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Formulation and Evaluation of Self-Regulated Insulin Delivery System Based on poly(HEMA-co-DMAEMA) Hydrogels

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In the present work a self-regulated insulin delivery system based on the hydrogel poly(2-hydroxyethyl methacrylate-co-N,N-dimethylaminoethyl methacrylate) with entrapped glucose oxidase, catalase and insulin was developed and evaluated both by *in vitro* and *in vivo* studies. The hydrogels were characterized by FTIR, DSC, SEM and elemental analysis. The swelling studies were carried out in different pH and glucose solutions. The mesh size of the hydrogels and diffusion coefficient of water and insulin in different glucose solution was calculated. The effect of the crosslinking agent (ethylene glycol dimethacrylate) concentration (0–2% w/w) on swelling and insulin release was studied. The equilibrium swelling and insulin release was found to depend on the external glucose concentration and dimethylaminoethyl methacrylate content of the hydrogels. The *in vivo* studies indicated that the entrapped insulin was stable and was effective in reducing the blood glucose of streptozotocin induced diabetic rats. The histopathological studies revealed that there was no fibrous tissue encapsulation after 56 days of implantation.

Keywords: insulin; self-regulated; swelling; biocompatibility; hydrogels

1 Introduction

Diabetes mellitus refers to a group of metabolic diseases characterized by chronic hyperglycemia, due to defects in insulin secretion, insulin action, or both, associated with the development of long-term vascular and neuropathic complications (1). According to the American Diabetes Association Diabetes mellitus can be classified into four etiologic types: type 1 diabetes, which is either autoimmune mediated (type 1a) or idiopathic (type 1b) destruction of the insulin-producing beta cells of the pancreas and is characterized by absolute insulin deficiency; type 2 diabetes, characterized by a combination of insulin resistance and inadequate compensatory insulin secretory response. Other specific types of diabetes include genetic defects in beta cell function and insulin action, diseases of the exocrine pancreas, endocrinopathies, and drug or chemically induced insulin deficiency and gestational diabetes mellitus (2). Due to the extensive degradation of orally administered insulin by proteases in the gastrointestinal tract, insulin can currently be given only by subcutaneous injection (3). The disadvantages of subcutaneous insulin injection include the unphysiological site of administration (which results in high insulin concentrations in the peripheral blood relative to those achieved in the portal vein), the local degradation of insulin in the subcutaneous depot (i.e., bioavailability $<100\%$), the high variability in absorption, and the relatively slow absorption from the subcutaneous tissue. Together these disadvantages lead to suboptimal pharmacodynamic properties of the applied insulin, which does not allow it to mimic the complex physiological insulin secretion pattern (4). There has been much interest in the development of self-regulated delivery systems that releases insulin in response to elevated glucose levels. A self-regulated delivery system is designed to release the insulin in response to changes in glucose concentration (6, 7). There are different types of self-regulated insulin delivery systems based on sensing mechanism and the type of devices used to develop the delivery systems. One such type makes use of hydrogels loaded with glucose oxidase (GOD). This type of system utilizes GOD as the glucose sensor and pH sensitive hydrogel as the insulin release controller. In such a system, glucose is oxidized to gluconic acid, catalyzed by GOD (8) as shown here:

$$\text{Glucose} + O_2 + H_2O \longrightarrow \text{gluconic acid} + H_2O_2$$

Because of the above reaction, pH inside the microenvironment decreases with the increase in the glucose concentration. This causes an increase in the volume of the pH sensitive hydrogel, which results in the release of entrapped insulin. Since the above reaction consumes oxygen, the pH decrease

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in the device is limited by the presence of oxygen, which is low compared to glucose concentration. Also, the formation of hydrogen peroxide deactivates GOD, therefore, catalase is added which converts hydrogen peroxide to oxygen.

$$H_2O_2 \longrightarrow \frac{1}{2}O_2 + H_2O$$

Traitel et al. (8) have studied the poly(HEMA-co-DMAEMA) hydrogel as insulin delivery systems in simulated *in vivo* conditions. The *in vivo* studies were carried out for 28 days. In the present study, an attempt has been made to characterize the poly(HEMA-co-DMAEMA) hydrogel by swelling studies, DSC, FTIR, Scanning Electron Microscopy (SEM), *in vitro* and evaluate by *in vivo* studies for a longer duration of time. The diffusion coefficient of water and insulin in different glucose concentration has been investigated. The hydrogel implants was tested *in vivo* in rats (8 weeks) to study the performance of the self-regulated device in varying the glucose concentration and also to investigate the biocompatibility of the device.

2 Experimental

2.1 Materials

The monomers, 2-hydroxyethyl methacrylate (HEMA), N,N-dimethylaminoethyl methacrylate (DMAEMA) and the crosslinking agent ethylene glycol dimethacrylate (EGDMA), were obtained from Aldrich Inc., USA. The monomers and crosslinking agent were distilled to remove the inhibitors. The initiators, ammonium persulfate and sodium metabisulphite were obtained from Rankem, India. Insulin (from porcine pancreas, Sigma St. Louis, USA), glucose oxidase (from *Aspergillus niger*, Sisco, India), catalase (HiMedia, India) and dextrose (Rankem, India) were used without purification. Double distilled water was used throughout the experiment.

2.1 Preparation of pH and Glucose Sensitive Hydrogels

pH sensitive hydrogel based on poly(2-hydroxyethyl methacrylate-co-N,N-dimethylaminoethyl methacrylate), poly(HEMA-co-DMAEMA), were prepared by mixing distilled monomers HEMA, DMAEMA and the crosslinking agent ethylene glycol methacrylate (EGDMA) in water in different ratios (Table 1). When the homogenous solution was obtained, aqueous solutions of initiators ammonium persulfate (0.5% w/v) and sodium metabisulphite (0.25% w/v) were added and the polymerization process was carried out at room temperature. Physically crosslinked hydrogels were prepared by a similar method but without the addition of EGDMA. The hydrogels were made glucose sensitive by incorporating glucose oxidase and catalase enzymes (unit ratio 1:11) during the preparation. Insulin was added to the monomer solution before mixing the initiators. The hydrogel was then dried at room temperature in a vacuum desiccator. The dried film was cut using a cork borer of 1 cm diameter to get circular implants of thickness 0.075 ± 0.0035 cm.

Table 1. The composition of poly(HEMA-co-DMAEMA) hydrogels

Formulation code	Monomer conc. HEMA (% w/w)	Monomer conc. DMAEMA (% w/w)	EGDMA (% w/w of total monomer conc.)
M1	80	20	1
M2	70	30	1
M3	60	40	1
M4	50	50	1
M5	40	60	1
M6	30	70	1
M7	20	80	1
M5a[a]	40	60	0
M5b	40	60	2
M6a[a]	30	70	0
M6b	30	70	2

[a]Physically crosslinked hydrogels.

2.2 Swelling Studies

The swelling media for pH-sensitive hydrogels (matrices without enzymes and insulin) were phosphate buffer solutions (PBS) of different pH of 5.0, 6.0, and 7.4. For glucose sensitive hydrogels (with immobilized glucose oxidase and catalase) the swelling medium was PBS of pH 7.4 containing different glucose concentrations of 50 mg/dl, 200 mg/dl and 400 mg/dl. Hydrogels were placed in a glass beaker containing 50 ml of the swelling medium and the beakers were placed in a shaking incubator at 37°C and 100 rpm. The hydrogels were weighed periodically throughout the experiment. The swelling process was characterized by the weight swelling ratio $q = W_s/W_d$, where W_s is the weight of the swollen hydrogel and W_d is the weight of initially dried hydrogel. The swelling studies were carried out in triplicate. The data obtained from the swelling studies was fitted into the Berens–Hopfenberg differential Equation (1):

$$M_t/M_\infty = \{1 - A\exp(-k_2 t)\} \quad (1)$$

where A and k_2 are constants calculated from the slopes and intercepts of the plot of $\log(1 - M_t/M_\infty)$ vs. time t at times later than those corresponding to $M_t/M_\infty = 0.6$ (12).

2.3 Mesh Size Calculation

The mesh size (ξ) of the hydrogels swollen in different glucose solution was calculated by Equation (2), $\xi = C_n^{1/2} Q^{1/3} N^{1/2} l$, where Q is the equilibrium volume swelling ratio at a particular pH, N is the number of repeating units between two crosslinks, l is the carbon–carbon bond length

in A° ($l = 1{:}54$) and C_n is the characteristic ratio, 14.4 in the case of a methacrylate chain. The volume swelling ratio, Q, was determined from the weights of the sample in air and heptane using the following relation:

$$Q = W_s^a - W_s^h / W_d^a - W_d^h \quad (2)$$

where W is the weight of the polymer samples, the superscripts a and h are for measurements in air and heptane, respectively, and the subscripts s and d are for swollen and dry states, respectively (9).

2.4 FT-IR, DSC and Scanning Electron Microscopy Studies

FT-IR measurements were performed on the dry state of materials (Perkin-Elmer, L1185247) in the range of 400–4000 cm^{-1}.

Differential scanning calorimetry (DSC) was conducted in nitrogen atmosphere using a DSC7 calorimeter (Perkin-Elmer) in sealed aluminum pans. Thermograms covered a range of 20 to 200°C with heating and cooling rates of 10°C/min. The composition of the poly(HEMA-co-DMAEMA) was determined using a Perkin–Elmer 2400 CHN Elemental Analyzer.

The surface morphology of dried hydrogels was determined using a scanning electron microscope (JEOL 6320). The hydrogel samples were mounted on the base plate and coated with gold using vapor deposition techniques. The surface was then scanned using a magnification of 5000.

2.5 In vitro Release

Release experiments were performed with glucose-sensitive hydrogels. The hydrogels were placed in a 100 ml glass beaker containing 50 ml PBS at pH 7.4 with glucose concentrations of 50 mg/dl, 200 mg/dl and 400 mg/dl. The beakers were placed in a shaking incubator maintained at 37°C and 100 rpm. The samples were withdrawn every 24 h and insulin concentration was measured using reversed-phase high performance liquid chromatography method (RP-HPLC) (10). To maintain uniform concentration of glucose in the media, fresh glucose solutions (pH 7.4 PBS) were replaced at the end of every 24 h during the release studies. The RP-HPLC consisted of C-18 column; the mobile phase was 0.2 M sodium sulphate (pH 2.3) and acetonitrile in the ratio of 74:26. The flow rate was 1 ml/min and the insulin was detected using UV detector at 214 nm.

2.6 Mathematical Analysis of Water Uptake and Drug Release

Analysis of the swelling behavior of hydrogels in different glucose solution of 50 mg/dl, 200 mg/dl and 400 mg/dl was carried out using Equation (3):

$$M_t/M_\infty = 4(Dt/\pi\delta^2)^{1/2} \quad (3)$$

where D is the water diffusion coefficient, δ the half thickness of the hydrogel, M_t the amount of water uptake at time t and M_∞ is the water uptake at equilibrium stage. Diffusion coefficients of insulin through the hydrogels at 50 mg/dl, 200 mg/dl and 400 mg/dl of glucose concentration (pH 7.4 PBS) were calculated from Equations (3) and (4).

$$M_t/M_\infty = 1 - 8/\pi^2 \exp(-\pi^2 Dt/4\delta^2) \quad \text{for} \quad 0.4 < M_t/M_\infty < 1 \quad (4)$$

where D is the diffusion coefficient of insulin through the hydrogel, δ is the half thickness of the hydrogel, M_t the amount of insulin released at time t, M_∞ is the maximum amount of insulin released (11).

2.7 In vitro Degradation

The hydrogels without insulin and enzymes were placed in 10 ml phosphate buffer (pH 7.4, 37°C) containing lysozyme enzyme (1 mg/ml). The PBS was changed for all the samples every day. Hydrogels were taken out at 7, 14, 21 and 28 days, washed with distilled water and air dried for 72 h. The resulting dry weights were recorded and the percentage weight loss was calculated.

2.8 In vivo Release Studies and Biocompatibility Studies

Wistar strain male Albino rats, weighing 225 ± 20 g, obtained from the Animal Department of the J.S.S. Medical College, Mysore were used. The animals were divided into three groups. The rats were first injected with 0.4 ml of a streptozotocin (STZ) solution (50 mg STZ/2 ml of 0.9 wt/vol% NaCl at pH 4) to the rat's tail veins in order to induce diabetes. A week after STZ injection, the blood glucose levels were checked in order to confirm that the rats became diabetic, defined as having a minimum of 300 mg/dl glucose in the blood. The rats were anesthetized by injection of Ketamine 80 mg/kg body weight. After anesthetizing, the backs of the rats were shaved, and the surgery carried out for two groups with one group kept as diabetic control. A single incision, 1.5–2 cm long, was made on their backs; blunt-scissor dissection was then used to create a lateral implant site by tunneling immediately beneath the skin. The implant was then inserted a distance from the incision and sutured. Following the surgical procedure, the rats were periodically tested for blood glucose levels using a glucometer. Blood samples were taken from the tail. At the end of the experiment, the rats were sacrificed and the implants were retrieved after 56 days for observation of tissue encapsulation.

2.9 Statistical Calculations

Data were expressed as mean $\pm$ S.D. Statistical significance was determined by a Newman–Keuls test after ANOVA

using GraphPad Prism, Version 3.0 (GraphPad Software, Inc., San Diego, CA). P values <0.05 were considered significant.

3 Results and Discussions

3.1 Swelling Studies

Design of this type of self-regulated insulin delivery systems may involve the development of hydrogels that are glucose-sensitive and have certain desirable swelling and release properties. The stimulus-sensitive behavior of ionic hydrogels may be due to the polyelectrolytic nature of the polymer carriers. Polyelectrolytic chains contain ionizable moieties which protonate or deprotonate depending on the surrounding conditions and such hydrogels exhibit a transition in swelling from a collapsed to a highly swollen state. Cationic hydrogels contain tertiary amine groups which protonate when the pH is decreased below the pK_a of the ionizable groups. The hydrogels swell when protonated and deswell when deprotonated. In the present work, DMAEMA was used as the cationic comonomer of the pH-sensitive hydrogels for drug delivery applications. The transition pH could be altered to lower pH values by incorporating more hydrophobic groups in the mesh. DMAEMA shows ionization at pH of 8.0 and a lower transition pH is desirable to ensure that there is no inadvertent release of incorporated drug. Therefore DMAEMA was copolymerized with HEMA to obtain a self-regulated insulin delivery system. The pH-dependent swelling properties of the hydrogel implants were studied in phosphate buffer solutions with pH values of 5.0, 6.0 and 7.4. Figures 1 and 2 show the equilibrium swelling of hydrogels as a function of pH and glucose solutions, respectively.

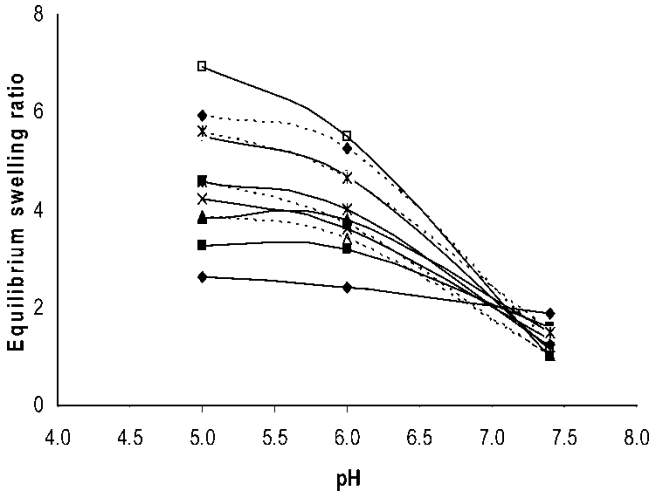

Fig. 1. Equilibrium swelling of hydrogels in pH 5.0, 6.0 and 7.4 buffer solutions. The equilibrium swelling behavior of hydrogels of various composition (—◆—) M1, (—■—) M2, (—▲—) M3, (— × —) M4, (— * —) M5, (—○—) M6, (—□—) M7, (— * —) M5a, (···△···) M5b, (···◆···) M6a, (···■···) M6b was carried out in pH 5.0, 6.0 and 7.4 buffer solutions.

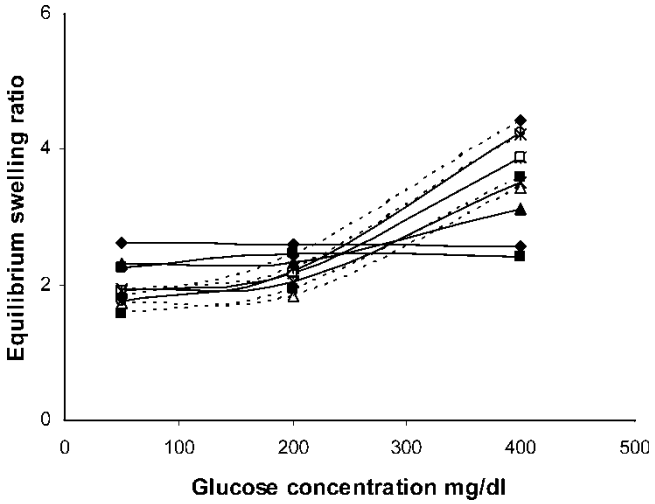

Fig. 2. Equilibrium swelling of hydrogels in pH 7.4 phosphate buffer solution containing 50 mg/dl, 200 mg/dl and 400 mg/dl glucose solution. The equilibrium swelling behavior of hydrogels of various composition (—◆—) M1, (—■—) M2, (—▲—) M3, (— × —) M4, (—*—) M5, (—○—) M6, (— * —) M7, (–□–) M5a, (···△···) M5b, (···◆···) M6a, (···■···) M6b was carried out in pH 7.4 phosphate buffer solution containing different glucose concentrations.

From the equilibrium studies, it can be seen that the hydrogels M1, M2, M3 (Table 1) did not show pH dependent swelling, as well glucose dependent swelling. For further *in vitro* release studies, these three hydrogels were omitted. The data obtained from the swelling studies were fitted into the Berens–Hopfenberg differential Equation (1). The values of A were in the range of 0.25–0.98 in different pH and glucose solution indicating the existence of different mechanism of water transport (both diffusion controlled and relaxation controlled) through the hydrogels. The diffusion coefficient of water through the hydrogels was found to depend on the external pH and glucose concentration (Table 2). The maximum diffusion coefficient was observed in pH 5.0 and also in pH 7.4 PBS containing glucose concentration of 400 mg/dl. At pH 5.0 the cationic monomer exists in protonated form causing the hydrogel to swell. At 400 mg/dl of glucose concentration the pH reduction inside the hydrogels due to activity of enzymes is maximum which results in protonation of the cationic monomer. The development of charge on the polymer backbone causes the hydrogel to swell allowing the water to diffuse into it. At pH 7.4 and glucose concentration of 50 mg/dl the protonation takes place to lesser extent as a result the hydrogel undergoes less swelling, which results in lower values of diffusion coefficient. Also, the diffusion coefficient of water was found to depend on DMAEMA content and crosslinking agent concentration in polymer. Physically crosslinked hydrogels (M6a and M5a) showed higher diffusion coefficient values than the corresponding chemically crosslinked hydrogels (M6, M6b, M5 and M5b).

Table 2. Diffusion coefficient of water in different pH and glucose solutions

Formulations	Diffusion coefficient (10^{-6} cm^2/min)			Diffusion coefficient (10^{-6} cm^2/min)		
	pH 5.0	pH 6.0	pH 7.4	50 mg/dl	200 mg/dl	400 mg/dl
M1	8.09	8.87	11.56	11.75	9.18	8.08
M2	9.12	9.30	10.77	10.60	9.17	8.29
M3	9.00	9.81	10.28	9.24	8.71	9.67
M4	10.78	10.14	7.57	8.54	7.48	9.70
M5	10.64	10.61	7.52	8.15	8.05	10.02
M5a	12.96	11.37	7.91	9.69	9.44	13.17
M5b	9.64	6.48	6.15	6.68	7.59	9.50
M6	11.63	10.92	9.49	8.66	8.85	10.77
M6a	14.26	12.09	10.1	10.36	10.82	14.76
M6b	9.96	9.43	7.90	7.70	8.50	9.00
M7	12.85	11.12	7.51	7.44	10.01	11.41

The diffusion coefficient of water through the hydrogels was obtained by plotting $\log[\pi^2/8(1 - M_t/M_\infty)]$ vs. t (Equation 4). The slope of the plot was $\pi^2 D/2.303 \times 4\delta^2$ from which the diffusion coefficient D was calculated.

The mesh size calculated from Equation (2) for the hydrogel implants was <30 A° when swollen in 50 mg/dl glucose solution and was 75–90 A° and 110–170 A° when swollen in 200 mg/dl and 400 mg/dl glucose solution. The mesh size of the hydrogel implant depended on the DMAEMA content in the hydrogel and also on the cross-linking agent concentration of the hydrogel.

3.2 FT-IR, DSC and Scanning Electron Microscopy Studies

FT-IR measurements were performed on the dry state of materials (M6, M6a, and M6b). This technique revealed the presence (vibration bands at 1700–1650 cm^{-1}) of unreacted double bonds of the free monomer present in materials (data not shown). The presence of unreacted methacrylates is dependent on the amount of crosslinker used. The more the crosslinker used, the fewer free double bonds were present. The DSC studies for the samples (M6, M6a, and M6b) indicated a shift in endothermic peaks to higher temperatures for hydrogels prepared with a higher concentration of the crosslinking agent (Figure 3). These results indicated that increase in cross-linking increases the polymer chain rigidity and hence, higher energy is required to stretch the highly cross-linked polymer than the loose network. The presence of additional crosslinks decreased the chain mobility, which resulted in higher T_g values (M6b > M6 > M6a). An Elemental Analyzer was used for the determination of carbon (C), hydrogen (H) and nitrogen (N) contents in the copolymers synthesized. Molar fractions (mol%) of comonomer units (m_1 and m_2) in HEMA–DMAEMA copolymers using elemental analysis data were calculated according to Equation (6), $m_2 = M_1/\{(A_N/B) - \Delta M 10^{-2}\}$, where M_1 is the molecular weight of HEMA unit; A_N is the atomic weight of N; B is the content (%) of N in the copolymers; $\Delta M = M_1 - M_2$ (M_2 is the molecular weight of the DMAEMA unit) (13). The monomer feed composition and the copolymer composition determined by elemental analysis is given in Table 3.

The SEM studies showed that the hydrogels were microporous in nature (Figure 4). The physically crosslinked hydrogel (M5a, Figure 5) showed a more porous nature. When the crosslinker concentration was increased to 2% w/w of monomer the hydrogels showed fewer pores (Figure 6).

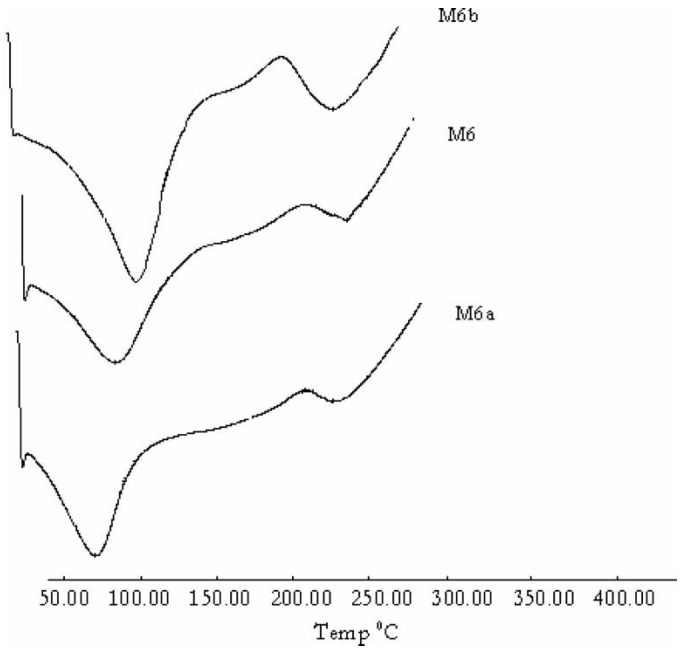

Fig. 3. DSC thermograms of poly(HEMA-co-DMAEMA) hydrogels. The thermogram was obtained while heating with the heating rate of 10°C/min.

Table 3. Elemental analysis data for determining the copolymer composition of poly(HEMA-co-DMAEMA) hydrogels

	Monomer Feed composition (mol%)		Copolymer composition (mol%)[a]	
Formulations	m_1 (HEMA)	m_2 (DMAEMA)	m_1 (HEMA)	m_2 (DMAEMA)
M1	84.84	15.16	86.77	13.23
M2	76.84	23.16	83.30	16.70
M3	68.05	31.95	75.48	24.52
M4	58.78	41.22	67.75	32.25
M5	48.89	51.11	59.51	40.49
M6	37.90	62.10	47.37	52.63
M7	26.36	73.64	39.54	60.46
M5a	48.89	51.11	59.88	40.12
M5b	48.89	51.11	57.93	42.07
M6a	37.90	62.10	48.62	51.38
M6b	37.90	62.10	44.68	55.32

[a]Molar fractions (mol%) of comonomer units (m_1 and m_2) in poly(HEMA-co-DMAEMA) was calculated using Equation 6.

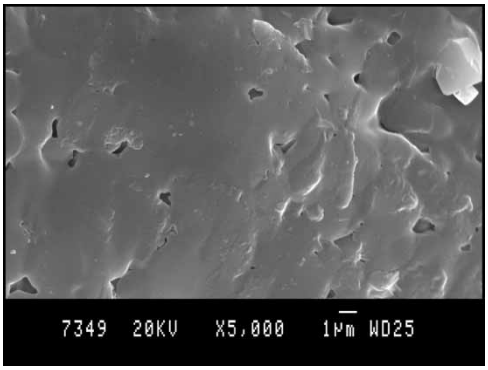

Fig. 4. Scanning electron micrograph of dried hydrogel (M5).

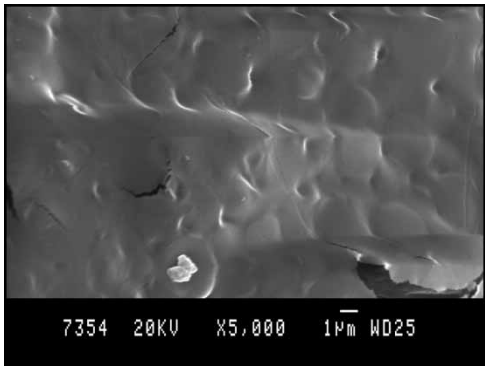

Fig. 6. Scanning electron micrograph of dried hydrogel (M5b).

3.3 In vitro Release Studies

The hydrogel implants showed a complete release of insulin in 12–15 days when placed in PBS containing 400 mg/dl glucose solution. In PBS with glucose concentration of 200 mg/dl, the

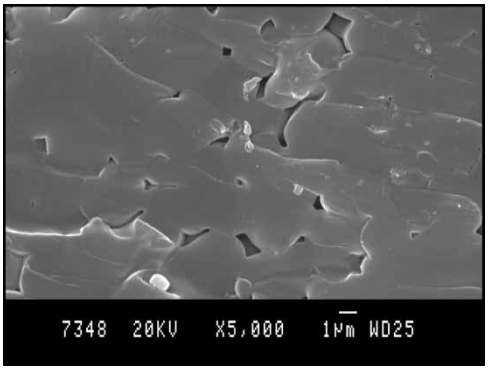

Fig. 5. Scanning electron micrograph of dried hydrogel (M5a).

Table 4. Diffusion coefficient of insulin in different glucose solutions through the hydrogels at early stages

	Diffusion coefficient ($\times 10^{-8}$ cm^2/min)		
Formulations	50 mg/dl	200 mg/dl	400 mg/dl
M4	0.650	1.383	2.800
M5	0.667	1.550	3.400
M5a	0.683	1.917	4.100
M5b	0.583	1.317	2.683
M6	0.700	1.900	3.917
M6a	0.683	2.033	5.400
M6b	0.583	1.450	3.600
M7	0.667	1.983	4.833

The diffusion coefficient of insulin through the hydrogels was obtained by plotting M_t/M_∞ vs. $\sqrt{t}$ for $0 < M_t/M_\infty < 0.6$ (Equation 3). From the slope, diffusion coefficient was calculated by the using the relation $D = m^2\pi\delta^2/16$.

Table 5. Diffusion coefficient of insulin in different glucose solutions at later stages

Formulations	Diffusion coefficient ($\times 10^{-8}$ cm^2/min)		
	50 mg/dl	200 mg/dl	400 mg/dl
M4	0.360	0.952	2.367
M5	0.362	1.085	2.433
M5a	0.342	1.240	2.850
M5b	0.318	0.740	2.400
M6	0.337	1.460	3.650
M6a	0.332	1.717	4.283
M6b	0.318	0.953	3.000
M7	0.338	1.967	5.383

The diffusion coefficient of insulin through the hydrogels was obtained by plotting $\log[\pi^2/8(1 - M_t/M_\infty)]$ vs. t for $0.4 < M_t/M_\infty < 1$ (Equation 4). The slope of the plot was $\pi^2 D/2.303 \times 4\delta^2$ from which the diffusion coefficient D was calculated.

release was 85–100% and in 50 mg/dl solution 45–50% of insulin release was seen in 30 days, respectively. The fraction of insulin released was fitted to the equation:

$$M_t/M_\infty = kt^n \quad \text{for} \quad 0 < M_t/M_\infty < 0.6$$

where M_t is the insulin released at time t, M_∞ is the maximum insulin released, k is the characteristic constant of the hydrogel, and n is the characteristic exponent describing the penetrant mechanism. For planar geometry, the value of $n = 0.5$ indicates a Fickian diffusion mechanism, for

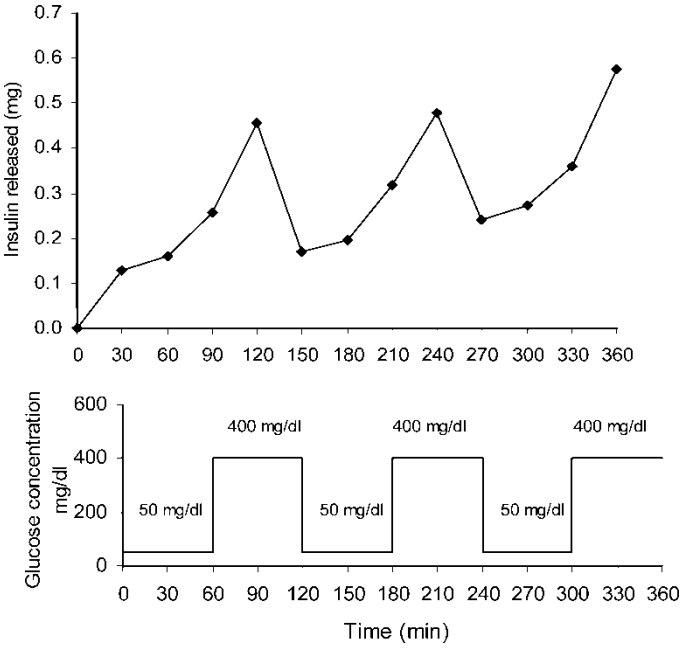

Fig. 7. Glucose responsive insulin release from hydrogel implant (M6). The implant was first placed in 50 mg/dl glucose solution for 1 h and then transferred to 400 mg/dl. The external glucose solution was changed every 1 h for 3 cycles.

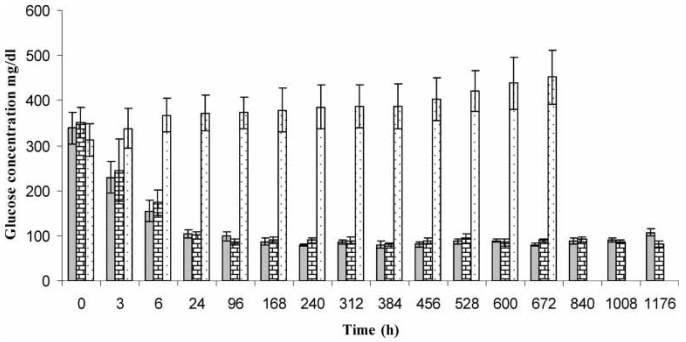

Fig. 8. The blood glucose levels of rats during the experiment. The blood glucose levels at time 0 indicates the glucose levels when the matrices were implanted in the rats for the implants M5, M6 and Control.

$0.5 < n < 1.0$ indicates non-Fickian or anomalous transport, and $n = 1$ implies case II (relaxation controlled) transport. The constant n and k was calculated from the slope and intercepts of the plots of $\log(M_t/M_\infty)$ vs. log t. The values of n calculated was in the range of 0.86–0.93, which indicated that the release of insulin followed non-Fickian or anomalous transport. The diffusion coefficient of insulin through the hydrogels was calculated using Equation (3) for $0 < M_t/M_\infty < 0.6$ and Equation (4) for $0.4 < M_t/M_\infty < 1$. The diffusion coefficient of insulin (molecular weight 5900, hydrodynamic radius 16A°) was calculated for the swollen and collapsed states of the hydrogels (Tables 4 and 5). The calculated diffusion coefficient was found to depend on both DMAEMA content and crosslinking agent concentration in the hydrogel. The diffusion coefficient observed in physically crosslinked hydrogels was higher than the corresponding chemically crosslinked hydrogels. The diffusion coefficient values at the early

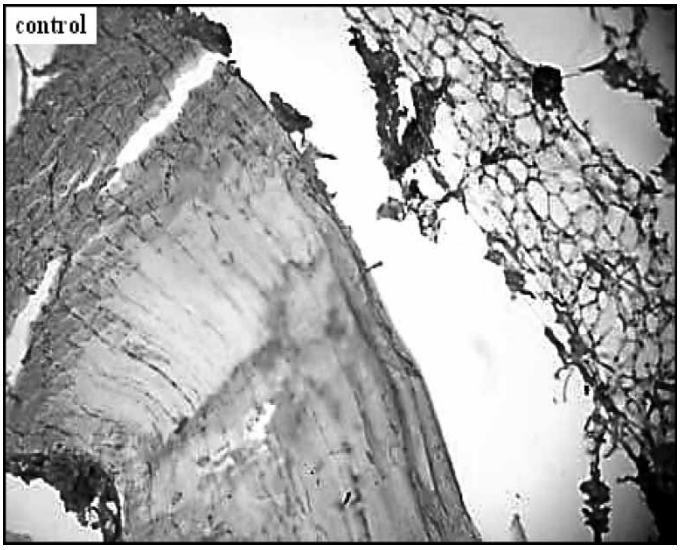

Fig. 9. Histopathology of the tissue surrounding the control.

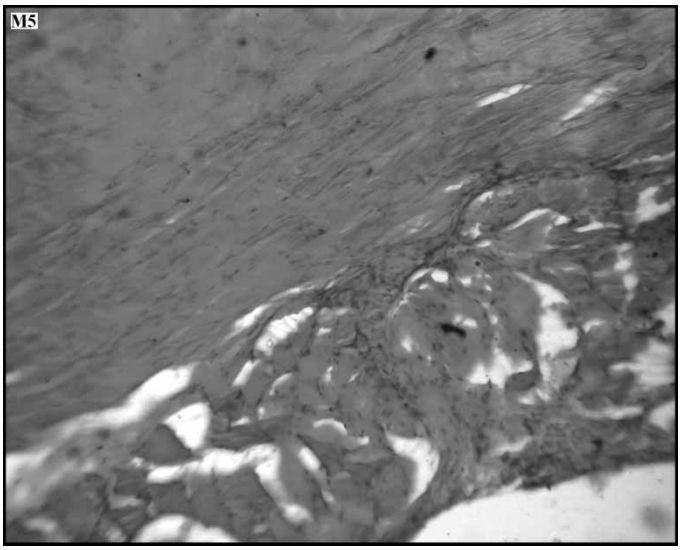

Fig. 10. Histopathology of the tissue surrounding the formulation M5.

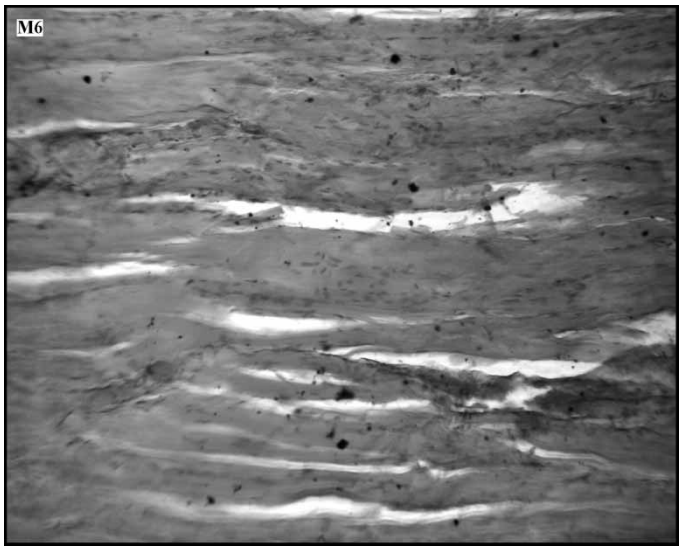

Fig. 11. Histopathology of the tissue surrounding the formulation M6.

stages was much higher compared to later stages because the drug molecules at the surface of the device are released, those in the center of the device have to migrate longer distances to be released, which takes a longer time. This increased diffusion time results in a decrease in the release rate from the device with time. The glucose concentration of 400 mg/dl produced significant swelling of the hydrogels with mesh size greater than the diameter of the insulin hexamer (the more stable state of insulin). As a result, incorporated insulin was released quite easily from the hydrogels at higher glucose concentration of 200 mg/dl and 400 mg/dl than at 50 mg/dl.

Glucose responsive insulin release studies were performed on the hydrogel implant in different glucose concentrations to ensure that the materials responded reversibly to glucose changes in the environment. It is necessary that the hydrogels swell when the external glucose concentration is high and then collapses to the original equilibrated state once the glucose concentration is low. This would ensure that the release of insulin is cut off as soon as there is a deficit of glucose in the environment. The glucose responsive insulin release studies shed light on the reversibility of the swelling/deswelling process occurring in the hydrogel network. It is necessary for the swelling process to be reversible to ensure that the release of insulin can be initiated and cut off easily. Figure 7 shows the glucose responsive insulin release nature of the hydrogel implant. The external glucose concentration was varied from 50 mg/dl to 400 mg/dl. It is evident from the graph that the insulin release depended on the external glucose concentration. The *in vitro* degradation studies indicated that there was no significant ($p > 0.05$) weight loss of the implants when placed in PBS containing lysozyme enzyme indicating the non-degrading nature of the hydrogels.

3.4 *In vivo Studies and Biocompatibility Studies*

The *in vivo* studies were carried out for two hydrogels implants M5 and M6 for 8 weeks. The animals belonging to control

Table 6. Effect of self-regulated implant on the blood glucose, serum urea, serum creatinine and lipid profile of diabetic rats

Tests	Diabetic control	M6	M5
Blood sugar	468.33 ± 45.44	92.83 ± 12.58[a,b]	94.17 ± 13.26[a,b]
Serum urea	86.17 ± 7.19	31.17 ± 3.43[a,b]	34.50 ± 7.82 [a,b]
Serum creatinine	0.85 ± 0.04	0.65 ± 0.04[a,b]	0.63 ± 0.04[a,b]
Cholesterol	215.00 ± 20.03	90.33 ± 5.32[a,b]	99.50 ± 15.02[a,b]
Triglycerides	133.83 ± 6.79	69.33 ± 4.76[a,b]	71.00 ± 11.78[a,b]
HDL	28.83 ± 4.17	50.00 ± 7.64[a,b]	51.33 ± 5.32[a,b]
LDL	132.33 ± 9.48	49.50 ± 10.25[a,b]	54.50 ± 7.29 [a,b]
VLDL	29.67 ± 2.94	19.83 ± 3.97[a,b]	18.50 ± 2.43 [a,b]

Each value represents mean ± S.D (n = 6).
[a] $p < 0.001$ vs. control.
[b] $p > 0.05$ M6 vs. M5.

group showed severe hyperglycemia and were sacrificed after 4 weeks. The animals treated with implants showed a significant ($p < 0.001$) reduction in blood glucose level compared to diabetic control. The groups treated with hydrogels M5 showed 43% and 102% reduction of blood glucose within 3 h and 6 h, respectively. The implant M6 showed 48% and 120% reduction of blood glucose within 3 h and 6 h, compared to the blood glucose level before implantation. Both the hydrogels maintained steady glucose level in a normal range of 80 − 125 mg/dl for 56 days (Figure 8). The difference in blood glucose levels of animals with implants M5 and M6 was statistically insignificant ($p > 0.05$). To evaluate the biocompatibility of the implants, the implantation site was observed, and the surrounding tissue was harvested for histopathological analysis. Macroscopic evaluation of the implantation site revealed no inflammatory reaction (redness or swelling). After 56 days the animals were sacrificed and the implants recovered to study the biocompatibility of the hydrogel. The histopathological studies revealed the absence of fibrous tissue formation around the implants indicating the biocompatibility of the formulations (Figures 9–11). The serum creatinine, urea and lipid profile of diabetic control and the treated animals are shown in Table 6. The treated animals showed statistically significant ($p < 0.001$) changes in the serum creatinine, urea, cholesterol, triglycerides, HDL, LDL and VLDL.

4 Conclusions

The poly(HEMA-co-DMAEMA) showed pH and glucose dependent swelling. The mesh size of the hydrogel was found to depend on the external pH and glucose concentration and also on the DMAEMA content of the hydrogel. The diffusion coefficient of water and insulin were found to depend on the cationic monomer (DMAEMA) content of the hydrogel. The hydrogel implants showed a complete release of insulin in 12–15 days when placed in PBS containing 400 mg/dl glucose solution. In PBS, with a glucose concentration of 200 mg/dl, the release was 85–100% and in 50 mg/dl solution 45–50% of insulin release was seen in 30 days, respectively. The pulsatile insulin release studies showed that the release of insulin from the hydrogel was modulated by external glucose concentration. The hydrogels were biocompatible as indicated by the histopathological studies.

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