Dynamic Behavior of Glucose-Responsive Poly(methacrylic acid-*g*-ethylene glycol) Hydrogels

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ABSTRACT: Glucose-sensitive poly(methacrylic acid-*g*-ethylene glycol) (P(MAA-*g*-EG)) gels were synthesized by copolymerizing methacrylic acid and poly(ethylene glycol) monomethacrylate in the presence of activated glucose oxidase. The dynamic and equilibrium swelling behavior of the gels was examined as a function of pH. At low pH values the gels were in a collapsed state due to complexation between carboxyl and etheric groups. At high pH values, the gels swelled to approximately 20 times their dry weights. The glucose oxidase containing gels exhibited a higher rate of expansion than the non glucose oxidase containing gels as the former had fewer entanglements. Relaxation times were determined for the swelling and deswelling processes, and a time-dependent swelling behavior was obtained for partial swellings in different pH values.

Introduction

Hydrogels are hydrophilic polymer networks that can absorb large amounts of water but remain insoluble because of the presence of cross-links, entanglements, or crystalline regions.[1,2] Hydrogels can be sensitive to the conditions of the external environment in the presence of thermodynamically active functional groups. The swelling behavior of these gels may be dependent on pH, temperature, ionic strength, or solvent composition. In particular, there has been increased research interest in the development of glucose-sensitive polymeric systems which deliver appropriate amounts of insulin in response to changing glucose levels so as to mimic the natural response of the body.[3] These systems could lead to better control of blood glucose levels in diabetic patients. This approach would involve a glucose oxidase substrate reaction that results in a pH change, triggering a pH-sensitive polymer to respond to the change and expand or contract. Typically, glucose reacts with oxygen in the presence of glucose oxidase forming gluconic acid and, thus, decreasing the pH of the environment. Insulin is released from this system with the change in the size of the pores of the polymer.

The materials of interest in this research were complexing poly(methacrylic acid-*g*-ethylene glycol) hydrogels, henceforth designated as P(MAA-*g*-EG). Interpolymer complexes are formed in these hydrogels due to hydrogen bonding between the hydrogens of the carboxylic group of the PMAA and the oxygens on the ether groups of the PEG chains.[2,4] At low pH values, there is sufficient protonation of the carboxylic acid groups, causing complexes to form and resulting in a collapse of the gel due to increased hydrophobicity in the polymer network. At high pH values, complexes break as the carboxylic groups become ionized.

Previously, Osada and Takeuchi[5] examined interactions between poly(methacrylic acid) (PMAA) and poly(ethylene glycol) (PEG). They observed dilation and contraction of the system due to reversible complexation of PMAA with PEG. For example, in the presence of a small amount of PEG, a PMAA membrane could contract and dilate many times to over 90% of its length. PEG with low molecular weight of 600 and 1000 produced rapid syneresis and the gels easily attained equilibrium.[6] PEG of molecular weight 2000 yielded a rapid and pronounced contraction. Klier et al.[7] were the first ones to investigate the preparation and characterization of P(MAA-*g*-EG) networks. These gels were prepared by the copolymerization of MAA with PEG−monomethacrylate in the presence of tetraethylene glycol dimethacrylate. Their swelling depended on solution pH, temperature, copolymer composition, and network structure. Nuclear Overhauser enhancement measurements indicated that graft copolymers of MAA and PEG formed complexes for a wider range of concentration and PEG molecular weights than the two ungrafted homopolymers. Copolymer networks were also found to swell to a lower extent than homopolymer networks due to complex formation between PEG and PMAA segments. Bell and Peppas[4] have done extensive work to characterize this swelling behavior by performing equilibrium swelling studies as a function of pH and oscillatory swelling studies as a function of time and pH. At low pH, the mesh sizes of the various samples were small, between 3 and 9 Å, whereas at high pH in the uncomplexed or expanded state they were in the range 240 to 350 Å.

In oscillatory swelling studies, the swelling ratio of the gels was monitored as a function of time as the pH of the surrounding solution was changed from acidic to basic over several cycles. In a basic solution, the gel swelled in a linear manner and did not approach equilibrium over the timescale of the experiment. When transferred to an acidic solution, the gel collapsed abruptly. Mesh sizes of the networks were also calculated under oscillatory conditions. Maximum mesh sizes in the expanded states ranged from 11 to 27 Å, whereas in the collapsed states they ranged from 4 to 9 Å.

Glucose-sensitive hydrogels can be produced by the incorporation of a pH-sensitive hydrogel with immobilized glucose oxidase. A "squeezing gel" is expected when incorporating P(MAA-*g*-EG) with glucose oxidase, as shown in Figure 1. At high concentrations of glucose, the glucose oxidase catalyzed glucose reaction with oxygen produces gluconic acid, resulting in a decrease in the pH of the environment. Thus, these hydrogels are expected to collapse abruptly with decrease in pH.

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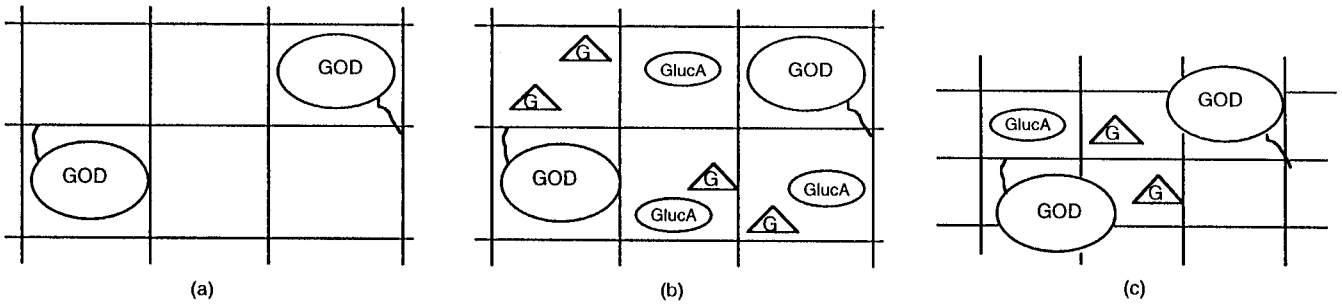

Figure 1. Mechanism of action of glucose-sensitive P(MAA-*g*-EG) gels with glucose oxidase (GOD), glucose (G), and gluconic acid (GlucA): (a) in the absence of glucose, at physiological pH, the hydrogel is in the swollen state; (b) in the presence of glucose, the glucose oxidase−glucose reaction produces gluconic acid causing a decrease in the pH; (c) the hydrogel collapses in response to the decrease in pH. Diagram was drawn by analogy to mechanistic interpretation of Horbett et al.[15]

Such a collapse could "squeeze out" any incorporated drugs including insulin. Alternatively, as the glucose concentration would decrease by the action of the released insulin, less gluconic acid would be produced resulting in an increase in pH of the environment. The hydrogel would be expected to swell with this change in pH. Due to the swelling/collapsing behavior of this system, insulin could be released in a pulsatile manner. A basal dose could be released from the gel in the swollen state at normal glucose concentrations, whereas an increased dose could be released as the gel collapses at high glucose concentrations.[8]

The main goal of this research was to examine the preparation and characterization of glucose-sensitive P(MAA-*g*-EG) hydrogels. Specifically, the objectives of this research were (i) to synthesize P(MAA-*g*-EG) hydrogels that exhibit hydrogen bonding leading to swelling/syneresis, (ii) to investigate the equilibrium and dynamic swelling response of the hydrogels to varying pH conditions, (iii) to investigate the glucose sensitivity of the hydrogels by studying their equilibrium and dynamic swelling response to varying glucose concentration, (iv) to compare the swelling behavior of glucose-sensitive and non-glucose-sensitive gels, and (v) to study the time-dependent swelling response of the glucose-sensitive gels to varying pH.

Experimental Part

Synthesis of Poly(methacrylic acid-*g*-ethylene glycol) Hydrogels. Graft P(MAA-*g*-EG) copolymers were synthesized by free radical polymerization of methacrylic acid (Aldrich Chemical Co., Milwaukee, WI) and methoxy-terminated poly(ethylene glycol) monomethacrylate (Polysciences, Inc., Warrington, PA; PEG MW of 400). The methacrylic acid was purified by vacuum distillation at 66 °C and 30 mm Hg. A 50:50 weight ratio of repeating units of MAA to repeating units of PEG was used. Tetraethylene glycol dimethacrylate (TEGDMA) (Aldrich Chemical Co., Milwaukee, WI) was used as the cross-linking agent and added in the amount of 1 wt % of total monomer. The reaction mixture was then diluted with a 50:50 weight ratio of ethanol and water in the amount equivalent to the total monomer weight. A 50:50 weight ratio of the redox initiators ammonium persulfate (J.T. Baker, Inc., Phillipsburg, NJ) and sodium metabisulfite (J. T. Baker, Inc., Phillipsburg, NJ) was added in the amount of 0.8 wt % total monomer.

In a typical experiment, 1.2 g of PEG monomethacrylate, 8.8 g of methacrylic acid, and 0.1 g of TEGDMA were mixed and diluted with 5 g of ethanol and 5 g of distilled/deionized water. This dilution was selected to lower the viscosity of the polymerizing solution and inhibit autoacceleration of the polymerization. The solution was then placed in a glovebox which was purged with nitrogen for 45 minutes. Nitrogen was bubbled through the polymerization mixture for 20 min to remove oxygen which acts as a free radical scavenger. Subsequently, 0.04 g of ammonium persulfate and 0.04 g of sodium metabisulfite were added as the redox initiators. The polymerizing solution was stirred well for 20 min using a magnetic stirrer and poured into a glass petri dish cover containing 0.7 mm thick teflon spacers. A petri dish bottom was then placed over the solution and spacers so as to create a thin film of polymer between the petri dishes. An additional petri dish cover was then placed on top. The arrangement was sealed with duct tape, removed from the nitrogen glovebox, and the mixture was allowed to polymerize at room temperature for 24 h. Upon completion of the reaction, the ensuing polymer was removed from the petri dish and cut into thin disks of 12 mm diameter.

Synthesis of Glucose-Sensitive Hydrogels. Glucose-sensitive gels of P(MAA-*g*-EG) were synthesized in two steps: activation of the enzyme and polymerization using a procedure adapted from those developed by Valuev and Platé.[9]

In the enzyme activation step, a buffering solution of sodium carbonate was prepared by dissolving 300 mg of sodium carbonate in 5 mL of deionized water. Next, 0.1 g of glucose oxidase type VII from *Aspergillus niger* (Sigma Chemical Co., St. Louis, MO) was added to the buffer solution. Catalase from *Aspergillus niger* (Sigma Chemical Co., St. Louis, MO) was then added to the solution in the amount of 350 μL. An ice bath was prepared to chill this solution to 4 °C. Then 2 μL of acryloyl chloride (Aldrich Chemical Co., Milwaukee, WI) was added. The solution was mixed for 1 h with a magnetic stirring bar. It was important that the solution remained at a temperature of 4 °C or below during this hour because acryloyl chloride reacts vigorously. This glucose oxidase solution served as a solvent in the polymerization reaction.

The monomers used for the preparation of the glucose-sensitive hydrogels were methacrylic acid (Aldrich Chemical Co., Milwaukee, WI) and methoxy-terminated poly(ethylene glycol) monomethacrylate (Polysciences, Inc., Warrington, PA; PEG MW of 400). The methacrylic acid was purified by vacuum distillation. A 50:50 ratio of MAA repeating units: PEG repeating units was used. Tetraethylene glycol dimethacrylate (TEGDMA) (Aldrich Chemical Co., Milwaukee, WI) was added as the cross-linking agent in the amount of 1 wt % total monomer. In a typical experiment, 8.8 g of methacrylic acid, 1.2 g of PEG monomethacrylate, and 0.1 g TEGDMA were mixed and diluted with 5 g of ethanol and 5 g of glucose oxidase solution. The solution was then placed in a glovebox. The glovebox was purged with nitrogen for 45 min. Nitrogen was then bubbled through the polymerization mixture for 20 min to remove oxygen. Next 0.04 g of ammonium persulfate and 0.04 g of sodium metabisulfite were added as the redox initiators. The polymerizing solution was stirred well for 20 min and poured into a glass petri dish cover containing 0.7 mm thick teflon spacers. A petri dish bottom was then placed over the solution and spacers so as to create a thin film of polymer between the petri dishes. An additional petri dish cover was then placed on top. The arrangement was sealed with duct tape, removed from the nitrogen glovebox, and the mixture was allowed to polymerize at room temperature for 24 h. Upon completion of the reaction, the ensuing polymer was removed from the petri dish and cut into thin

disks of 12 mm diameter. The samples were then stored at −20 °C to retain enzymatic activity.

Test for Enzymatic Activity. The glucose oxidase containing P(MAA-*g*-EG) hydrogels were tested for enzymatic activity in a solution of 100 mg of dextrose, anhydrous (J. T. Baker, Inc., Phillipsburg, NJ) per 100 mL of deionized water. The initial pH of the solution was measured with a pH meter (model 399A; Orion Research, Inc., Cambridge, MA). A polymer disk was placed in the solution for 15 min and the final pH of the solution was measured. A significant drop in the pH, caused by glucose oxidase catalyzing the reaction of glucose in solution to produce gluconic acid, indicated whether or not the glucose oxidase remained active during the polymerization.

Swelling Studies. Equilibrium swelling studies were performed at various pH values to initially characterize the swelling behavior for the glucose-sensitive P(MAA-*g*-EG) hydrogels. Dimethyl glutaric acid/sodium hydroxide buffer solutions of pH 3.2, 4.0, 4.8, 5.4, 5.8, 6.0, and 7.0 were prepared by combining 100 mL of a 0.1 M solution of 3,3-dimethylglutaric acid (Sigma Chemical Co., St. Louis, MO) and appropriate amounts of a 1 N sodium hydroxide solution (Sigma Chemical Co., St. Louis, MO) and diluting to 1 L with distilled/deionized water. The ionic strength was kept constant for all solutions at $I = 0.1$ M by adding appropriate amounts of sodium chloride (Fisher Scientific Co., Fair Lawn, NJ) to each solution during preparation.

Dried samples were weighed and placed in 50 mL of a solution of particular pH at 37 °C. The samples were blotted and weighed, and the solutions were changed daily until the weight of the sample did not change by more than 0.01 g over a 24 h period. This equilibrium study was repeated three times. For the oscillatory studies, a polymer disk of 0.5 mm thickness was equilibrated in the pH 4.0 solution at 37 °C. Then the disk was placed in the pH 7.0 solution (still at 37 °C) for 45 min. The sample was blotted and weighed every 5 min. After 45 min, the sample was placed back into a new pH 4.0 solution for 45 min. The sample was again blotted and weighed every 5 min. This 90 min cycle of pH 7.0 to pH 4.0 was repeated two additional times. Three sets of oscillatory swelling data were collected for both the pH- and the glucose-sensitive hydrogels.

Equilibrium swelling studies were also performed in glucose solutions of 80, 200, and 500 mg/dL. The initial pH of each solution was measured. Dry disks of 0.5 mm thickness were then weighed, and placed in glucose solutions. The polymers were blotted and weighed and the pH of each glucose solution was monitored as a function of time until equilibrium was achieved. Swelling studies were also carried out to determine the behavior of glucose-sensitive P(MAA-*g*-EG) hydrogels to varying pH conditions for different time intervals. A polymer disk of 0.5 mm thickness was first equilibrated in a pH 3.2 buffer solution. The sample was blotted and weighed and then placed in a pH 4.0 solution for a time interval of 10 min. At that time, the sample was blotted and weighed again and transferred to a pH 4.8 solution for an additional 10 min. This procedure was continued for pH 6.0 and 7.0 buffer solutions. This experiment was repeated in the same manner using time intervals of 30 min, 3 h, and 24 h to show the time-dependent expansion of glucose-sensitive P(MAA-*g*-EG). Another polymer sample of 0.5 thickness was equilibrated in a pH 7.0 buffer solution. The sample was then blotted, weighed, and placed in a pH 6.0 solution for a time interval of 10 min. Then, the polymer was blotted and weighed and placed in a pH 4.8 solution for 10 min. This procedure was continued for pH 4.0 and 3.2. This experiment was also repeated in the same manner for time intervals of 30 min, 3 h, and 24 h.

Results and Discussion

Synthesis of Glucose-Sensitive Hydrogels. Glucose oxidase containing hydrogels of P(MAA-*g*-EG) were synthesized following (i) activation of the enzymes and (ii) polymerization. The purpose of the activation of glucose oxidase and catalase was to provide structures that allowed the enzymes to be physically attached to P(MAA-*g*-EG) during the polymerization step. In the procedure that was followed, the acrylate group of the acryloyl chloride attached to the enzymes at the site of amines on the enzymes. This formed a peptide bond either at the N-terminus of a polypeptide or on an amino acid side group, allowing the enzymes to maintain activity.[9] It was important to maintain the solution at a temperature of 4 °C or below during its 1 h of mixing because of the vigorous nature of the reaction.

The actual polymerization of the glucose oxidase containing P(MAA-*g*-EG) involved adding the activated glucose oxidase/catalase solution to the polymerization solution as a solvent. The hydrogels produced from this technique appeared to be homogenous and seemed to have polymerized completely. Because of the bright yellow color of the glucose oxidase, the polymer could be examined to determine how the glucose oxidase was attached during the polymerization. Continuous yellow coloring, upon visual inspection, throughout the polymer indicated that the glucose oxidase was evenly distributed and that the attachment procedure likely went well. It was important that the glucose oxidase/catalase solution was immediately used in the polymerization step. Storage of the enzyme solution before use in the polymerization resulted in nonuniform attachment of the enzymes. A yellow coloring only at the surface of the polymer film indicated that the enzymes were concentrated mostly on the surface and that the glucose oxidase did not attach uniformly in the polymer or may not have attached at all. When the enzyme solution was used immediately in the polymerization, the resulting hydrogels appeared homogeneous and completely polymerized.

In this polymerization procedure it was necessary to bubble nitrogen through the reaction mixture for at least 20 min to get complete polymerization. This polymerization also occurred at room temperature whereas the standard P(MAA-*g*-EG) required a polymerization temperature of 37 °C.

The glucose oxidase containing hydrogels were tested to determine if the glucose oxidase remained active throughout the activation and polymerization techniques used. A disk of 0.5 mm thickness caused a drop of 3 pH units when placed in a 100 mg/dL solution of glucose for 15 min. Over this 15 min interval, the glucose in the surrounding solution reacted in the presence of glucose oxidase to produce gluconic acid according to the following reaction:

$$\text{glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

These results indicate that the glucose oxidase remained active throughout the activation of enzymes and polymerization procedures.

Catalase was also incorporated into the gels as it catalyzes the conversion of hydrogen peroxide to oxygen and water:

$$2H_2O_2 \xrightarrow{\text{catalse}} O_2 + 2H_2O$$

The incorporation of catalase is important in order for the glucose oxidase to continue to react in the presence of glucose to produce gluconic acid. In the absence of catalase, the glucose oxidase reaction would be limited because of the disappearance of oxygen in the environment. Albin et al.[10] reported situations in which their glucose oxidase based system became oxygen limited

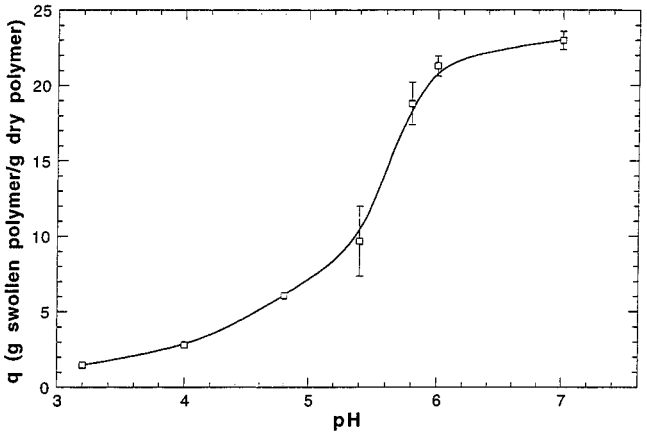

Figure 2. Equilibrium weight swelling ratio of glucose oxidase containing P(MAA-*g*-EG) hydrogels as a function of pH at 37 °C.

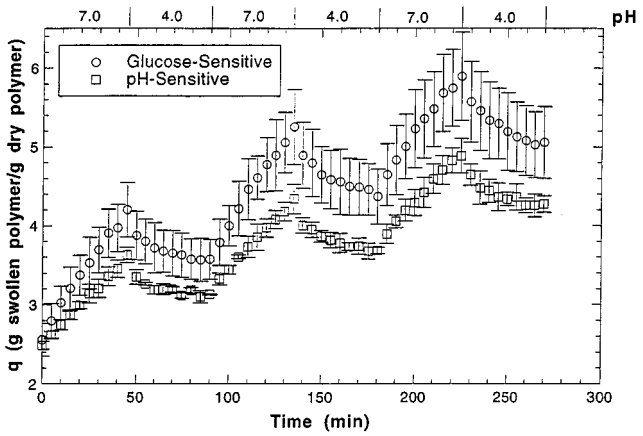

Figure 3. Oscillatory swelling behavior of glucose-sensitive and pH-sensitive P(MAA-*g*-EG) hydrogels as a function of time and pH at 37 °C as the gels were swollen at pH values of 7 and 4.

and consequently functioned as an oxygen sensor rather than a glucose sensor. Catalase served to replenish this supply of oxygen as well as decrease the amount of hydrogen peroxide, which could be dangerous under physiological conditions.

Swelling Studies. Oscillatory and equilibrium swelling studies were performed with both the pH-sensitive and the glucose-sensitive P(MAA-*g*-EG) hydrogels. The results show the effects of pH and glucose concentration on the swelling behavior of the gels.

The equilibrium swelling behavior of glucose-sensitive P(MAA-*g*-EG) hydrogels was investigated over the pH range of 3.2 to 7.0. The weight equilibrium swelling ratio, *q*, was calculated for each pH value by

$$q = \frac{W_s}{W_d} \tag{1}$$

where W_s is the weight of the swollen matrix and W_d is the weight of the dry matrix.

Figure 2 shows the equilibrium swelling behavior of thin disks of the glucose oxidase containing P(MAA-*g*-EG) gels. At low pH values of 3.2 and 4.0, the gel was in a collapsed state due to complexation or hydrogen bonding between the carboxylic acid groups of PMAA and the oxygens of the ether groups of the PEG chains. At high pH values of 5.8, 6.0, and 7.0, the gels swelled to approximately 20 times their dry weights. This high degree of swelling is attributed to breakage of most of the complexes.

Hydrogen bonds were broken at higher pH values as the carboxylic groups became ionized. As a result, the gel began to swell to a high extent as electrostatic repulsion was increased within the network. Larger error bars were calculated at higher pH values. This was due to the fact that the gels were very sensitive to changes in the environment once the pH was above a value where significant decomplexation occurred. Therefore, any slight difference in pH of the buffer solutions could cause a significant difference in the degree of swelling. The transition region of the swelling ratio vs pH curves (such as that of Figure 2) is roughly between pH values of 4.6 and 5.8. In the specific case, the exact transition pH was 5.2, as determined by the inflection point of the curve.

The swelling/syneresis behavior of glucose oxidase containing P(MAA-*g*-EG) gels was studied under varying pH conditions to characterize their dynamic behavior (see Figure 3). This behavior was also compared with the swelling/syneresis behavior of non glucose oxidase containing P(MAA-*g*-EG). In these studies, the pH of the environment was changed from pH 7.0 to pH 4.0 every 45 min. The weight swelling ratio, *q*, was calculated for each data point using eq 1.

Figure 3 shows the behavior of thin disks of the gels under these varying conditions. In the initial solution of pH 4.0, the gels were in their collapsed state due to complexation. The weight swelling ratios for both the glucose oxidase containing and non glucose oxidase containing gels were approximately 2.5 g of swollen polymer/g of dry polymer. When the samples were placed into a pH 7.0 solution, hydrogen bonds were broken as the carboxylic acid groups became ionized. This resulted in swelling of the gels. The swelling ratios increased at a fairly constant rate in the pH 7.0 solution for both gel types. The glucose-sensitive gels exhibited a higher rate of expansion than the standard gels. Because of the pendent groups of glucose oxidase and catalase, the glucose-sensitive gels had fewer entanglements and could expand faster than the standard gels. It is also shown in Figure 3 that there was an initial rapid collapse for both types of gels when they were placed in pH 4.0 solutions. The remaining collapse period of 40 min at pH 4.0 was rather slow. When the gels were initially placed in the pH 4.0 solutions, complexes formed rapidly, causing the gel to collapse quickly. The glucose-sensitive and pH-sensitive gels appeared to have similar collapse (complexation) behavior although their rates of swelling (decomplexation) differed. In both cases, the gels did not return to their initial equilibrium state at pH 4.0 after 45 min. This caused the swelling ratios to shift to slightly higher values with each repeated cycle. However, the swelling ratios increased (pH 7.0) and decreased (pH 4.0) by the same relative amount in each cycle. If the gels were allowed to remain in a pH 4.0 solution for a longer period of time, the initial equilibrium swelling ratio could be achieved in the repeated cycles.

Analysis of the chain relaxation time of these hydrogel systems can be performed using these oscillatory swelling results. This analysis was based on the application of the Boltzmann superposition principle.[11] The principle is based on the idea that output is a linear function of various inputs such that the total effect of applying several inputs is the same as the cumulative effect of applying each one separately. This principle states that the output is a weighted integral of the inputs over the time history of the samples studied.

In the case of the hydrogel system of interest, the change in pH can be represented by the change in the concentration of hydrogen ions. The application of the Boltzmann superposition principle can then be described as

$$\epsilon(t) = \int_0^t L(t-\tau)\frac{\partial[\mathrm{H}^+]}{\partial\tau}\,\mathrm{d}\tau \qquad (2)$$

where $\epsilon(t)$ is the time dependent strain (output), $[\mathrm{H}^+]$ is the concentration of hydrogen ions (input), τ is an integration variable, and $L(t-\tau)$ is the mechanochemical compliance of the gel.

When this principle is extended to an isotropic gel swelling in three dimensions, we can write

$$Q(t) = \frac{V_s(t)}{V_d} = \frac{l^3}{l_o{}^3} = \frac{(l_o+\Delta l)^3}{l_o{}^3} = [1+\epsilon(t)]^3 \qquad (3)$$

where Q is the volume swelling ratio, V_s is the polymer volume in the swollen state, V_d is the polymer volume in the dry state, l_o is the original length, and l is the length. In general, the volume swelling ratio could be calculated from the weight swelling ratio, q, as follows:

$$Q = 1 + \frac{\rho_p}{\rho_w}(q-1) \qquad (4)$$

Here, ρ_p is the density of the dry polymer and ρ_w is the density of water. Finally, from eqs 3 and 4 we obtain

$$Q(t) = \left[1 + \int_0^t L(t-\tau)\frac{\partial[\mathrm{H}^+]}{\partial\tau}\,\mathrm{d}\tau\right]^3 \qquad (5)$$

In the simplest analysis of the viscoelastic behavior, the mechanochemical compliance was assumed to be time-independent, yielding

$$Q(t) = \left[1 + L\frac{\Delta[\mathrm{H}^+]}{\Delta t}t\right]^3 \qquad (6)$$

In more general terms, the mechanochemical compliance can be expressed as a function of relaxation times of the polymer chains, θ

$$L(t) = \int_0^\infty \theta\, \mathrm{L}(\theta)[1-\mathrm{e}^{-t/\theta}]\frac{\mathrm{d}\theta}{\theta} \qquad (7)$$

In our case, the relaxation time was determined from the data of Figure 3 by combining eqs 5 and 7. An approximation of the average relaxation times of the polymer chains was obtained by plotting ln $Q^{1/3}$ as a function of time.[12] The slope of this curve yielded the reciprocal of the average relaxation time, θ. Each region was analyzed separately to compare relaxation times between the expansion and contraction of the hydrogels. Table 1 shows the results from this analysis.

It may be noted that this approximation may only be used for hydrogels which respond quickly to changes in the surrounding environment. The initial collapse (first 5 min of collapse) and final collapse (remaining 40 min of collapse) regions for each cycle of the oscillatory swelling studies were analyzed in this manner for both the glucose-sensitive and pH-sensitive gels. It can be seen from the table that average relaxation times were higher during expansion than during the initial collapse. This emphasizes that there was an initial rapid collapse of the gels, followed by a slower period of collapse with much higher average relaxation times. When the glucose-sensitive and pH-sensitive gels were compared, it was noted that the pH-sensitive gels exhibited longer relaxation times during the expansion period due to the absence of the large enzymes, glucose oxidase and catalase. The initial collapse periods of the two types of gels were virtually the same for each cycle. Although the presence of the enzymes affected the rate of expansion, the initial collapse occurred at the same rate for both gel types.

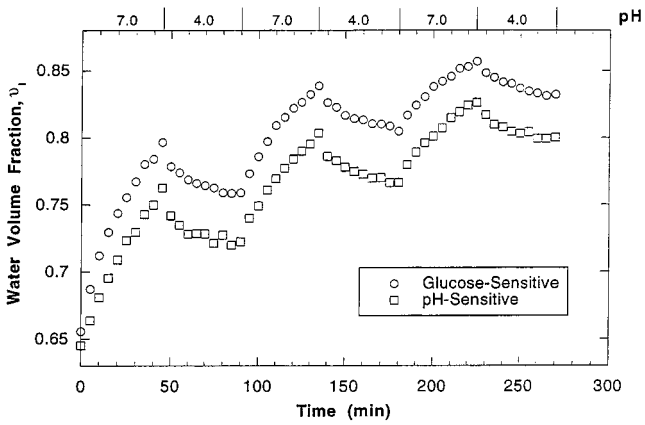

Figure 4. Change of water volume fraction of glucose- and pH-sensitive P(MAA-*g*-EG) hydrogels as a function of time and pH at 37 °C as the gels were swollen at pH values of 7 and 4.

Table 1. Average Relaxation Times of Glucose-Sensitive and pH-Sensitive P(MAA-*g*-EG) Gels Determined from Oscillatory Swelling Results

			θ (min)	
cycle	pH	response	glucose-sensitive	pH-sensitive
1	7.0	expansion	270	345
1	4.0	initial collapse	177	178
1	4.0	collapse	1641	2541
2	7.0	expansion	343	412
2	4.0	initial collapse	202	202
2	4.0	collapse	1292	1481
3	7.0	expansion	451	466
3	4.0	initial collapse	260	287
3	4.0	collapse	1251	1939

The oscillatory swelling results were also analyzed in terms of the water volume fraction, v_1, which can be determined from the volume swelling ratio by

$$v_1 = 1 - \frac{1}{Q} \qquad (8)$$

Figure 4 shows the water volume fraction as a function of pH and time for the glucose- and pH-sensitive gels. The gels contained approximately 65% water in their initial equilibrated state. When placed in a pH 7.0 solution, the glucose-sensitive gels began to take up water at a constant rate for 45 min, reaching a water volume fraction of 0.8. The pH-sensitive gels also swelled at a constant rate but only took up water to a fraction of 0.77. When placed in the pH 4.0 solutions, both gels collapsed, excluding approximately 4% of the water out of the system. This behavior was observed again in the following two cycles. These results emphasize the faster rate of expansion but similar rate of collapse when comparing the glucose- and pH-sensitive gels.

The change in network swelling in response to pH can be translated to a change in the mesh size of the gel. The mesh size can be described in one dimension by the correlation length, ξ, the distance between two adjacent

cross-links. In determining the mesh size from oscillatory swelling results, the Peppas−Merrill[13] equation was used to calculate the experimental molecular weight between cross-links

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{\left(\frac{\bar{v}}{V_1}\right)[\ln(1 - v_{2,s}) + v_{2,s} + \chi_1 v_{2,s}^2]}{v_{2,r}\left[\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} - \frac{1}{2}\left(\frac{v_{2,s}}{v_{2,r}}\right)\right]} \quad (9)$$

In this equation, $\bar{M}_c$ is the average molecular weight between cross-links, $\bar{M}_n$ is the average molecular weight of the uncross-linked polymer, $\bar{v}$ is the specific volume of the polymer (0.984 cm^3/g for PMAA and 0.898 cm^3/g for PEG), V_1 (18.1 cm^3/mol) is the molar volume of water, $v_{2,r}$ is the polymer volume fraction after cross-linking but before swelling (the relaxed polymer volume fraction), $v_{2,s}$ is the polymer volume fraction after equilibrium swelling (swollen polymer volume fraction), and χ_1 is the Flory polymer−water interaction parameter (0.5987 for PMAA and 0.55 for PEG). Because random copolymers were used in these studies, weighted averages of the values for the homopolymers were employed in eq 9 and the equations that follow.

The average molecular weight of the uncross-linked polymer was calculated by[14]

$$\bar{M}_n = M_o \frac{k_p[M]}{(f k_d k_t [I])^{1/2}} \quad (10)$$

where M_o (=86) is the molecular weight of the monomer unit, [M] = 0.00474 mol/cm^3 and [I] = 1.8 × 10^{-5} mol/cm^3 are the concentrations of monomer and initiator, k_p (=670 L/mol·s) is the kinetic constant of propagation, k_t (=2.1 × 10^6 L/mol·s) is the kinetic constant of termination, k_d (=0.0165 s^{-1}) is the kinetic constant of the decay of the initiator, and f (=0.5) is the efficiency of the initiator. The calculated value of $\bar{M}_n$ was approximately 15 500.

The swollen polymer volume fraction was determined from the volume swelling ratio, Q

$$v_{2,s} = \frac{1}{Q} \quad (11)$$

The relaxed polymer volume fraction was determined using the equation

$$v_{2,r} = \frac{V_p}{V_{g,s}} \quad (12)$$

where V_p is the volume of the dry polymer and $V_{g,s}$ is the volume of the polymer containing the solvent that was used during the cross-linking process. The volume of the dry polymer was calculated by

$$V_p = \frac{W_a - W_h}{\rho_h} \quad (13)$$

where W_a is the weight of the polymer in air, W_h is the weight of the polymer in heptane, and ρ_h (=0.6837 g/cm^3) is the density of heptane. The term $V_{g,s}$ was found in a similar manner

$$V_{g,s} = \frac{W''_a - W''_h}{\rho_h} \quad (14)$$

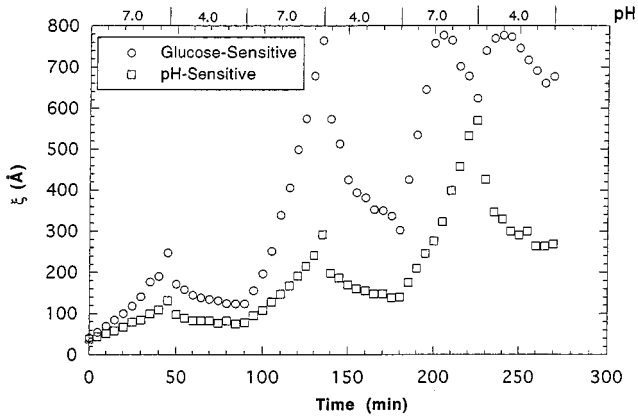

Figure 5. Change of mesh size of glucose-sensitive and pH-sensitive P(MAA-*g*-EG) hydrogels as a function of time and pH at 37 °C as the gels were swollen at pH values of 7 and 4.

Here, W''_a and W''_h designate the weight of the gel, before drying, in air and heptane, respectively.

After determining $\bar{M}_c$, the number of links along the polymer chain, n, was then calculated by

$$n = \frac{2\bar{M}_c}{M_r} \quad (15)$$

where M_r is the molecular weight of the repeating unit. After n was determined, the root mean squared end-to-end distance of the polymer chain in the freely jointed state was calculated as

$$(\bar{r}^2)^{1/2} = l n^{1/2} \quad (16)$$

In this equation, l is the bond length along the polymer chain (1.54 Å for carbon−carbon bonds). The root mean squared end-to-end distance of the polymer chain in the unperturbed state was calculated by

$$(\bar{r}_o^2)^{1/2} = \sqrt{C_n}(\bar{r}^2)^{1/2} \quad (17)$$

where C_n is the characteristic ratio of the polymer (based on mole fractions of PMAA and PEG where C_n is 14.6 for PMAA and 3.8 for PEG). The mesh size, ξ, of the polymer was then calculated by

$$\xi = v_{2,s}^{-1/3}(\bar{r}_o^2)^{1/2} \quad (18)$$

Mesh sizes of the P(MAA-*g*-EG) gels are shown in Figure 5. When initially equilibrated at pH 4.0, both the glucose- and pH-sensitive samples were in collapsed states with mesh sizes of approximately 40 Å. When first exposed to pH 7.0 conditions, the mesh size of the glucose-sensitive gel increased to about 250 Å after 45 min. The mesh size of the pH-sensitive gel only increased to approximately 130 Å. The less entangled chains of the glucose-sensitive gels were able to relax faster creating a larger distance between adjacent cross-links. In the first swelling cycle, the percent changes in the mesh sizes after 45 min were 500% for the glucose-sensitive gel and 250% for the pH-sensitive gel. When placed back into a pH 4.0 solution, the gels exhibited an initial rapid decrease in the mesh size due to rapid complexation in the network. The glucose-sensitive gel showed a decrease of 75 Å, whereas the mesh size of the pH-sensitive gels decreased by 35 Å. For both gel types, the initial collapse in the first 5 min after being placed in a pH 4.0 solution resulted in a 30%

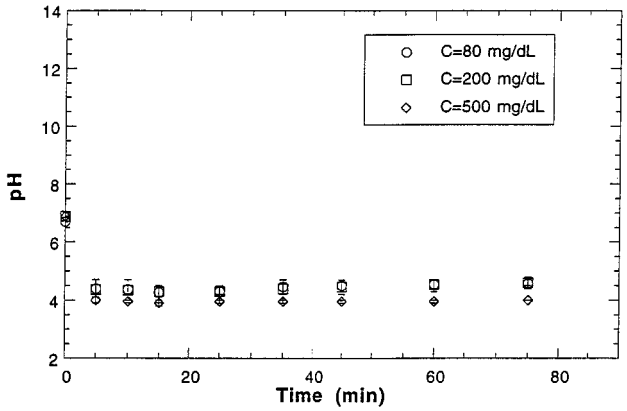

Figure 6. Change in pH of three different glucose solutions as a function of time after immersion of dry glucose-sensitive P(MAA-*g*-EG) network samples at 37 °C.

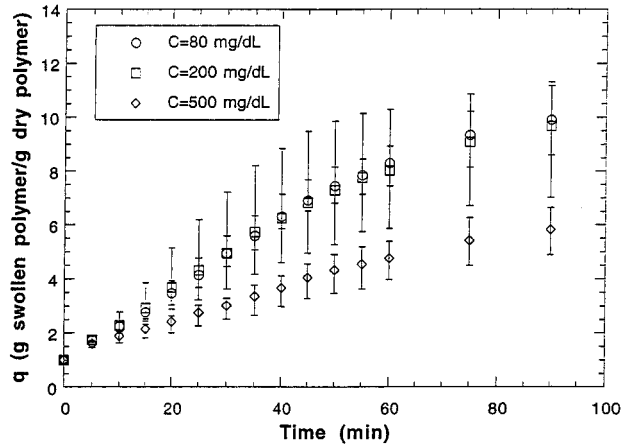

Figure 7. Change of weight swelling ratio of glucose-sensitive P(MAA-*g*-EG) samples as a function of time after immersion in three different glucose solutions at 37 °C.

decrease in the mesh size. Similar changes in the mesh sizes of the gels were noted in the subsequent cycles.

Glucose-Responsive Swelling Experiments. The ability of glucose-sensitive P(MAA-*g*-EG) hydrogels to respond to glucose was examined by swelling these gels in solutions with glucose concentrations of 80, 200, and 500 mg/dL. These concentrations were used to represent normal blood glucose (80 mg/dL) to hyperglycemic conditions (200−500 mg/dL). Initially dry, glucose-sensitive disks were placed in the various glucose solutions. An initial drop in the pH was observed in all glucose solutions (see Figure 6). This drop in the pH was attributed to glucose oxidase on the surface of the polymer disks which could catalyze the reaction of glucose to gluconic acid.

The dynamic swelling behavior of the same glucose-sensitive P(MAA-*g*-EG) is shown in Figure 7. The disks were swollen from an initially dry state ($q = 1$). An rapid initial water uptake was observed for all samples when placed in glucose solutions. However, the samples at the high glucose concentration of 500 mg/dL swelled at a slower rate than those in the lower glucose concentrations. This rate was approximately 0.063 g of swollen polymer/g of dry polymer-min as calculated from the slope over the first 60 min. Samples placed in the lower glucose concentrations had a tendency to swell at a faster initial rate of 0.120 g of swollen polymer/g of dry polymer-min.

The swelling behavior of preswollen gels as they came to equilibrium is shown in Figure 8. Although the

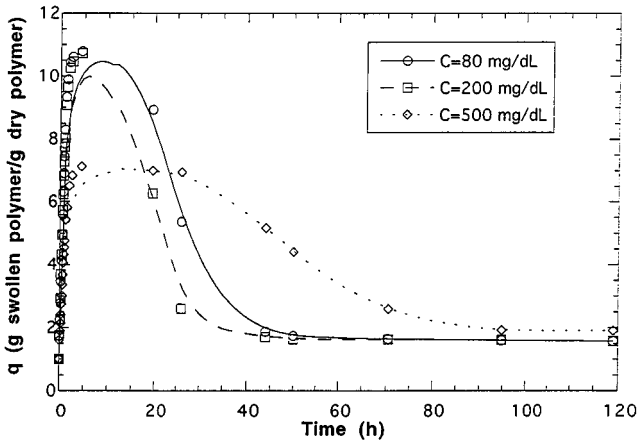

Figure 8. Weight swelling ratios of glucose-sensitive P(MAA-*g*-EG) hydrogels as a function of time in response to swelling in glucose solutions at 37 °C.

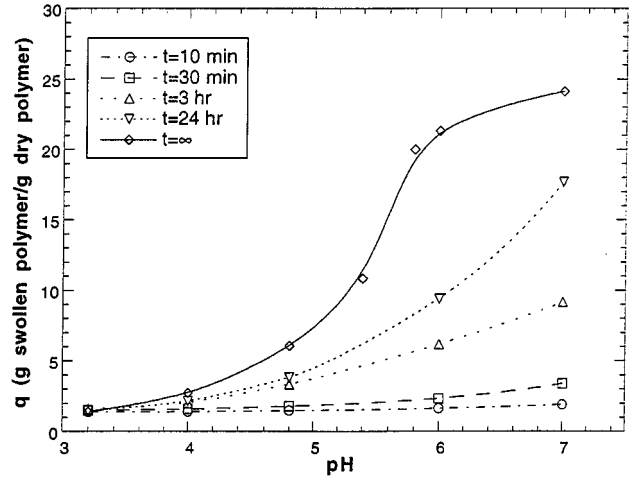

Figure 9. Gradual weight swelling ratio change as a function of pH for glucose-sensitive P(MAA-*g*-EG) hydrogels swollen at 37 °C in successive pH solutions starting from the deswelling state at a pH = 3.2 and changing the swelling time by 10 min (○), 30 min (□), 3 h (△), or 24 h (▽). Data at equilibrium swelling ($t = \infty$, ◇) are also reported.

samples initially swelled to a high extent, collapse of the networks began after approximately 5 h. For the lower glucose concentrations, interior glucose oxidase further catalyzed the reaction of glucose to gluconic acid, resulting in a further decrease in pH and faster rate of collapse. A slower collapse occurred for the 500 mg/dL glucose concentration because only a slight decrease in the pH was observed. Of course, all these data indicate only the general swelling/deswelling response of the gels. For the development of a diabetic device, the characteristic thickness of the samples can be reduced, leading to a squared time reduction, i.e., fast response.

Time-Dependent Swelling Experiments. The swelling behavior of glucose-sensitive P(MAA-*g*-EG) hydrogels was studied as a function of pH for varying time intervals. These studies were performed to analyze the response of the gels to gradual changes in pH. Figure 9 shows the results obtained from a sample that was initially equilibrated in a buffer solution of pH 3.2 and then exposed to a gradual increase in pH. Equilibrium swelling ratios of the sample at different pH values are plotted in a curve indicated by $t = \infty$. These values were obtained after equilibration for 4 days. However, when the same sample was exposed to an increase in pH for only 10 min, there was only a slight increase observed in the swelling ratio. As the time

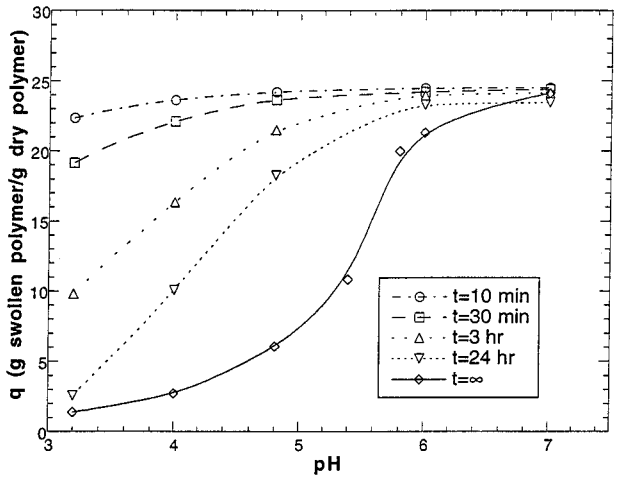

Figure 10. Gradual weight swelling ratio change as a function of pH for glucose-sensitive P(MAA-*g*-EG) hydrogels swollen at 37 °C in successive pH solutions starting from the swelling state at a pH = 7.0 and changing the swelling time by 10 min (○), 30 min (□), 3 h (△), or 24 h (▽). Data at equilibrium swelling ($t = \infty$, ◇) are also reported.

interval was increased to 30 min, there was a noticeable change in the swelling ratio. A large increase in the degree of swelling was not noted until a time interval of 3 h between changes in pH was used. With a time interval of 24 h, the gel reached a final swelling ratio that was 75% of its equilibrium swelling ratio. These results indicate that the gels respond slowly to a gradual increase in the pH.

A glucose-sensitive P(MAA-*g*-EG) sample was similarly exposed to a gradual decrease in pH after being equilibrated at pH 7.0 to better analyze its deswelling behavior. Figure 10 shows the results of this deswelling process. Again, the equilibrium degree of swelling is represented by the curve with the indication $t = \infty$. Even with the small time interval of 10 min, a noticeable decrease in the swelling ratio was observed. By the end of the 24 h interval experiment, the gel had collapsed quite close to its complexed, equilibrated state at pH 3.2. When the sets of curves are examined together, it is seen that the gels collapse faster in response to a gradual decrease in pH than they expand in response to a gradual increase in pH for the same time intervals. This supports the idea that the rate of contraction of the gels is higher than the rate of swelling, as was observed in the oscillatory swelling studies and in previous work.[4]

Conclusions

The focus of this research was to synthesize graft copolymers of P(MAA-*g*-EG) with immobilized glucose oxidase to yield a glucose-sensitive polymer, and to characterize the pH- and glucose-sensitive swelling behavior. Important parameters controlling the behavioral type of glucose-responsive hydrogels included the equilibrium and dynamic degrees of swelling, network mesh size, and diffusive characteristics. Glucose-sensitive P(MAA-*g*-EG) hydrogels were synthesized by the activation of glucose oxidase and then polymerization in the presence of the activated enzyme. When placed in contact with a glucose solution, these gels produced a significant decrease in the pH of the environment, indicating that the glucose oxidase had remained active throughout the synthesis procedure.

The pH-dependent swelling behavior was characterized in equilibrium swelling studies. At low pH values, the gels were in a collapsed state due to complexation. At high pH values, the gels swelled to approximately 20 times their dry weights. The transition pH of the gels was estimated to be 5.2.

Oscillatory swelling studies were performed to characterize the swelling/syneresis behavior of glucose-sensitive and non-glucose-sensitive gels. Glucose-sensitive gels showed a higher rate of expansion than non-glucose-sensitive gels at high pH. Because of the presence of the large enzymes glucose oxidase and catalase, the glucose-sensitive gels were less entangled, resulting in a more rapid expansion. The percent changes in the mesh sizes after 45 min at pH 7.0 were 500% for the glucose-sensitive gel and 250% for the non-glucose-sensitive gel. The glucose-sensitive and non-glucose-sensitive gels had similar rates of collapse. Both gels exhibited a 30% decrease in their mesh sizes during the first 5 min at pH 4.0. Although the presence of the enzymes affected the rate of expansion, the initial collapse occurred at the same rate for both gel types.

The response of glucose-sensitive P(MAA-*g*-EG) hydrogels was examined for various glucose concentrations. An initial drop in the pH was observed for all glucose concentrations, which was attributed to the possible glucose oxidase on the surface of the polymer disks which could catalyze the reaction of glucose to produce gluconic acid. The drop in pH was more significant for higher glucose concentrations, resulting in a lower initial rate of swelling. An additional drop in the pH occurred for the lower glucose concentrations as the gels approached equilibrium. The additional decrease in pH occurred because interior glucose oxidase became available to further catalyze the reaction of glucose to gluconic acid due to the initial degree of swelling.

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