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Glucose-sensitivity of glucose oxidase-containing cationic copolymer hydrogels having poly(ethylene glycol) grafts

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Abstract

Glucose oxidase and catalase were immobilized on poly(diethylaminoethyl methacrylate-g-ethylene glycol) gels by copolymerization of the constituent monomers and the functionalized enzyme solutions. The hydrogels were prepared in the form of discs and microparticles. The amount and the activity of enzymes immobilized in the matrix were determined. The hydrogels were tested for their response to glucose by exposing microparticles to varying concentrations of glucose. The generation of gluconic acid as a result of the reaction of glucose with oxygen was investigated as a function of polymer parameters, such as crosslinking ratio and enzyme loading. Pulsatile variation of the glucose concentration was used to confirm the glucose-dependent swelling properties of these hydrogels. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Glucose oxidase; Immobilization; Microparticles; Poly(ethylene glycol)-grafted

1. Introduction

Glucose-sensitive membranes and hydrogels can be used effectively to achieve feedback-controlled release of insulin. These polymeric gels demonstrate a reversible swelling behavior in response to glucose concentrations in the surrounding medium. The resulting changes in the mesh size modulate release of insulin physically imbibed in the gels. Since insulin diffusion through these gels is dependent on the changes in mesh size caused by the varying concentration of glucose in the medium, these gels are effective in bringing about feedback-responsive release.

Glucose oxidase (GOD) immobilization on polymers has been investigated in detail in attempts to produce materials for the construction of new types of glucose sensors [1]. Work has also been published on the use of GOD for stimulation of pH-responsive release from polymer matrices and membranes [2]. GOD has been successfully immobilized on a wide variety of polymers, such as polyacrylates [3], polymethacrylates [4], polyethylene [5], polypyrroles [6], silica [7], and poly(vinyl alcohol) [8,9]. The activity of immobilized enzymes is an important parameter that determines the performance of the hydrogel membrane. In most cases, there is a significant loss of enzyme activity due to harsh methods employed during immobilization and polymerization.

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Stability of these enzymes is also a major concern as irreversible degradation might occur with time [9].

The next important criterion determining the feasibility of these gels in insulin delivery is their optimal performance under physiological conditions. Heller and co-workers [10,11] observed that the glucose-sensitivity of poly(ortho esters) could be improved by incorporating tertiary amines into the structure. Albin et al. [12] studied GOD-immobilized polyacrylamide gels and poly(dimethylaminoethyl methacrylate) (PDMAEM) gels in the form of macroporous and microporous matrices. The kinetics of solute transport in these membranes was found to be limited by the solubility of oxygen in the surrounding medium.

The studies conducted by Goldraich and Kost [13] on GOD-immobilized copolymers of 2-hydroxyethyl methacrylate (HEMA) and dimethylaminoethyl methacrylate (DEAEM) crosslinked with tetra-(ethylene glycol) dimethacrylate (TEGDMA) indicated very slow deswelling rates. These gave rise to significant gel irreversibilities as the gels were unable to recover their original conformation when transferred between two solutions containing varying concentrations of glucose. This was further confirmed by Ishihara and co-workers using GOD-immobilized polyamides [14,15] and polymethacrylates [16]. The slow collapse was attributed to the small range of pH variation (between 6.2 and 6.6) attained in the matrix.

In our previous work [17,18], we studied the pH sensitivity of poly(diethylaminoethyl-g-ethylene glycol) (P(DEAEM-g-EG)) hydrogels. It was shown that these hydrogels had a distinct transition behavior, characterized by a steep change in swollen volume at pH 7.0. Below this pH, the hydrogels exhibited high swelling ratios. Above the transition pH, the hydrogels were relatively collapsed with much smaller swelling ratios. Both of these states were related to their corresponding mesh sizes The ionization of the tertiary amine side groups under these conditions resulted in the swelling of the polymer through two mechanisms: (i) increase in the hydrophilicity of the polymer and (ii) electrostatic repulsion between the positively charged groups. In the absence of glucose, the pH in the microenvironment of the gels was found to remain constant at 7.4 and the gel remained collapsed. For applications, two different geometries were studied, namely, discs and microparticles. Of course, the dynamics of the pH-sensitive swelling/deswelling behavior was considerably faster in the case of microparticles.

In our research, GOD was immobilized in the hydrogel to impart glucose-sensitivity to the matrix. To improve the rate of reaction and, therefore, the swelling rates within the gel matrix, one option was to immobilize catalase in the hydrogel along with GOD. Low conversions and reduced rates of reactions might be due to the inhibitory effect of hydrogen peroxide that is formed as a byproduct of the glucose oxidation reaction. Catalase reduces hydrogen peroxide and eventually removes it from the system. By this reaction, some oxygen is recovered and made available for the formation of acid. Thus, an efficient redox system is set up within the gel which operates to minimize unwanted products and provide maximum availability of reactants for the formation of hydrogen ions [12].

Incorporation of poly(ethylene glycol) grafts on the main chains of the hydrogel was an innovative approach based on previous work done in our laboratory [19]. These grafts were expected to retard the degradation of enzymes and proteins within the gel. For an internally implantable device, the presence of certain ‘stealth’ groups helps to minimize the immunoreaction and subsequent rejection by the body. PEG is known to have such stealth properties, which can help maximize the lifetime of the hydrogel in the body [20].

In this work, the glucose sensitivity of P(DEAEM-g-EG) hydrogels was investigated. The effect of a glucose stimulus and the eventual decrease in the pH of the hydrogel on swelling were studied. The change in the swelling ratio and the mesh size under a glucose stimulus were determined.

2. Experimental

The preparation of discs and microparticles of glucose-sensitive P(DEAEM-g-EG) hydrogels involves the copolymerization of functionalized enzymes, methacrylate comonomers and crosslinking agent. GOD and catalase were functionalized before they were added to the monomer mixture. This was done by dissolving 0.01 g of GOD and 175 μl of

catalase (both obtained from Sigma, St. Louis, MO, USA) in 5 ml of pH 7.4 phosphate buffer solution. To this solution, 2 μl of acryloyl chloride (Aldrich, Milwaukee, WI) was added to functionalize amino groups of the enzymes. The reaction was allowed to take place at 4°C in an ice bath for 1 h.

The crosslinked polymer matrix was prepared by using diethylaminoethyl methacrylate (DEAEM, Aldrich), poly(ethylene glycol) monomethacrylate (PEGMA, Polysciences, Warrington, PA, USA) and the crosslinking agent tetraethylene glycol dimethacrylate (TEGDMA, Aldrich). In a typical polymerization process, 3.5 ml of DEAEM was copolymerized with 1.2 g of PEGMA 1000 and 68 μl of TEGDMA. This resulted in a copolymer containing 10 methacrylate repeating units per graft of PEG and a crosslinking ratio $X=0.01$ mol/mol of comonomers. The mixture was then acidified with 1.5 ml of 0.01 N HCl. This decreased the pH of the monomer solution to 6.5, which was necessary to prevent phase separation in the enzyme–comonomer system. Next, 1.5 ml of the functionalized enzyme solution was added with constant stirring. Discs of GOD-immobilized P(DEAEM-g-EG) were prepared by photopolymerization of the above monomer mixture along with 1 wt% cyclohexyl phenyl ketone (Irgacure 164® Ciba–Geigy, Hawthorn, NY, USA) as photoinitiator.

Microparticles were prepared by dispersing the aqueous monomer mixture in silicone oil (Dow 200 Fluid, Dow, Midland, MI, USA). The silicone oil was thoroughly purged with nitrogen before the monomer mixture was added. Redox initiators, sodium metabisulfite and ammonium persulfate (both from Mallinckrodt, Paris, KY, USA) were dissolved in DI water and added to the dispersion. The polymerization was performed under nitrogen for 45 min at room temperature. The details of the polymerization procedure have been discussed in our previous paper [17].

The particles were washed in water and filtered several times until they were oil free. Finally, they were stored in a pH 7.4 PBS solution for further use. Particles were prepared having crosslinking ratio, $X=0.005$–0.04 mol crosslinker/mol comonomer, GOD content, E, ranging between 1.5×10^{-4} and 6.6×10^{-4} g GOD/g of polymer and PEG graft lengths, M_{PEG}, of 200, 400 and 1000.

The rate of hydrogen ion formation was determined by monitoring the change in pH of the medium observed in the presence of the disks or microparticles. Unless otherwise indicated, 200 mg/dl of glucose in 100 ml of saline solution were used. A quantity of 0.05 g of discs or dry microparticles was suspended in the medium for reaction to occur. The microparticles have an average diameter of 160 μm. Samples studied had crosslinking ratio, $X=0.02$ and 0.04 mol/mol of comonomer, polymer graft length of 200 and enzyme loading of 3.3×10^{-4} and 6.6×10^{-4} g GOD/g of polymer.

The amount of enzyme immobilized was determined by analyzing the wash solutions from freshly prepared polymer discs for UV absorbance. Wash solutions from enzyme-free samples were used for calibration and baseline adjustments. The absorbance at 280 and 450 nm were used to estimate the total protein and GOD content in the wash solutions. The activity of the enzyme–polymer system was compared to that of the free enzyme system by monitoring the time-dependent decrease in the pH of both the systems in a saline solution containing 200 mg/dl of glucose.

Swelling studies were performed to characterize the glucose-sensitive properties of the hydrogel microparticles. The swelling medium used for these experiments was saline solution having ionic strength 0.1 M. Dextrose (JT Baker, Phillipsburg, NJ, USA) was used to prepare saline solutions containing different concentrations of glucose. It was necessary to monitor the pH of the surrounding solution during the experiment. Hence, the use of a buffer solution as a swelling medium was not feasible. Also, in these studies, we were interested in observing the rates of swelling and deswelling. It is known [21] that cationic polymers (without glucose oxidase) have a slow response in the presence of strong anions, such as Cl^-. Thus, the rates obtained from swelling studies in saline are representative of those occurring under physiological conditions.

The swelling ratios of individual microparticles were studied using a slide assembly described in our earlier work [18]. Briefly, it consisted of two slides separated using a nylon mesh spacer. A few microparticles were injected in the space between the slides and allowed to distribute themselves within the nylon mesh. The assembly had an inlet and an outlet

for the swelling media. The images of the microparticles were observed through a light microscope and recorded using a camera.

For the pulsatile swelling studies, the particles were first equilibrated in a glucose-free saline solution of pH 7.4. Next, saline solutions having 200 mg/dl of glucose were pumped through the slide assembly for 5 min. The particles were then subjected to a glucose-free saline solution for the next 5 min. This cycle was repeated several times to demonstrate the reversibility of the swelling–deswelling process. Pulsatile swelling studies were also performed on gel microparticles having crosslinking ratios of 0.02, 0.03 and 0.04. All swelling experiments were conducted at 37°C.

3. Results and discussions

The behavior of glucose-sensitive P(DEAEM-g-EG) hydrogels in a glucose environment was studied. As these hydrogels would operate under feedback conditions, the swelling properties were expected to depend on the glucose concentration. In our earlier studies, the swelling rates of hydrogel discs were found to be considerably slow. Microparticles of the same materials proved to be not only effective but also sufficiently controllable, so that insulin release from them could be easily modulated. The dynamic and pulsatile swelling studies were performed primarily on microparticles, in keeping with the favorable rates of swelling that were obtained from them. Pulsatile swelling studies on discs proved to have severe diffusional limitation with the hydrogels unable to recover their collapsed state even at the end of deswelling periods of 60 min.

The swelling behavior may not be the rate controlling step in the release process. Though diffusional limitations can be overcome using microparticles, the reaction of glucose to produce gluconic acid might be crucial in establishing the release rates. This is especially true for small microparticles (<50 μm) where the characteristic times of swelling were found to be less than 30 s. The reaction rate in the hydrogel was determined by the amount and the activity of the enzymes. Thus, it was necessary to study the immobilization efficiency and the activity of immobilized GOD and catalase to fully understand the working of the hydrogel system.

The quantity of enzyme immobilized in the system was determined by spectrophotometric methods. From the absorbance of the wash solution at 280 and 450 nm, the quantities of total protein and GOD which were retained in the hydrogel after the washing process were determined. The efficiency of immobilization was calculated as the ratio of the amount of enzymes immobilized to the total amount added during polymerization. The immobilization efficiency was estimated to be 0.83 for GOD and 0.70 for catalase. The efficiency of immobilization of GOD was higher compared to that of catalase because of the relative smaller molecular weight of the former.

The chemical activity of these enzymes was a major consideration for establishing the efficiency of these systems. The glucose reaction kinetics generated by the enzyme-immobilized hydrogel systems was compared to that obtained using free enzymes dissolved in solution. It must be noted that in case of the free enzymes, the diffusional limitations were nonexistent because the enzymes were dissolved in the reaction medium. On the other hand, in the dry hydrogel, the reactants and the products could encounter diffusion limitations within the mesh. To remove these limitations, the hydrogel disc was used in its equilibrium swollen state. As a result, the diffusion coefficients of the reacting species in the hydrogel were equal to those in water. Thus, the reaction occurring within the hydrogel was isolated and studied as the rate-controlling step.

Fig. 1 shows the variation of pH with time under the action of free enzymes and the enzyme-immobilized hydrogel system in a saline solution having a glucose concentration of 200 mg/dl. The rate of decrease of pH was found to be higher in the free system compared to the immobilized system. This is due to the fact that some of the activity of the enzymes was lost during the polymerization. It is to be noted that, despite the decreased activity of the enzymes, the pH in the hydrogel was reduced to 3.5. The equilibrium swelling curves for these hydrogels reported in our earlier paper [17] show that this value is below the critical pH. Therefore, we can conclude that the hydrogel will swell under the action of glucose.

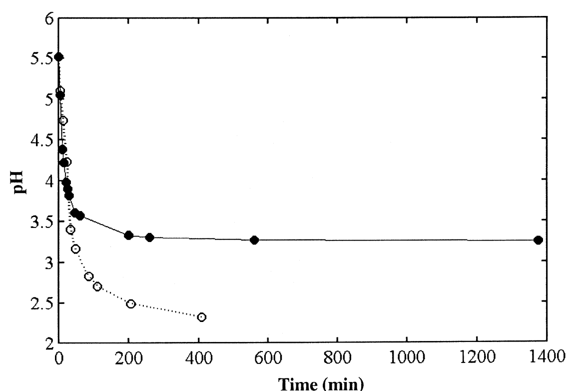

Fig. 1. Kinetics of decrease in pH and hydrogen ion formation in enzyme-immobilized gel and free enzymes in solution under the action of 200 mg/dl of glucose. The hydrogel disc used contained $X=0.02$, $E=3.3\times10^{-4}$ g/g polymer and $M_{PEG}=200$. The dotted lines and the symbols (○) are for the free enzyme system The continuous lines and the symbols (●) are for enzyme immobilized hydrogel.

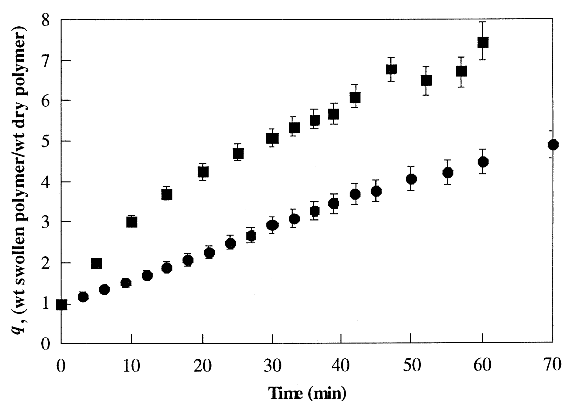

Fig. 2. Kinetics of swelling in P(DEAEM-g-EG) hydrogels having $X=0.02$, $E=3.3\times10^{-4}$ g/g polymer and $M_{PEG}=200$ under in the influence of 200 mg/dl of glucose. The data show the enhancement in rate in presence of catalase (■) compared to gels without catalase (●).

The equilibrium swelling characteristics of P(DEAEM-g-EG) hydrogels were investigated to demonstrate the variations in swelling for different concentrations of glucose. As the glucose concentration of the swelling medium increased from zero to 20 mg/dl, the hydrogel transformed from a collapsed state to a swollen state. This indicated that the swelling of these hydrogels was stimulated by the presence of glucose. However, in this closed system where there is no flow, the hydrogen ions accumulated in the system and reduced the pH until the gel was fully swollen. Thus, even for very small concentrations of glucose, the hydrogel attained high swelling ratios.

Our calculations show that a single disc of weight 0.05 g contained 1.9×10^{-4} mol of DEAEM repeating units. For complete ionization of all the diethyl groups and for the reduction of the solution pH to 6.8, 34.2 mg of glucose were required. Thus, hydrogels placed in solutions having glucose concentrations greater than 34.2 mg/dl would be fully swollen under equilibrium conditions in closed systems.

Dynamic swelling studies were conducted to investigate the rate of gel swelling as a function of changes in glucose concentration in the surrounding medium. The effect of catalase on the swelling rate of these hydrogels was established. Fig. 2 shows the variation in swelling due to the presence of catalase. It was observed that the swelling rates obtained from the catalase-loaded gels were significantly higher than those without catalase. In addition, it was found that the equilibrium swelling ratio of the catalase-loaded gels was higher than that of the gels without any catalase. The action of catalase can be attributed to two factors: (i) catalase converted hydrogen peroxide to water and oxygen aided the kinetics of the swelling and (ii) catalase contributed to increased osmotic pressure in the gel interior, thus increasing the driving force for water to diffuse into the mesh. These two effects can be differentiated by calculating the parameters which characterize the swelling kinetics.

The time constants and initial rates of swelling of these two gel samples are shown in Table 1. The time constant was estimated by fitting an exponential function of time to the swelling data. It was defined

Table 1
Time constants and swelling rates derived from swelling data of P(DMAEM-g-EG) gels with or without catalase

Sample	Time constant, τ (min)	Initial rate of swelling (min^{-1})	Gain, K (mg/dl of glucose)$^{-1}$
With catalase	31.2	0.2019	0.0315
Without catalase	56.5	0.0705	0.0199

as the time required for the gel to attain 62.3% of its equilibrium swollen weight. As is evident from the data, the ratio of the rate of initial swelling was 2.81. The ratio of the gains was found to be 1.58. If catalase had contributed to the increase in the osmotic pressure alone, the time constant ratio would be expected to be equal to the ratio of the gains. However, the time constant decreased by 1.8 due to the presence of catalase. This proves that rate of swelling was enhanced by 80% due to the reactivity of catalase.

The effect of different concentrations of glucose on a batch of hydrogel particles having nominal crosslinking ratio, $X=0.02$, $E=3.3\times10^{-4}$ g/g polymer and $M_{PEG}=200$ was studied. Fig. 3 shows the change in pH in the solutions as a result of 50, 100 and 200 mg/dl of glucose. As expected, the pH reduction corresponding to 200 mg/dl solution was maximum. The pH reductions were progressively lower in the 100 and 50 mg/dl solutions. The final value of the pH reflected the net conversion of glucose under the action of the microparticles.

An understanding of the role of the parameters involved in the reaction was obtained using the Michaelis–Menten kinetic equation often used to model enzymatic reactions. The equation is given as

$$V_0 = \frac{V_{max} C_{G,0}}{K_s + C_{G,0}} \quad (1)$$

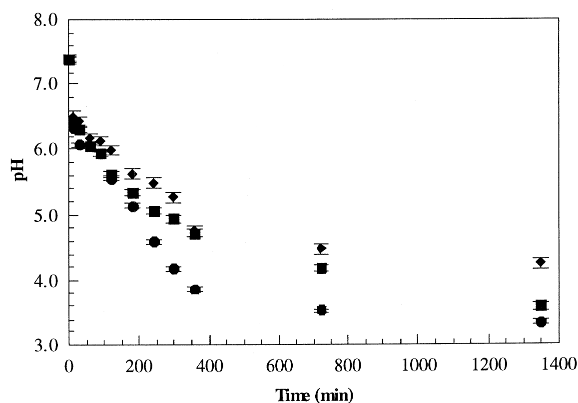

Fig. 3. Change in the pH of glucose solutions due to the oxidation of glucose to gluconic acid by GOD immobilized in P(DEAEM-g-EG) gels. The reduction in pH is shown for 200 (●), 100 (■) and 50 mg/dl (♦) of glucose.

where, V_0 is the initial rate of reaction, $C_{G,0}$ is the initial glucose concentration in the system and V_{max} and K_s are kinetic constants which are estimated from the rate data. Three different types of fitting of the initial kinetic data were used to calculate the values of V_{max} and K_s independently (Table 2) [22]. The term V_{max} increases proportionally with the amount of enzyme in the system. In the original expression of the Michaelis–Menten equation, V_{max} is incorporated as a term for the quantity (or mol) of enzymes taking part in the reaction. On the other hand, K_s, in terms of pure Michaelis–Menten kinetics, is an intrinsic property of the enzyme remaining unaffected by external factors. However, in the immobilized system, the change in diffusional characteristics of the gel by swelling, or otherwise, can affect the value of K_s.

The crosslinking ratio, the enzyme loading and the PEG chain length are some of the parameters which can be varied to modulate the rate of swelling of these hydrogels. Fig. 4 shows the effects of the crosslinking ratio and enzyme loading on the variation of hydrogen concentration in the saline solution containing 200 mg/dl of glucose. The data were plotted for the base case where the nominal crosslinking ratio $X=0.02$ and the enzyme loading was 3.3×10^{-4} g/g of polymer. The sample was studied in the presence and absence of glucose. A second sample having $X=0.04$ and the same enzyme loading was also investigated. The third sample had twice the amount of enzyme immobilized into the polymer as the base case. In the absence of glucose, small changes did occur due to an equilibration process of the hydrogels when there was a redistribution of the ions in the particles and the solution by Donnan equilibrium. However, these changes were too small to cause significant variations in the pH of

Table 2
Kinetic parameters calculated from $[H^+]$ formation data for different initial concentrations on glucose

Fitting	$V_{max}\times10^{-10}$ (mg/dl min)	$K_{s,}$ (mg/dl)
Lineweaver–Burk	7.27	0.0214
Eadie Hoftsee	8.21	0.0254
Hanes	7.74	0.0233
Average	7.74	0.0234

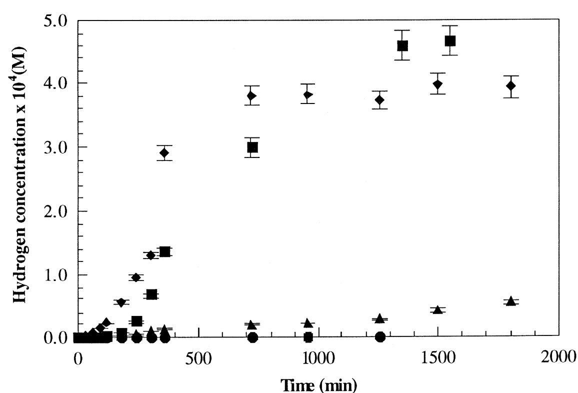

Fig. 4. Kinetics of the hydrogen ion concentration due to P(DEAEM-g-EG) microparticles under the action of 200 mg/dl of glucose in the saline solution. The change in the hydrogen concentration is shown for gels with different crosslinking ratio, X, and enzyme concentrations, E: namely, $X=0.02$ mol/mol, $E=3.3\times10^{-4}$ g GOD/g polymer (■); $X=0.02$ mol/mol, $E=3.3\times10^{-4}$ g GOD/g polymer without glucose (●); $X=0.04$ mol/mol, $E=3.3\times10^{-4}$ g GOD/g polymer (▲); and $X=0.02$ mol/mol, $E=6.6\times10^{-4}$ g GOD/g polymer (♦).

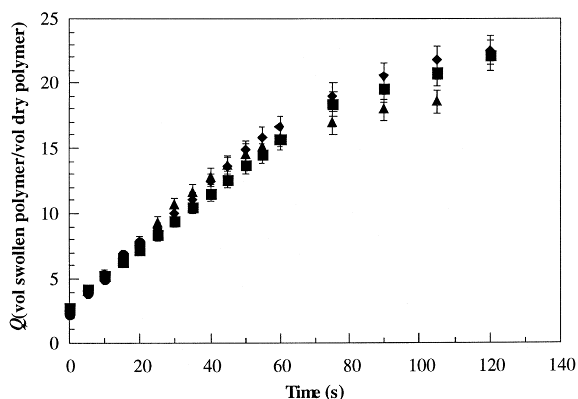

Fig. 5. pH-responsive swelling characteristics of single P(DEAEM-g-EG) microparticles containing different amounts of enzymes. The microparticles used were of the size range 160±10 μm and had crosslinking ratio $X=0.02$, and $M_{PEG}=1000$. The swelling response is shown for microparticles with no enzymes (●), $E=1.67\times10^{-4}$ g/g polymer (■), $E=3.3\times10^{-4}$ g/g polymer (♦), $E=6.6\times10^{-4}$ g/g polymer (▲).

the solution. In the presence of glucose, however, there were significant changes in the pH of the medium.

The rate of hydrogen ion formation was significantly reduced when the crosslinking ratio in the gels was doubled. This effect was attributed to the diffusional limitations of glucose through the mesh. The flux of glucose into the mesh contributed to the overall rate of the hydrogen formation. An increase of the amount of GOD and catalase in the hydrogels led to an increase of the initial rate of reaction. This was explained by the fact that the presence of a larger number of enzyme molecules provided more reaction centers within the matrix. However, the final conversion of glucose for these particles was lower than the base case. The reaction catalyzed by catalase might be rate-limiting in this case. The rate of production of hydrogen ions was enhanced by the increased immobilization. However, the lower immobilization efficiency of catalase resulted in the accumulation of hydrogen peroxide as the reaction proceeded. Under these conditions, the deactivation of GOD was responsible for the early decrease in rate. Thus, though initially the reaction was relatively fast, the reaction yielded a lower glucose conversion.

The swelling of the hydrogels under the action of pH in the absence of glucose for gels containing different amount of enzyme is shown in Fig. 5. Polydispersed particles having $X=0.02$ were equilibrated in PBS of pH 7.4 and transferred to pH 5.0. It is evident that the initial rate of swelling did not vary from sample to sample for such low concentrations of enzymes. However, as the hydrogel swelled to its equilibrium value small variations appeared in the swelling nature. These variations were due to the differences in the relaxational nature of the hydrogels induced by the large enzyme molecules on the backbone chains. The diffusional characteristics of the gels remained unchanged by the incorporation of such small quantities of enzymes into the network.

The dynamic reversible nature of swelling was further investigated by studying the pulsatile swelling response of the hydrogels when the glucose concentration varied between 0 and 200 mg/dl. The results are shown in Fig. 6. Initially, the hydrogel showed a transient response behavior. After the first cycle in pH, the response settled down to a steady pulsatile behavior, though not approaching equilibrium. The swelling ratios reached at the end of each swelling cycle were equal within experimental error. This proved that the swelling behavior of these hydrogels were reversible. Thus, after the initial transient response, the release from these hydrogels

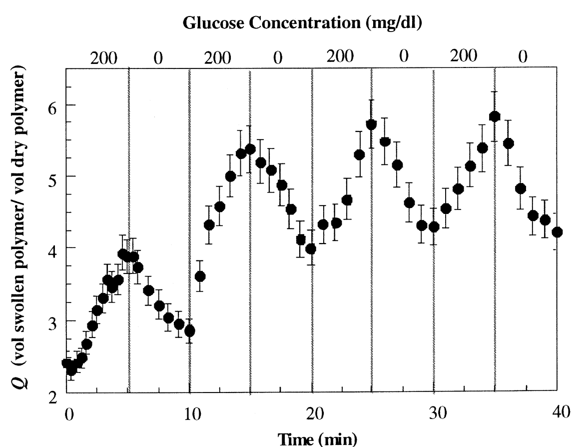

Fig. 6. Variation of the volume swelling ratio of 160-μm particles with time due to a pulsatile concentration of glucose in the swelling medium. These particles have a nominal crosslinking ratio, $X=0.02$, and enzyme loading, $E=3.3\times10^{-4}$ g/g of polymer. The concentrations of glucose used in this study are shown on top of the plot.

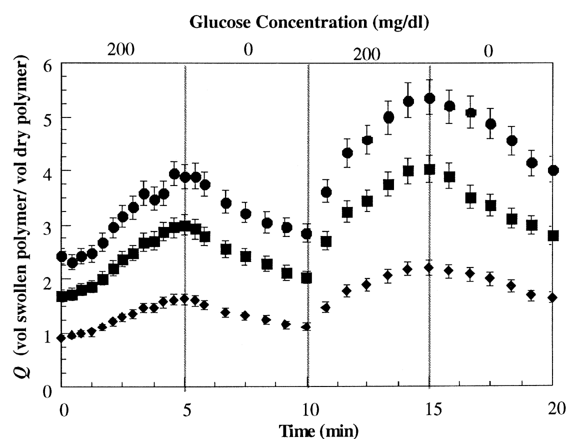

Fig. 7. Pulsatile swelling studies on P(DEAEM-g-EG) microparticles for changes in glucose concentrations in the swelling medium. The results have been shown for different nominal crosslinking ratio of the polymer: namely, $X=0.02$ (●), $X=0.03$ (■), $X=0.04$ (♦).

can be expected to reach values repeatable over several cycles of glucose variations.

The local rates of change of hydrogen ion concentration were estimated from the constants calculated earlier using Eq. (1). These calculations showed that the rate of change of $[H^+]$ ion concentration in a single particle could be as high as 1×10^{-5} M/min. The mesh sizes were calculated for the data obtained using equations relating the mesh size, ξ, in Å to the volume swelling ratio, Q, and the number of links between two tie points and the carbon–carbon bond length in Å [17]. After the steady state was reached, the mesh size settled down to values between the two limits, 105 and 93 Å in the swollen state and the collapsed state, respectively. These variations in the mesh size were enough to generate a difference in insulin diffusion coefficient ranging from 1.09×10^{-6} cm^2/s in the swollen state and 9.66×10^{-7} cm^2/s in the collapsed state. It is evident that variation in the diffusion coefficient of insulin was not significant. The reason behind this is that the cycle time of 10 min was too short for the hydrogels to reach their final equilibrium swelling. Such short cycling times are not typical in the body, where the average half cycles of high blood glucose concentrations could range between one and several hours. However, these experiments do prove that the hydrogels can relax back to their original configurations even when they were exposed to a high frequency of blood glucose variations.

The variations of the pulsatile response of gels having different values of the crosslinking ratio are shown in Fig. 7. Gels having higher crosslinking densities had a lower swelling ratio. This is in keeping with our earlier observation that the diffusion of glucose was hindered by smaller mesh sizes. Single particles were observed under the action of changes in glucose concentration. For $X=0.02$, the maximum swelling ratio was greater than 5 in the 5 min period. The swelling of the gel having $X=0.03$ reached a value around 4 during the period, while for $X=0.04$, the swelling ratio was only 2. The maximum and the minimum mesh sizes encountered during this experiment are shown in Table 3. In-

Table 3
Maximum and minimum mesh sizes of glucose-sensitive of P(DEAEM-g-EG) microparticles undergoing pulsatile swelling

Crosslinking, X (mol/mol of monomer)	Maximum mesh size ξ_{max} (Å)	Minimum mesh size ξ_{min} (Å)
0.02	105.0	94.9
0.03	74.3	67.2
0.04	53.7	48.6

creasing the crosslinking density from 0.02 to 0.03 and 0.02 to 0.04, decreased the average mesh size by 29 and 51%, respectively.

4. Conclusions

The glucose-sensitive characteristics of glucose oxidase and catalase containing P(DEAEM-g-EG) gels were investigated. It was established that the enzymes in the gels were active and could produce an acidic environment within the hydrogel causing the network to swell. Catalase was found to increase the rate of swelling of the gel significantly. The effects of the parameters, such as crosslinking ratio and enzyme loading, on the kinetics of the reaction were established. Higher enzyme loading resulted in faster initial rate of reaction but a lower final conversion. The gels demonstrated a reversible swelling in response to pulsatile variations in glucose.

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