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Preparation and dynamic response of cationic copolymer hydrogels containing glucose oxidase

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Abstract

Glucose-sensitive hydrogels were prepared by the copolymerization of diethylaminoethyl methacrylate (DEAEM), poly(ethylene glycol) monomethacrylate (PEGMA) and functionalized glucose oxidase (GOD) and catalase in solution. Tetra(ethylene glycol) dimethacrylate (TEGDMA) was used as the crosslinking agent. The equilibrium and dynamic swelling properties were studied to investigate the pH-sensitive swelling behavior of the gels. The effects of crosslinking ratio and enzyme content on the swelling properties were studied. The hydrogels demonstrated critical swelling behavior as the pH approached 7.0. The mesh sizes and the diffusion coefficients were determined. Dynamic swelling studies were performed and the results were analyzed using a first-order model to calculate the time constants of swelling and syneresis. Pulsatile swelling studies revealed the reversible nature of the swelling/deswelling process.

Keywords: Cationic hydrogel; Glucose-sensitivity; Equilibrium swelling

1. Introduction

1.1. Hydrogels

Hydrogels have been studied extensively as materials for controlled delivery of macromolecular drugs. Environmentally sensitive hydrogels have been investigated as viable alternatives to traditional carriers for drug delivery [1]. The use of hydrogels offers a considerable amount of flexibility in terms of the amounts and the rates of drug release. In addition, their typical biocompatibility renders them promising candidates for internally implantable devices [2]. Site-specific drug delivery [3] and tissue targeting [4] have also utilized hydrogels successfully.

Ionic hydrogels swell or deswell depending on certain external conditions, such as, pH [5], temperature [6], ionic strength [7] and buffer composition [7]. Such changes can be also brought about by external force fields, such as pressure [8], ultrasounds and electromagnetic radiation [9]. Swelling/deswelling may be due to phase changes [6], hydrophobic–hydrophilic interchange or ionization [7] that occurs due to variations in the environmental conditions. In both cases, the thermodynamics and the kinetics of the swelling process are affected by several other factors, including the ionic strength of the medium, buffer composition and presence of salts [7].

pH-sensitive hydrogels can be anionic or cationic depending on the nature of the ionizable moieties on their backbones. Cationic hydrogels were studied extensively by Siegel and associates [7,10–13] who prepared hydrogels from copolymers of cationic monomers, such as dimethylaminoethyl methacrylate (DMAEM) or diethylaminoethyl methacrylate (DEAEM) with methyl methacrylate (MMA). At pH values over the pK_a of the cationic groups, the copolymers were hydrophobic and excluded water from the system whereas at pH values lower than the pK, the amine groups protonated to form NH_3^+. As a result, the gels became hydrophilic and absorbed water.

The formation of charged groups on the polymer backbone affects the osmotic balance between the hydrogel and the surrounding medium. Thus, the swelling dynamics was found to be very dependent on the ionic strength and the type of ions of the surrounding medium [11]. It was observed that Donnan exclusion of hydrogen ions from the gel was a rate-limiting factor in water sorption. Exclusion could be overcome by the incorporation of weakly acidic buffering ions in the surrounding medium [12]. The

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nonionized buffer molecules were capable of carrying the hydrogen ions to the swollen gel, thus overcoming Donnan equilibrium. Common examples of such buffers are citrates and phosphates.

The equilibrium swelling characteristics of these materials show a sharp transition between their swollen and collapsed states [5,7]. The effect of the methacrylate chain length on the equilibrium swelling was also studied. Increasing the chain length amounted to an increase of the hydrophobic nature of the polymer. Though the critical pH was not affected, the maximum amount of water imbibed decreased significantly with increase in hydrophobicity. Also, an increase in the MMA:DEAEM ratio had a significant effect on both the value of the critical pH and the uptake of water.

Hariharan and Peppas [14] and am Ende et al. [15] studied the swelling behavior of cationic hydrogels as carriers for drug delivery, DEAEM and diethylaminoethyl acrylate (DEAEA) were used as the cationic comonomers and were copolymerized with hydroxyethyl methacrylate (HEMA). The equilibrium water uptake was a strong function of the ionic strength of the medium, decreasing as the ionic strength was increased. Dynamic swelling studies showed that the rate of uptake of water increased with a decrease of pH.

1.2. Glucose-sensitive hydrogels

Glucose-sensitive gels have been prepared by several researchers by physical or chemical entrapment of glucose oxidase (GOD) into various polymers, such as poly(ortho esters) [16], poly(vinyl alcohol) [17], polyamides [18,19], polymethacrylates [20] and polyacrylates [21]. GOD reacts with glucose to form gluconic acid, which triggers the pH-sensitive swelling/deswelling of the hydrogel. Such glucose-responsive materials have been tested under different conditions.

For example, Albin et al. [22,23] studied the use of cationic polymers, namely, polyacrylamide gels and poly(dimethylaminoethyl methacrylate) gels, as glucose-sensitive matrices. Macroporous gels showed greater glucose sensitivity than nonporous or microporous gels [22]. A model of release was proposed to take into account the kinetics of glucose reaction [23]. The kinetics of transport in the membranes was limited by the solubility of oxygen in the surrounding medium. Klumb and Horbett [24] have proposed a new geometry for the device which would overcome oxygen dissolution limitations.

Further characterization of GOD-immobilized copolymers of HEMA and DMAEM crosslinked with tetraethylene glycol dimethacrylate (TEGDMA) was done by Goldraich and Kost [25]. The dependence of swelling properties on crosslinking ratio and comonomer ratio was established.

In previous work done in our group [26], GOD-immobilized poly(methacrylic acid-*g*-ethylene glycol) hydrogels were investigated for pH sensitivity. In this case, the hydrogels were expected to release insulin by a rapid squeezing effect. Pulsatile pH–swelling studies showed a rapid collapse. In a later paper [27], the equilibrium and the dynamic swelling behavior of these anionic grafted hydrogels was reported. The characteristic swelling behavior was attributed to the formation of reversible complexes within the matrix.

In this work, we prepared and characterized a series of poly(ethylene glycol)-grafted cationic hydrogels containing immobilized GOD. The equilibrium and dynamic swelling of the hydrogels were studied under varying pH conditions.

2. Experimental

2.1. Synthesis

Copolymerization was carried out by mixing predetermined quantities of the two comonomers, DEAEM (Aldrich, Milwaukee, WI) and poly(ethylene glycol) monomethacrylate (PEGMA, containing PEG units of different molecular weights, 200, 400 and 1000; Polysciences, Warrington, PA). In each case, the ratio of methacrylate repeats to PEG grafts was kept at a constant value of 10:1 to 50:1.

The crosslinking agent used was TEGDMA (Aldrich, Milwaukee, WI). Hydrogels containing nominal crosslinking ratios of $X = 0.005$–0.04 mol of TEGDMA/mol of monomers were prepared (see also Fig. 1). In a typical synthesis procedure, 3.5 ml of DEAEM was mixed with 1.9 g of PEGMA containing PEG units of molecular weight 1000. This mixture gave a polymer containing a 10:1 ratio of methacrylate repeating units to PEG grafts. For a network having $X = 0.01$, 68 μl of TEGDMA was added to the comonomer mixture.

Functionalized enzyme solutions of GOD and catalase (both from Sigma, St. Louis, MO) were used for the immobilization procedure. Acryloyl chloride (Aldrich, Milwaukee, WI) was used as the binding agent according to the technique reported by Platé et al. [28]. In a typical functionalization process, 0.01 g of GOD and 175 μl of catalase were dissolved in 10 ml of phosphate buffer solution of pH 7.4. Finally, 2 μl of acryloyl chloride was added. The solution was then allowed to react for 2 h in an ice bath.

The monomer solution had a pH of 9.5. At such high pH values, the enzymes tended to precipitate out of solution. To prevent this from happening, 1.2 ml of hydrochloric acid (Fisher, Fairlawn, NJ, 0.1 N) was added to the comonomer mixture. To the acidified solution, 1.5 ml of the functionalized enzyme solution was added.

The monomer solution was then transferred to nitrogen atmosphere (glove box) and mixed with 1 wt% of the photoinitiator, 2-hydroxy cyclohexyl phenyl ketone (Irgacure 164®, Ciba-Geigy, Hawthorn, NY). The mixture was then pipetted between glass slides separated by Teflon® spacers at 0.1 cm thickness. The system was then placed under a UV

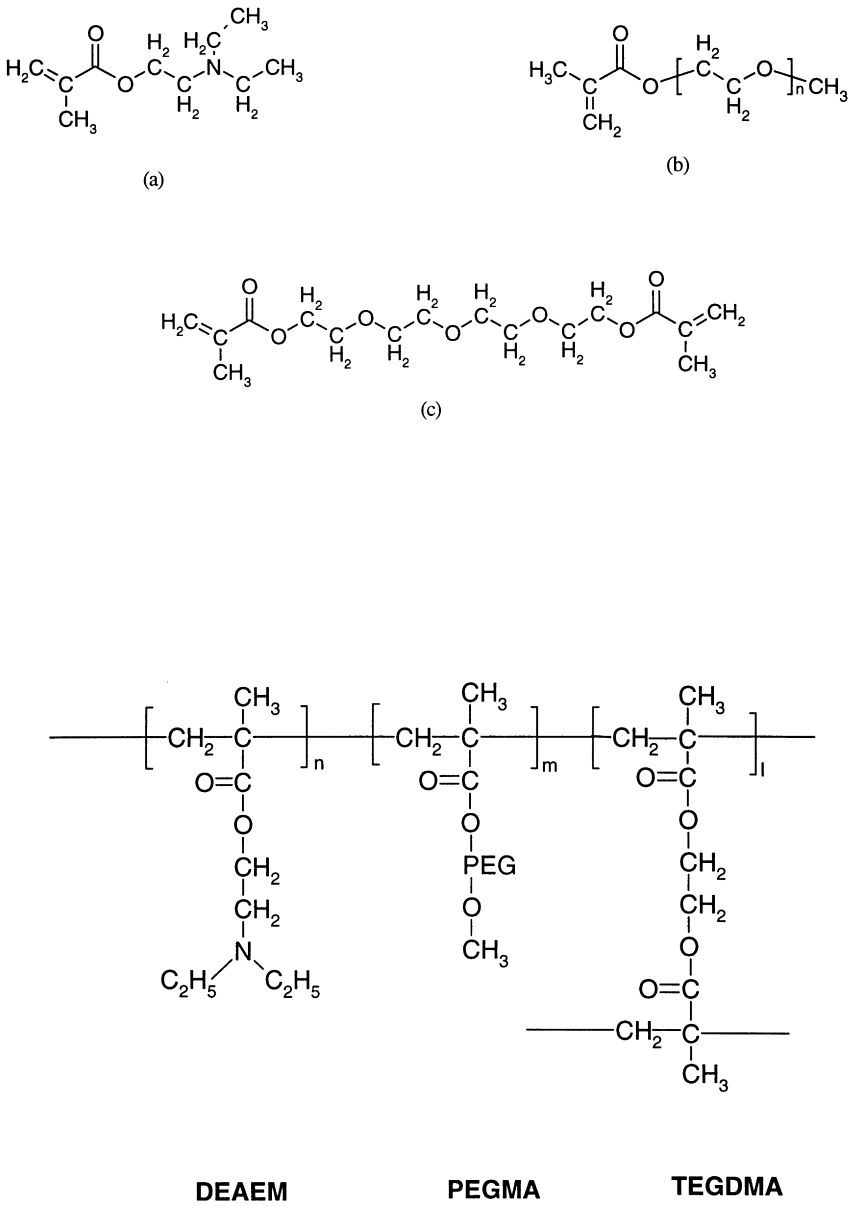

Fig. 1. Comonomers and crosslinking agents used in the formation of the P(DEAEM-*g*-EG) hydrogels. (a) DEAEM; (b) PEGMA; and (c) TEGDMA.

source (UltraCure 100, EFOS, 1 mW/cm^2) and photopolymerized for 45 s.

Thus, films of GOD-immobilized poly(diethylaminoethyl methacrylate-*g*-ethylene glycol) (henceforth designated as P(DEAEM-*g*-EG)) were obtained. Discs of 1 cm diameter were cut out of these films, wasted for 3 days and dried in a vacuum oven for 2 days.

2.2. Equilibrium swelling studies

The pH-dependent swelling properties of the hydrogel discs were studied in phosphate buffer solutions with pH values between 5.0 and 8.0. For lower pH values of 3.2–5.0, dimethyl glutaric acid (Sigma, St. Louis, MO) with sodium hydroxide was also used. To maintain near-physiological conditions, the ionic strength of the buffer was maintained at 0.1 M by adding sodium chloride.

Equilibrium swelling characteristics were obtained by exposing polymer samples to different pH solutions and calculating their swelling ratio as a function of pH. Dried polymer discs were weighed and placed in buffer solutions of different pH values ranging from 4.5 to 8.0 at 37°C. The samples were weighed and the weight swelling ratio was calculated using the equilibrium swollen and the dried sample weights. To calculate the volume swelling ratios, each sample was weighed in air and in heptane in the dry state and also in the fully swollen state [15].

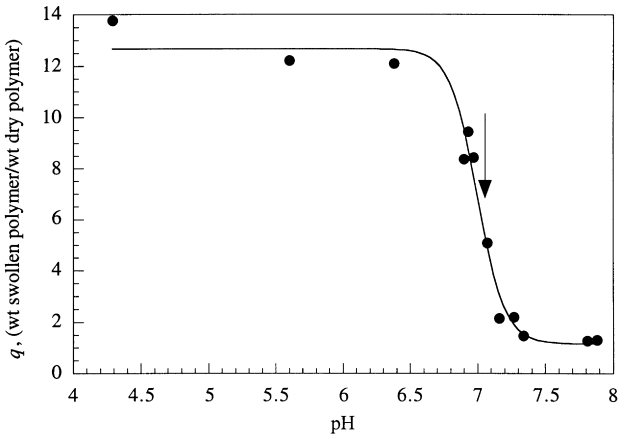

Fig. 2. Equilibrium weight swelling characteristic ratio of P(DEAEM-*g*-EG) hydrogels immobilized with GOD and catalase as a function of swelling pH at 37°C. Hydrogel samples were prepared with a 50:50 molar ratio of DEAEM:PEGMA (of PEG molecular weight 200), 5.413×10^{-4} g of GOD/g of polymer and $X = 0.02$ mol TEGDMA/mol monomers. The arrow indicates the location of the transition pH.

2.3. Dynamic swelling experiments

To study the dynamic swelling behavior, a single dry disc was weighed and immersed in a phosphate buffer solution of pH 5.0, 6.8 and 7.4 at 37°C. The sample was taken out at regular intervals of time and weighed until it approached a constant water content.

The pulsatile response of P(DEAEM-*g*-EG) discs was tested with in various pH solutions. Hydrogel discs were equilibrated at a pH of 6.2. An arbitrary sequence of step changes was used to analyze the response of these materials. The pH was changed between 5.0 and 7.4. The discs were taken out and weighed at regular intervals.

3. Results and discussion

3.1. Hydrogel design

Design of stimuli-sensitive hydrogels for insulin delivery involves the development of matrices that are glucose-sensitive and have certain desirable swelling and release properties. For implantable systems, it is important that the hydrogels have 'stealth' properties to prevent the immune system from recognizing them as foreign objects.

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. Such gels exhibit a transition in swelling when they change from a collapsed to a highly swollen state. Of course, cationic hydrogels contain tertiary amine groups which protonate at low pH. When the pH is decreased below the pK_a of the ionizable groups, protonation occurs resulting in the formation of positive charges along the backbone chain.

In our work, DEAEM was used as the cationic comonomer of the pH-sensitive gels for drug delivery applications. The transition pH could be altered to lower pH values by incorporating more hydrophobic groups in the mesh. A diethyl-substituted amine group imparts more hydrophobicity than a dimethyl-substituted amine. DEAEA shows ionization at pH of 8.0. A lower transition pH is desirable to ensure that there is no inadvertent release of incorporated drugs. Therefore, the use of a methacrylate monomer was more effective in our case.

In possible applications in contact with blood, it is important to impart stealth properties to the gels. Thus, grafts of PEG were attached to the backbone chain. PEG is known to reduce the immunoreaction of the body against polymers [29]. The molecular weight of PEG in the grafts was varied between 200, 400 and 1000. Glucose-sensitivity was imparted by the presence of GOD, which is responsible for the oxidation of glucose at physiological pH. Thus glucose is converted to gluconic acid:

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2 \qquad (1)$$

Hydrogen must be removed from the hydrogel. Under physiological conditions, the reaction is limited by the solubility of oxygen in the surrounding medium. To overcome both of these problems catalase was immobilized [23] into the matrix as it reduces the hydrogen peroxide to water and oxygen. Thus, we ensure that hydrogen peroxide is effectively eliminated from the system.

Gluconic acid production results in an increase of hydrogen ion concentration in the microenvironment. This triggers the pH-sensitive swelling of the ionic polymers due to ionization of their carboxyl group. Insulin diffusion is restricted by size exclusion. Upon swelling, the mesh size increases significantly enabling the insulin molecules to diffuse out of the matrix under the influence of a concentration gradient.

3.2. Equilibrium swelling studies

The equilibrium swelling properties of the P(DEAEM-*g*-EG) hydrogels were studied in order to elucidate their pH-sensitive swelling and identify the transition pH where the network converted from a relatively collapsed structure to a fully swollen gel. Gel swelling was quantified using the equilibrium weight swelling ratio which was defined as:

$$\text{Weight swelling ratio, } q = \frac{\text{Weight of swollen polymer sample}}{\text{Volume of dry polymer sample}} \qquad (2)$$

The equilibrium volume swelling ratio for the same system

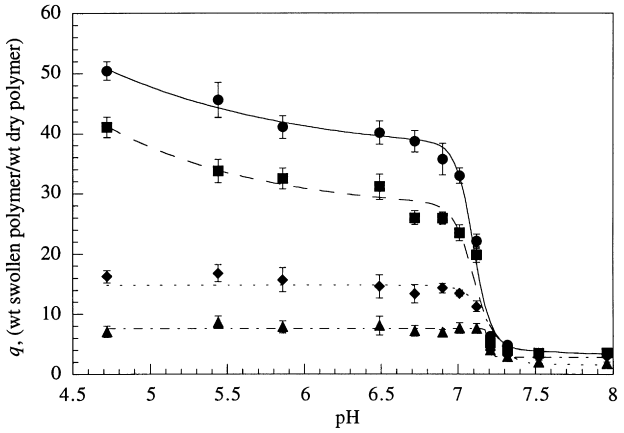

Fig. 3. Effect of crosslinking ratio on equilibrium weight swelling ratio of P(DEAEM-*g*-EG) hydrogels immobilized with GOD and catalase at 37°C. Hydrogels were prepared with 50:50 molar ratio of PEG molecular weight 200), $3.3x10^{-4}$ g GOD/g of polymer. The graphs shown are for $X = 0.005$ (●), 0.01 (■), 0.02 (◆) and 0.04 (▲).

was defined as:

Volume swelling ratio, Q

$$= \frac{\text{Volume of swollen polymer sample}}{\text{Volume of dry polymer sample}} \tag{3}$$

Fig. 2 shows a typical equilibrium weight swelling behavior P(DEAEM-*g*-EG) hydrogels. Weight swelling ratios were determined for pH values ranging from 5.0 to 8.0. Above a pH of 7.0, the swelling ratio of hydrogel was small, between 2.0 and 3.0, and the hydrogel was in the collapsed state. Below pH of 7.0, the hydrogel experienced a significant change. The transition pH value was determined by fitting a hyperbolic tangent curve to these equilibrium swelling data. The inflection point of the curve was identified as the transition pH. For this specific sample, the transition pH was found to be located at 7.06.

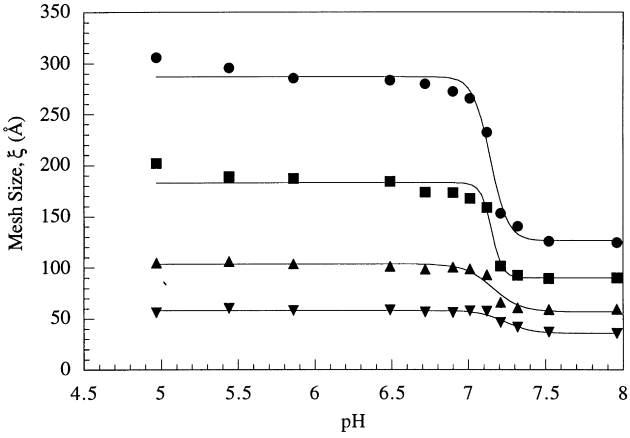

Fig. 4. Effect of initial crosslinking ratio on the mesh size of P(DEAEM-*g*-EG) gels at 37°C as a function of time under the same preparation conditions as in Fig. 3. The graphs shown are for $X = 0.005$ (●), 0.01 (■), 0.02 (▲) and 0.04 (▼).

The characteristic network parameter controlling solute transport is the mesh size, ξ. The mesh size varies with water composition of the gel. The mesh size was calculated by the following equation [30]:

$$\xi = C_n^{1/2} Q^{1/3} N^{1/2} l \tag{4}$$

Here, Q is the equilibrium volume swelling ratio at a particular pH, N is the number of repeating units between two crosslinking, l is the carbon–carbon bond length in Å ($l =$ 1.54 Å) and C_n is the characteristic ratio, 14.4 in 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:

$$Q = \frac{w_s^a - w_s^h}{w_d^a - w_d^h} \tag{5}$$

Here, 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.

The mesh size of the gels tested was calculated in the swollen and the collapsed state. It was found that $\xi_{\text{collapsed}}$ was equal to 45 Å whereas ξ_{swollen} was 250 Å for P(DEAEM-*g*-EG) containing 50:50 DEAEM:PEG ratio, with molecular weight of PEG of 2.0 and $X = 0.02$. Thus the mesh size of the networks increased considerably. Under these conditions, solutes of size much smaller than the mesh size could diffuse easily through the network.

Fig. 3 shows the effect of the nominal crosslinking on the equilibrium swelling characteristics of P(DEAEM-*g*-EG) hydrogels. Samples having crosslinking ratio $X = 0.005$, 0.01, 0.02 and 0.04 mol/mol were equilibrated in different pH solutions. Below the transition pH, the equilibrium swelling ratios were not very sensitive to crosslinking density. However, above the transition pH, the maximum swelling ratio for $X = 0.005$ was 50, whereas for $X = 0.04$, it was 8. The large variations of the swelling ratios were reflected in mesh sizes that were calculated from these experimental results as shown in Fig. 4. It is seen that for gels with $X =$ 0.005, the maximum mesh size obtained was 300 Å whereas for gels with $X = 0.04$, the maximum mesh size was equal to 45 Å. Thus, an incorporated drug such as insulin might be expected to be released quite easily from the loosely crosslinked matrix and considerably more slowly from the crosslinked one.

Therefore, we determined the diffusion coefficient, D, of a solute of radius, r, which can be related to the mesh size by the following equation [30]:

$$D \cong \left(1 - \frac{r}{\xi}\right) e^{[-Y/(Q-1)]} \tag{6}$$

Here, Y is a constant taken to be equal to 1 for most polymeric systems. The diffusion coefficient of insulin (molecular weight 5900, hydrodynamic radius 16 Å) was calculated for the swollen and collapsed states of the

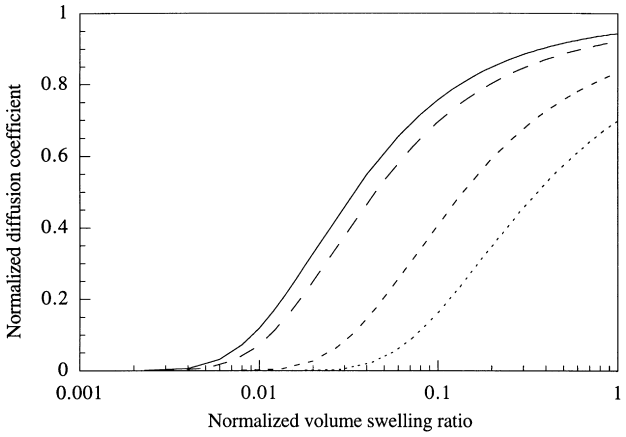

Fig. 5. Normalized insulin diffusion coefficient through P(DEAEM-*g*-EG) at 37°C as a function of the volume swelling ratio normalized with respect to the maximum volume swelling ratio. The diffusional behavior is for gels with initial crosslinking ratio of $X = 0.005$ (—), $X = 0.01$ (– –), $X = 0.02$ (– – –) and $X = 0.04$ (· · ·).

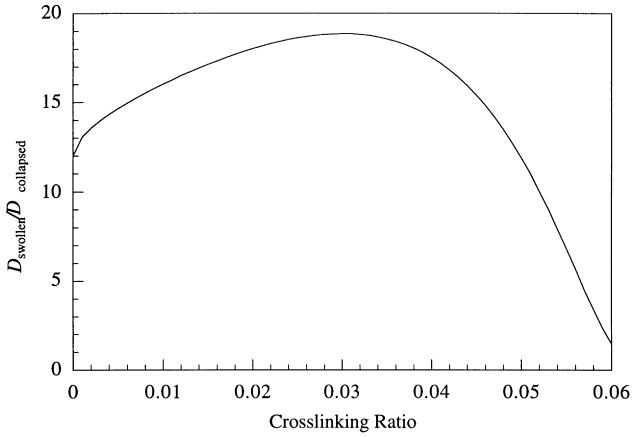

Fig. 6. Ratio of insulin diffusion coefficients in the swollen and collapsed states as a function of the nominal crosslinking ratio of P(DEAEM-*g*-EG) gels, otherwise prepared under the conditions of Fig. 3.

P(DEAEM-*g*-EG) gels and the ratio was $D_{swollen}/D_{collapsed} = 21$. Thus, solute diffusion through the network would be greatly enhanced by the swelling process.

Although the size of insulin is smaller than the mesh size of the gel in the collapsed state, the PEG grafts and the entanglements of the network could prevent insulin from diffusing out in the fully collapsed state. If the pH of the microenvironment of the gel is very close to the pH transition, a very small increase in the pH could induce the complete collapse of the gels and a total cut off of the diffusion of drug through the mesh.

The insulin diffusion coefficient through P(DEAEM-*g*-EG) gels under various equilibrium swelling conditions is shown in Fig. 5. The values of the insulin diffusion coefficient were calculated from Eq. (6) given the mesh size of the gel at a certain pH. The diffusion coefficient was normalized with respect to the diffusion coefficient of insulin in water. The volume swelling ratio was normalized with respect to the volume swelling ratio obtained at a pH of 4.7. It is evident that in the collapsed state the insulin diffusion coefficients were considerably small. As the mesh size increased, the normalized diffusion coefficients approached asymptotically a value of 1.0. As expected, the value of the diffusion coefficients at maximum swelling decreased with increase of the nominal crosslinking ratio. This reflects the ease of insulin transport through the gels having crosslinked ratio of $X = 0.005$.

Table 1 summarizes the ratios of the insulin diffusion coefficients in the collapsed and the swollen states. Fig. 6 illustrates the variation of the ratio of diffusion coefficients in the swollen state and in the dry state. For pH < 7.0, the equilibrium swelling ratios were fit to second-order polynomials of the crosslinking ratios. The ratio of the insulin diffusion coefficients showed a maximum at a nominal crosslinking ratio of 0.03. Above this value, the ratio decreased drastically. This was due to the fact that diffusion was severely retarded due to the tight network structure. It must be noted that the mesh sizes obtained were for networks without PEG grafts. Hence, the diffusion coefficients were conservative estimates of values obtained from network with grafts. The PEG grafts were expected to reduce the surface area open for transport, and consequently the diffusion coefficient.

The effect of GOD loading on the swelling ratio of P(DEAEM-*g*-EG) hydrogels is shown in Fig. 7. Higher enzyme concentration affected the mechanical stability of the hydrogels making them soft and fragile. However, GOD loading did not affect the pH-sensitivity. The equilibrium swelling characteristics showed the usual swollen behavior in low pH values while maintaining the collapsed nature at

Table 1
Crosslinking characteristics mesh size and the ratio of solute diffusion coefficients in the swollen and the collapsed states of P(DEAEM-*g*-EG) gels

Crosslinking density X (mol TEGDMA/mol monomers)	Mesh size $\xi_{collapsed}$ (Å)	Mesh size $\xi_{swollen}$ (Å)	$D_{swollen}/D_{collapsed}$
$X = 0.005$	305.69	124.32	14.62
$X = 0.010$	201.86	89.98	16.12
$X = 0.020$	104.76	63.43	17.47
$X = 0.040$	56.18	35.75	19.73

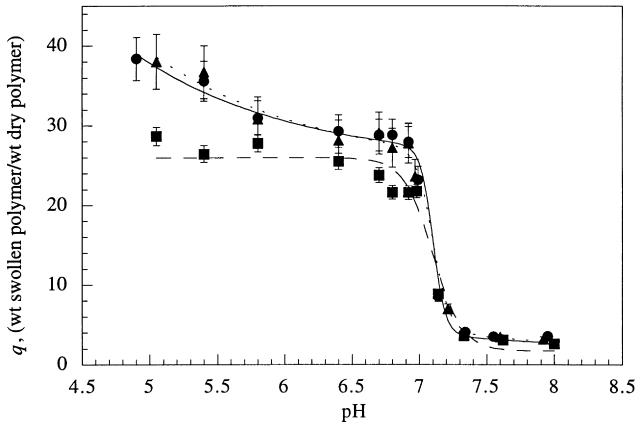

Fig. 7. Effect of GOD loading on the equilibrium weight swelling ratios of P(DEAEM-*g*-EG) gels (prepared as in Fig. 3) versus pH at 37°C. The gels contained 5.1×10^{-4} (●), 10.2×10^{-4} (■) and 20.4×10^{-4} g of GOD/g of polymer (▲).

high pH. The transition pH was also unaffected by varying concentrations of enzymes.

3.3. Dynamic swelling studies

Dynamic swelling studies were performed on both, dry and equilibrated P(DEAEM-*g*-EG) discs to investigate the rate at which the hydrogels responded to any changes in pH in the external medium. It is also necessary to calculate the characteristic time of response of these materials

Fig. 8 shows the kinetics of swelling of discs from the fully dry state. Results are presented for three values of pH, 5.2, 6.8 and 7.4. These values were selected because the pH of blood under normal conditions is 7.4, whereas the hydrogel matrix is expected to attain a pH of 5.2 under the action of glucose. P(DEAEM-*g*-EG) gels had a smaller time constant at a pH = 5.2 as can be seen from Table 2. This was because the swelling driving force was larger than at pH

Table 2
Time constants and variation of swelling ratio of P(DEAEM-*g*-EG) gels between the swollen and collapsed states

pH	Time constant τ (min)	Change in swelling, Δq
7.4	17.2	0.94
6.8	181.8	13.15
5.2	86.8	17.65

of 6.8. From this experiment, it was concluded that the *rate of swelling* of the hydrogels varied with the pH of the surrounding medium. Since, the pH generated in the microenvironment of the hydrogel was dependent on the concentration of glucose in its surroundings, the response of the hydrogel was proportional to the glucose concentration. In a higher glucose environment, the hydrogel swelled to a larger extent, releasing insulin at a higher rate than in a medium containing a lower concentration of glucose.

The time constants of the dynamic swelling responses of the gels were calculated using a first-order approximation to the swelling response. The step response to a first-order linear system is given by

$$q(t) = KM\{1 - \exp(-t/\tau)\} \quad (7)$$

Here, K is the gain in the system, M is the size of the step change that was imposed on the hydrogel, and τ is the time constant of this first-order linear system. Note that M is the number of units of pH change to which the hydrogel is subjected; it is positive for an increase in the pH and negative for a decrease in pH.

The response of the P(DEAEM-*g*-EG) hydrogels to different pH levels from an equilibrated swollen state is shown in Fig. 9. Two samples of these hydrogels were swollen at pH 6.6 and then transferred, one to a lower pH and the other to a higher pH. The two step changes were of equal magnitude. The dynamic response of the hydrogels show that though the pH changes were equal in magnitude,

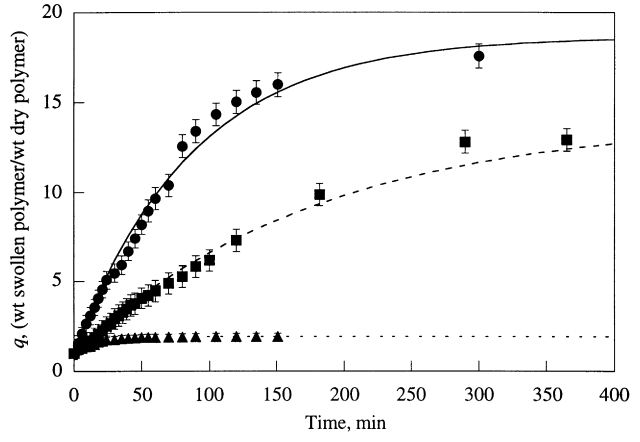

Fig. 8. Dynamic swelling of dry P(DEAEM-*g*-EG) discs at 37°C in solutions pH 5.2 (●), 6.8 (■) and 7.4 (▲). The P(DEAEM-*g*-EG) discs were prepared as in Fig. 3. The curves indicate fitting of the data with time constants and gains as shown in Table 2.

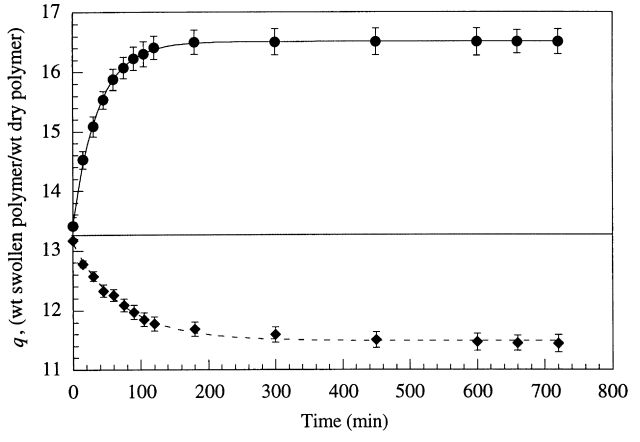

Fig. 9. Positive and negative step responses of P(DEAEM-*g*-EG) hydrogel discs of 37°C prepared under the conditions of Fig. 3. The hydrogels were equilibrated at pH 6.6 and subjected to positive (●) and negative (◆) pH inputs of 0.8 pH units. The curves represent the first-order model responses.

Table 3
Analysis of the oscillatory (step) response of P(DEAEM-*g*-EG) gels

Direction of change	Time constant τ (min)	Change in swelling Δq_∞	Absolute gain K
Positive	38.0	3.07	3.83
Negative	72.8	1.64	2.05

the response of the hydrogels was different in each case. The sample transferred from pH 6.6 to 5.2 swelled as a result of the pH change. The magnitude of change in the swelling incurred was 3.07 unit. The corresponding change in the swelling ratio for the deswelling gel transferred from pH of 6.6 to 7.4 was found to be 1.64 units. This shows that the response of the hydrogel is asymmetric, indicating that the system dynamics is nonlinear in nature.

The time constant of the system was measured by fitting the first-order relation shown in Eq. (7) to the response of the hydrogel samples are shown in Table 3. The fit in each case had correlation with coefficient r^2 between 0.99 and 0.995. The time constants obtained showed that the swelling response was much faster than the deswelling response. The swelling time constant was 38 indicating slow release dynamics. The deswelling time constant was around 72 min indicating that the mechanism responsible for cease of insulin release was also kinetically hindered.

3.4. Pulsatile swelling results

Pulsatile swelling studies were performed on the hydrogel discs to ensure that the materials responded reversibly to pH changes in the environment. It is necessary that the materials swell under the transition pH so that insulin or any other molecule be released immediately in the surrounding medium whenever there is an excess of glucose in the environment. On the other hand, it is also imperative that the gels collapse to the original equilibrated state once the glucose is removed from the surroundings. This would ensure that the release of insulin is cut off as soon as there is a deficit of glucose in the environment. The pulsatile swelling 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.

Table 4
Time constants of elastic response of swelling and deswelling of P(DEAEM-*g*-EG) gels

Section	Swelling/deswelling	Time constant τ (min)
I	Deswelling	4.56
II	Swelling	11.77
III	Deswelling	18.65
IV	Swelling	12.59
V	Deswelling	4.49
VI	Swelling	77.80
VII	Deswelling	15.74

Fig. 10 shows the pulsatile swelling nature of the polymer network. It is evident from the graph that the swollen gels reverted to their relatively collapsed network whenever the pH was increased from 5.8 to 7.2. Also, as the pH was decreased from 7.4 to 5.8, the swelling ratio increased to indicate increased water sorption. The change in swelling ratio was instantaneous coinciding with the change in pH, both, for the swelling, as well as the deswelling cycles. From a device point of view, it can be concluded that the release of insulin within the body could be initiated as soon as excess glucose is detected in the surrounding medium. This would make the control of blood glucose levels easier with the concentrations rapid attainment of normoglycaemic levels.

The mesh sizes were calculated using Eq. (5) at each stage of the swelling process. The time constant in each cycle was calculated using Eq. (7) (see Table 4). These experiments can be used to establish reversibility of nature

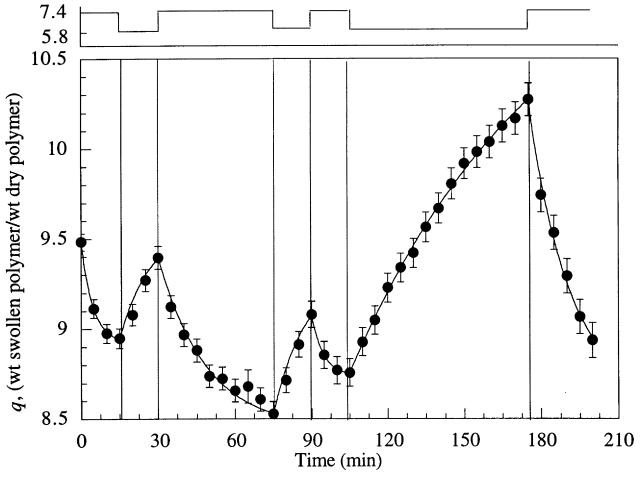

Fig. 10. Pulsatile swelling behavior of P(DEAEM-*g*-EG) gels at 37°C prepared as in Fig. 3. The pH was varied between pH values of 5.8 and 7.4.

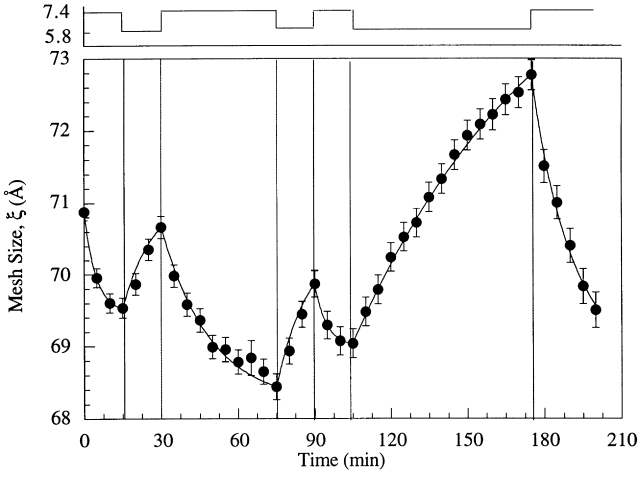

Fig. 11. Calculated mesh sizes of P(DEAEM-*g*-EG) gel discs under varying pH conditions at 37°C.

of response and the also the effect of previous response history on the present response. Fig. 11 shows expected changes in the mesh sizes that were calculated from the swelling ratios obtained from Fig. 3. The mesh size increased with decreases in pH and vice versa. It is also seen that the change in the mesh size was small, ranging between 68 and 72 Å. However, the time intervals between pH changes chosen here were rather small (15 min). Given enough time, the changes in the swelling ratio should be enough to generate a large change in the mesh size capable of either, triggering the release of insulin or shutting off the release depending on the presence or absence of glucose in the surrounding medium.

4. Conclusions

pH-Sensitive hydrogels were synthesized from cationic monomers diethylaminoethyl methacrylate using PEG as grafts on the backbone chains. GOD and catalase were immobilized on the polymer to impart glucose-sensitivity to the hydrogels. Hydrogel discs were studied for pH-sensitivity. Equilibrium swelling studies indicate that the hydrogels showed a strong pH-dependent swelling behavior. The transition between the swollen and the collapsed states was at a pH of 7.0. The dynamic swelling behavior indicated that the hydrogels had a complex and nonlinear swelling and deswelling behavior. It was also observed that the hydrogels showed pulsatile swelling behavior which can be effective in the stimulus-sensitive release of insulin.

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