Enzymatically Degradable and pH-Sensitive Hydrogels for Colon-Targeted Oral Drug Delivery. I. Synthesis and Characterization

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ABSTRACT: Cylindrical hydrogels, composed of starch and poly(acrylic acid), were synthesized, and their swelling behavior was studied as a function of the pH of the medium. The gels underwent a sharp transition from Fickian swelling behavior (swelling exponent $n = 0.30$) to non-Fickian swelling behavior ($n = 0.96$) as the pH of the swelling medium changed from 2.0 to 7.4. The hydrogels also underwent partial enzymatic degradation in an amylase-containing medium of pH 7.4 at 37°C. The effects of the enzyme concentration in the swelling media, the amount of starch present in the gel, the initial water content, the degree of crosslinking, and the diameter of cylindrical hydrogels on the degradation behavior were studied. The degradation of the gels followed Michaelis–Menten kinetics, and the value of the Menten constant was 41.62×10^{-2}. The gels exhibited minimum swelling in an acidic pH medium through the formation of a complex hydrogen-bonded structure and underwent enzymatic degradation in a medium of pH 7.4 (i.e., simulating intestinal fluid) along with chain-relaxation-controlled swelling. Therefore, the gels have potential for colon-targeted drug delivery. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 3630–3643, 2004

Key words: degradation; swelling

INTRODUCTION

The oral administration of protein and peptide drugs seems to be a significant solution to problems, such as a rapid increase and a subsequent rapid decrease in blood serum concentration levels and a need for frequent injections, that are caused by parenteral medication. Therefore, the major challenge in the field of protein delivery is to develop a device that can deliver a drug while maintaining its concentration level for a considerable time period inside the therapeutic region and reduce the number of doses to be administered. Although oral administration is a convenient route, it is difficult to achieve. One of the major obstacles to oral delivery is the digestion of proteins by gastric and pancreatic enzymes present in the stomach and small intestine.[1] pH-sensitive hydrogels,[2–5] consisting of carboxylic groups containing monomers, seem to be the best remedy because they keep the encapsulated protein drug almost protected in the highly acidic environment of the stomach (pH 1–2) by exhibiting minimum swelling and release most of the drug in a medium of slightly alkaline pH (i.e., the pH of intestinal fluid) by swelling to the maximum. Hence, these gels may be used for the treatment of colonic diseases such as colon cancer, inflammatory bowel disorder, and Chron's disease.

Although pH-sensitive hydrogels, to some extent, have been able to protect encapsulated drugs in highly acidic media by exhibiting minimum swelling due to the formation of a compact structure through hydrogen-bonding interactions among the un-ionized carboxylic groups, they have not been able to provide zero-order release in a medium of colonic pH (i.e., pH 7–8), which is desired by pharmaceutical scientists working in the field of drug delivery. The reason is that to achieve zero-order release at the target site (i.e., in the colon at pH 7–8), the degree of crosslinking should be low.[6] However, hydrogels with a low degree of crosslinking may release an appreciable quantity of the drug in a medium of lower pH (i.e., in gastric simulating fluid), as observed in our previous work:[7] poly(acrylamide-*co*-maleic acid) gels, although providing zero-order release in a medium of pH 7.4 for nearly 3 h, also released nearly 33% of the total drug loaded in a medium of lower pH. This ultimately results in the digestion of the protein drug by gastric juice along with a subsequent loss of drug activity before the device enters the colon.

Because of the unique physiological characteristics of the large intestine, oral drug delivery to the colon can also be achieved through the use of colonic micro-

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flora, which mainly consist of anaerobic or facultative anaerobic microorganisms that produce a variety of enzymes in the colon.[8] This has led to the discovery of a new class of drug delivery vehicles, consisting of natural and synthetic polymers, that release drugs through enzymatic degradation. In the recent past, natural polymers such as starch,[9,10] dextran,[11] guar gum,[12] sodium alginate,[13] and chitosan[14] and azo-crosslinked synthetic polymers such as poly(acrylic acid)[15] and poly(acrylamide-*co*-allyl methacrylate ester)[16] have been developed for colonic drug delivery through enzymatic degradation. The major drawback of these gels is that they take a longer time to degrade (and hence to deliver the drug). For example, dextran-based cylindrical gels[17] have been found to degrade (and, therefore, to release the drug bovine immunoglobulin) over a period of 25–80 days.

In this work, we have developed a novel hydrogel system through the aqueous polymerization of acrylic acid (AAc) in the presence of starch, which produces a polymer network that demonstrates pH-dependent swelling behavior along with enzymatic degradation in the presence of enzyme α-amylase, which is produced in the colon. In a medium of acidic pH, the device exhibits a compact bonded structure that swells to the minimum, whereas in an α-amylase-containing medium of slightly alkaline pH, it undergoes the enzymatic degradation of its one constituent starch. The degradation of starch results in the formation of macropores, through which an encapsulated drug may be released with zero-order kinetics. Therefore, the proposed device demonstrates minimum swelling in a medium of acidic pH through the formation of a compact hydrogen-bonded structure and thus may provide almost complete protection to the loaded drug. However, it undergoes enzymatic degradation along with chain-relaxation-controlled swelling in simulating intestinal fluid of pH 7.4 and so is expected to release the drug in a zero-order pattern. Therefore, the proposed device operates through a combined mechanism involving enzymatic degradation and pH-dependent swelling.

EXPERIMENTAL

Chemicals

The monomer AAc (Research Lab, India) was distilled under reduced pressure[18] to remove the inhibitor before use. Soluble starch (Merck, Mumbai, India), the crosslinker *N*,*N*′-methylene bisacrylamide (MB; Sigma, St. Louis, MO), and the initiator potassium persulfate (KPS; Merck) were used as received. Double-distilled water was used in the experiments.

Preparation of the cylindrical hydrogels

The cylindrical hydrogels were prepared through the free-radical polymerization of AAc in the presence of starch in an aqueous medium. For example, to prepare a hydrogel with an initial water content of 77%, we dissolved soluble starch in water to produce a 1.6% solution (w/v). To 4 mL of this solution, 15 m*M* AAc, 324 μM MB, and 111 μM KPS were added, and the resulting mixture was poured into poly(vinyl chloride) straws, each with a diameter of 5.30 mm, kept in an electric oven (Tempstar, India) for a period of 2 h at 70°C. After the polymerization was finished, the resulting semitransparent cylindrical gels were taken out of the straws, cut into small pieces, each 2.54 $\pm$ 0.10 cm long, and then washed with distilled water for the removal of the unreacted salts. Finally, the gels were placed in a dust-free chamber at 30°C until they were dried completely. The hydrogel samples are labeled HG (x/y), where x denotes the amount of starch in the feed mixture (%) and y represents the crosslinking ratio (i.e., moles of crosslinker/moles of monomer acid in the reaction mixture). For example, the aforementioned sample is designated HG (1.28/0.021).

Swelling studies

The completely dried, preweighed hydrogel samples were placed in a 250-mL solution of a swelling medium of the desired pH (ionic strength = 0.01*M*) containing a precalculated quantity of the enzyme α-amylase at the physiological temperature of 37°C. The swollen gels were taken out at regular time intervals, wiped superficially with filter paper, weighed accurately, and then placed in the same bath.

The mass measurements were continued until the attainment of a constant mass for each sample. The percentage of mass swelling (% S_M) was obtained with the following expression:

$$S_M = [(m_t - m_0)/m_0] \times 100 \quad (1)$$

where m_0 and m_t are the initial mass and mass at time t, respectively.

The following equation was used to determine the nature of the swelling process:

$$F = M_t/M_\infty = kt^n \quad (2)$$

where M_t and M_∞ denote the amounts of the solvent diffused into the polymer matrix at time t and at equilibrium, respectively; k is the gel characteristic constant; and swelling exponent n describes the type of diffusion. For cylindrical gels, n = 0.45–0.50 corresponds to a Fickian diffusion process, whereas $0.50 < n < 1.0$ indicates anomalous or non-Fickian diffusion. This equation is applicable to the initial stage of swelling, and a plot of ln F verses ln t gives straight lines up to almost a 60% increase in the mass of the gel.

The diffusion coefficient D ($cm^2\ min^{-1)}$ was calculated with the following equation:[18]

$$D = 0.049/(t/4l^2)_{1/2} \quad (3)$$

where t is the time at which the swelling is one-half the equilibrium value and l is the radius of the cylindrical sample. All the experiments were carried out in triplicate, and the average values are given.

Enzymatic degradation studies

Completely dried (and, in some studies, freshly prepared) and preweighed hydrogel samples were placed in a phosphate buffer of pH 7.4 with an ionic strength of 0.01*M* that contained a definite amount of the enzyme α-amylase at the physiological temperature of 37°C. After definite time intervals, 5-mL aliquots were taken out and analyzed for the amount of reducing sugar by a method described elsewhere.[19] After each measurement, the hydrogels were placed in a fresh buffer solution. To stabilize α-amylase, 0.132 g L^{-1} calcium was already added to the phosphate buffer.[20]

RESULTS AND DISCUSSION

Swelling in the amylase-containing medium

The proposed hydrogel system consists of two constituents, one of which (i.e., starch) is enzymatically degradable, whereas the other [i.e., poly(acrylic acid)] is pH-sensitive. Therefore, the device is supposed to function via a coupled mechanism involving pH-sensitive swelling and enzymatic degradation. In a lower pH medium, the hydrogel exhibits minimum swelling because of the formation of intermolecular hydrogen bonding among macromolecular poly(acrylic acid) chains, whereas in a medium of colonic pH, it undergoes enzymatic degradation because of the presence of starch within the gel.

To determine whether the degradation of starch would enhance the rate of water uptake because of the formation of macropores within the polymer network, we allowed HG (1.28/0.021) to swell in two buffer solutions of pH 7.4 (ionic strength = 0.01*M*), one containing a definite amount of the enzyme (1.3 IU/mL) and the other enzyme-free. The results, depicted in Figure 1, clearly suggest that the degradation of starch does not cause only statistically significant enhancement in the extent of swelling of the gel in comparison with the swelling of the hydrogel in the enzyme-free buffer medium. To further confirm this finding, we synthesized a number of hydrogels that differed in composition, and we studied their swelling kinetics in enzyme-free and enzyme-containing buffer media of pH 7.4 (data not shown). For nearly all the samples, the water uptake was almost the same in both media, and this confirmed that the degradation of starch did not cause any appreciable increase in the water uptake. This can be attributed to the fact that the amylase-induced degradation of starch, a hydrophilic polymer, tends to reduce the swelling capacity of the degrading gel. However, the formation of macropores due to the degradation of starch may tend to enhance the water uptake. In this way, the two opposite tendencies nearly nullify each other, and this may result in almost the same water uptake of the hydrogels in enzyme-free and enzyme-containing media.

The initial swelling rates, as calculated from the initial linear plots, were found to be 2.70 and 3.10%/min in the blank and enzyme-containing media, respectively. The closeness of the two values also supports the experimental findings. Likewise, the average values of D for the water uptake in the two media were evaluated to be 17.28×10^{-6} and 18.43×10^{-6} $cm^2\ min^{-1}$, respectively; this also indicates that enzymatic degradation does not cause any significant enhancement in the water uptake in the two media. Finally, n was found to be 0.86 and 0.96, respectively, and this indicated that the swelling was mainly governed by a chain-relaxation-controlled mechanism.

pH-dependent swelling behavior

The basic requirement for a drug delivery system, working via swelling-dependent mechanism, to be used as a colon-targeted device is that it must exhibit minimum swelling (and hence drug release) in a lower pH medium (i.e., gastric fluid), whereas maximum swelling should be observed in a medium of slightly alkaline pH (i.e., the colon). However, as this hydrogel system is supposed to protect the drug in the acidic environment through a complex hydrogen-bonded structure and release most of the entrapped drug in a medium of colonic pH (i.e., pH 7.4) through enzymatic degradation, the behavior of hydrogel HG (1.28/0.021) was investigated in media of different pHs. Our main interest was whether the samples would exhibit minimum swelling in the lower pH by forming compact hydrogen-bonded structures. Therefore, the dynamic uptake of water of sample HG (1.28/0.021) was studied in buffer media of pHs 2.0, 4.0, and 7.4 with ionic strengths of 0.01*M* at the physiological temperature of 37°C. The results, as depicted in Figure 2, reveal that the water uptake at different times is low in media of pHs 2.0 and 4.0, but it becomes appreciably high for swelling in a pH 7.4 medium. This can be explained by the fact that in a pH 2.0 medium, the carboxylic groups attached along the polymer backbone are in an almost un-ionized state, thus imparting a nonpolyelectrolyte character to the gel. As a result, the ion osmotic swelling pressure, $\Pi_{ion} = RT\ \Sigma(C_i^g - C_i^s)$, where C_i^g and C_i^s are the molar concentration of mobile ions in the gel phase and solution phase, re-

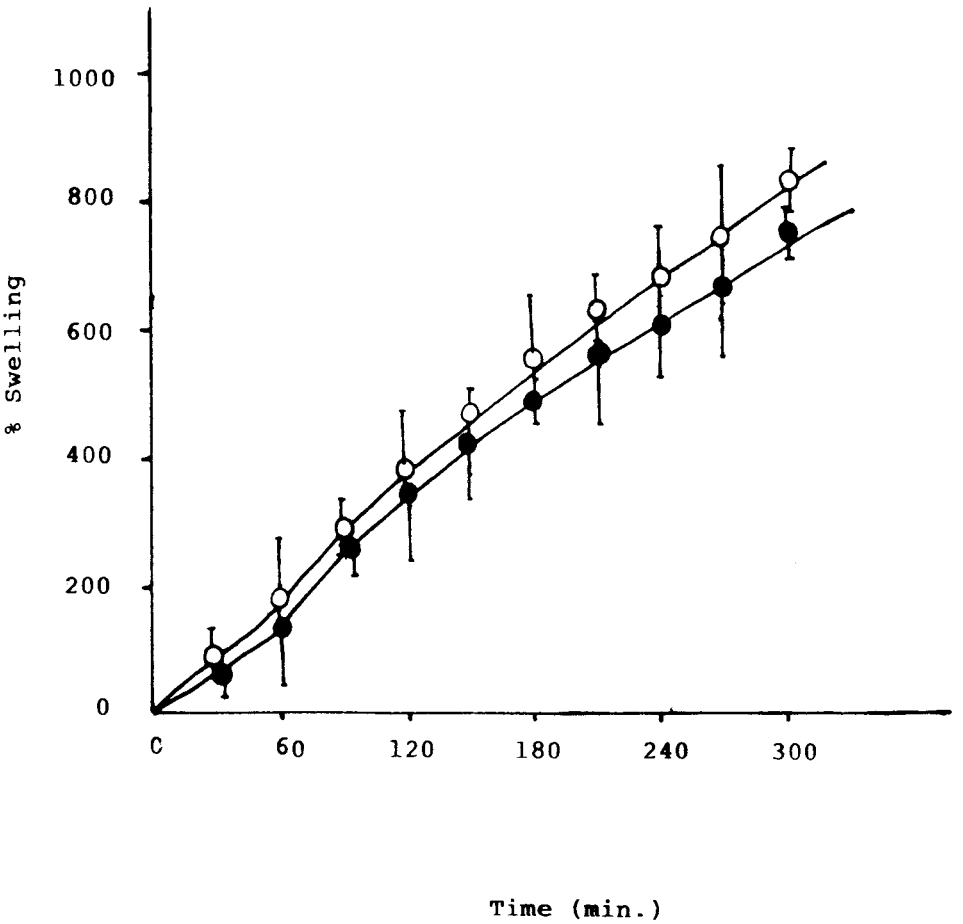


Figure 1 Dynamic swelling behavior of hydrogel sample HG (1.28/0.021) in (●) amylase-free and (○) amylase-containing media (pH 7.4) with an ionic strength of 0.01*M* at 37°C.

spectively, is nearly ineffective. Moreover, the existence of hydrogen-bonding interactions between —COOH groups in the polymer matrix [Scheme 1(A)] results in a complex structure[21] within the network, and so the movements of polymeric segments is restricted. This also accounts for the minimum swelling of the gel in a medium of pH 2.0. For the gel in a medium of pH 4.0, almost the same explanation can be offered, with the slight modification that because there is little ionization of —COOH groups, the osmotic swelling pressure becomes operative, and at the same time, the complexity of the hydrogen-bonded structure is also reduced [Scheme 1(B)].

However, when the sample is placed in a medium of pH 7.4, the almost complete ionization of —COOH groups present within the polymer network not only increases the ion osmotic swelling pressure to a great extent but also enhances the relaxation of macromolecular chains because of repulsion among similarly charged —COO^- groups. These two factors ultimately result in a greater increase in the water uptake. Similar observations have been reported previously.[22] Finally, the values of n, as determined from the double logarithmic form of the equation $M_t/M_\infty = kt^n$, were found to be 0.30, 0.46, and 0.96 in media of pHs 2.0, 4.0, and 7.4, respectively. These values clearly suggest that a transition occurs from Fickian swelling behavior to anomalous (almost zero-order) swelling behavior as the pH of the swelling medium changes from 2.0 to 7.4. The increase in the swelling of the gel in a medium with a slightly alkaline pH may enhance its enzymatic degradation because of increased mesh size. However, the release of the drug is mainly supposed to take place through the enzymatic degradation of starch.

The results obtained from γ-scintigraphic studies on guar-gum tablets with 99-m Tc-DTPA as a tracer in human volunteers[23] reveal a mean gastric emptying time of 1.08 ± 0.11 h and a mean colonic arrival time of 2.83 ± 0.33 h. This means that the small intestine transit time is likely to be 1.75 ± 0.25 h, which suggests that the oral formulation should enter the colon 1.75–3.75 h after administration. Relying on these data, we exposed a sample for 2 h to a medium of pH 2.0, for 2 h to a medium of pH 4.0, and for 6 h to a medium of pH 7.4, thus mimicking the transition of the formulation from the stomach to the colon. The results, as

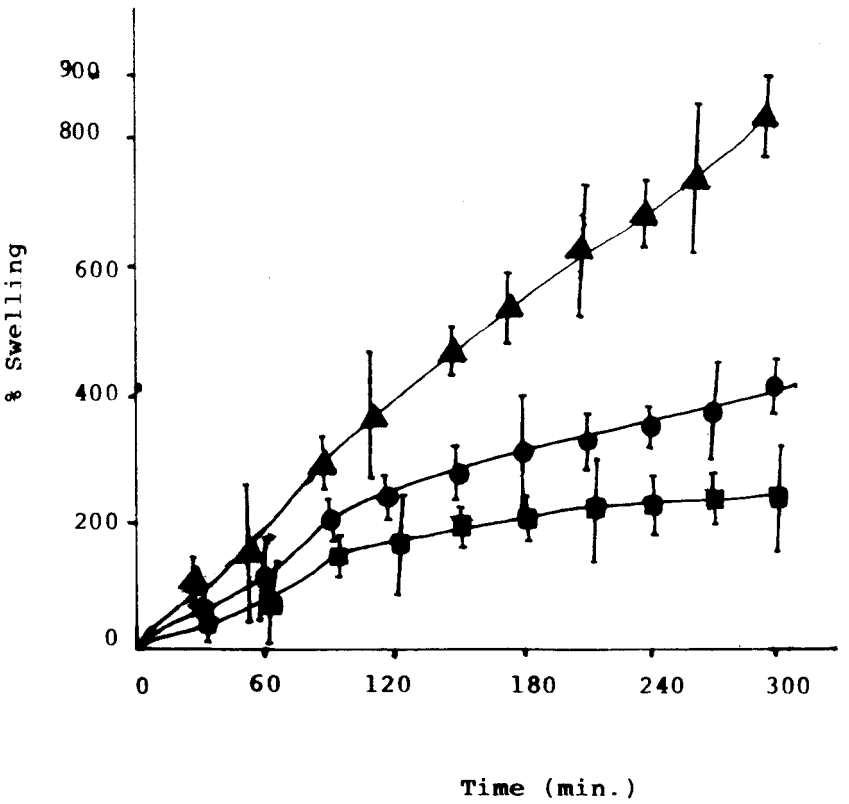


Figure 2 Dynamic swelling behavior of hydrogel sample HG (1.28/0.021) in media of (■) pH 2.0, (●) pH 4.0, and (▲) pH 7.4 with an ionic strength of 0.01*M* at 37°C.

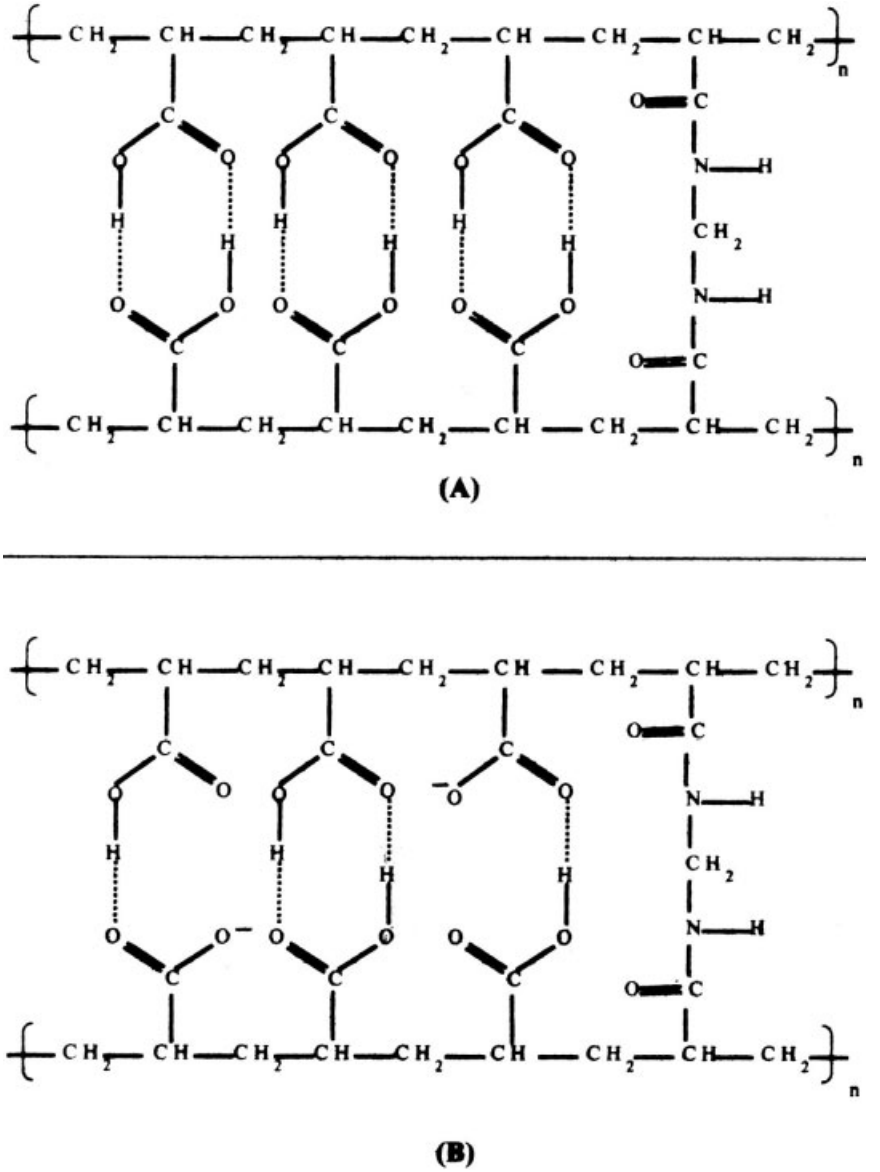
depicted in Figure 3, clearly indicate that out of the total swelling of 1233% in the first 10 h, the hydrogel swells only 7.2% in the first 2 h in the medium of pH 2.0 and 12.3% in the next 2 h in the medium of pH 4.0; the rest of the swelling occurs in the buffer medium of pH 7.4. This indicates that for the initial period of 10 h, the hydrogel swells by nearly 20% in the first 4 h before its entry into the medium of pH 7.4. Therefore, a very low extent of swelling in the first 4 h suggests that the gel may be able to protect the encapsulated drug from the gastric enzyme before its entry into the colon.

Effect of the enzyme concentration on degradation

During the enzymatic degradation of starch-based hydrogels, reducing oligosaccharides are released from the polymer matrix. Figure 4 gives a representative plot of the dynamic release of reducing oligosaccharides from the degrading hydrogel sample HG (1.28/0.021) in buffer media of pH 7.4 containing various amounts of enzymes (0.32–1.95 IU/mL of degradation medium) at the physiological temperature of 37°C. As the concentration of the enzyme in the external solution increases, the amount of reducing sugars coming out also increases. This is expected, because with an increase in the enzyme content, the binding sites available for starch molecules to undergo degradation through glycosidic linkages also increase in number. In addition, it is clear from Figure 4 that at a higher concentration of the enzyme (i.e., 1.95 IU/mL), the increase in the concentration of the reducing sugar is almost linear with time over a period of at least 6 h. However, as the enzyme concentration in the degradation medium decreases, the increase no longer remains linear. Through the selection of an appropriate enzyme concentration, it is possible to obtain a zero-order release of oligosaccharides from the degrading polymer; this is a simple result of degradation through a zero-order pattern. Similar results have been reported elsewhere.[24] Araki et al.[25] measured the *in vitro* amylase concentration in feces of five healthy volunteers and reported an average value of approximately 5.18 ± 2.90 IU/mL. It is clear that the amylase activity used in this study falls within the proper range.

The degradation rates, as calculated from the linear parts of the release profiles (expressed in μ mol g^{-1} gel min^{-1}), increase with the concentration of α-amylase in the solutions. The final amount of reducing oligosaccharides depends on the enzyme concentration and varies from 2407 to 5752 μ mol g^{-1} of gel for the entire enzyme concentration range of 1.95–0.32 IU/mL.

Figure 5 presents the degradation rate as a function of the concentration of amylase in the degradation



Scheme 1 (A) In a medium of pH 2.0, the almost un-ionized —COOH groups interact through hydrogen bonding to give a complex structure. (B) In a medium of pH 4.0, because of partial ionization of —COOH groups, the structure loses its complexity to some extent.

media. An almost linear relationship exists between the degradation rate and enzyme concentration in the external media. This agrees with the Michaelis–Menten enzyme kinetics.[26] Similar observations were reported by Hennink et al.[27] for the enzymatic degradation of dextran hydrogels by the enzyme dextranase.

Finally, we also studied the degradation kinetics of a freshly prepared gel sample of HG (1.28/0.021) as a function of time in swelling media of pH 7.4 containing various amounts of the enzyme (0.32–1.30 IU/mL) at the physiological temperature of 37°C. The results, as depicted in Figure 6, reveal that as the concentration of α-amylase in the release media increases, the amount of starch degraded at different time intervals also increases. Now, the correlation between the history of the gel (i.e., a freshly prepared or dry sample) and the extent of degradation can possibly be best made by a comparison of the degradation profiles of dry and freshly prepared samples, as shown in Figures 4 and 6, respectively, studied for the same range of enzyme concentrations in the degradation media. For a given enzyme concentration, the amount of oligosaccharides released from a dry sample is more than that from a fresh hydrated sample. This can be explained as follows.

In a freshly prepared hydrogel sample with an initial water content of 77%, although the diffusion of enzyme molecules into the polymer matrix is fast, the number of actives sites (i.e., glycosidic linkage) available in a small-volume element of the matrix for deg-

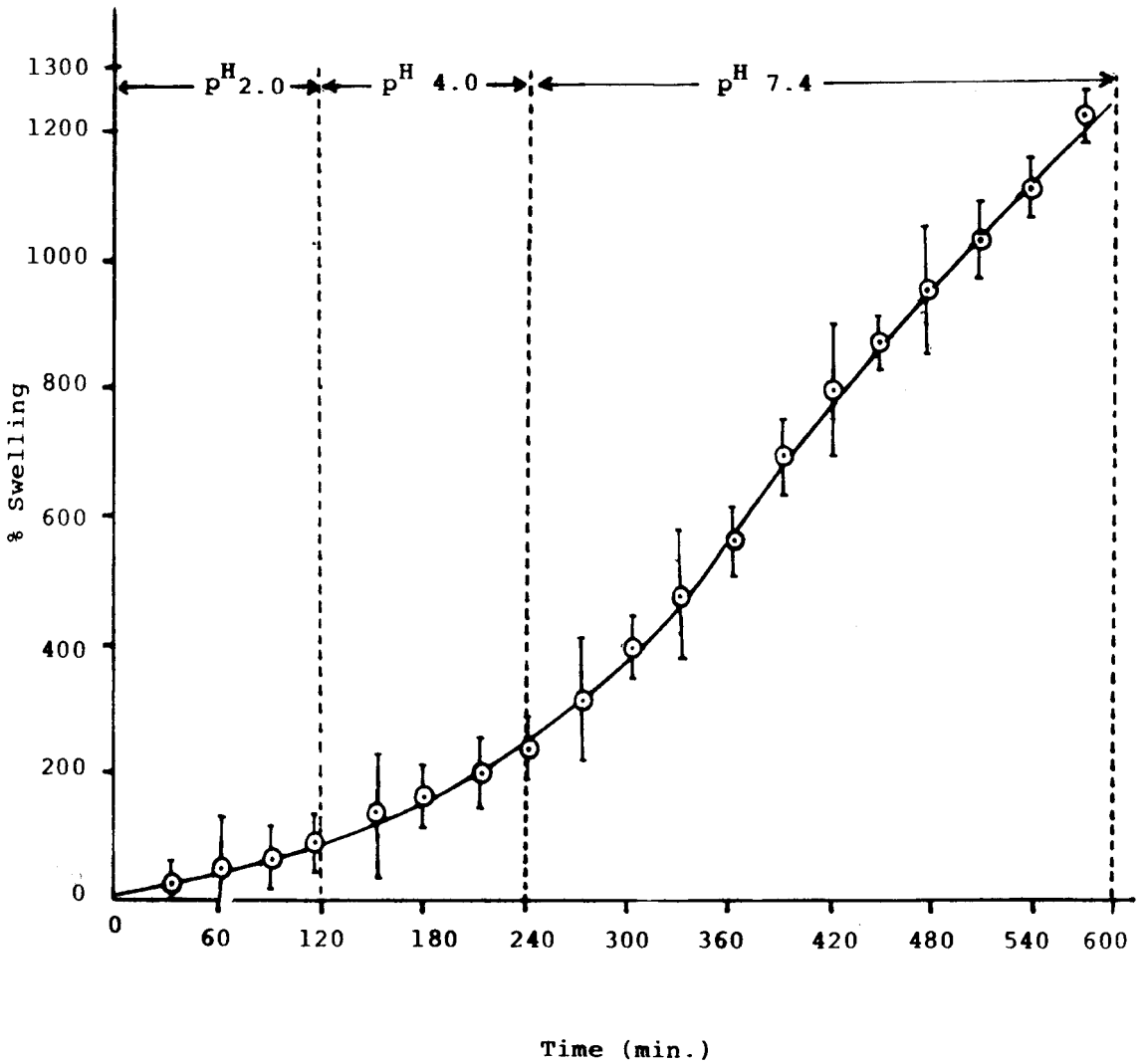


Figure 3 Dynamic water uptake of hydrogel sample HG (1.28/0.021) in media of various pHs with an ionic strength of 0.01*M* at 37°C.

radation is smaller. In other words, for a small-volume element in the hydrogel, the number of starch molecules available for degradation is small because there is already a large amount of water present in the sample (note that the water content is nearly 77%). However, the situation is quite different with a dry sample. Here, the number of starch molecules available in a small-volume element is naturally large in comparison with that of a hydrated fresh sample. Therefore, more starch is degraded by incoming amylase molecules. This accounts for the smaller extent of degradation of a freshly prepared hydrogel sample in comparison with a dry one. The degradation rates (i.e., the amount of sugar released/g of gel min^{-1}) for fresh samples in media with a given range of enzyme concentrations are between 0.44 and 2.43 μ mol/g of gel/min, whereas the degradation rate for dry samples in the same range of enzyme concentrations is between 2.55 and 8.88 μ mol/g of gel/min, as calculated from the profile depicted in Figure 4.

Effect of the starch concentration on degradation

The kinetics of enzymatic degradation are affected by the concentration of the substrate present in the degrading hydrogel system. To investigate this, we synthesized hydrogels containing the same amount of AAc but various concentrations of starch (in the range of 0.32–1.6% in the feed mixture), and we studied their degradation as a function of time in an α-amylase-containing buffer medium of pH 7.4 at the physiological temperature of 37°C. The results thus obtained clearly reveal that the amount of sugars released at different times increases with the starch content within the gel. This can be attributed to the fact that with an increase in the starch content within the degrading gel, more and more glycosidic linkages un-

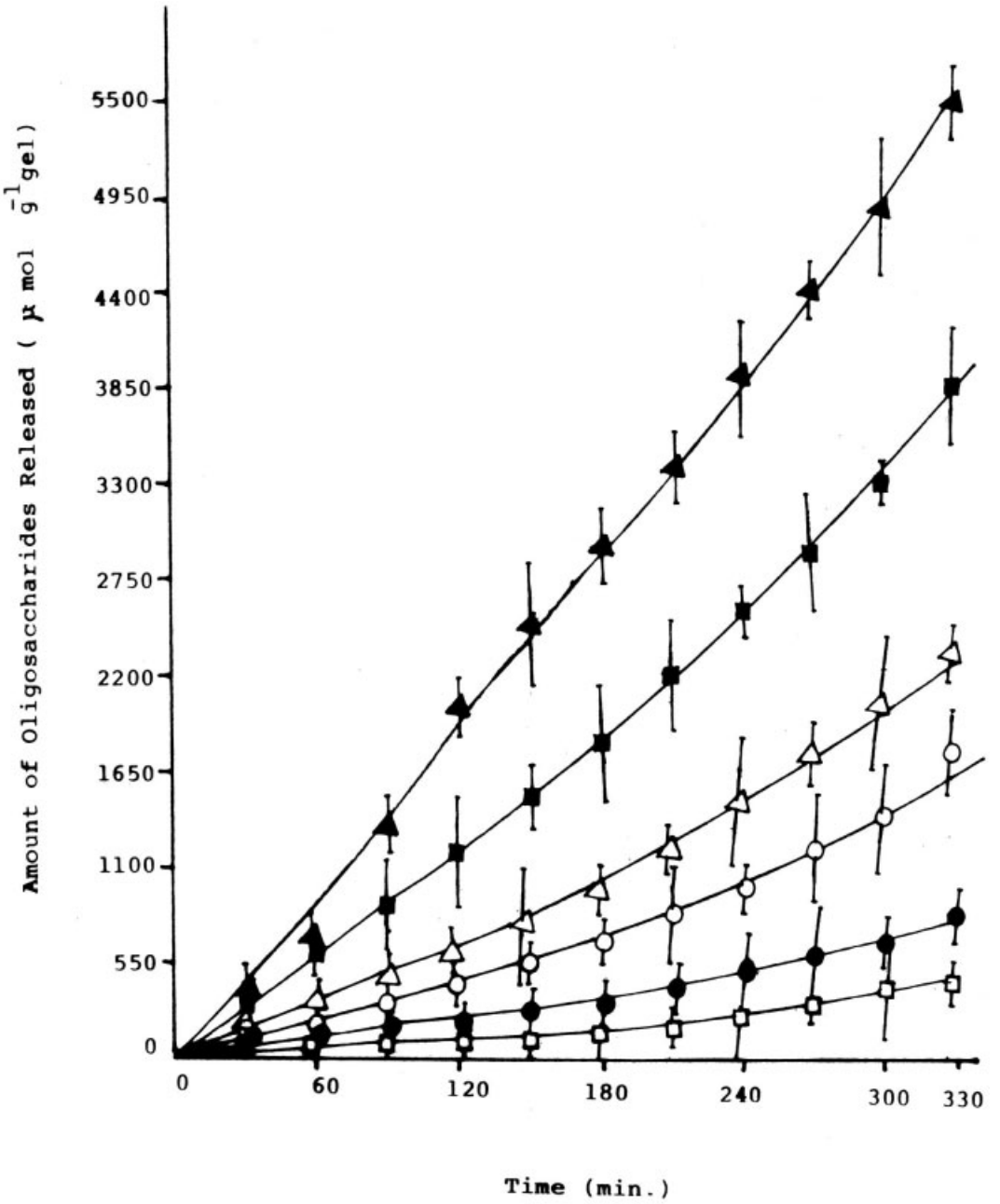


Figure 4 Cumulative release of oligosaccharides as a function of time from hydrogel sample HG (1.28/0.021) in media of pH 7.4 with enzyme concentrations of (□) 0.32, (●) 0.65, (○) 0.97, (△) 1.30, (■) 1.60, and (▲) 1.95 IU/mL at 37°C.

dergo enzymatic cleavage, and this results in greater degradation with a subsequent release of oligosaccharides from the matrix. The initial release rates (r), as calculated from the linear parts of the curves, range from 8.70 to 12.25 μ mol g^{-1} gel min^{-1} for starch contents of 0.32–1.6% in the feed mixture. Finally, the Michaelis–Menten constant (K_M) and the maximum swelling rate (V_{max}) were evaluated with the help of the slope and intercept of a linear plot of $1/[r]$ versus $1/[S]$, (i.e., the reciprocal of the degradation rate vs the reciprocal of the starch concentration), as depicted in Figure 7. K_M and V_{max} were found to 41.62×10^{-2} and 5.55×10^{-3} g min^{-1}, respectively.

Effect of the degree of crosslinking on degradation

The swelling capacity of a hydrogel is affected by the degree of crosslinking. Normally, as the degree of crosslinking increases (i.e., crosslinking ratio), the swelling capacity decreases.[28] To observe the effect of the crosslinking ratio on the degradation of the gel, we synthesized control samples with various crosslinking ratios of 58.4×10^{-3}, 45.4×10^{-3}, and 25.9×10^{-3}, and we studied their degradation kinetics in an α-amylase-containing buffer medium of pH 7.4. The results, shown in Figure 8, clearly reveal that the amount of degraded starch (i.e., the amount of released oligosac-

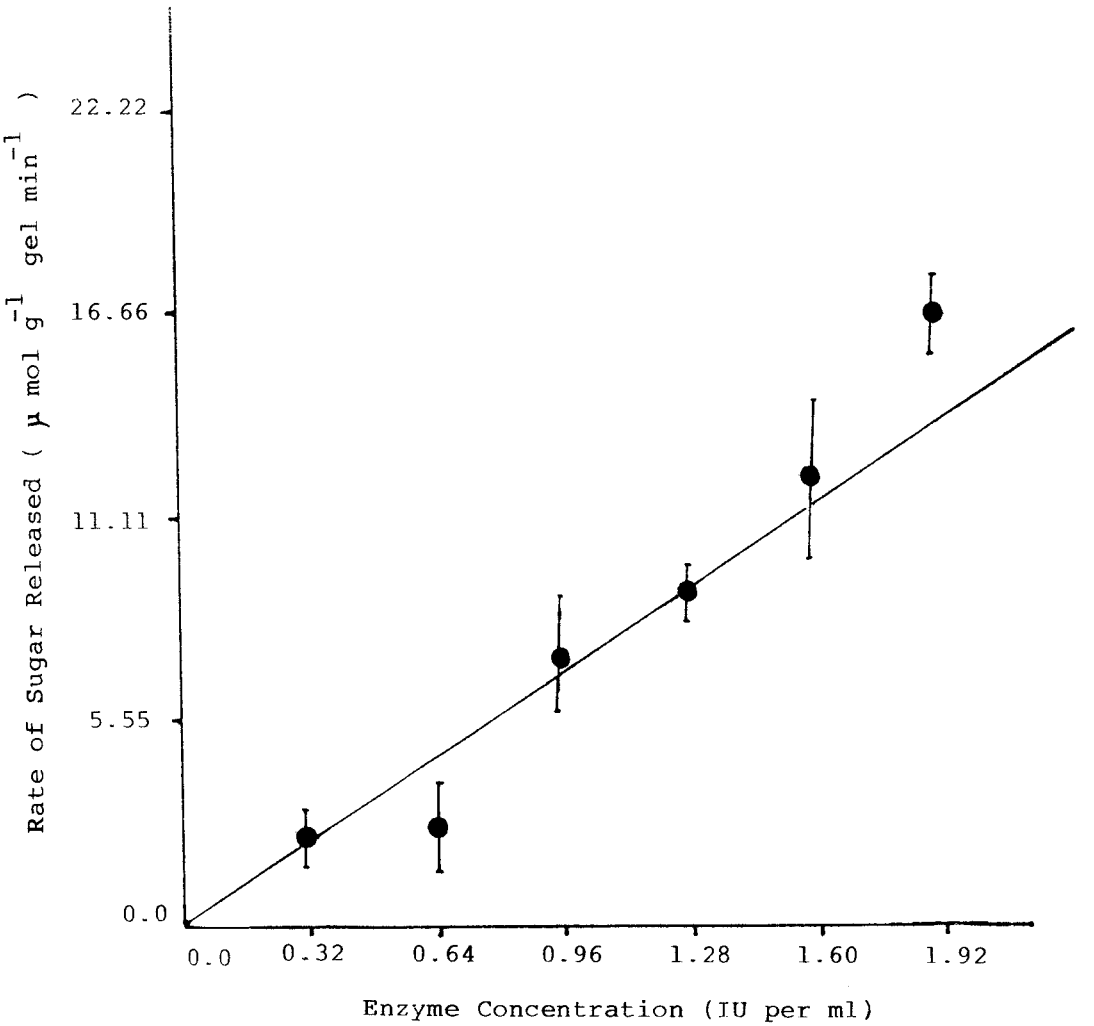


Figure 5 Plot of the initial degradation rate versus the enzyme concentration in degradation media of pH 7.4 at 37°C.

charides) at different times decreases with an increase in the crosslinking ratio. At first sight, it appears that the increase in the degree of crosslinking should cause an increase in the degradation rate because as the crosslinking density increases, the structure becomes more compact, and this brings the degradable starch macromolecule much closer; therefore, for a given enzyme concentration, the chances of degradation are greater. However, the results contradict this presumption. In fact, the mesh size of the network appears to play a major role in governing the degradation behavior. Thus, the observed results can be well attributed to the fact that for a highly crosslinked network, the mesh size is sufficiently small and results in a slower diffusion of α-amylase molecules into the polymer matrix. Moreover, in a highly crosslinked structure, the structure of the polymer matrix is so compact that the number of glycosidic linkages in the substrate molecules is high enough for cleavage by enzyme molecules.[29] This also accounts for the slower degradation. Finally, the enzyme may not be able to hydrolyze the glycosidic linkage after binding to the substrate in gels with highly crosslinked networks. All these factors ultimately result in slower degradation. Similar results have been reported previously.[30] Thus, our results show that the crosslink density is an important factor in the enzymatic degradation of starch-based hydrogels.

Effect of the initial water content on degradation

As stated earlier, the average transit time for a oral formulation to reside in the stomach and small intestine is approximately 4 h. This means that the delivery vehicle enters the colon 4 h after its oral administration. Therefore, the amount of water, already absorbed by the device before its entry to the colon, may prove to be a significant factor in controlling its enzymatic degradation behavior in the colon. To investigate this aspect in some detail, we synthesized hydrogel samples containing the same amount of starch (1.32% in the feed mixture) but different water contents (65.9,

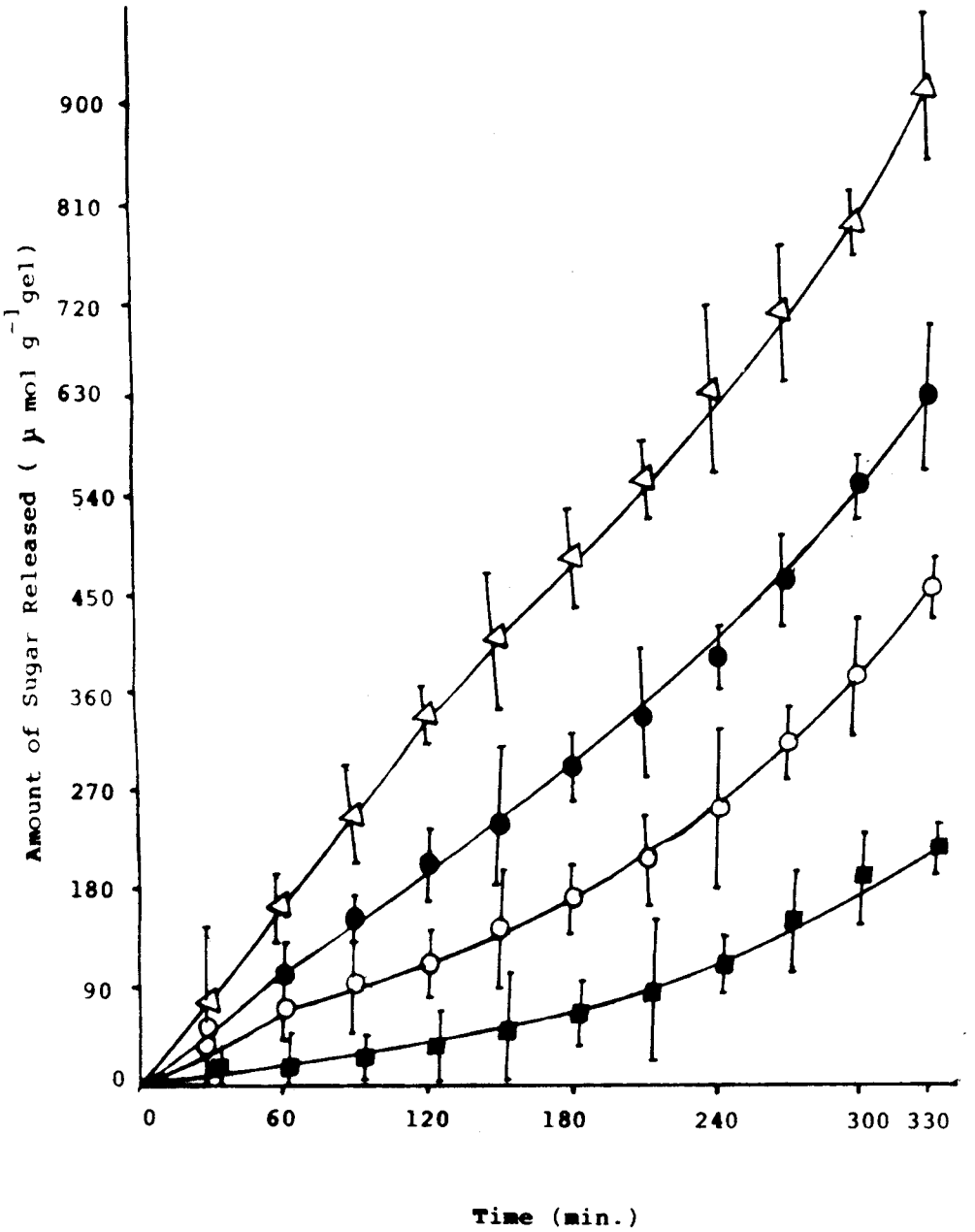


Figure 6 Cumulative release of oligosaccharides as a function of time from hydrated hydrogel samples in media of pH 7.4 with enzyme concentrations of (■) 0.32, (○) 0.65, (●) 0.97, and (△) 1.30 IU/mL at 37°C.

74.7, and 83.7%), and we studied their degradation kinetics in an amylase-containing buffer medium of pH 7.4 at the physiological temperature of 37°C. The results show that the amount of starch degraded (i.e., the amount of sugar released from the degrading device) at different times increases with an increase in the water content of the hydrogel. This can be explained by the fact that with an increase in the water content, the mesh size within the polymer network also increases,[31] and so the enzyme molecules diffuse into the network at a greater rate, thus causing an enhancement in the amount of starch degraded, which is indicated by the increased amount of sugar coming out of the degrading gels.

Effect of the sample thickness on degradation

One of the basic requirements of a oral drug delivery vehicle is that the blood serum concentration should be below the toxic level and around the effective level during the release of the drug at the target site.[32] In other words, the drug should be released at a predetermined rate. In this study, the device is supposed to release the drug via a coupled mechanism involving

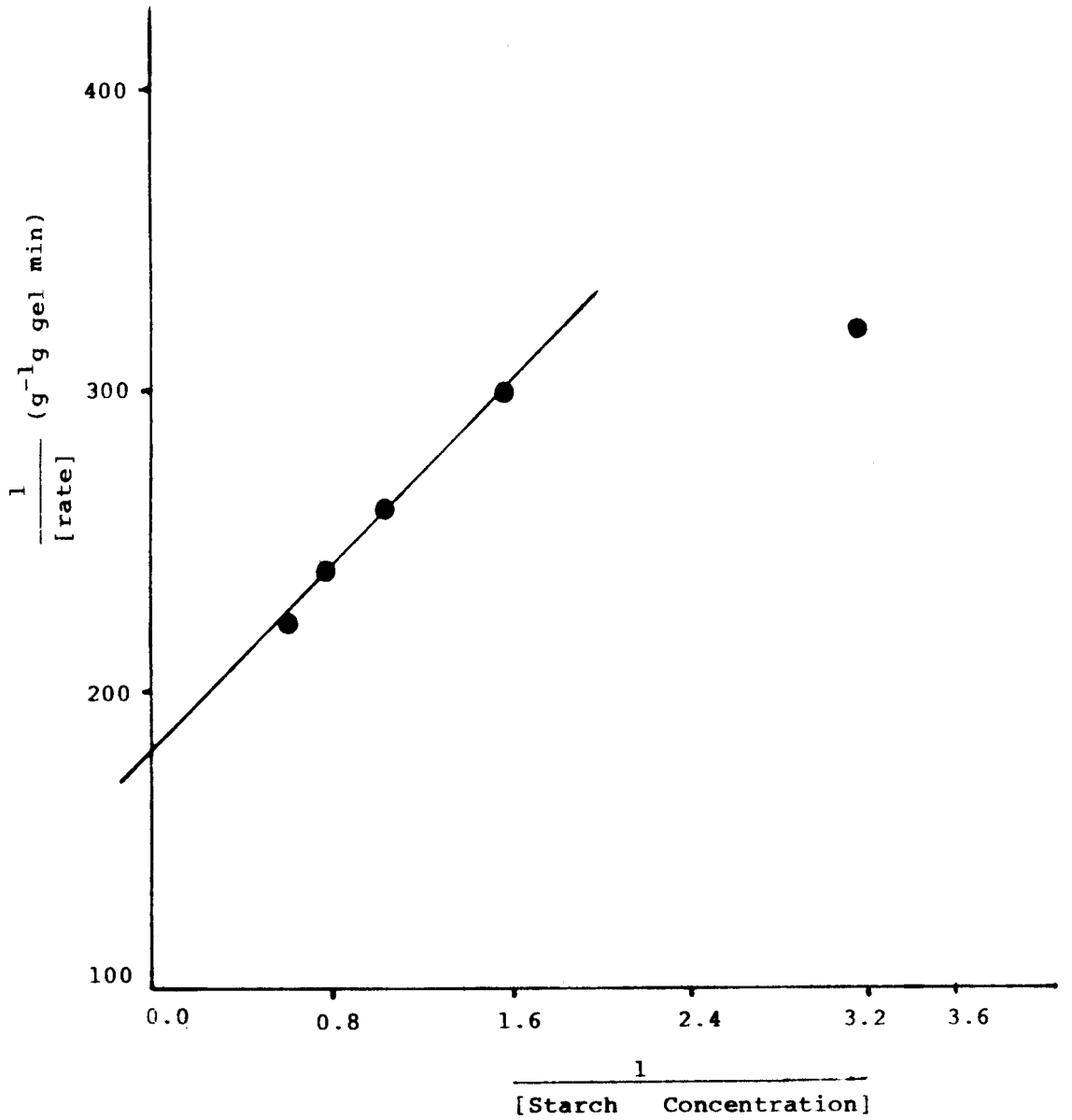


Figure 7 Plot of the reciprocal of the initial degradation rate versus the reciprocal of the starch concentration for the evaluation of K_M.

swelling and degradation (as observed in the study), and then the desired release rate can be achieved through the control of the swelling and degradation, which depend on the thickness of the sample. To test this, we synthesized hydrogels with diameters of 1.40–3.60 mm and studied their degradation in a buffer medium of pH 7.4 (amylase concentration = 1.3 IU/mL) at 37°C. The amount of starch degraded (i.e., sugar released) at different times increased as the diameter decreased (similar results were obtained for the swelling behavior). The observed increase in degradation can be explained by the fact that as the diameter of the cylindrical gels decreases, the surface area available for exposure to enzyme molecules (for a given mass of the gel) increases. Therefore, the number of substrate molecules available for the enzymatic cleavage of glycosidic bonds also increases. At the same time, because of enhanced swelling, the diffusion of amylase molecules into the bulk of the swelling gel also increases because of the increasing mesh size of the network. This finally causes more degradation of starch, as indicated by the increased rate of sugar release.

Therefore, it is clear that the desired release rate of a drug can be obtained through the control of the degradation rate of starch present in the device, which is regulated by the thickness of the delivery vehicle. For the proposed hydrogel system to be used for colon-targeted drug delivery, the device must undergo minimum swelling (and hence release a mini-

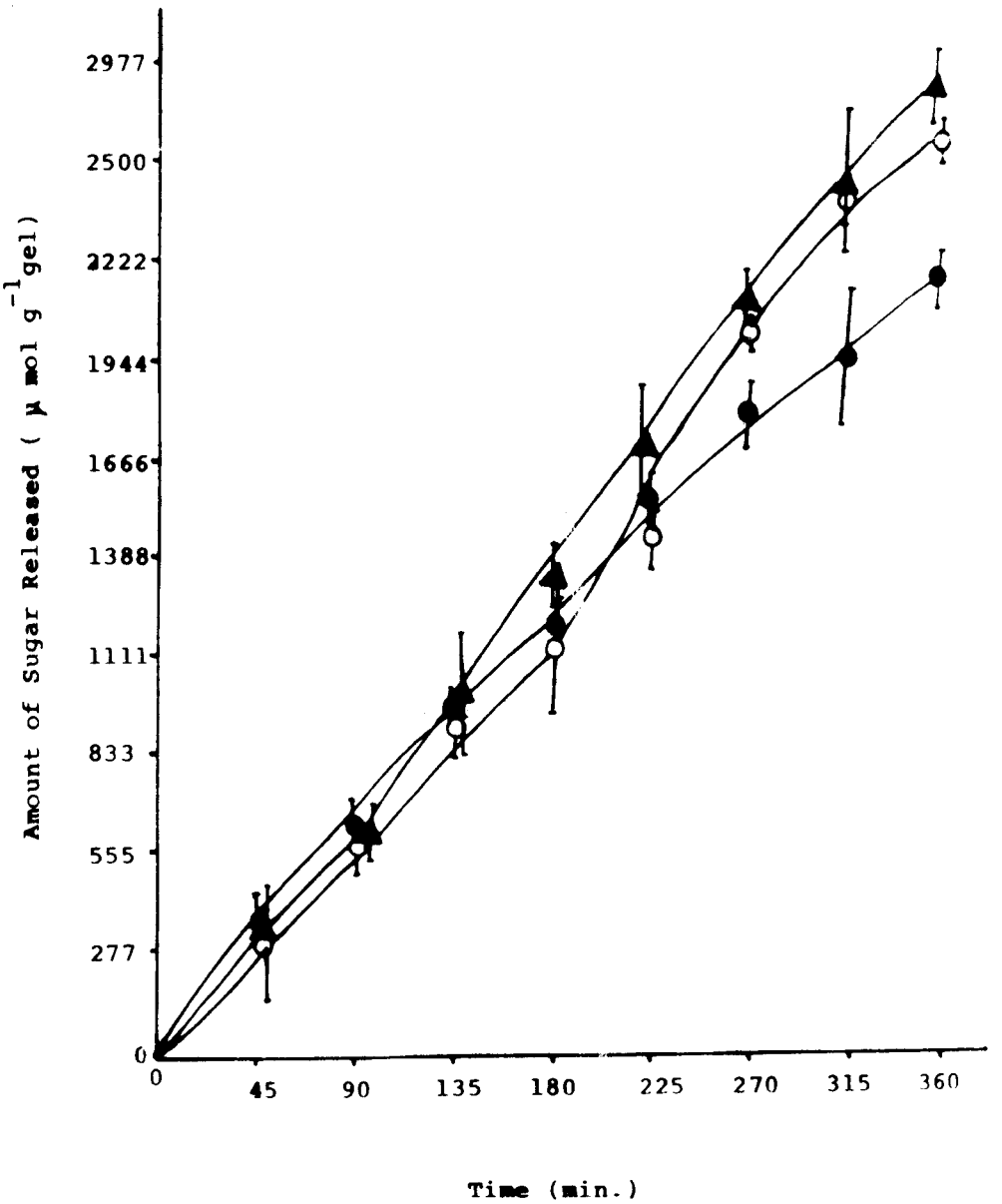


Figure 8 Cumulative release of oligosaccharides as a function of time from degrading gels with crosslinking ratios of (●) 58.4 $\times 10^{-3}$, (○) 45.4 $\times 10^{-3}$, and (▲) 25.9 $\times 10^{-3}$ in a medium of pH 7.4 at 37°C.

mum amount of the drug) before its entry into the colon, and so a device with larger diameter would be most suitable for this purpose.

Finally, a plot of the time required for 50% degradation ($t_{1/2}$) versus the square of the diameter yielded a straight line (see Fig. 9); this also supports the Tanaka–Fillmore theory.[33]

CONCLUSIONS

On the basis of these studies, carried out with a novel hydrogel system composed of starch and poly(acrylic acid), it can be concluded that the proposed device not only exhibits pH-sensitive swelling behavior but also undergoes enzymatic degradation by amylase present in the swelling medium. In an amylase-free medium of pH 2.0, the hydrogel exhibits Fickian swelling behavior ($n = 0.30$), whereas in an amylase-containing medium of pH 7.4, the swelling patterns becomes non-Fickian or almost zero-order ($n = 0.96$) because of the degradation of starch molecules with the subsequent formation of micropores and chain relaxation in controlled swelling. The degradation increases with the amount of starch present in the gels and with the amylase concentration in the external medium. The process of degradation obeys the Michaelis–Menten equation, and K_M is 41.62 $\times 10^{-2}$. The initial water content in the polymer network also affects its enzy-

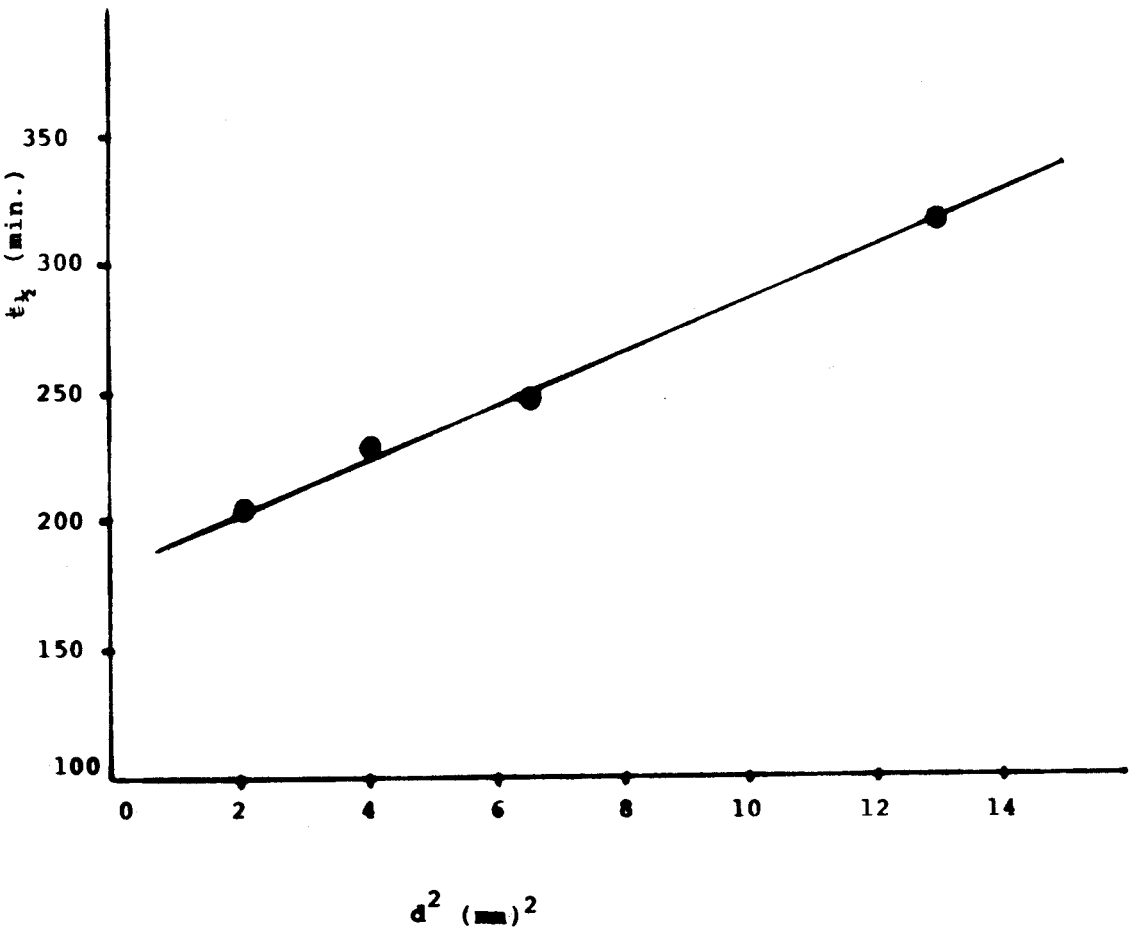


Figure 9 Plot of $t_{1/2}$ and the square of the diameter (d^2) in a medium of pH 7.4 at 37°C.

matic degradation. Finally, the thickness of the cylindrical hydrogels is an effective parameter in controlling the degradation and swelling rate of the gels.

With the data obtained from studies of various effects, as discussed previously, it is now possible to get a picture of the overall mechanism followed by the hydrogels. The device, when placed in an amylase-free medium of lower pH (i.e., gastric fluid), demonstrates minimum swelling because of the formation of a complex hydrogen-bonded structure; this proves its ability to protect any encapsulated drug in an acidic environment. However, when the hydrogel is put in an amylase-containing medium of pH 7.4 (i.e., simulating intestinal fluid), the solvent enters the polymer matrix and is followed by an invasion of enzymes molecules into the matrix. As a result, the gel begins to swell with the simultaneous degradation of starch present in the gel. The degradation causes the formation of macropores (i.e., water-filled pores) through which the drug is supposed to come out. However, the degradation of starch does not cause any significant enhancement of the swelling. Conversely, the swelling of the device influences the degradation of starch. In fact, the diffusion of the solvent (along with enzyme molecules) causes the hydrogel to swell, and the enzyme present in the release media induces the degradation of starch. In this way, a direct relationship may be established between the swelling of the hydrogel and the diffusion of enzyme molecules into the matrix with the subsequent degradation of starch present within the matrix. Hence, all three phenomenon appear to be jointly responsible for the degradation of the swelling hydrogel.

Although the proposed device is partially degradable and may not be disposed from the body in a easy way, it exhibits almost complete swelling and degradation over a period of 24 h, which is the average transit time for a oral formulation to pass through the whole gastrointestinal tract. This implies that the device, when loaded with a suitable protein drug, will release the drug over the same time period of 24 h. Moreover, fully enzymatically degradable gels, based on the natural polymer dextran,[17] have been reported to release the drug bovine immunoglobulin through enzymatic degradation during a period ranging from 25 days to more than 80 days. Finally, the synthesis of the enzymatically degradable gels studied by us does not involve the use of any organic solvents or highly

toxic reagents, unlike the synthesis of azo-polymeric hydrogels,[34] the synthesis of which involves the use of organic solvents, the degradation products of which (e.g., aromatic amines) are very toxic in nature.

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