Flow Through Diffusion Cell Method: A Novel Approach to Study *In Vitro* Enzymatic Degradation of a Starch-Based Ternary Semi-Interpenetrating Network for Gastrointestinal Drug Delivery

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ABSTRACT: A ter-polymeric semi-IPN has been synthesized by aqueous polymerization of methacrylamide in the presence of polyethylene glycol (PEG) and natural polysaccharides starch, and its enzymatic degradation has been studied in the phosphate buffer medium of pH 6.8 at the physiological temperature 37°C. With the increase in content of enzyme in the external solution and starch in the hydrogel, the degradation is enhanced while the extent of degradation is lowered with the increase in the amount of PEG in the hydrogel. The initial water content also affects the degradability of the polymer matrix. The degradation follows Michaelis–Menten kinetics and K_M was found to be 3.92 $\times 10^{-5}$ mol dm^{-3}. The hydrogel exhibits different degradation behavior when studied by "traditional degradation method" (TDM) and "flow through diffusion cell" (FTDC) method. The degradability is suppressed in FTDC method because of the absorption of amylase molecules onto filler particles. Finally the nature and size of the filler particles also affects the degradation behavior of hydrogels. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 2975–2984, 2006

Key words: degradation; enzyme; Michaelis–Menten kinetics; flow through diffusion cell

INTRODUCTION

Recent past has witnessed a tremendous research work on oral drug delivery of conventional drugs for treatment of colonic disorders like inflammatory bowel disorder (IBD), infectious diseases, constipation, and colon cancer.[1] Colonic delivery is also useful for systemic absorption of protein and peptide drugs, because of less hostile environment prevailing in the colon compared with stomach and small intestine. Various approaches have been used for colon-targeted drug delivery, which include pH-dependent swelling-controlled systems,[2] delayed release delivery systems,[3] intestinal pressure-controlled colon delivery capsules,[4] and enzymatically-degradable systems that utilize various enzymes produced by intestinal flora.[5]The intestinal microflora produce a number of enzymes like α-amylase, dextranase, glucosidase, pectinase etc.[6] in the colon. This has led to the discovery of a new class of drug delivery systems based on natural polysaccharides like starch,[7] guar gum,[8] and dextran,[9] which are degradable by their respective enzymes present in the colon. These polymers are not affected by the highly acidic environment of stomach and presence of gastric and pancreatic enzymes in stomach and small intestine. These nontoxic and biodegradable polysaccharides have potential of delivering the drug specifically to the colon through their enzymatic degradation.

Starch-based polymers have been studied as potential materials to be used in several biomedical applications.[10–11] They were found to be biocompatible and noncytotoxic[12] and have shown great processing versatility being proposed for applications such as drug delivery carrier systems,[13] hydrogels and partially degradable bone cements[14] materials for bone replacement/fixation or fillers for bone defects,[15] and porous structures to be used as scaffolds in tissue engineering of bone and cartilage.[16] The main enzymes involved in starch hydrolysis are α-amylases, which act upon large polymers of starch at internal bonds and cleaves them to short glucose polymers. α-amylase catalyzes the hydrolysis of internal α1–4 glucan bonds in polysaccharides containing three or more α1–4 linkages; it results in a mixture of maltose and glucose.

A thorough survey of the literature available reveals that in *in vitro* drug release studies, carried out for gastrointestinal drug delivery, the experimental conditions do not match with the *in vivo* human GI con-

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ditions. For example, in stomach water content is high and pH is sufficiently low, while in the large intestine water content and agitation intensity is low, and dosage form is surrounded by semi-solid mass (undigested food particles). However, these conditions are usually not considered in the experiments, which are generally carried out for studying *in vitro* degradation and drug release behavior of oral dosage forms. Therefore, attempt should be made to minimize the difference between the conditions maintained in *in vitro* degradation tests and actual *in vivo* GI conditions, so that the possible degradation behavior of hydrogel in GI tract can be predicted in a more realistic way. The major objective of the proposed study is to carry out the *in vitro* enzymatic degradation of the hydrogel system under such conditions, which are very close to the *in vivo* conditions of GI tract.

We have made a sincere attempt in this direction by carrying out enzymatic degradation of starch-based ternary semi-IPN by a novel approach called "flow through diffusion cell method." The experimental conditions involved in this method are very close to the *in vivo* GI conditions. So, we expect to get a more realistic degradation behavior of hydrogels in GI tract. The polymer matrix consists of a synthetic nontoxic monomer methacrylamide, crosslinked in the presence of starch and polyethylene glycol (PEG) to yield well-shaped cylindrical hydrogels, which can be degraded enzymatically with respect to its component starch. The constituent methacrylamide is a water soluble monomer whose hydroxypropyl derivatives have been frequently used to synthesize polymer–drug conjugates for drug delivery,[17–18] and it is reported to be biocompatible and nonimmunogenic.[19] Likewise, J. D. George et al.,[20] in a study to assess the potential for orally administrated methacrylamide to cause developmental toxicity in outbred albino, swiss mice revealed that nearly 60 mg/kg/day methacrylamide was found to be at No-Observed Adverse Effect Level(NOAEL) for both maternal and developmental toxicity. Likewise, PEG is also a nontoxic and biocompatible polymer and variation in its amount within the polymer matrix may influence the degradability of the semi-IPN. In addition to this, PEG has great capability of forming hydrogen bonds in the acidic pH. Therefore, we expect that presence of PEG within the polymer network should provide a compact structure to the gel in the artificial gastric fluid through strong H-bonding interactions, thus providing protection to the encapsulated protein/peptide drug in acidic fluid of stomach. Finally, the variation in degree of crosslinking of polymethacrylamide is also expected to affect the degradability of the hydrogels.

EXPERIMENTAL

Materials

The monomer methacrylamide (MAAm; molar mass 85.11 g mol^{-1}, density 1.10–1.12 g cm^{-3} at 20°C) was supplied by Merck, Dermstadt, Germany and was recrystallized in methanol to remove the inhibitor. The enzyme α-amylase (Amyl; activity 1300 IU/g) from *Bacillus Subtilis* was purchased from Research Lab, Pune, India and was used as received. The crosslinker *N,N′*-methylene bisacrylamide (MB; molar mass 158. g mol^{-1}, Research Lab, Pune, India) and the initiator potassium persulphate (KPS; Research Lab, Pune India) were of analytical grade. Polyethylene glycol (PEG.; molecular weight 20,000, HiMedia, Mumbai, India) and soluble starch (St; average molar mass 5 $\times$ 10^5, Research Lab, Pune India) were used as received. The double distilled water was used throughout the investigations.

Synthesis of hydrogels

The cylindrical hydrogel samples were prepared by carrying out free-radical polymerization of MAAm in the presence of PEG and starch in the aqueous medium using MB as the crosslinking agent. For example, to synthesize hydrogel with the initial water content 76.5%, 1.0 g of methacrylamide and 0.4 g of PEG were dissolved in freshly prepared 1.6% (w/v) solution of starch to give a total volume of 5 mL. To this 0.05 g of the crosslinker MB was dissolved followed by the addition of 0.02 g of initiator KPS. The reaction mixture was transferred into PVC straws, each of diameter 5.3 mm, and kept in an electric oven (Tempstar, India) at 60°C for a period of 2 h. After the polymerization was over, the cylindrical gels were taken out of straws, cut into small pieces each of length 2.50 $\pm$ 0.02 cm, washed extensively with distilled water to remove unreacted salts, and then dried in dust-free chamber till they attained constant weight. The length, diameter, and mass of samples were found to be 16.8 $\pm$ 0.8 mm, 4.16 $\pm$ 0.02 mm, and 0.148 $\pm$ 0.01 g respectively. Although there was variation in mass of the samples, it did not affect the accuracy of results, because calculations were made for 1 g of the polymer. The sample shall be denoted by HG (*X*) where *X* is percent starch content in the feed mixture. For example, the sample, described above, will be designated as HG (1.6). Here, it is worth mentioning that in preliminary studies, we had synthesized a number of hydrogel samples with varying concentrations of starch, PEG, methacrylamide, and crosslinker MB, and the weight of the final product was taken in dry state to determine the percent gelation as follows:

$$\% \text{ Loading} = [\text{Weight of dry polymer/Total weight of constituents in feed mixture}] \times 100$$

Finally, a range of concentrations of different components used in semi-IPN was selected, which yielded

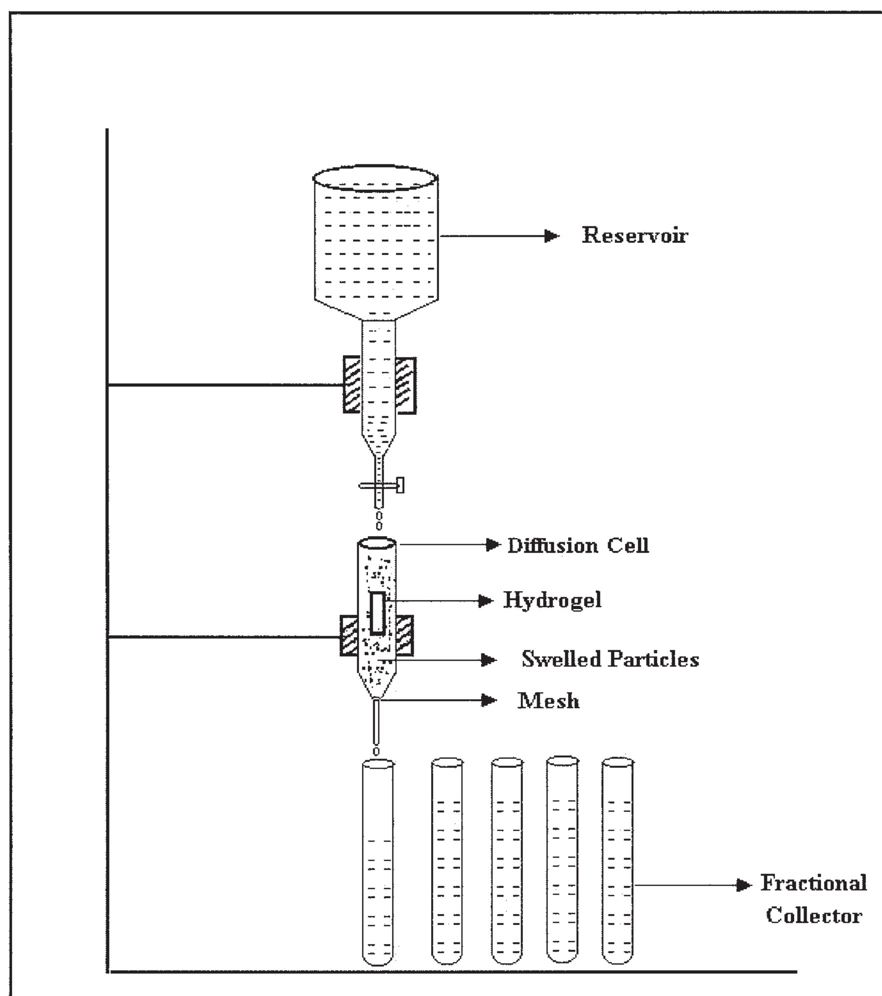

Figure 1 Schematic diagram of apparatus employed for "flow through diffusion cell" method.

almost 100% gelation. Therefore, the composition of various constituents in the feed mixture can be taken as the final composition of polymer matrix.

FTIR spectra

The hydrogel samples HG(1.6) were characterized by recording infrared spectra using a Perkin–Elmer 1600 Fourier Transform IR spectrophotometer with a resolution of 4 cm^{-1} and averaged over 32 scans. The dry hydrogel sample was crushed with potassium bromide and pellets were formed under a hydraulic pressure of 600 kg cm^{-2}.

Enzymatic degradation study

To study the enzymatic degradation of blank hydrogel samples by traditional method, completely dried (and in some cases freshly prepared also) hydrogel sample was put in 25 mL of amylase-containing phosphate buffer of pH 6.8 at the physiological temperature 37°C under constant stirring at 50 rpm. After definite time intervals, 5 mL aliquot was taken out and analyzed for the amount of reducing sugar by the method described previously.[21] After each measurement, hydrogel sample was taken out of the degradation medium, wiped superficially to remove loosely bound solvent on the surface, and then placed in the fresh buffer solution. The removal of surface water from the degrading hydrogel minimizes the "bleeding or washing out" effect. To stabilize the enzyme α-amylase, definite amount of $CaCl_2$ (0.132 g L^{-1}) was already added to the phosphate buffer[22].

As stated in the "Introduction" section we made an attempt to modify the above traditional degradation method (TDM) so as to make experimental conditions look much closer to the *in vivo* GI conditions. This new, modified method shall be called flow through diffusion cell (FTDC) method, which has been depicted in the Figure 1.

A cylindrical cell with a cone-shaped bottom (in accordance with the dissolution test method 3 of Jap-

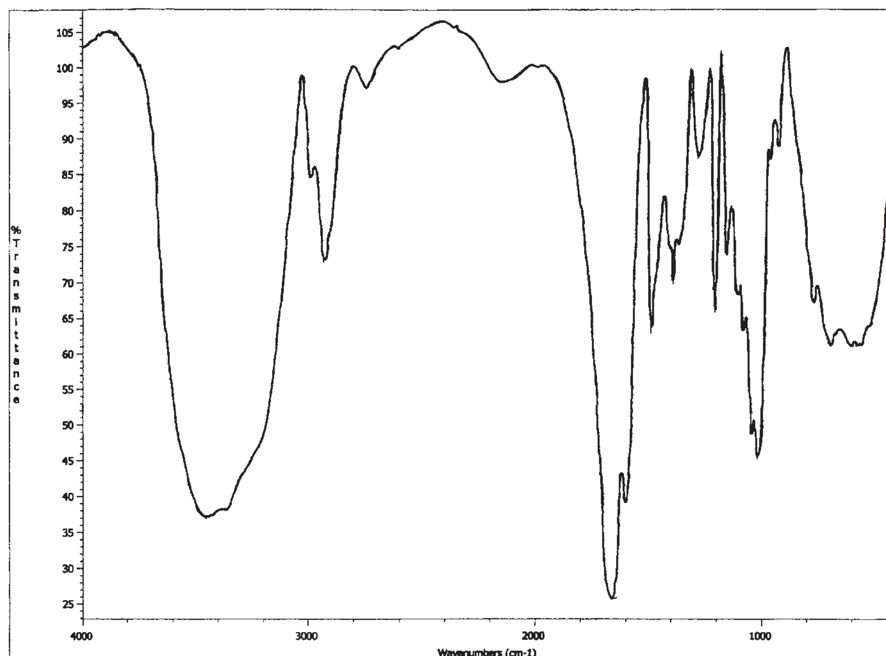

Figure 2 IR spectra of polymeric hydrogel sample.

anese pharmacopoeia) was filled with swollen polyacrylamide hydrogel particles (initially sieved to 600 μm) as filler. The test medium (HCl of pH 1.2 as JP 1st fluid, and enzyme containing phosphate buffer of pH 6.8 as JP 2nd fluid) is dropped from the upper side of the cell at a constant flow rate and the outflow from the bottom of the cell is collected in fractions at different time intervals. Since the dropped medium drains through the swollen gel particles into the bottom and the volume of the medium in the cell remains consistently small, this mimics the presence of undigested food particles and the low water content conditions of large intestine.

RESULTS AND DISCUSSION

FTIR spectral analysis

In Figure 2 a broad and intense band in the range 3400–3500 $cm^{-1,}$ indicates the presence of amide groups of methacrylamide and hydroxyl groups of PEG, starch, and bound water that is present in hydrgel (due to O—H stretching). Characteristic peak of methylene groups appears at 2935.8 cm^{-1} (due to symmetrical stretching of υ_s $CH_{2)}$ and at 2747.4 cm^{-1} (due to asymmetrical stretching of υ_{as} $CH_{2)}$. A band near 1606 cm^{-1} shows the amide—I (NH_2—I) bonding vibration. The occurrence of C═O stretching vibration at 1667 cm^{-1} indicates the presence of carbonyl groups of methacrylamide and *N*,*N*′-methylene bisacrylamide. The spectra also shows a prominent band at 2156.7 cm^{-1}, which could be attributed to the presence of —C—N group of crosslinking agent.

Therefore, the FTIR spectra of IPN confirms the presence of starch, PEG, methacrylamide, and *N*,*N*′-methylene bisacrylamide in the polymer synthesized.

Effect of enzyme concentration on degradation

Starch, which is one of the constituents in the proposed hydrogel system, undergoes enzymatic degradation to yield oligosaccharides that are released from the degrading hydrogel. Figure 3 depicts the dynamic release of reducing oligosaccharides from the hydrogel samples HG (1.6) in the buffer media of pH 6.8

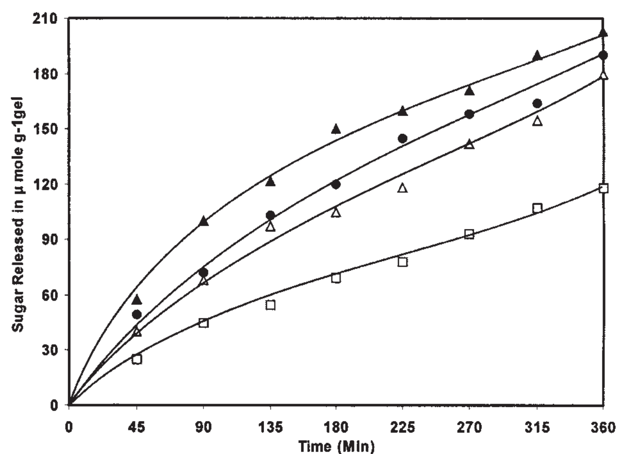

Figure 3 Amount of sugar released as a function of time from the degrading hydrogel in the phosphate buffer of pH 6.8 with enzyme concentration 3.5 (□), 5.0(Δ), 6.5 (●), and 8.0 (▲) IU/mL at the physiological temperature 37°C.

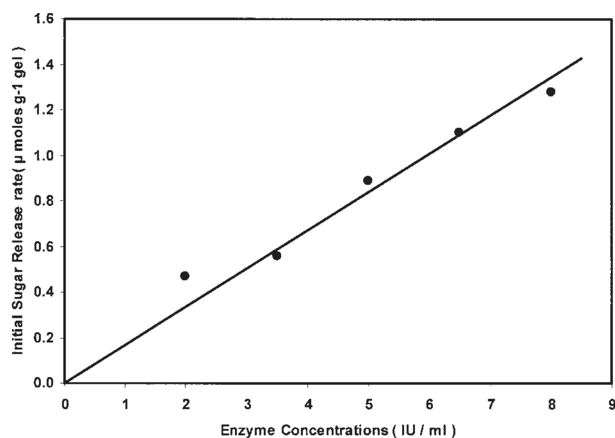

Figure 4 Enzyme concentrations versus initial release rates of sugar for the degradation of the hydrogel samples HG(1.6) in the amylase buffer media of pH 6.8 with amylase concentration 5.0 IU/mL at the physiological temperature 37°C.

containing varying amounts of α-amylase in the range of 3.5 to 8.0 IU/mL at the physiological temperature 37°C. It is clear from the Figure 3 that as the concentration (or activity) of enzyme increases, the amount of reducing sugars coming out of the polymer matrix also increases. The results are quite expected because with the increase in the enzyme content, the binding sites available for starch molecules to undergo degradation through glycosidic linkage also increases, thus causing an increase in the amount of reducing sugars diffusing out of the degrading gels. A close look at the Figure 3 also reveals an interesting fact. For the hydrogel samples immersed in the degradation media with amylase activity 6.5 and 8.0 IU/mL, the release profiles are almost linear over a time period of nearly 6 h, thus indicating a "zero-order" release of reducing sugars. This suggests that by selecting an appropriate enzyme activity in the degradation media, it may be possible to obtain a zero-order degradation kinetics, which also explore the possibility of obtaining a zero-order release of encapsulated drug. Similar type of results have also been reported elsewhere.[23] The total amount of reducing oligosaccharides released from the degrading hydrogels was also found to increase with the enzyme activity and varied from 10 μmol g^{-1} gel to 205 μmol g^{-1} gel for the entire range of enzyme concentration studied.

The degradation rates, as calculated from the initial part of the release profiles (expressed as μmol/g gel/min), were also plotted against the initial amylase activity in the degradation media, as depicted in the Figure 4. It is clear that there exits a linear relationship between the degradation rates and enzyme concentrations, which is a simple consequence of Michaelis–Menten enzyme kinetics[24]. Similar results have also been reported by W.E. Hennink et al.[25], for the enzymatic degradation of dextran hydrogels.

Effect of starch concentration on degradation

The enzymatic degradation of hydrogel should also be influenced by the amount of substrate present in the polymer matrix. To investigate this, various hydrogel samples containing varying concentrations of starch (in the range of 0.32–1.60% w/v) were synthesized and their degradation was studied as a function of time in the α-amylase-containing phosphate buffer media (amylase concentration 5.0 IU/mL) of pH 6.8 at the physiological temperature 37°C. The results, as depicted in the Figure 5 clearly indicate that the amount of sugars released at different time intervals increases with the starch content within degrading hydrogels. This may be attributed to the fact that with the increase in the starch concentration within the degrading gels, more and more glycosidic linkages undergo enzymatic cleavage, thus resulting in an increase in the extent of degradation with subsequent release of oligosaccharides.

To evaluate the Michaelis constant $K_{M,}$ the Lineweaver and Burk equation was employed, according to which

$$\frac{1}{r} = \left[\left(\frac{1}{r_{\max}}\right) + \left(\frac{K_M}{r_{\max}[S]}\right)\right]$$

where r denotes the release rates, $r_{\max}$ is the maximum rate, and $[S]$ represents the substrate concentration. The release rates $r_{1,}$ were determined from the linear part of the curves obtained in Figure 5 and their values were found to be in the range 0.26–0.89 μmol/g gel/min for the entire range of starch concentration studied. Finally a graph was plotted between $1/r_1$ (i.e., reciprocal of release rates of sugars, which measures the degradation rate of starch) and $1/[S]$ (i.e., recipro-

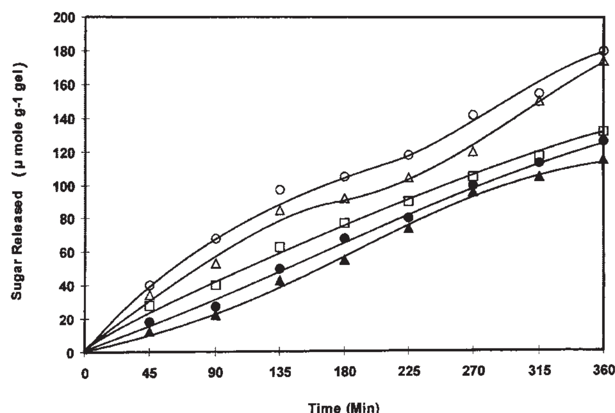

Figure 5 Amount of sugar released as a function of time from the degrading hydrogels with percent starch content 0.32 (▲), 0.64(●), 0.96 (□), 1.28 (Δ), and 1.60 (*o*) in the amylase-containing phosphate buffer of pH 6.8 with enzyme concentration 5.0 IU/mL at the physiological temperature 37°C

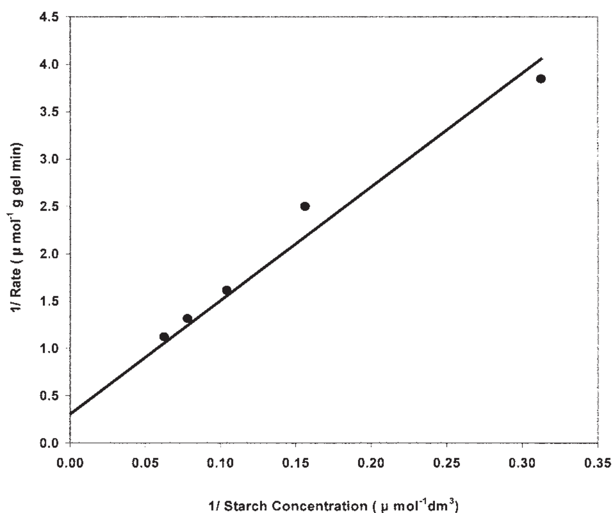

Figure 6 1/Sugar release rate versus 1/Starch concentration plot for the evolution of Michaelis constant K_M.

cal of starch concentration), which yielded a straight line as depicted in the Figure 6. Using the slopes and intercept values the Michaelis constant K_M and the maximum rates r_{max} were evaluated to be 3.92×10^{-5} mol dm^{-3} and 3.27 μmol min^{-1} respectively. The smaller value of K_M is indicative of the higher substrate–enzyme affinity. Here, it is important to note that the Michaelis–Menten kinetics is basically applied for the homogeneous systems (i.e., soluble enzyme acting on a soluble substrate). However, in the present study, the application of this model on the enzymatic hydrolysis of solid substrate (i.e., starch molecules within the swollen hydrogel) may involve diffusion and boundary layer effects. But, as the degradation study has been carried out under constant stirring conditions, these effects are almost minimized. However, there is some probability of variation in the adsorption characteristics of amylase on the substrate due to change in surface structure of substrate as the hydrolysis has been carried out under optimum conditions (i.e., in the pH 6.8 at 37°C).

Effect of initial water content on degradation

The object of the present study is to explore the possibilities of using the proposed hydrogels system as oral drug delivery system and it is expected to undergo enzymatic degradation in the colon. However, when a dosage form is administered orally, it resides for nearly 4 h in the stomach and small intestine prior to its entry to the colon. It means that the formulation must have absorbed some water (or fluid) before its enters into colon for delivering the drug through the amylase-induced degradation. In other words, it is more logical that degradation of hydrogels should be carried out with the hydrated sample and not with the dry sample. Therefore, hydrogel samples containing same quantity of starch (i.e., 1.6% in the feed mixture) but with different initial water content such as 76.5, 79.6, 82.0, and 83.9% were synthesized and the enzymatic degradation of freshly prepared samples was studied in the amylase-containing phosphate buffer (amylase activity 5.0 IU/mL) of pH 6.8 at the physiological temperature 37°C. The results as depicted in the Figure 7 clearly indicate that as the water content in the hydrogel increases, the quantity of starch degraded (which is measured in the terms of amount of sugar released) also increases. This can be attributed to the fact that with the increase in the water content, mesh size within the polymer matrix also increases.[25] This causes the enzyme molecules to enter into the degrading polymer matrix with faster rate, which is indicated by increased amount of reducing sugars coming out of the hydrogels. Therefore initial water content of hydrogel is an important parameter in degradation studies.

Effect of PEG concentration on degradation

The proposed hydrogel system is a ter-polymeric semi-IPN in which chemical crosslinking of polymethacrylamide chains and physical crosslinking via formation of entanglements among polymeric segments have resulted in the formation of hydrogel. In addition to this, H-bonding interactions among PEG macromolecular chains are also present. Therefore, it is expected that variation in the amount of PEG in the hydrogel should affect its degradability. To investigate this aspect, hydrogel with varying concentrations of PEG, ranging from 3 to 12% (w/v), were synthesized and their degradation was studied in the phosphate buffer medium of pH 6.8 at 37°C. The results, as depicted in the Figure 8, clearly indicate that as the

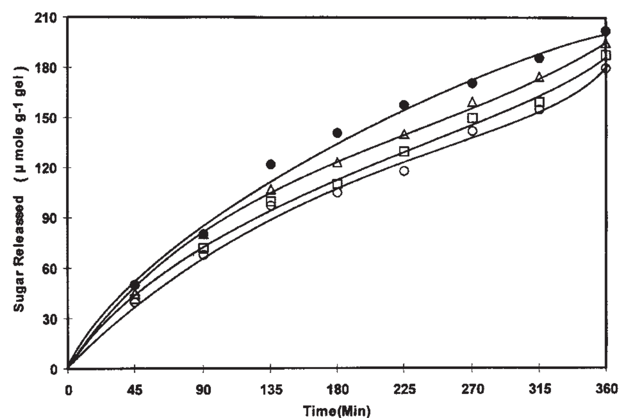

Figure 7 Amount of sugar released as a function of time from the degrading hydrogels with percent initial water content 76.50(*o*), 79.68 (□), 82.06 (Δ), and 83.94(●) in the buffer media of pH 6.8 with enzyme concentration 5.0 IU/mL at 37°C.

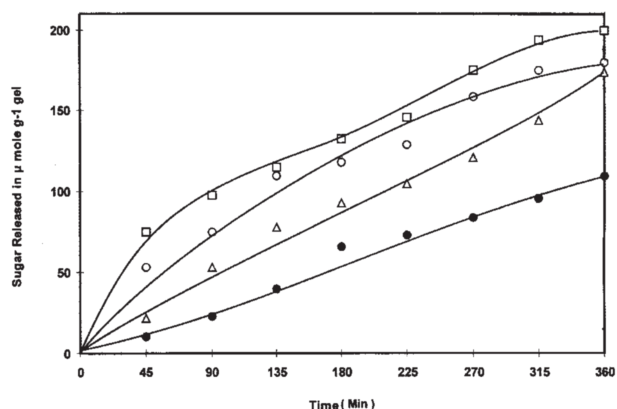

Figure 8 Amount of sugar released as a function of time from the degrading hydrogels sample HG(1.6) with percent PEG content 3.0 (□), 6.0(*o*), 9.0 (Δ), and 12.0(●) in the amylase-containing buffer media of pH 6.8 at 37°C. The amylase concentration in the degradation media is 5.0 IU/mL

concentration of PEG in the hydrogels increases, the amount of oligosaccharides released at different time intervals decreases, thus indicating a fall in the extent of degradation. This can be well attributed to the fact that as the amount of PEG in the polymer network increases, its entanglements with other polymeric segments also increases, thus producing a much more dense polymer network. The increasing number of H-bonding interactions among PEG chains also helps in producing the compact network. As a result the diffusion of enzyme molecules into the polymer network is hindered or becomes slow, thus making the degradation process slower. In this way the degradation of gel (and hence drug releasing capacity also) can be controlled by varying the amount of PEG in the hydrogels.

Degradation study by TDT and FTDC method

Until now, the degradation behavior of hydrogel has been studied under sink conditions (where the sample sinks in the degradation medium). However, these studies can not act as a basis for predicting the degradability of the proposed hydrogel system in GI tract because the conditions maintained in the above studies do not match with the *in vivo* GI conditions. For example, in large intestine the agitation intensity is quite low, water content is small, and oral dosage form is surrounded by wet semi-solid mass etc. To incorporate these conditions in the *in vitro* degradation studies, the degradation behavior of hydrogel was studied by the modified method called flow through diffusion cell (FTDC) method.(please see experimental section).

Figure 9 depicts a comparative depiction of degradation behavior of hydrogel sample HG(1.6) in the α-amylase-containing phosphate buffer media of pH 6.8 as studied by the traditional degradation method and flow through diffusion cell (FTDC) method with swollen gel particles(crosslinking ratio 0.025, particle size 600 μm) used as fillers. It is very clear from the Figure 9 that the two methods yield almost different degradation profiles. The amount of reducing sugars released (or amount of starch degraded by amylase) at different time intervals is more in the traditional degradation method while a slower degradation is observed in FTDC method. The slower degradation (as indicated by decreased rate of diffusion of reducing sugars) may probably be explained on the basis of adsorption of enzyme molecules onto swollen polyacrylamide gel particles. To study the degradation, the cylindrical sample was put into the swollen gel particles at the depth of nearly 1 cm. It means that the enzyme-containing buffer medium, dripping from the top of the diffusion cell, has to pass through the 1 cm-long column of swollen gel particles before its reaches the polymer sample. During their passage, the amylase molecules may probably be adsorbed on the gel particles and hence the activity of amylase in the buffer solution decreases. Hence, this may cause slower degradation of hydrogel sample. One more interesting point about the Figure 9 is that the curve obtained in FTDC method exhibits biphasic nature. A close look at the profile reveals that in the initial phase, the extent of degradation (as indicated by the amount of sugar released) is almost negligible for first 2 h and after that, appreciable amount of sugar is released thus indicating a faster degradation. The initial slower or almost negligible degradation may be attributed to the adsorption of enzyme molecules from the dripping buffer medium on the swollen gel particles as mentioned above. However, after nearly all the adsorption sites available on the surface of swollen gel particles have been occupied by amylase molecules,

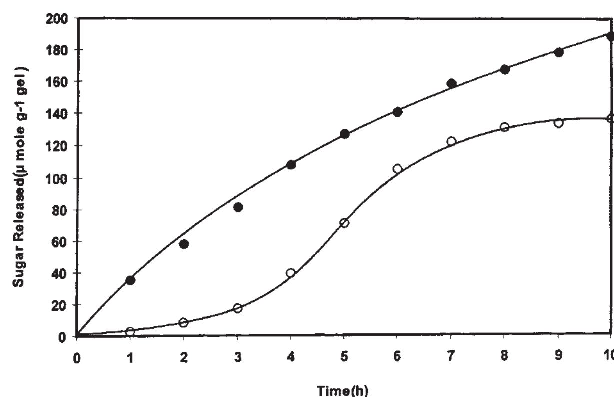

Figure 9 Amount of sugar released as a function of time from the degrading hydrogel samples HG(1.6) as studied by TDM(●) and FTDC (*o*) (with crosslinked swollen polyacrylamide gel particles (initial sieved to 400–600 μm) as fillers in the amylase-containing buffer media of pH 6.8 at 37°C. The amylase concentration is 5.0 IU/mL

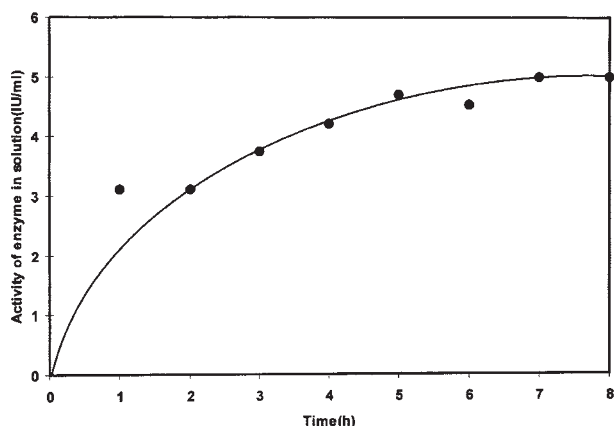

Figure 10 Amount of enzyme retained in the dripping buffer solution of pH 6.8 as a function of time because of absorption onto crosslinked swollen gel particles in FTDC method.

the extent of adsorption decreases and hence greater number of enzyme molecules are now available in the phosphate buffer medium dripping from the top of the diffusion cell. This causes faster degradation of the polymer sample. However, to further confirm this, we investigated the adsorption of amylase molecules on the swollen gel particles. In a separate study, the diffusion cell was filled with swollen gel particles upto the height of 1 cm and the amylase-containing medium was dripped from the top with the same flow rate. the solution was collected at the bottom and it was analyzed for the amylase concentration by the method described elsewhere.[26] The amount of amylase left in the medium was plotted against time and the curve obtained has been shown in the Figure 10. It is very clear that concentration of enzyme in the medium being collected decreases because of passage of solution through the gel particles. This indicates that the enzyme molecules must have adsorbed on the filler particles, thus causing a decrease in their concentration in the medium, which leaves the filler column at the bottom. This suggests that less number of amylase molecules are available in the phosphate buffer for the degradation of starch in the polymer sample. A close look at Figure 10 also reveals that initially the concentration of enzyme in the solution, collected at the bottom, is very low; thus suggesting a high degree of adsorption of amylase from the flowing degradation medium onto gel particles. However, after some time, the concentration of enzyme in the buffer medium, being collected, increases; thus indicating that degree of adsorption has now decreased. This may be due to fact that as the adsorption process continues, the number of sites available for adsorption of gel particles decreases and after 7 h the concentration of enzyme in the media attains almost its initial value (i.e., 5.0 IU/mL) as indicated in the Figure 10. Thus, it can be concluded that adsorption of amylase molecules on the filler particles may be responsible for slower degradation of hydrogel in FTDC method. Finally, the extremely slower degradation of hydrogel in the first 3 h in FTDC method may be attributed to the fact that in the initial stage most of the amylase molecules, present in the dripping medium, are adsorbed onto gel particles and hence number of amylase molecules in the medium becomes so small that it causes almost minimum degradation of the gel. Later on, as the degree of adsorption decreases, concentration of enzyme molecules in the buffer medium increases. This accounts for enhanced degradation in the later stage.

Effect of particle size of filler on degradation

The size of the semi-solid mass in the large intestine may affect the enzymatic degradation of oral dosage form in many ways. To investigate this aspect, the swollen gel particles of varying size, ranging from 1.83 to 5.85 mm were filled in the diffusion cell and the degradation of the buried hydrogel samples was studied by FTDC method with amylase-containing phosphate buffer of pH 6.8 dripping down the cell at a constant flow rate at 37°C. The results, as depicted in the Figure 11 clearly indicate that as the size of the filler particles in the diffusion cell increases, the degradation of hydrogel(as measured in the terms of amount of sugars released) also increases. This may be attributed to the fact that the particles with smaller size have greater surface area exposed to the amylase-containing buffer and hence greater amount of enzyme may be adsorbed on the swollen gel particles. This may cause reduction in the concentration of enzyme available to cause degradation of hydrogel, thus finally lowering the extent of degradation. In addition

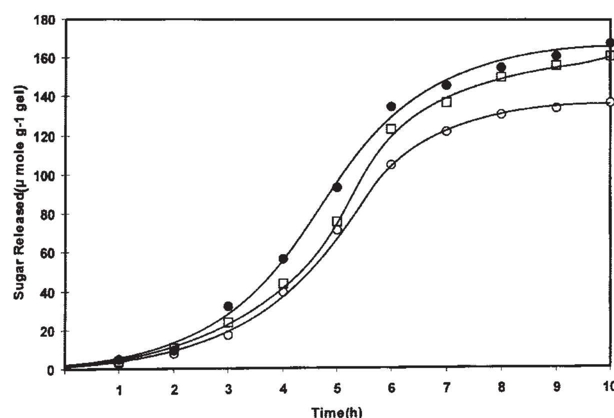

Figure 11 Amount of sugar released as a function of time from the degrading hydrogel samples HG(1.6) as studied by FTDC with filler particles of size 5.85 mm(●), 3.10 mm(□), and 1.82 mm (*o*) at the physiological temperature 37°C.

to this, the smaller sized particles occupy greater volume in the space available in the diffusion cell. This reduces the area of direct contact between the polymer matrix and buffer solution. This ultimately offers less opportunity for the enzyme molecules to get in contact with hydrogel sample, thus lowering the extent of degradation. Finally, due to rather compact packing of small sized particles in the vicinity of the polymer matrix, narrow water channels are available for buffer solution to reach the hydrogel sample. This also contributes to observed decrease in the degradation. On the other hand, the big sized gel particles, due to smaller surface area, adsorb less quantity of enzyme from the dripping phosphate buffer and hence comparatively more enzyme is retained in the buffer, which ultimately causes greater degradation of polymer matrix. Moreover, the big sized filler particles provide wider water channels through which enzyme molecules can reach the hydrgel samples and reducing sugars can diffuse out.

Finally, the extremely low extent of degradation in the first 3 h may be attributed to the fact that in the beginning the amylase molecules present in the degradation media are readily adsorbed onto filler particles to a greater extent and hence enzyme retained in the buffer causes a little degradation of hydrogel. In this way, we see that size of filler particles in the diffusion cell may influence the enzymatic degradation of hydrogel sample in FTDC method.

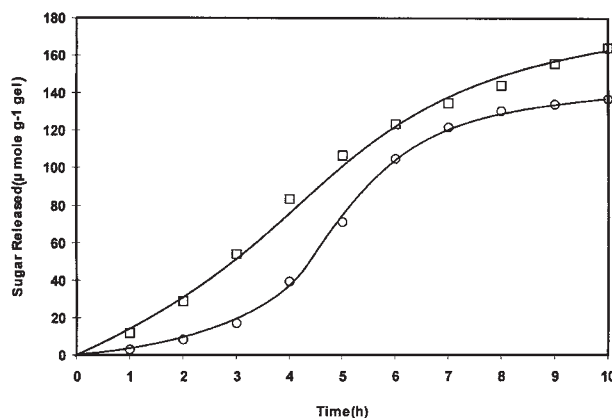

Figure 12 Amount of sugar released as a function of time from the degrading hydrogels sample HG (1.6) as studied by FTDC with glass beads of size 1.86 mm (□) and swollen gel particles of size 1.82 mm (*o*) at the physiological temperature 37°C.

Effect of nature of solid mass on degradation

The nature of the solid mass surrounding the oral dosage form in the large intestine may influence its degradation. If the solid mass has greater tendency to adsorb enzyme molecules, then it will lower the degradation of the gel. Moreover, if the solid mass has hydrophobic nature, it may provide more opportunity for medium to interact with oral dosage form, thus enhancing its degradation. To investigate this aspect, the diffusion cell was filled with glass beads (representing hydrophobic mass) and the degradation of hydrogel sample was studied by FTDC method with amylase-containing medium dripping from the top at a constant flow rate. Figure 12 depicts a comparison of the two profiles, one obtained with the swollen gel particles and other with glass beads. It is clear from the Figure 12 that the sample demonstrates faster degradation in the presence of glass beads whereas in the presence of gel particles the degradation (as observed by release of reducing sugars) is found to be slow. The observed finding may be explained on the basis of the fact that the glass beads are hydrophobic in nature and hence their surface possess water repelling property. Hence, the dripping medium does not interact with beads surface, but it interacts strongly with the polymer sample. This may provide greater opportunity for enzyme molecules to diffuse into polymer sample and cause enzymatic degradation of starch. Moreover, the adsorption of enzyme molecules onto glass beads is almost nil, which was also confirmed in a separate study (data not shown). Therefore, this also helps in enhancing the degradation process. In addition to this, the presence of glass beads forms wider and almost fixed water channels in the vicinity of the oral formulation. This also helps in making degradation process faster. On the other hand, the hydrophilic swollen gel particles have greater tendency to adsorb amylase molecules (as has been discussed previously) and therefore the number of enzyme molecules, available for degradation, becomes fever. As a result, a slower degradation is observed. Here it is worth mentioning that the purpose of taking glass beads as surrounding mass is just to have some idea about the degradation of starch-based hydrogels sample in the presence of wet semi-solid mass which is hydrophobic in nature and possess weaker tendency to adsorb enzyme molecules.

CONCLUSIONS

From the above study, it can be concluded that the enzymatic degradation of proposed hydrogel increases with the amount of enzyme present in the degradation medium as well as the amount of substrate present in the polymer matrix. The degradability of proposed hydrogel is also influenced by the amount of PEG in the polymer matrix. The system obeys the Michaelis–Menten kinetics. The degradation profiles, obtained by traditional degradation method (TDM) and flow through diffusion cell (FTDC) method differ appreciably from each other.

The extent of degradation is decreased when studied by FTDC method has been attributed to the ad-

sorption of enzyme onto swollen polyacrylamide gel particles used as fillers. The nature of the filler particles (hydrophobic/hydrophilic) also influences degradation behavior.

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