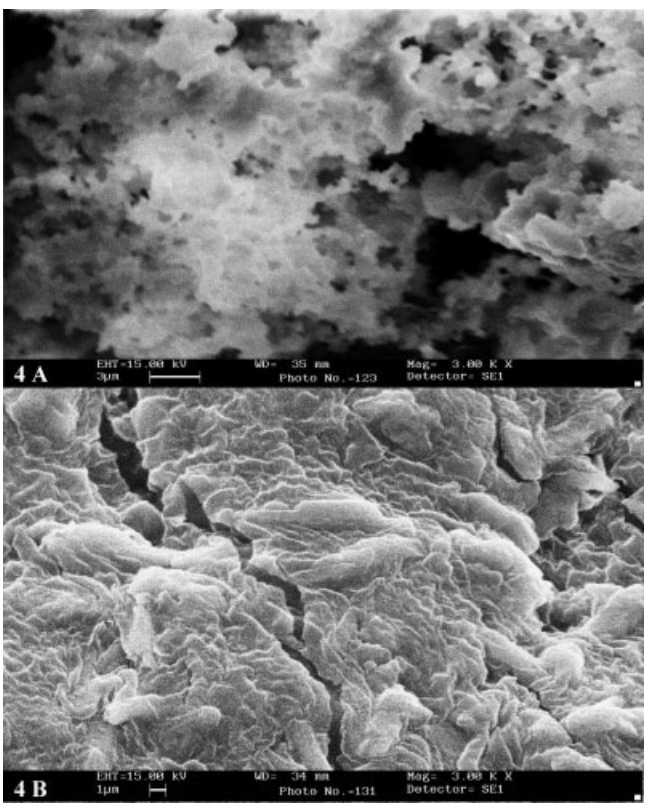


Figure 4 SEM analysis of microparticles based on PEGDMA4000 at pH 2.5 (4A), and at pH 7.4 (4B).

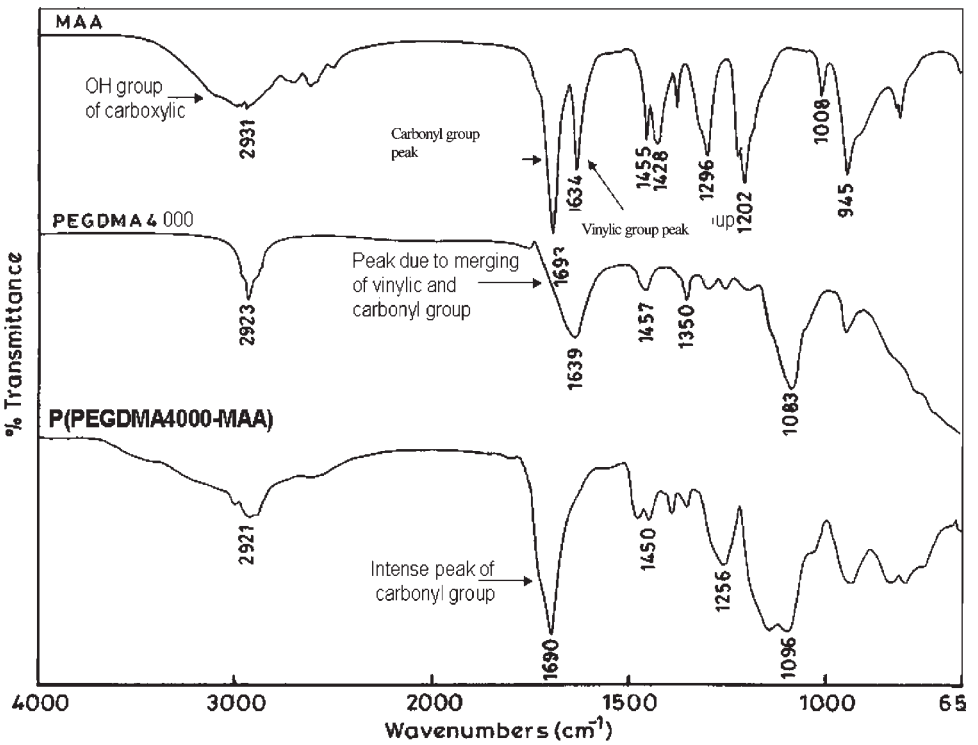


Figure 5 FTIR spectra of MAA, PEGDMA4000 and poly(PEGDMA4000-MAA) microparticles.

between carboxylic acid groups of MAA with the etheric groups of PEG (hydrogen bonding). It was also observed from the figures that the size of the copolymeric microparticles increased with increasing molecular weight of PEGDMA at pH 2.5 and partial coalescing of particles was also observed with PEGDMA 1000 and PEGDMA 4000 because of higher swelling (Fig. 8). Chances of particle aggregation and coalescing of hydrophilic particle is possible because of complete drying of samples before taking SEM pictures.[18] No particle morphology but a continuous film, as a result of coalescing of microparticles, is observed when samples were dried at basic pH 7.4 probably due to ionization and very high swelling of particles as observed in Figures 1B, 2B, 3B, and 4B.

FTIR analysis

FTIR spectra of MAA, PEGDMA4000 and the microparticles of poly(PEGDMA4000-MAA) feed ratio 1 : 2 respectively, are given in Figure 5. MAA had characteristic absorption peaks at 1634 cm^{-1} for vinylic groups and 1693 cm^{-1} for carboxylic group and a band from 3000 to 3450 cm^{-1} for OH group of carboxylic, PEGDMA showed a broader peak at 1639cm^{-1} because of merging of carbonyl and vinylic peaks. In case of poly(PEGDMA4000-MAA) microparticles, IR absorption peak of carbonyl group at 1690 cm^{-1} was intensified due to carboxylic acid groups of PEGDMA and MAA.

Swelling studies

The swelling characteristics of poly(PEGDMA400-MAA), poly(PEGDMA1000-MAA) and poly(PEGDMA 4000-MAA) microparticles are shown in Figure 8. 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 increase in molecular weight of PEGDMA used for synthesis and pH of the surrounding medium. Basically, at low pH inter/intra particle hydrogen bonding takes place in the microparticles which works 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. On the other hand, increase in the PEGDMA chain length (molecular weight) increase the hydrophilicity of the respective microparticles and consequently increased the degree of swelling. Due to this reason, poly (PEGDMA4000-MAA) microparticles showed the highest degree of swelling, while the poly (PEGDMA400-MAA) microparticles showed the lowest degree of swelling.

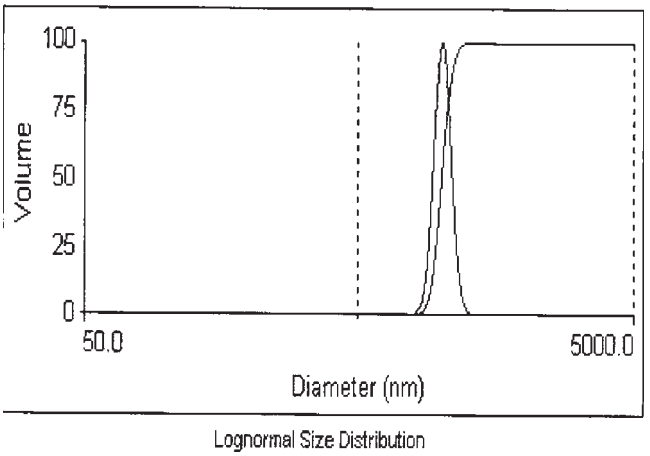


Figure 6 Particle size analysis of poly (PEGDMA1000-MAA) microparticles at pH 7.4.

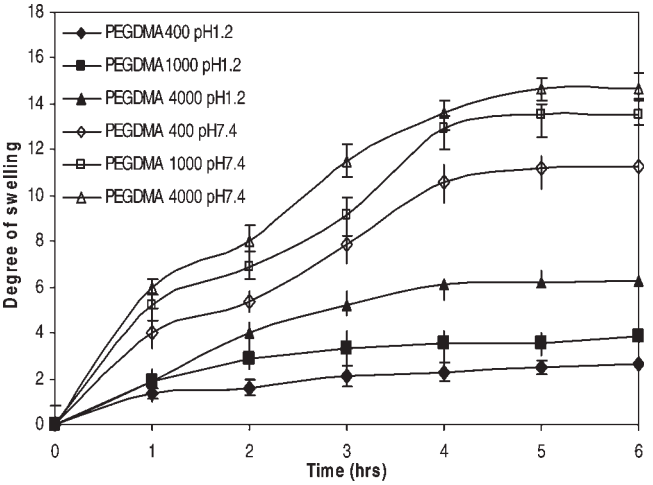


Figure 8 Swelling characteristics of poly(PEGDMA-MAA) microparticles at pH 1.2 and 7.4.

Insulin loading of microparticles

Insulin loading into the microparticles was carried out at pH 7.4, where the mesh size of the gel carrier was large enough due to ionization of the 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 of poly(PEGDMA-MAA) microparticles. Basically insulin loading into the gel carriers depends on various factors like molecular weight pf PEGDMA used during microparticles synthesis, molecular composition of the microparticles, mesh size of the network and the pH of the surrounding media. 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 as shown in Figure 9. Low crosslinking density and high degree of swelling is responsible for high loading efficiency of insulin in poly (PEGDMA4000- MAA) microparticles.

In vitro insulin release

Cumulative insulin release from microparticles based on various molecular weight PEG dimethacrylates and MAA at 37°C as a function of pH and duration of exposure is shown in Figure 10. 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 aforementioned. 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

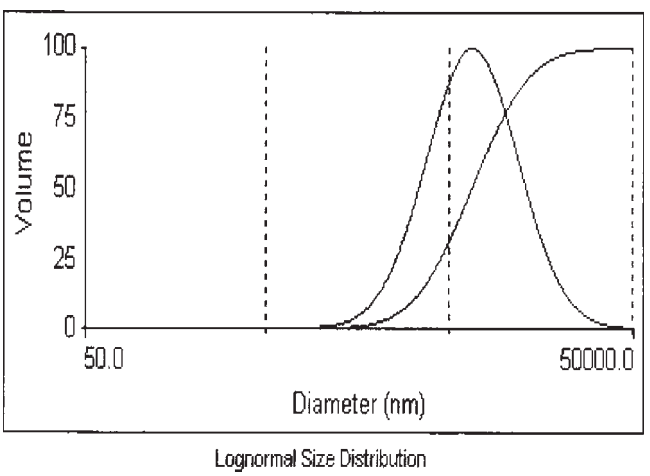


Figure 7 Particle size analysis of poly(PEGDMA1000-MAA) microparticles at pH 2.5.

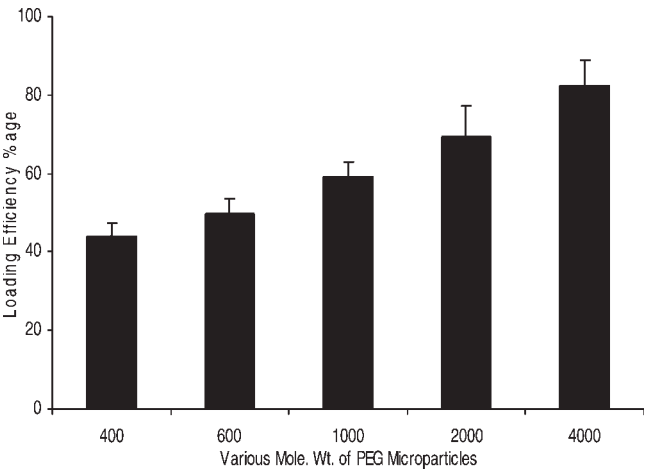


Figure 9 Loading efficiency percentage of poly(PEGDMA-MAA) microparticles using various molecular weights PEGDMA.

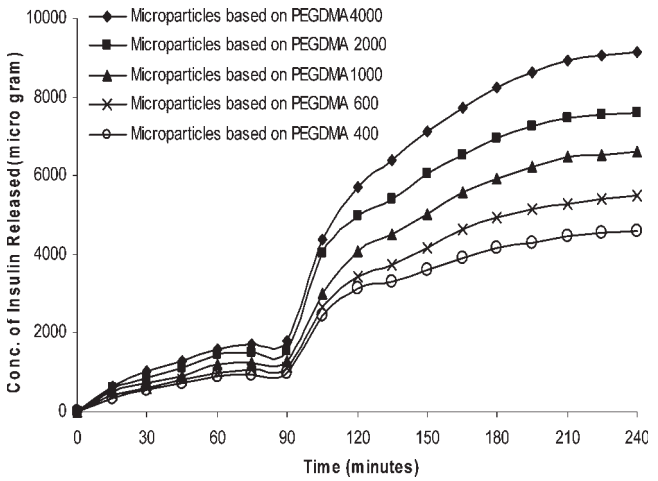


Figure 10 In vitro cumulative release of insulin from poly (PEGDMA-MAA) microparticles based on various molecular weight PEGDMA at pH 2.5 (from 0 to 90 min) and 7.4 (from 90 to 240 min) at 37°C.

that ~ 35% of left insulin released from the microparticles within first 15 min while the rest released within next 150 min (2.5 h) at pH 7.4. Rate of release of insulin increases with increase in molecular weight but pattern of release remain same in all the poly(PEGDMA-MAA) microparticles.

Animal studies

Effect of oral administration of poly(PEGDMA4000-MAA) microparticles loaded with 50 IU/kg insulin dose on over night fasted diabetic rabbits is shown in Figure 11. Insulin loaded microparticles reduced the blood glucose level by 78% within first 4 h of the treatment and maintained the same for next 2.5 h and then started rising slowly and approached the control value. The effect of insulin-loaded microparticles lasted for at least 10 h after the oral administration. Control animals fed with poly(PEGDMA-MAA) microparticles without insulin loading showed almost no change in the blood glucose level during the experiments.

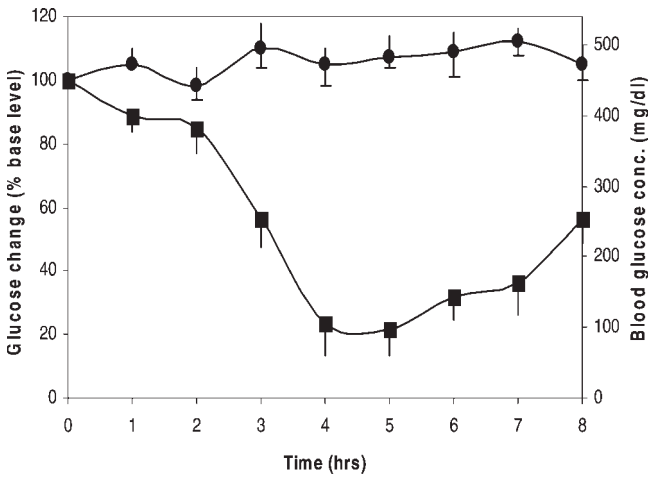


Figure 11 Hypoglycemic effect of orally administered poly(PEGDMA4000-MAA) microparticles to fed diabetic rabbits: (-●-) poly(PEGDMA4000-MAA) microparticles without insulin loading to control animals, (-■-) poly (PEGDMA4000-MAA) microparticles loaded with of 50 IU/kg insulin dose.

CONCLUSIONS

Various molecular weights PEGDMA were synthesized successfully as confirmed by proton NMR studies. Copolymeric microparticles synthesized using various molecular weights PEGDMA and MAA were found to be hydrophilic and pH sensitive. Poly-(PEGDMA4000-MAA) microparticles showed the highest loading efficiency and minimum insulin release in acidic medium while sustained and sufficient release was observed in basic medium as required in gastrointestinal tract. Poly(PEGDMA4000-MAA) showed the efficiency to reduce the blood glucose level in diabetic rabbits and the effect was lasted for 8–10 h. Insulin loaded crosslinked poly (PEGDMA-MAA) hydrogel microparticles have strong potential to be used as a convenient, safe, and effective oral delivery system for diabetic patients.

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