Synthesis and Characterization of a Porous Poly(hydroxyethylmethacrylate-*co*-ethylene Glycol dimethacrylate)-Based Hydrogel Device for the Implantable Delivery of Insulin

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ABSTRACT: Poly(hydroxyethylmethacrylate-*co*-ethylene glycol dimethacrylate) [poly(HEMA-*co*-EGDMA)]-based hydrogel devices were synthesized by a free-radical polymerization reaction with 2-hydroxyethylmethacrylate as the monomer, different concentrations of ethylene glycol dimethacrylate (EGDMA) as the crosslinking agent, and ammonium persulfate/*N*,*N*,*N*′,*N*′-*tetra*-methyl ethylenediamine as the free-radical initiator. The porosity of the poly(HEMA-*co*-EGDMA) hydrogels was controlled with water as the porogen. The Fourier transform infrared spectrum of poly(HEMA-*co*-EGDMA) showed absorption bands associated with —C=O stretching at 1714 cm^{-1}, C—O—C stretching vibrations at 1152 cm^{-1}, and a broad band at 3500–3800 cm^{-1} corresponding to —OH stretching. Atomic force microscopy studies showed that the hydrogel containing 67% water had pores in the range of 3500–9000 nm, whereas the hydrogel containing 7% water did not show measurable pores. The hydrogel synthesized with 1% EGDMA showed 50% thallium-201 release within the first 30 min and about 80% release within 60 min. *In vitro* insulin-release studies suggested that the hydrogel with 27% water showed sustained release up to 120 min, whereas the hydrogels with 47 and 67% water showed that nearly all of the insulin was released within 60 min. Hydrogel devices synthesized with 27% water and filled with insulin particles showed sustained release for up to 8 days, whereas the hydrogels synthesized with 47 and 67% water released insulin completely within 3 days of administration. Animal studies suggested that the hydrogel devices synthesized with 27% water and filled with insulin-loaded particles (120 IU) were able to control blood glucose levels for up to 5 days after implantation. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: biomaterials; copolymers; hydrogels

INTRODUCTION

Hydrogels can be defined as polymers that have the ability to swell in water or aqueous systems without dissolving in them.[1] The development of hydrogels started in the beginning of the 1960s with the production of 2-hydroxyethylmethacrylate (HEMA)-based polymers,[2] which had a swelling capacity of 40–50%. These highly hydrophilic polymers were mainly used in sanitary products, such as diapers,[3] materials for wound closure,[4,5] intraocular and contact lenses,[6–9] soil improvement, and vegetal growth.[10]

Much of the existing work has been carried out on polyacrylates and their derivatives; the composition of such polymers can be easily changed to influence the permeability and diffusion patterns of the hydrogel. For this reason, it has been possible to synthesize hydrogels that can be used to immobilize a great variety of compounds, including drugs,[11,12] proteins,[13,14] and even cells.[15] In this way, different systems with different applications can be obtained.

The controlled delivery of therapeutic agents with acrylate polymers has become a popular method of drug administration in recent years. Drug administration can range from the taking of a daily multivitamin to a detailed combination approach to treating cancer or diabetes. These controlled delivery systems have several advantages over conventional delivery methods. When a pharmaceutical agent is encapsulated within, attached to, or loaded on a polymer or lipid, drug safety and efficacy can be greatly improved. For example, implantable drug-delivery devices can reduce the chance for both underdosing and overdosing, reduce the number of necessary administrations, be more localized, make the active

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agents serve a better use, and increase patient compliance.

Polyacrylates are frequently used as implants. Their rubberlike flexibility when hydrated (which minimizes the mechanical damage of the surrounding tissues) and low surface tension (which minimizes cell adsorption and adhesion) are its main advantages for such applications.[16] Although hydrogels have a number of nonbiomedical applications (e.g., in agriculture), their use in the field of medicine and pharmacy is promising.[17] Part of this success is due to some important properties of hydrogels, including their ability to absorb aqueous solutions without losing shape or mechanical strength. These characteristics are commonly found in many natural constituents of the human body, such as muscles, tendons, and cartilage. Moreover, hydrogels usually exhibit good biocompatibility with blood, body fluids, and tissues. Unlike other hydrogels, HEMA-based hydrogels are soluble in water; other polymers exhibit only limited solubility.[18,19] This phase behavior allows the formation of a macroporous spongelike structure when the hydrogels are reacted in dilute solutions of monomers.[20] Porous forms of poly(hydroxyethylmethacrylate-*co*-ethylene glycol dimethacrylate) [poly(HEMA-*co*-EGDMA)] have also been studied for potential applications in implants, drug-delivery applications, soft tissue replacement, breast augmentation, and nasal cartilage replacement.

In this article, we describe the synthesis of polymeric hydrogel implants based on 2-HEMA and their characterization via the evaluation of properties such as swelling capacity, glass-transition temperature (T_g), pore size, and *in vitro* release of thallium-201 as a model compound. Insulin-loaded poly (HEMA-*co*-EGDMA)-based hydrogel devices were also evaluated in diabetic male New Zealand rabbits after implantation.

EXPERIMENTAL

Materials

HEMA, ethylene glycol dimethacrylate (EGDMA), *N,N,N′,N′-tetra*-methyl ethylenediamine (TEMED), and ammonium persulfate (APS) were obtained from Merck Darmstadt, Germany. Water, methanol, and acetonitrile (high-performance liquid chromatography grade) were supplied by Ranbaxy Chemicals (Delhi, India). 2,2′-Azobisisobutyronitrile (AIBN) was obtained from Acros Organics (NJ), whereas sodium sulfate, sodium hydroxide pellets, and alloxan were obtained from Central Drug House (Delhi, India). Monocomponent human insulin (rDNA origin) with a concentration of 100 IU/mL from Eli Lilly and Co. was used as received. Thallium-201 was supplied by the Board of Radiation and Isotope Technology, Bhabha Atomic Research Centre (Mumbai, India). The insulin-loaded poly(PEGDMA4000–MAA) microparticles were synthesized earlier by our group.[21]

Experimental animals

Male New Zealand white rabbits weighing 2.5–3.0 kg were provided by the Experimental Animal Facility of the Institute of Nuclear Medicine and Allied Sciences (Delhi, India). The study protocol was reviewed and approved by the Institutional Animal Ethics Committee.

Synthesis of the poly(HEMA-*co*-EGDMA)-based hydrogel

Polymeric hydrogels based on HEMA were synthesized by bulk polymerization with 0.4% AIBN as the free-radical initiator and variable amounts (1–4%) of EGDMA as the crosslinking agent.[22] The reaction mixtures were poured into cylindrical polypropylene (PP) molds approximately 5 mm in diameter and equipped with a Teflon rod suspended vertically at the center and approximately 1.5 mm in diameter at one end and with an elastomeric piston at the other end of the cylinder. After 3 h of reaction at 60°C, the hydrogel was pushed out of the PP mold with the piston and washed repeatedly with deionized water to remove the unbound monomers.

In the same manner, poly(HEMA-*co*-EGDMA)-based hydrogels were also synthesized with 0.6% APS/0.8% TEMED (percentage of monomer concentration) as the redox free-radical initiator. Different concentrations of EGDMA (1–5%) as the crosslinking monomer and water (0–60%) as the porogen (pore-generating solvent) were also added to the reaction mixture, poured into the PP mold, and kept at room temperature for 15–20 min. The hydrogels were taken out from the mold, postcured at 80°C for 2 h, and washed repeatedly with deionized water to remove the unbound monomers.

Characterization of the poly(HEMA-*co*-EGDMA)-based hydrogels

Polymeric hydrogels based on HEMA were synthesized with 1–5% EGDMA as the crosslinker and 0–80% water as the porogen. The characterization of the poly(HEMA-*co*-EGDMA) hydrogels was carried out with three samples.

Fourier transform infrared (FTIR) analysis

Attenuated total reflectance–FTIR spectroscopy of vacuum-dried samples of the poly(HEMA-*co*-EGDMA) hydrogels synthesized with different percentages of

EGDMA as the crosslinker was recorded on a PerkinElmer Spectrum One spectrometer (Waltham, USA). FTIR spectroscopy of all of the samples were recorded in the transmittance mode.

Thermal characterization

Differential scanning calorimetry (DSC) studies were done with a PerkinElmer DSC7 instrument in the temperature range 50–380°C in static air at a heating rate of 10°C/min. Vacuum-dried samples (5 ± 2 mg) were used.

A TA 2100 thermal analyzer having a 951 TG module was used for the thermal from Perkin Elmer, Massachusetts, USA characterization of the poly (HEMA-*co*-EGDMA) polymeric hydrogels in dry powder form. Thermogravimetric analysis (TGA) of 10 ± 2-mg samples were done in the temperature range 50–850°C in an N_2 atmosphere (flow rate = 60 cm^3/min) at a heating rate of 20°C/min.

Swelling studies

The swelling characteristics of the polymeric poly (HEMA-*co*-EGDMA) hydrogels were determined by immersion of the dried test samples separately in distilled water solutions at 37°C. At specified time intervals, the samples were removed from the swelling media and blotted with a piece of paper for 5 s to absorb excess water on their surfaces. The swelling percentage (Q_s) of the test samples was calculated with eq. (1):

$$Q_s = [(W_s - W_d)/W_d] \times 100 \quad (1)$$

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

Atomic force microscopy (AFM)

Surface morphological studies of the polymeric hydrogels synthesized with 1% EGDMA and different amounts of water (0–60%) were carried out with scanning probe microscopy (SPM; SPM–AFM, Molecular Devices and Tools for Nanotechnology (NT-MDT), Moscow, Russia). All of the hydrogels were freeze-dried for 24 h, and a small piece of sample was observed under AFM.

In vitro release of thallium-201 from the poly(HEMA-*co*-EGDMA) hydrogel

Thallium-201 was taken as a model compound (small radioactive isotope) to observe the release profile from the poly(HEMA-*co*-EGDMA) hydrogels crosslinked with different percentages of EGDMA. Activities of 200 μCi were loaded in the poly (HEMA-*co*-EGDMA) hydrogels separately, and the open end of the hydrogel device was sealed with a cynoacrylate adhesive. The hydrogel devices were kept in 20 mL of saline water (a 0.9*N* NaCl solution) as surrounding media. The hydrogel devices were taken out, and the activity released into the surrounding media was checked at fixed time intervals with a γ counter (Electronics Corp. India, Ltd., Mumbai, India).

In vitro release of insulin

Loading and *in vitro* release of insulin from the poly(HEMA-*co*-EGDMA) hydrogels

The poly(HEMA-*co*-EGDMA) hydrogels synthesized with 1% EGDMA as the crosslinker and variable amounts of water as the porogen (0–60%) were found to exhibit desirable properties and, hence, were selected for insulin loading and release studies.

Two milliliters of concentrated insulin (100 IU/mL) was loaded into the poly(HEMA-*co*-EGDMA) hydrogel device separately, and the open end of the hydrogel device was sealed with a cynoacrylate adhesive. The hydrogel devices were kept in 20 mL of a 0.1*N* HCl solution as the surrounding media. The surrounding media (20 mL of a 0.1*N* HCl solution) was replaced periodically. A small volume (50 μL) was taken from the surrounding media at fixed time intervals, and the insulin release from the hydrogel devices was evaluated with reverse-phase high-performance liquid chromatography.

Loading and *in vitro* release of the insulin-loaded microparticles from the hydrogel devices

Poly(PEGDMA4000–MAA) pH-sensitive microparticles synthesized in our laboratory by suspension polymerization with poly(ethylene glycol) dimethacrylate 4000 and methacrylic acid as the monomer were loaded with insulin per Kumar et al.[21] Insulin-loaded poly(PEGDMA4000–MAA) copolymeric microparticles with 120 IU of insulin were loaded into the poly(HEMA-*co*-EGDMA) hydrogel device, and the open end of the hydrogel device was sealed with a cynoacrylate adhesive. The hydrogel devices were kept separately in 20 mL of a 0.1*N* solution of HCl as the surrounding media. A small volume (50 μL) was taken from the surrounding media at fixed time intervals, and the insulin release was evaluated with reverse-phase high-performance liquid chromatography to determine the insulin concentration eluted from the hydrogel devices into the surrounding media.

In vivo studies

An insulin-loaded poly(HEMA-*co*-EGDMA) hydrogel device was used for *in vivo* release studies. Diabetes was induced in the male experimental rabbits

TABLE I
Poly(HEMA-*co*-EGDMA) Hydrogels Synthesized with 0.4% AIBN as the Initiator

	EGDMA		Physical properties of the hydrogel device	
HEMA (g)	Monomer (%)	Volume (μL)	In mold	In water after 24 h swelling
4.95	1	50	Transparent, hard	Poor strength
4.9	2	100	Transparent, hard	Good strength
4.85	3	150	Transparent, hard	Cracks appeared on the surface
4.8	4	200	Transparent, brittle	Cracked within water
4.75	5	250	Transparent, brittle	Cracks, poor flexibility

with a single dose of alloxan (150 mg/kg of body weight) dissolved in sterile water injected intravenously, and their glucose levels were checked for diabetic conditions after 48 h. Animals with glucose levels at 300 mg/dL or greater were used for further study.[23] Twelve diabetic rabbits were randomly selected, divided into three groups, and housed in separate cages. The rabbits were anesthetized with 40 mg/kg thiopentane (Ketalar, Parke-Davis, Hospira, Illinois, USA) intraperitoneally. In the middle upper left of the dorsum, just at the left of the middle line, a tricotomy was performed, followed by a skin incision, which allowed access to the subcutaneous space where the hydrogel implant was inserted after it was washed with pure ethanol and air-dried. The skin was sutured with 3/0 silk. The same procedure was also done without implantation of any polymer (the positive control). Insulin-loaded poly(HEMA-*co*-EGDMA) hydrogel devices were implanted subcutaneously in the rabbits in such a way that one end of the hydrogel device protruded outside the skin so we could insert a known amount of insulin at regular intervals into the device. Animal studies were carried out in two phases. Animals in the first group were taken as controls, in which poly(HEMA-*co*-EGDMA) hydrogel devices without any insulin were implanted subcutaneously.

In vivo insulin-release studies from the poly(HEMA-*co*-EGDMA) implantable devices

A second group of animals were subcutaneously implanted with poly(HEMA-*co*-EGDMA) hydrogel devices in such a way that one end of the hydrogel device protruded outside the skin so we could inject a known amount of insulin at regular intervals.

In vivo insulin-release studies from the poly(HEMA-*co*-EGDMA) implantable devices containing insulin-loaded poly(PEGDMA4000–MAA) microparticles

A poly(HEMA-*co*-EGDMA) hydrogel device synthesized with 27% water as the porogen was loaded with poly(PEGDMA4000–MAA) microparticles (120 IU of insulin) and sealed with a cynoacrylate adhesive at the open end. A third group of diabetic rabbits was subcutaneously implanted with this insulin-loaded poly(HEMA-*co*-EGDMA) device, whereas the first group of diabetic animals were taken as a control, and the poly(HEMA-*co*-EGDMA) device was implanted without any insulin. Blood samples were collected from the ear vein of the rabbits every 24 h after implantation of the devices, and the blood glucose level was measured with an Accutrend blood glucometer (Roche, Mnnheim, Germany). The reduction in blood glucose concentration was obtained from the blood glucose concentration–time curves (percentage change from the initial concentration) of each rabbit with eq. (2):

$$\%\text{Change} = [(F - P_t)/F] \times 100 \quad (2)$$

where F is the fasting glucose level and P_t is the plasma glucose level at time t after the implantation of the poly(HEMA-*co*-EGDMA) device.

RESULTS AND DISCUSSION

Synthesis of the polymeric hydrogel device

Polymers can be prepared with a wide range of methods that lead to the control of the shape of the final product. Poly(HEMA-*co*-EGDMA) hydrogel devices were prepared by a bulk polymerization reaction in a spatially designed mold of a PP cylinder fitted with Teflon rod. It was observed in preliminary studies that the hydrogel devices synthesized with AIBN as the free-radical initiator were transparent and bubble free and had good mechanical properties that were independent of the crosslinker percentage, as shown in Table I. However, the hydrogel devices often cracked during removal from the mold because of their hard and brittle nature. Tranoudis and Efron[24] also reported the tensile and mechanical strengths of HEMA-based hydrogels and found similar results. The physical properties of the hydrogel devices in the mold and after 24 h of swelling in water are shown in Table I. The poly(HEMA-*co*-EGDMA) hydrogel devices synthesized with AIBN as the initiator cracked within 24 h of swelling in water, independent of the crosslinker percentage. It was probably due to the brittle nature of the

TABLE II
Poly(HEMA-*co*-EGDMA) Hydrogels Synthesized with APS/TEMED as the Initiator

	EGDMA		Physical properties of the hydrogel device	
HEMA (g)	Monomer (%)	Volume (μl)	In the mold	In water with over 24 h of swelling
5	0	0	Opaque, flexible	Opaque, poor mechanical properties
4.95	1	50	Opaque, flexible	Opaque, flexible, bubble-free
4.90	2	100	Opaque, hard	Opaque with reduced flexibility
4.85	3	150	Opaque, hard	Opaque with poor flexibility
4.80	4	200	Opaque, hard	Opaque, poor flexibility, and breaking
4.75	5	250	Opaque, brittle	Broke within 24 h of swelling

Hydrogel devices with 7% water of monomer concentration at room temperature.

hydrogel devices. The hydrogel device synthesized with AIBN as the initiator were suitable for the delivery of insulin from implants because of their brittle nature.

Hydrogel devices synthesized with APS/TEMED as the free-radical initiator were easily removed from the PP mold because of incomplete polymerization and the presence of water (used as the initiator solution), which acted as a plasticizer. Hydrogel devices synthesized with APS/TEMED as the initiator were found to have better physical properties in comparison to hydrogel device synthesized with AIBN. Thus, further studies were carried out with the APS/TEMED hydrogel devices. Poly(HEMA-*co*-EGDMA) hydrogel devices were collected easily from the PP mold and immersed into hot water (80°C) to remove any unreacted monomer. After 24 h of swelling in water, the hydrogel device synthesized with 0% EGDMA was found to be flexible but mechanically poor, as it broke down with the application of a little pressure. The polymeric hydrogels with 2 and 3% EGDMA were found to be mechanically strong after overnight swelling in water, but cracks appeared over the surface after 15 days. The polymeric hydrogel with 5% crosslinker showed cracks after just overnight swelling, as shown in Table II. This was probably due to an increase in the crosslinker percentage (EGDMA), which resulted in a brittle nature in the hydrogel device, which consequently cracked on the surface. The polymeric hydrogel with 1% EGDMA was flexible, opaque, and mechanically strong. It was found to be crack free on the surface, even after a longer duration of swelling. The polymeric hydrogel device with 1% EGDMA was found to be the best and was, thus, selected for further study.

The generation of pores in the poly(HEMA-*co*-EGDMA) hydrogel devices was achieved by a pore-generating solvent called a porogen (water), which was added during the polymerization reaction. Variable amounts of water (0–80% in addition to 7% water in the initiator solution) was added during the polymerization of HEMA containing 1% EGDMA as the crosslinking monomer (Table III). Hydrogel devices containing 20% water contents were milky white and flexible with optimal mechanical strength. The poly(HEMA-*co*-EGDMA) hydrogel devices having 40 and 60% water contents were milky white, spongy, and highly flexible but with poor mechanical properties. The hydrogel devices containing 80% water were also synthesized and found to be milky white, and spongy with a noticeably porous structure but were mechanically poor and were, thus, not used in further study. On the basis of the mechanical properties, the hydrogel device synthesized with 20% water as the porogen was found to be the best and

TABLE III
Poly(HEMA-*co*-EGDMA) Hydrogel Synthesized with Various Concentrations of Water as the Porogen

		Water content		Physical properties of the hydrogel device
HEMA (g)	EGDMA (μL)	g	%	
10	50	0	0	Opaque, flexible
9	50	1	10	Opaque, flexible
8	50	2	20	Milky white, flexible
6	50	4	40	Milky white, highly flexible, and with poor mechanical properties
4	50	6	60	Milky white, spongy, highly flexible, and with poor mechanical properties
2	50	8	80	Milky white, spongy, highly flexible, with poor mechanical properties, and noticeably porous

Synthesis of polymeric hydrogel devices with APS/TEMED at room temperature.

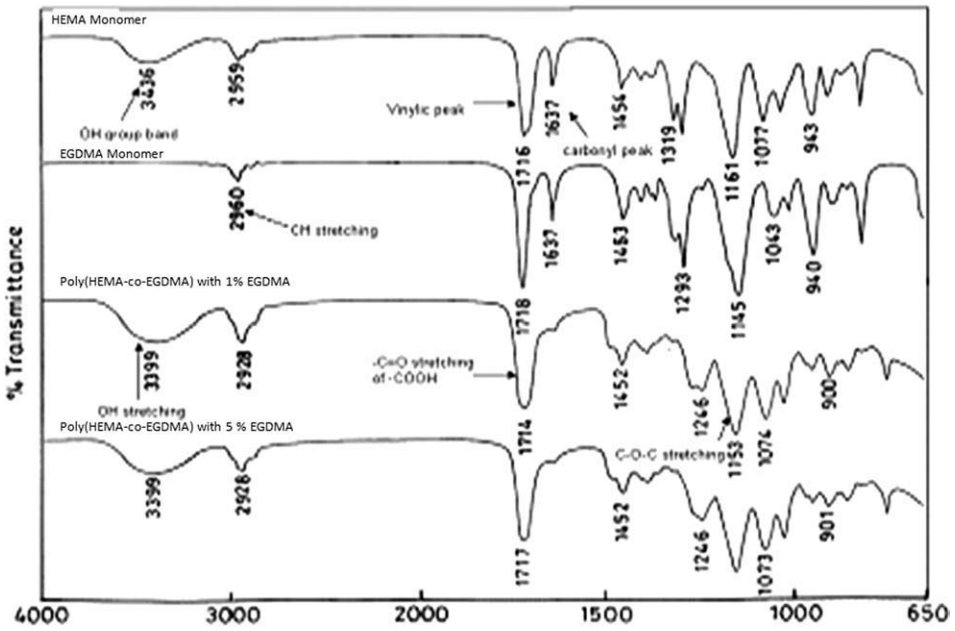

Figure 1 FTIR spectra of the HEMA monomer, EGDMA monomer, and poly(HEMA-*co*-EGDMA) hydrogel with 1 and 5% EGDMA.

was, thus, used for the animal studies. It was also reported earlier that the amount of water added to the reaction mixture produces most dramatic effect on the size of the pores in a poly(hydroxyethylmethacrylate) sponge. Chirila et al.[25] in 1998 demonstrated that when the water content was below 45–50%, the poly(HEMA-*co*-EGDMA) polymer chains remained soluble and did not form a two-phase system. When the reaction mixture's water content was increased, phase separation occurred, with excess water acting as a pore-forming agent. We further increased the water content; the number of water molecules excluded from the polymer phase increased to create larger voids between the polymer droplets.[26,27] It is well established that networks containing 85% or more water possess pore sizes that are large enough for cellular invasion.[28,29] Unfortunately, these high water solutions result in materials with characteristically weak mechanical properties and larger pore size distributions.

Characterization of the poly(HEMA-*co*-EGDMA)-based hydrogel devices

FTIR spectroscopy

An FTIR spectrum of hydrogel devices synthesized with different amounts (1–5%) of EGDMA is given in Figure 1. The FTIR spectrum of the HEMA monomer showed a band at 3436 cm^{-1} due to OH groups, a peak at 2959 cm^{-1} due to CH stretching, a vinylic peak at $\sim$ 1637 cm^{-1}, and C—O—C stretching at 1161 cm^{-1}, as shown in Figure 1. EGDMA showed a peak at 2960 cm^{-1} due to CH stretching, a vinylic peak at $\sim$ 1637 cm^{-1}, and C—O—C stretching at 1145 cm^{-1}. The FTIR spectrum of poly(HEMA-*co*-EGDMA) showed absorption bands associated with the —C=O stretching of carboxylic acid groups (—COOH) at 1714 cm^{-1} and C—O—C stretching vibrations at 1152 cm^{-1}. The appearance of a broad band at 3500–3800 cm^{-1} corresponded to —OH stretching vibrations of the poly(HEMA-*co*-EGDMA) hydrogel. The FTIR spectra of hydrogels with 1 and 5% EGDMA did not show any significant differences.

DSC

The T_g values of the poly(HEMA-*co*-EGDMA) hydrogel devices were found to increase increasing concentration of EGDMA, as shown in Figure 2. The lowest T_g was found to be 82.8°C for the hydrogel device having 1% EGDMA as a crosslinker, whereas the highest (95.5°C) was observed in the case of hydrogel devices containing 5% crosslinker. The hydrogels synthesized with 2, 3, and 4% EGDMA as the crosslinker had T_g values of 84.4, 86.4, and 91.7°C, respectively. An increase in crosslinker concentration (1–5%) increased T_g because of the increase in the crosslinking ratio in the hydrogel network; this also increased the brittle nature of the hydrogel system, as discussed previously.

TGA

TGA of the poly(HEMA-*co*-EGDMA) hydrogels with variable percentages of EGDMA was carried out. In the case of poly(HEMA-*co*-EGDMA), the initial

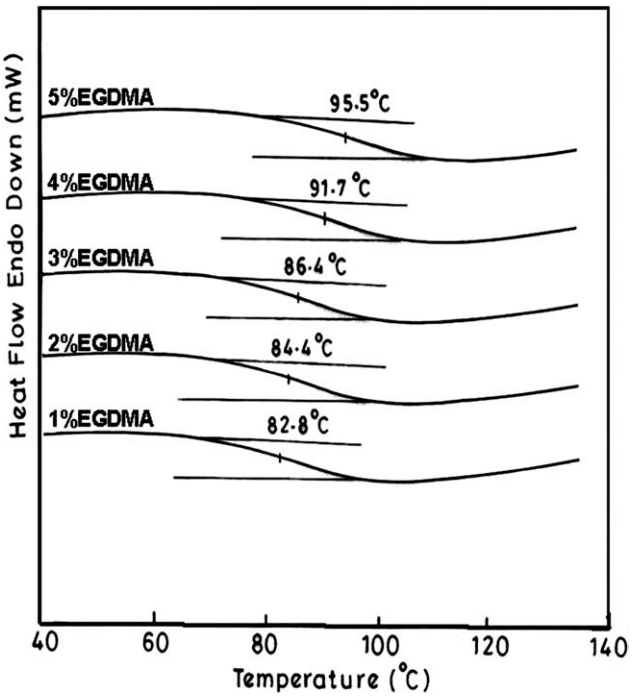

Figure 2 DSC thermograms of the poly(HEMA-*co*-EGDMA) hydrogel devices synthesized with different concentrations of EGDMA as a crosslinker.

weight loss was observed at 150°C and was probably due to the evaporation of residual moisture, independent of the EGDMA concentration. The subsequent weight loss observed at approximately 285°C was due to the onset of degradation in poly (HEMA-*co*-EGDMA). The onset degradation temperature increased with increasing crosslinker (EGDMA) concentration in the poly(HEMA-*co*-EGDMA) hydrogel because of an increase in the crosslinking density. The TGA value of the poly(HEMA-*co*-EGDMA) hydrogel synthesized with 1, 2, 3, and 5% EGDMA was found to be 285, 294, 302, and 312°C, respectively. The degradation taking place at this stage resulted from the main-chain degradation of poly (HEMA-*co*-EGDMA). The degradation was initiated by the release of carbon dioxide due to decarboxylation, as reported by other authors.

Swelling studies

Poly(HEMA-*co*-EGDMA) hydrogel devices synthesized with 1% EGDMA and 7% water (used to dissolve the initiators) showed the highest Q_s (53.1%), as shown in Figure 3. The lowest Q_s (15.2%) was observed in the hydrogel synthesized with 5% EGDMA. The decrease in Q_s was due to increases in the crosslinking density and hydrophobicity with increasing EGDMA concentration in the poly (HEMA-*co*-EGDMA) hydrogel. Similar results were reported earlier by other authors.[27]

AFM

AFM micrographs of the poly(HEMA-*co*-EGDMA) hydrogel devices with 1% EGDMA crosslinker and variable amounts of water (0–60%) are given in Figures 4–7. The poly(HEMA-*co*-EGDMA) device synthesized with 7% water (used to dissolve the initiator mixture) showed no pores on the surface, whereas roughness on the surface was clearly observed, as shown in Figure 4(A,B). The poly (HEMA-*co*-EGDMA) device synthesized with 27% water showed about 800-nm pores, which were unevenly distributed on the hydrogel surface, as shown in Figure 5(A,B). The hydrogel device synthesized with 47% water showed 2500–5000-nm pores that were unevenly distributed on the hydrogel surface, as shown in Figure 6(A,B). The polymeric hydrogel containing 67% water showed pore sizes in the range 3500–9000 nm, as shown in Figure 7(A,B). The pore size increased with increasing water content in the reaction mixture of the hydrogel device. The increase in pore size was due to the fact that water did not play any part in the polymerization reaction; instead, it was placed between the reaction matrices and created voids in the hydrogel, which appeared as pores thereafter. On the other hand, the pores were distributed unevenly on the surface of the hydrogel device, as water was nonuniformly distributed during the reaction in the PP mold.

In vitro release from the hydrogel devices

In vitro release of thallium-201 from the hydrogel devices

The *in vitro* release of thallium-201 from the poly (HEMA-*co*-EGDMA) hydrogel devices synthesized with different EGDMA percentages is shown in Figure 8. Hydrogel devices synthesized with 1% EGDMA as a crosslinker showed approximately a 50% release of activity within the first 30 min. It was

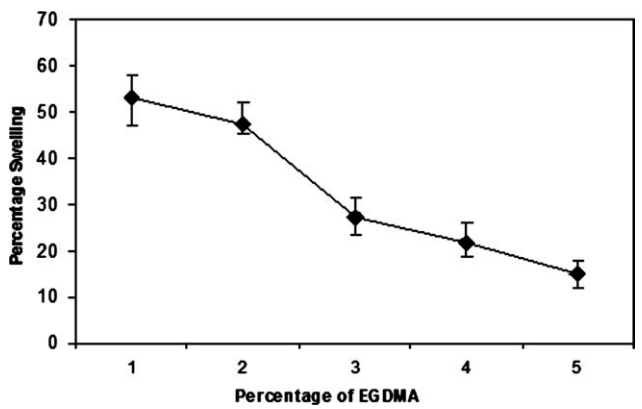

Figure 3 Q_s values of the poly(HEMA-*co*-EGDMA) hydrogel devices synthesized with different concentrations of EGDMA.

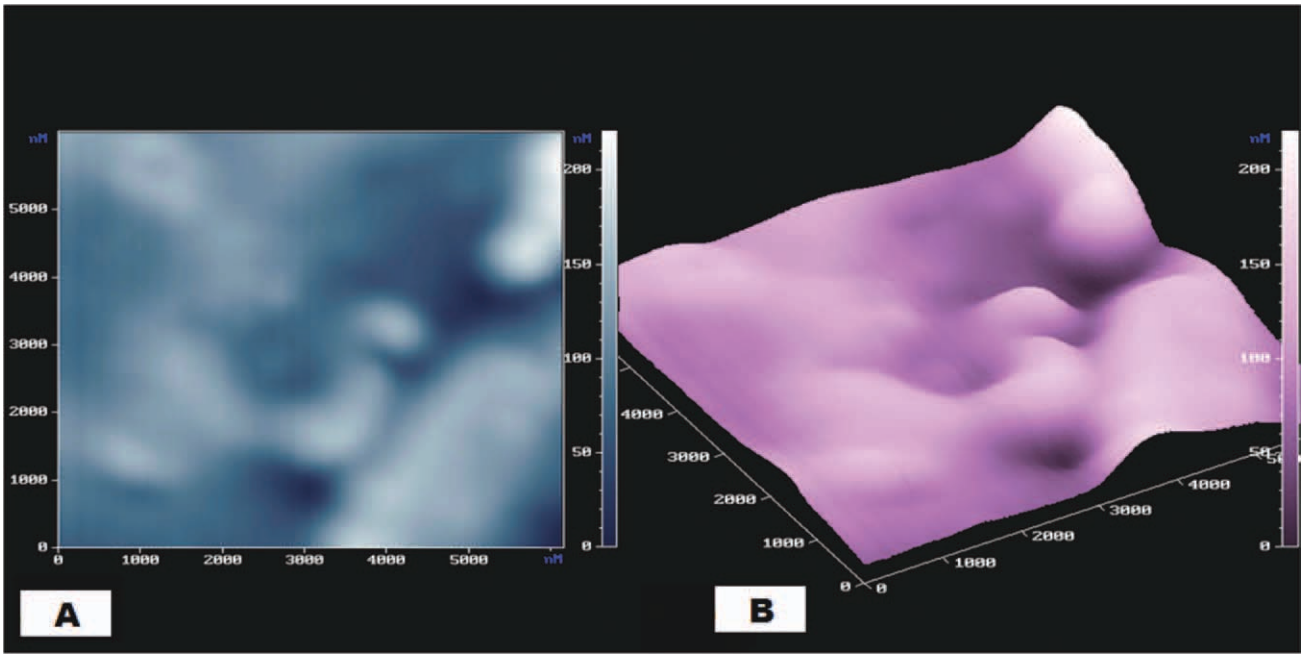

Figure 4 AFM micrograph of the poly(HEMA-*co*-EGDMA) hydrogel synthesized with a 7% water content: (A) two-dimensional and (B) three-dimensional views. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

also observed that 90% thallium-201 was released from the device within the next 45 min. The poly (HEMA-*co*-EGDMA) hydrogel devices synthesized with 3 and 5% EGDMA showed approximately 25% thallium-201 release within first 30 min, whereas only 45% thallium-201 release was observed within the next 105 min. The hydrogel devices synthesized with different concentrations of EGDMA showed sustained release of thallium-201 because increases in the crosslinker percentage in the reaction mixture also increased the crosslinking density of the hydrogel network; this allowed the controlled release of thallium-201 from the network. The maximum release rate was observed in the hydrogel device

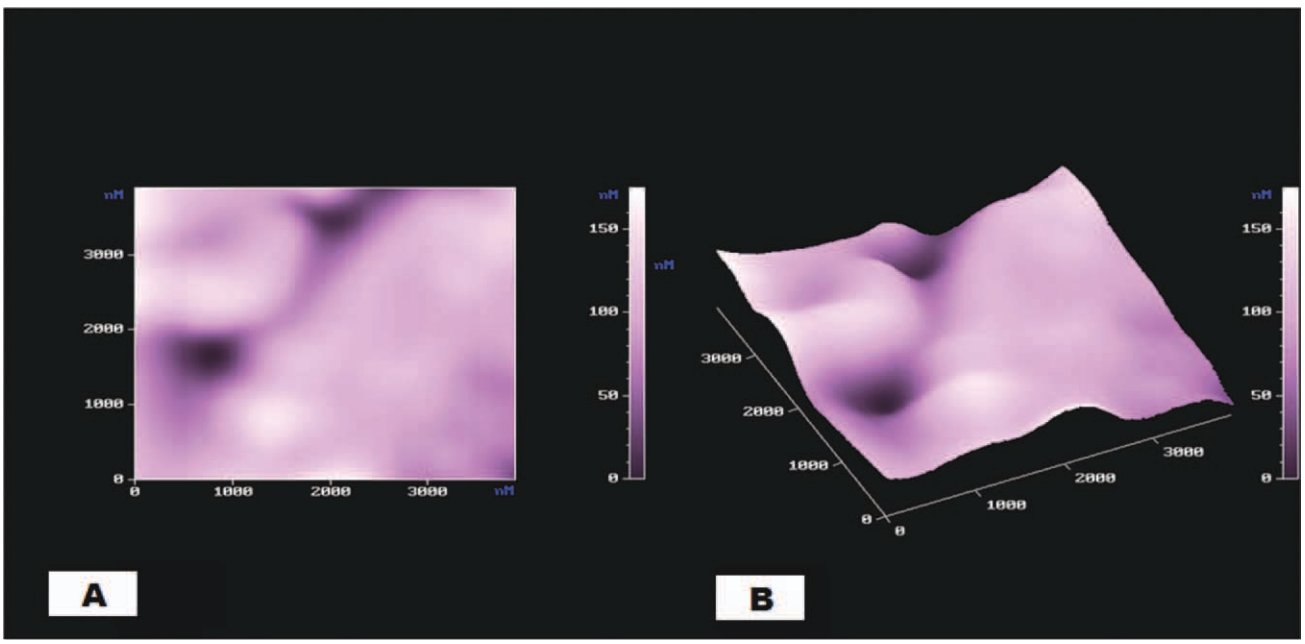

Figure 5 AFM micrograph of the poly(HEMA-*co*-EGDMA) hydrogel synthesized with a 27% water content: (A) two-dimensional and (B) three-dimensional views. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

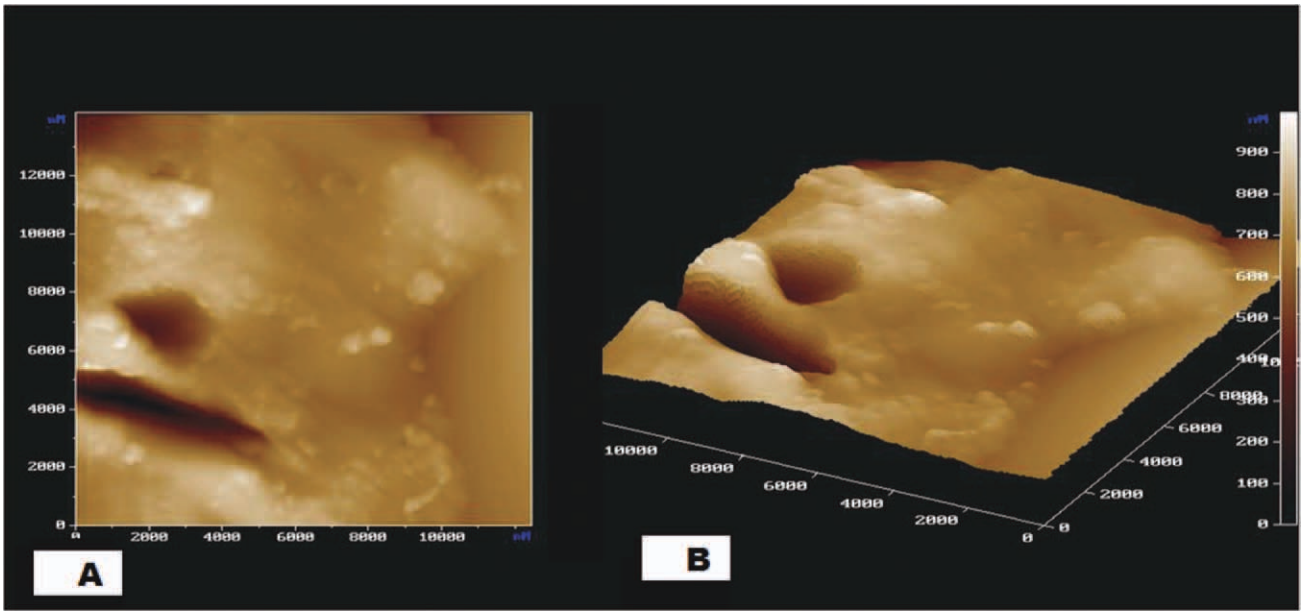

Figure 6 AFM micrograph of the poly(HEMA-*co*-EGDMA) hydrogel synthesized with a 47% water content: (A) two-dimensional and (B) three-dimensional views. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

synthesized with 1% EGDMA because of the lowest crosslinking density, whereas the hydrogel device synthesized with 5% EGDMA showed the lowest release rate (only 45% in 105 min) and released only 50% thallium-201 over a longer duration; thus, these hydrogel devices were not used for further study. The hydrogel devices synthesized with 1% EGDMA were found to be the best on the basis of thallium-201 release and were selected for further study.

Loading and *in vitro* release of insulin from the hydrogel device

The cumulative release values of insulin from the poly(HEMA-*co*-EGDMA) hydrogel devices synthesized with different concentrations (7–67%) of water as the porogen are given in Figure 9. The poly (HEMA-*co*-EGDMA) hydrogel device synthesized with 7% water (the amount of water used to prepare the initiator mixture) showed almost no release of

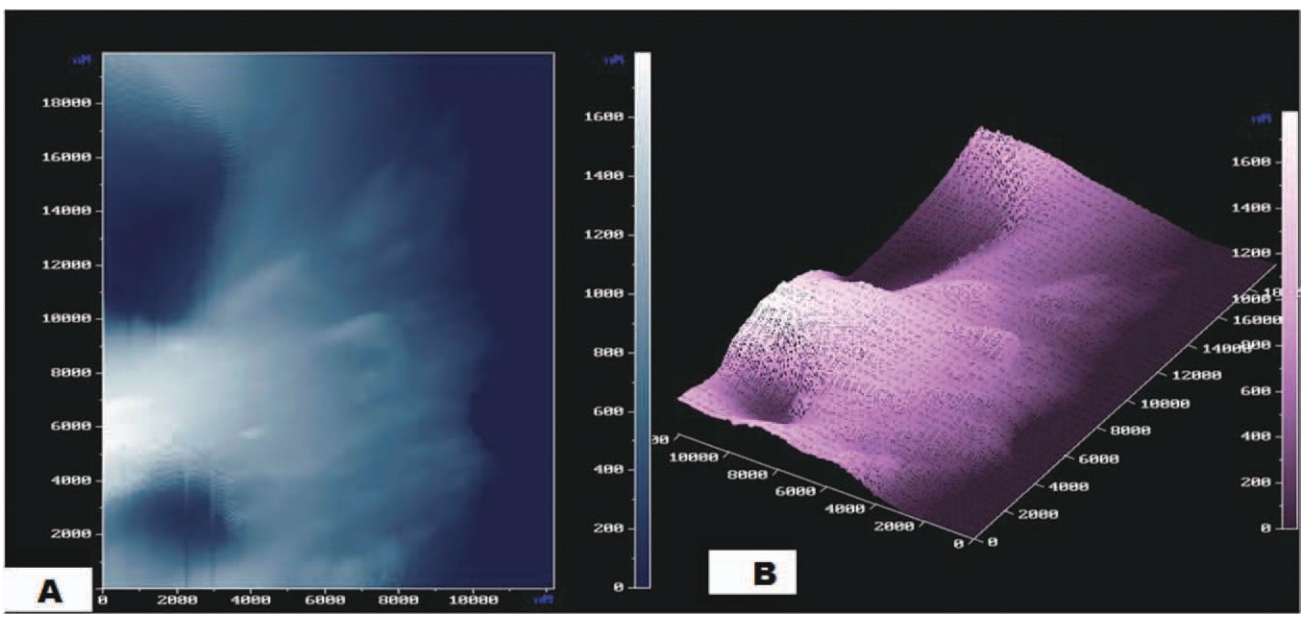

Figure 7 AFM micrograph of the poly(HEMA-*co*-EGDMA) hydrogel synthesized with a 67% water content: (A) two-dimensional and (B) three-dimensional views. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

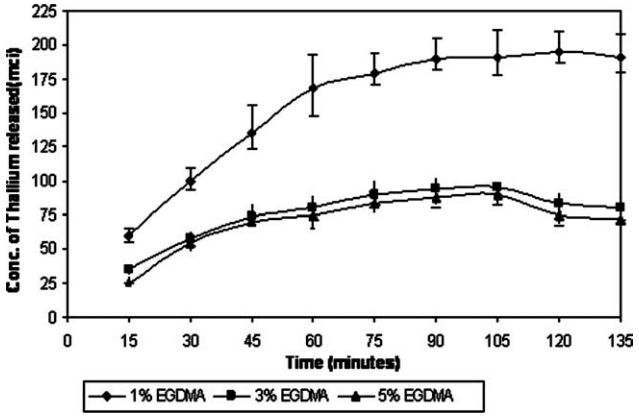

Figure 8 *In vitro* release of thallium-201 from the poly (HEMA-*co*-EGDMA) hydrogel device synthesized with different crosslinker percentages.

insulin in 165 min. The poly(HEMA-*co*-EGDMA) hydrogel devices synthesized with 47 and 67% water showed approximately 50% insulin release within the first 30 min and 90% insulin release within 60 min. The poly(HEMA-*co*-EGDMA) hydrogel device synthesized with 27% water showed approximately 18% insulin release within the first 30 min and only 50% insulin release in 60 min. The rest of the insulin took a longer time (165 min) to get released from the poly(HEMA-*co*-EGDMA) hydrogel device synthesized with 27% water, as shown in Figure 9. The poly(HEMA-*co*-EGDMA) hydrogel device synthesized with 1% EGDMA and 7% water showed the best release profile of thallium-201, whereas the insulin-release profile from the same gel was not satisfactory. This was probably due to the very large size of the insulin molecule in comparison to the thallium-201 molecule. The hydrogel devices synthesized with 7% water as the porogen did not show any observable pores on the surface, as evident from the AFM studies, so the release of the insulin was also lowest as per microscopic studies. On the other hand, the hydrogel devices synthesized with 47 and

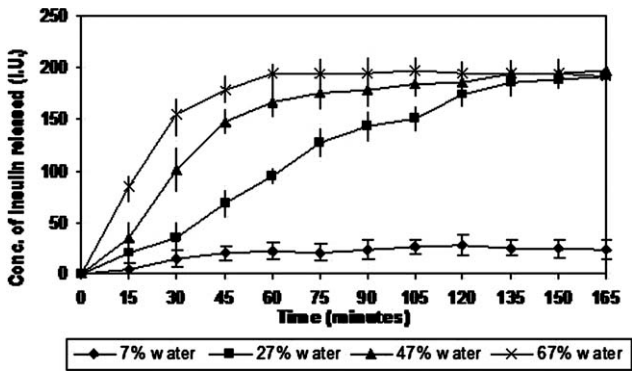

Figure 9 Cumulative release of insulin from the insulin-loaded poly(HEMA-*co*-EGDMA) hydrogel device synthesized with different concentrations of water as a porogen.

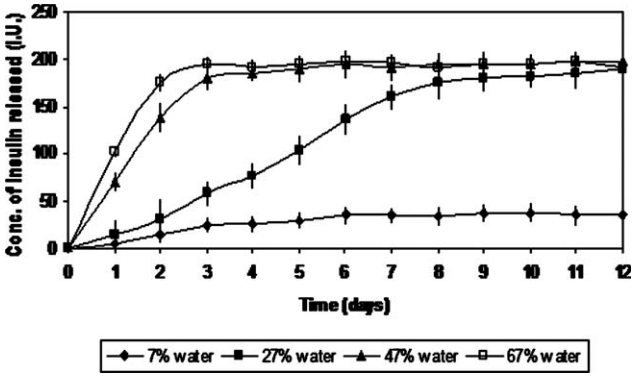

Figure 10 Cumulative release of insulin from insulin-loaded microparticles loaded in poly(HEMA-*co*-EGDMA) hydrogel devices synthesized with different concentrations of water as a porogen.

67% water as the porogen showed a burst release of insulin (50% in 30 min) due to surface pore size of about 5000 nm. The hydrogel devices synthesized with 27% water as the porogen were found to be optimal for further study because they showed good mechanical properties, optimal pore size, and *in vitro* release of insulin. The hydrogel devices synthesized with 27% water as the pore-generating solvent showed a sustained release of insulin, and hence, these were selected for further study.

Loading and *in vitro* release of insulin from the hydrogel device containing insulin-loaded microparticles

The cumulative release of insulin from the poly (HEMA-*co*-EGDMA) hydrogel device filled with insulin-loaded poly(PEGDMA4000–MAA) microparticles is shown in Figure 10. The poly(HEMA-*co*-EGDMA) hydrogel device synthesized with 7% water showed almost no release of insulin in 12 days. The poly(HEMA-*co*-EGDMA) hydrogel devices synthesized with 47 and 67% water showed 40–50% insulin release within the first 24 h and 80–90% insulin release in 48 h (Fig. 10). The poly(HEMA-*co*-EGDMA) hydrogel device synthesized with 27% water showed only 18% insulin release within 2 days and only 50% insulin release in 5 days. The rest of the insulin took a longer time (8–10 days) to be released from the poly(HEMA-*co*-EGDMA) hydrogel device synthesized with 27% water, as shown in Figure 10. The poly(HEMA-*co*-EGDMA) hydrogel device synthesized with 27% water showed a sustained release of insulin, which was maintained for at least 8 days, whereas the hydrogel devices containing 47 and 67% water released the insulin within 2–3 days. The hydrogel devices having 27% water showed a sustained release of insulin up to the 8th day of study. This was because the hydrogel devices containing 47 and 67% water had bigger

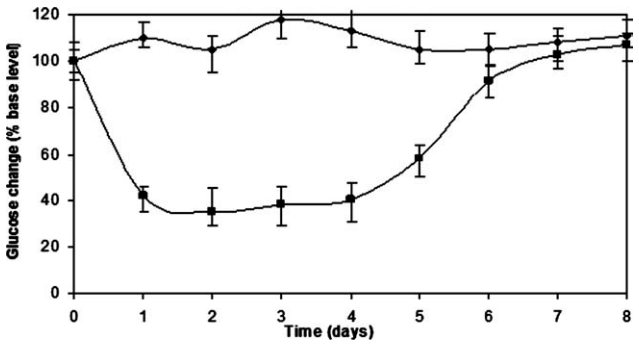

Figure 11 Hypoglycemic effect of the poly(HEMA-*co*-EGDMA) devices: (●) control animals implanted with a device without insulin and (■) animals implanted with poly(HEMA-*co*-EGDMA) device loaded with the poly (PEGDMA4000–MAA) microparticles containing 120 IU of insulin.

pore sizes in comparison to the device synthesized with 7% water; this was also evident from the microscopic studies. The hydrogel devices synthesized with 27% water as the porogen showed the optimum and regulated release of insulin and were, thus, selected for further study.

Animal studies

In vivo insulin-release studies from the poly(HEMA-*co*-EGDMA) implantable device

The poly(HEMA-*co*-EGDMA) hydrogel devices were implanted in the peritoneal cavities of the experimental animals. The results of the implantable poly (HEMA-*co*-EGDMA) device were not encouraging, and thus, studies were discontinued; these results were similar to those observed by other authors.[30] A burst release of insulin was observed from the porous poly(HEMA-*co*-EGDMA)-based hydrogel device probably because of the large and uneven pore sizes over the surface. On the other hand, there was a chance of infection because of skin discontinuity. Considering all of these parameters, we carried out the next set of experiments by implanting the whole device beneath the skin.

In vivo insulin-release studies from the poly(HEMA-*co*-EGDMA) implantable device with insulin-loaded poly(PEGDMA4000–MAA) microparticles

The hypoglycemic effects of the poly(HEMA-*co*-EGDMA) implantable device containing 120 IU of insulin loaded in poly(PEGDMA4000–MAA) microparticles is given in Figure 11. The first group of animals showed a 60% reduction in blood glucose level within the first 24 h (the 1st day), whereas the highest reduction (70%) in blood glucose level was observed on day 3 after implantation. The animals maintained the 70% reduction in blood glucose level up to the 4th day, whereas on the 5th day, a 40% reduction in blood glucose level was observed, and then, the blood glucose level approached the control value by the 6th day. The second group of animals (control animals) did not show any changes in their blood glucose levels, as shown in Figure 11. The 60% reduction in the blood glucose level observed on day 1 was due to an uninterpreted release of insulin from the polymeric hydrogel device, whereas the same was maintained up to day 4 after implantation. On day 5, an increase in the blood glucose level was observed, which was due to either the degradation of insulin inside the device or a blockage of micropores on the surface of the poly (HEMA-*co*-EGDMA) hydrogel device.

CONCLUSIONS

Hydrogel devices based on HEMA were found to be biostable and hydrophilic. Poly(HEMA-*co*-EGDMA) hydrogel devices synthesized with 1% EGDMA as the crosslinker and 27% water as the pore-generating solvent were found to be the best in view of the insulin release and were able to release insulin for up to 7 days.

Animal studies suggested that the same hydrogel device controlled the blood sugar level up to 5 days when filled with insulin-loaded poly(PEGDMA4000–MAA) microparticles. Detailed studies with high insulin-loaded poly(PEGDMA4000–MAA) microparticles filled in hydrogel devices showed the ability to control blood glucose levels for at least 1 month until the devices were withdrawn.

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