Enzyme-digestible swelling hydrogels as platforms for long-term oral drug delivery: synthesis and characterization

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A method was developed for synthesizing enzyme-digestible swelling hydrogels. Albumin molecules were modified using glycidyl acrylate to introduce vinyl groups. The functionalized albumin molecules participated as cross-linkers in the polymerization of vinyl monomers, such as acrylic acid or acrylamide. The extent of chemical modification of albumin was an important variable in controlling the cross-linking ability. The albumin in the synthesized hydrogels retained its property of enzymatic digestion by proteolytic enzymes. The kinetics of swelling and enzymatic digestion of the hydrogels were examined using various enzyme concentrations. It was observed that the digestion kinetics were largely determined by the relative concentrations of albumin and enzyme. The potential application of the enzyme-digestible swelling hydrogels as platforms for long-term oral drug delivery is discussed.

Of the many routes of controlled drug delivery, the oral administration has been considered to be the most convenient means to introduce drugs into the systemic circulation. One drawback of using oral dosage forms, however, is that they pass through the stomach and small intestine in a relatively short period of time. From various studies, it was suggested that transit time from mouth to caecum varies between 3 and 12 h[1,2]. Since most drugs are absorbed from the upper portion of the small intestine, duodenum and jejunum, the time interval from mouth to caecum is obviously too short for long-term oral controlled drug delivery, unless a drug is to be absorbed from the large intestine[3]. It is therefore desirable to decrease the rate of transit past the upper portion of the small intestine by retaining the dosage form in the stomach. For this reason, a few approaches have been taken to prolong the gastric retention time. The density and geometry of oral dosage forms were altered[4-10] or muco-adhesive polymers were used[11,12] to retard gastric emptying. These approaches have shown limited success[13].

It is known that the stomach selectively empties solids based on the size of various particles[14,15]. For example, large lumps of meat remain in the body of the stomach until reduced in volume, whereas ground beef or liver paste leaves quickly[16]. Thus, one of the possibilities of retaining oral dosage forms in the stomach is to make large size dosage forms. Since the dosage form should be small enough for easy swallowing, the best approach would be to design dosage forms which can swell in the gastric environment to

such an extent that the passage through the pyrolus is inhibited. In fact, a similar approach has been used successfully in animals to accomplish gastric retention[17]. The shape of drug delivery systems has been designed in such a way that the systems either unfold wings or unroll upon contact with gastric fluid. The application of these systems to humans, however, requires improvement. In humans, the dosage form has to be removed from the body after its use without any surgical procedure. There is therefore a need to solubilize the gastric retention devices in addition to accomplishing retention in the stomach. Here we describe a concept of enzyme-digestible swelling hydrogels that can be used to achieve both gastric retention and solubilization in the stomach.

MATERIALS AND METHODS

Alkylation of albumin

A solution of 5% human albumin (Sigma, fraction V) was prepared using phosphate–buffered saline which was diluted by a factor of 2 (1/2X PBS, pH 7.2). Glycidyl acrylate (Aldrich Chemical, Milwaukee, WI, USA) was added directly to the albumin solution while stirring. The extent of alkylation was a function of the amount of glycidyl acrylate and the reaction time[18,19]. Typically, 200 μl of glycidyl acrylate was added to 5 ml of the albumin solution while stirring at room temperature. After 5 h, 1 ml of 20% glycine solution was added and stirring was continued for another 30 min. This solution was then dialysed against 2 litres of

1/2X PBS. During 24 h of dialysis, the buffer was changed five times. After the protein was collected, the concentration of albumin solution was determined spectrophotometrically. Absorptivity[20] used for 0.1% albumin solution at 280 nm was 0.58. The degree of alkylation was determined by measuring the free amine groups of albumin using 2,4,6-trinitrobenzenesulphonic acid (Aldrich Chemical, Milwaukee, WI, USA)[21]. Unmodified albumin was used as a control and the degree of alkylation was expressed as the percentage of the total amine groups available for the titration.

Synthesis of albumin-cross-linked hydrogels

Water-soluble acrylic monomers, such as acrylamide (Bio-Rad) and acrylic acid (Aldrich Chemical, Milwaukee, WI, USA), were polymerized in the presence of functionalized albumin as a cross-linker. Monomer concentrations used were 15% (w/v) for acrylamide and 30% (v/v) for acrylic acid, respectively. These monomer concentrations were chosen on the basis of the rigidity and swelling ability of the synthesized gels. The concentration of albumin was varied from 0.5 to 3% (w/w) of the monomer. The concentration of ammonium persulphate was 1% (w/w) of the monomer. The mixture of monomer, cross-linker (functionalized albumin) and initiator was poured into the space between two glass plates using a syringe and a needle. The thickness of a spacer (Mylar film, Polysciences) was 0.6 mm. The mixture was then polymerized at 60°C for 1 h.

Polyacrylamide (PAM) gels were washed in de-ionized distilled water while poly(acrylic acid) (PAA) gels were washed in diluted HCl solution (pH 2) for at least 2 d, changing the washing solutions several times. The washed hydrogels were transferred into either PBS or simulated gastric fluid without pepsin and equilibrated at room temperature. The hydrogels in a sheet form were cut into discs using a cutting mold. The diameter of the disc was 1.3 cm and the thickness was varied from 1.5 to 3 mm depending on the type of hydrogel and washing solution. Some of the hydrogels were washed with only de-ionized distilled water, cut into discs, and air dried at the room temperature for several days. These discs were used for subsequent experiments, unless specified otherwise.

Swelling and enzymatic digestion of dried hydrogels

A dried hydrogel disc was placed into 5 ml of either PBS with trypsin or simulated gastric fluid with pepsin and allowed to swell at 37°C. Enzyme solutions were made immediately before use. The swelling ratio Q was determined from the relation:

$$Q = w^*/w$$

where w^* and w are the weights of wet and dry samples, respectively. The swelling of a dried hydrogel disc in the absence of enzymes was used as a control. The concentrations of pepsin (Sigma, 2850 units/mg protein) and trypsin (Sigma, 10 200 BAEE units/mg protein) ranged from 0.04 to 1.0 mg/ml. At timed intervals, each disc was carefully picked up using a forceps, excess water was blotted off and the weight measured using a polystyrene weighing dish. As enzymatic digestion proceeded, the gel structure became so loose that the gel behaved like a liquid. At this point (gel disruption point), the gel could not be picked up by the forceps, although not completely dissolved. After this point, the weight measurement was discontinued, but we continued visual examination of the gel boundary until the gel was completely dissolved.

Enzyme digestion of preswollen hydrogels

The hydrogels were equilibrated in PBS or simulated gastric fluid without pepsin at 37°C. These preswollen hydrogels were transferred into buffer solutions which contained either pepsin or trypsin. The change in the swelling ratio was measured at various enzyme concentrations as previously described.

RESULTS

Synthesis of albumin-cross-linked hydrogels

In free radical polymerization, polymers are produced if monomers have functionality $\geqslant 2$. If the functionality of a monomer is > 4, it serves as a cross-linker and a network is formed. The functionality can be derived from opening a double bond. Thus, protein molecules which have more than two alkenyl groups can be used, at least in theory, as cross-linkers in the polymerization of water-soluble vinyl monomers[18]. We observed that vinyl groups on albumin molecules indeed participated in the polymerization of acrylamide or acrylic acid and resulted in cross-linked hydrogels.

The extent of albumin modification had a direct influence on the cross-linking ability and the digestion by enzymes. *Figure 1* shows the degree of modification of amine groups of albumin by glycidyl acrylate. When < 10% of the total amine groups of albumin were modified, albumin molecules could not function as cross-linking agents. If the degree of modification was > 20%, albumin molecules lost the ability to be digested by enzymes, particularly trypsin. This is understandable, since the protease activity of trypsin is highly specific toward lysine and arginine[22, 23]. Therefore, about 15% of the total amine groups of albumin was modified for subsequent experiments.

Swelling behaviour of dried hydrogels in the presence of enzymes

Albumin cross-linked hydrogels swell in buffer solutions to such an extent that the swelling ratio can reach up to several hundred depending on the type and concentration of monomers and cross-linking density. In the absence of enzymes, dried gels swelled in buffer solutions to reach
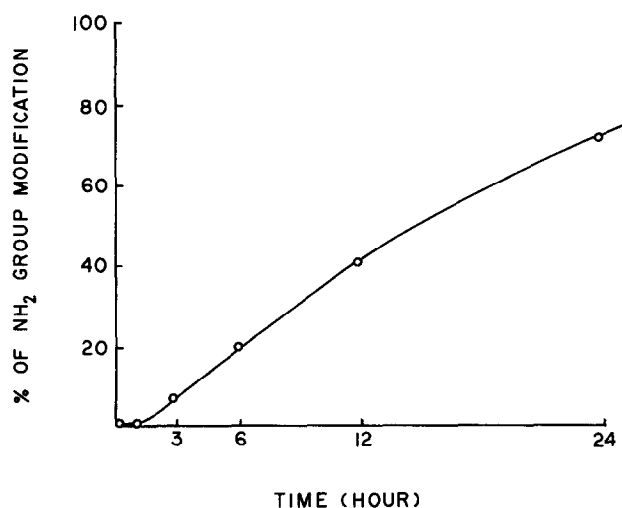
Figure 1   Degree of amine group modification by glycidyl acrylate as a function of time. To 5 ml of 5% albumin solution, 200 µl of glycidyl acrylate was added while stirring at room temperature. Free amine groups of albumin were titrated by 2,4,6-trinitrobenzenesulphonic acid.
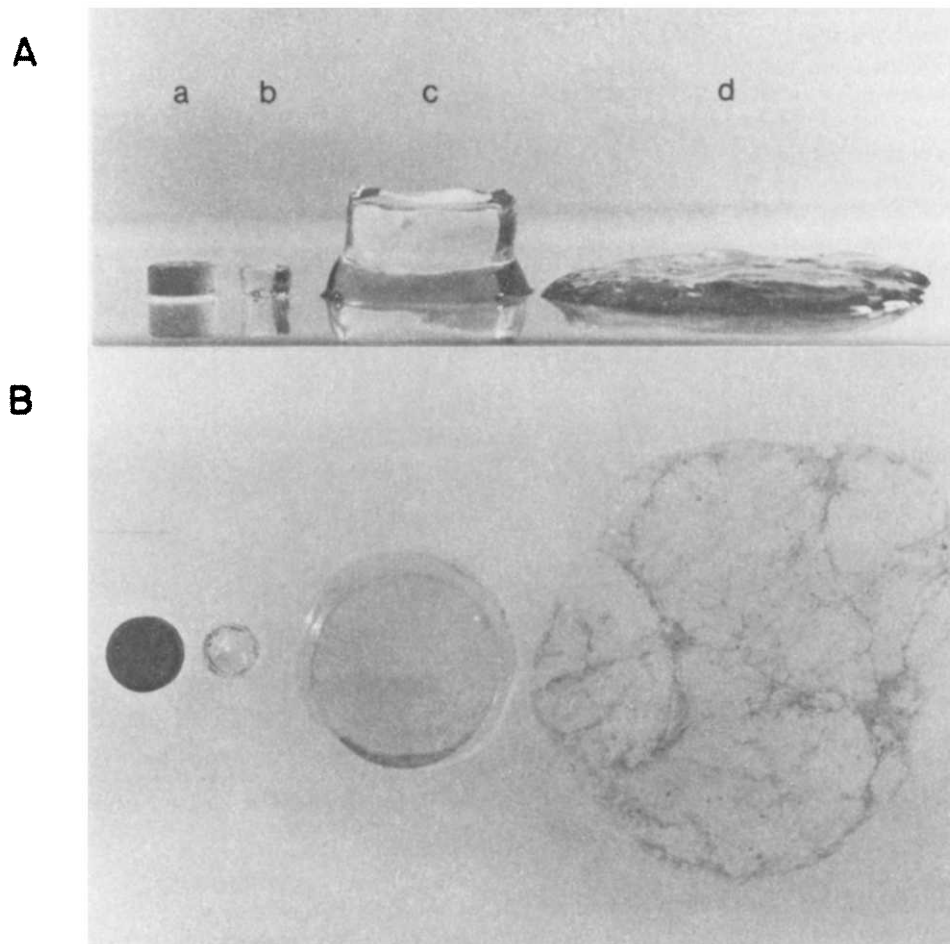
Figure 2   Side (A) and top (B) views of albumin-cross-linked polyacrylamide gels in various states. (a) Extra-strength Tylenol® tablet as a size indicator; (b) dried polyacrylamide gel; (c) polyacrylamide gel swollen in the simulated gastric juice without pepsin at 37°C; (d) polyacrylamide gel swollen in the simulated gastric juice with pepsin (570 units/ml) at 37°C for 24 h. The concentration of acrylamide was 15% (w/w) and that of albumin was 1% (w/w) of the monomer.

equilibrium states. In the presence of enzymes, however, gels swelled faster and to larger extents than in the absence of enzymes. The presence of enzymes resulted in the disruption of the gel structure and eventual complete dissolution of the gels. *Figure 2* shows albumin-cross-linked PAM gels swollen respectively in the absence and presence of pepsin. For this particular demonstration, gels were synthesized in such a dimension that the size of dried gels was comparable to that of a Tylenol® tablet (a in *Figure 2*). The digestion of the gel by pepsin is obvious (d in *Figure 2*). The concentrations of albumin and enzyme in the subsequent experiments were chosen to achieve the digestion of the hydrogels in a reasonably short period of time. In this way, complications arising from the slow inactivation of enzymes could be avoided.

The swelling kinetics of albumin cross-linked PAM gels in the presence of pepsin are shown in *Figure 3*. The occurrence of the transient maximum swelling is due to the incomplete enzymatic digestion of albumin molecules. As the albumin molecules are digested, the cross-linking density is reduced and more swelling occurs. As the enzymatic digestion proceeds further, the gel structure becomes so loose that the gels cannot maintain their shape and behave like a viscous liquid. The transient maximum swelling and the gel disruption occurred faster as the pepsin concentration was increased. Since the separation of the loosely cross-linked gels from the solution was not easy after the gel disruption point, the weight of the sample was not measured further. In solution, however, the gel boundary

was clearly observed and the complete dissolution of the gels could be visually identified. It was clear from observation that the enzymatic digestion occurred homogeneously throughout the gel. Thus, the diffusion of enzymes into the
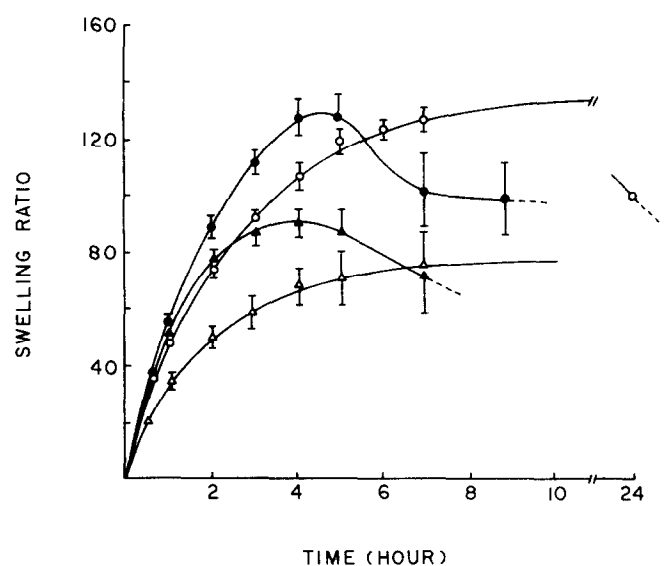

TIME (HOUR)

Figure 3   Swelling behaviour of albumin-cross-linked polyacrylamide gels in the absence and in the presence of pepsin at 37°C. The concentration of acrylamide was 15% (w/v) and that of albumin was 1% (w/w) of the monomer. The concentrations of pepsin were: 0 (△), 140 (○), 570 (●), and 2850 (▲) units/ml. Dotted lines indicate the transition to the disrupted gel state. (n = 4).

PAM gels does not appear to be rate-limiting in the digestion process. The swelling behaviour of PAM gels in the absence of enzymes was independent of the pH of the swelling solution. The enzymatic digestion behaviour of PAM gels by trypsin in PBS was similar to that by pepsin.

The swelling profiles of dried PAA gels in the absence and in the presence of enzymes were different from those of PAM gels. The swelling of PAA gels was strongly pH-dependent due to the presence of carboxyl groups as shown in *Figure 4*. The swelling behaviour of dried PAA gels in the presence of trypsin (pH 7.2) was different from that in the presence of pepsin (pH 1.2). The presence of trypsin did not show any appreciable increase in the swelling ratio compared to the control gels until 4 h of incubation. The mode of PAA digestion by trypsin, according to the visual observation, was unique compared to others. The PAA gels were dissolved by trypsin from the surface of the gel disc, while the core of the gel remained relatively intact. This surface digestion rather than the bulk digestion may be responsible for the small difference in the swelling of PAA gels in the presence or absence of trypsin. The iso-electric pH of trypsin[24] is known to be 10.5. This peculiar digestion behaviour is probably due to the binding of trypsin, which is positively charged at pH 7.4, to highly anionic carboxyl residues of the PAA gels. Electrostatic interaction may inhibit trypsin molecules from entering into the gel, especially when the gel is not fully swollen.

Digestion of preswollen hydrogels with enzymes

Enzymatic digestion was also examined using gels which were preswollen to equilibrium states in the absence of enzymes. *Figure 5* describes further changes of the swelling of PAA gels in the presence of trypsin. When the concentration of functionalized albumin was high (3% and 5% of the monomer), gels swelled further to reach transient
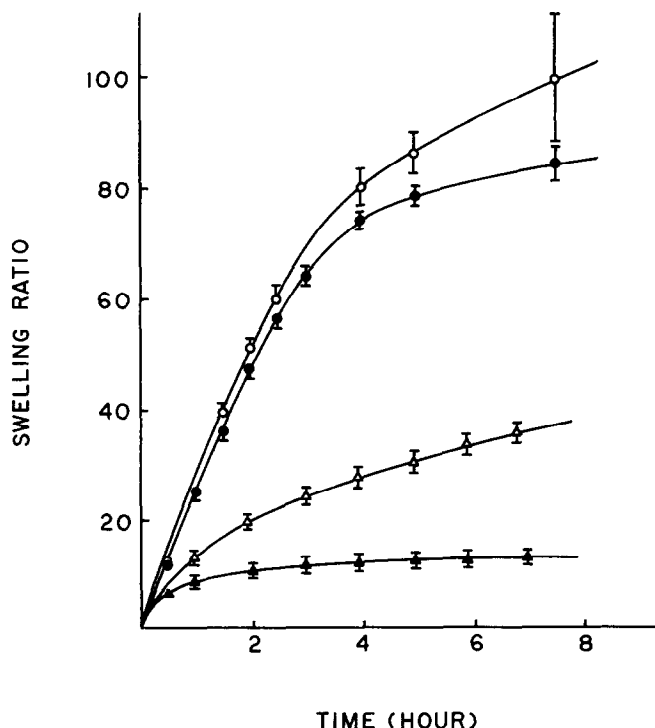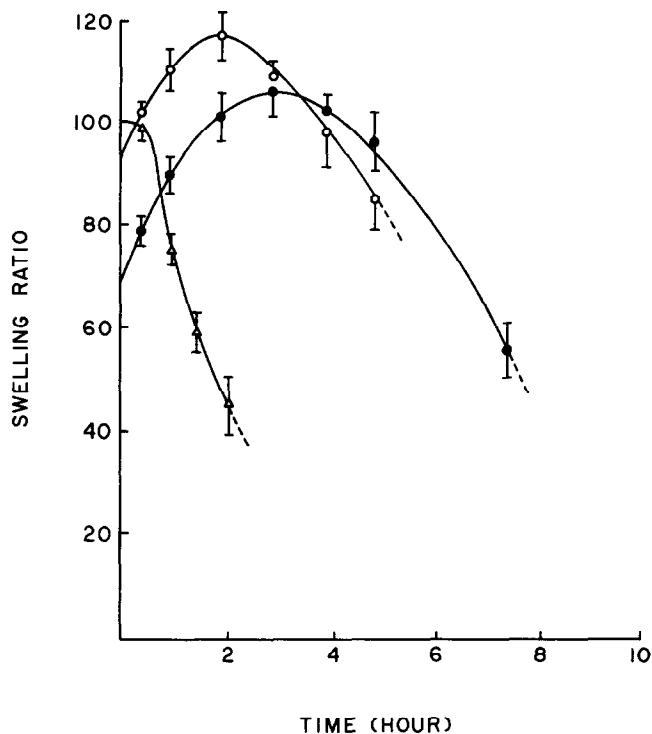

Figure 5   Changes in the swelling ratio of albumin-cross-linked poly(acrylic acid) gels in the presence of trypsin (51 BAEE units/ml) at 37°C. The albumin concentrations used for cross-linking were 1% (△), 3% (○) and 5% (●) of the monomer by weight. The gels were preswollen in PBS before enzymatic digestion. (n = 5).

maxima before they were collapsed by enzymes. At a low cross-linker concentration (1% of the monomer), no transient increase in gel size was observed and the gels began to reduce the swelling ratio immediately after enzymes were added. The PAA gels at three different cross-linker concentrations were digested at various trypsin concentrations. As the trypsin concentration increased, the transient maximum swelling occurred faster with a lower degree of swelling. The gel disruption point also occurred much earlier. When the cross-linker concentration was low (1% of the monomer), the dissolution rate increased. Examples are shown in *Figure 6*.

The digestion behaviour of the preswollen PAA gels by pepsin was similar to that by trypsin. *Figure 7* describes the digestion of PAA gels by pepsin. At lower pepsin concentrations (140–570 units/ml), the digestion rate was slow and the gels at high cross-linker concentration (3% and 5% of monomers) showed continuous increase up to 10 h. After 24 h these gels became completely soluble. When the concentration of pepsin was increased to 2300 units/ml, all PAA gels were disrupted in less than 5 h.

The swelling and digestion profiles of PAM gels were similar to those of PAA gels, except the swelling of the PAM gels was not dependent on pH (*Figure 8*). The general trend is that the digestion occurs faster and the transient maximum swelling is observed as the enzyme concentration is increased. Although pepsin and trypsin hydrolyse peptide links involving different amino acids[22, 23], both enzymes were able to digest modified albumin molecules used as cross-linkers.

TIME (HOUR)

Figure 4   Swelling behaviour of albumin-cross-linked poly(acrylic acid) gels in the absence (▲ and ●) and in the presence of pepsin (△) or trypsin (○) at 37°C. The concentrations of acrylic acid was 30% (v/v) and that of albumin was 3% (w/w) of the monomer. The concentrations of pepsin and trypsin were 570 units/ml and 510 BAEE units/ml, respectively. (n = 4).

General enzyme-digestion behaviour of hydrogels

Upon analysing the enzymatic digestion data of various hydrogels at different cross-linking densities and different enzyme concentrations, a general trend of the change in
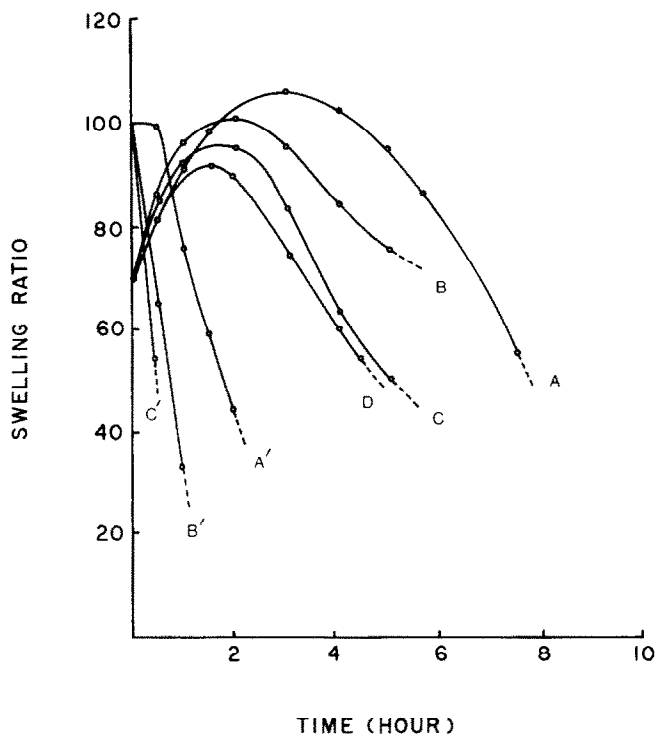
Figure 6   Changes in the swelling ratio of albumin-cross-linked poly(acrylic acid) gels in the presence of various trypsin concentrations. The trypsin concentrations were 51 (A and A'), 102 (B and B'), 510 (C and C') and 2040 (D) BAEE units/ml. The albumin concentrations used for cross-linking were 1% (A', B' and C') and 5% (A, B, C and D) of the monomer by weight. The gels were preswollen in PBS before enzymatic digestion. Data points are averages of five measurements.
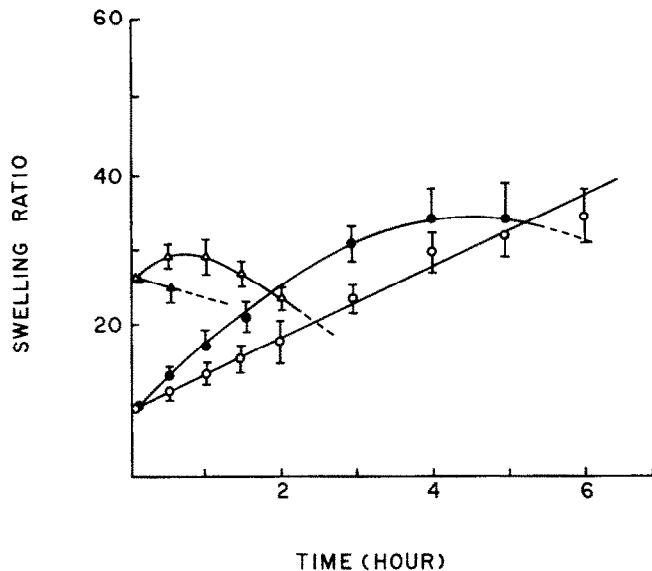

Figure 7   Changes in the swelling ratio of albumin-cross-linked poly(acrylic acid) gels at 37°C in the presence of pepsin at the concentration of 140 units/ml (O, △) and 2280 units/ml (●, ▲). The gels were cross-linked with 1% (▲, △) and 5% (●, O) of albumin and preswollen in the simulated gastric fluid without pepsin. (n = 4).

swelling ratio was observed as shown in *Figure 9*. When the digestion of albumin by enzymes was slow, the gels continued to swell more (A and B in *Figure 9*). This happened when the concentration of enzyme was very low or the cross-linking density was very high as seen in *Figures 7* and *8*. When the rate of enzymatic digestion and the rate of gel swelling is comparable, the transient maximum swelling (C-F in *Figure 9*) occurs as shown in *Figures 3* and *5-8*. As

the digestion occurred faster, the transient maximum occurred earlier and the extent of swelling was reduced. When the digestion rate was much faster than the swelling rate, gels started releasing polymer chains due to enzymatic digestion and no transient maximum was observed (G-I in *Figure 9*) as shown in *Figure 6*. The change in swelling ratios after enzyme addition was dependent on the type of monomer used, the concentration of albumin and the concentration of enzymes. Thus, hydrogels of any property can be made according to a special need.
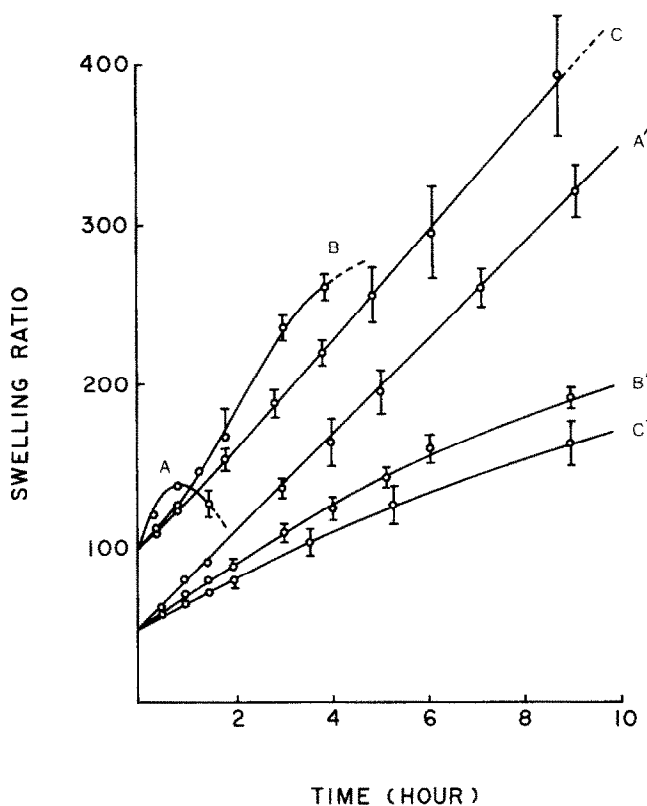

Figure 8   Changes in the swelling ratio of albumin-cross-linked poly-acrylamide gels at 37°C in the presence of various pepsin concentrations. The pepsin concentrations were 2280 (A and A'), 570 (B and B'), and 114 (C and C') units/ml. The albumin concentrations used for cross-linking were 1% (A, B and C) and 5% (A', B' and C') of the monomer by weight. The gels were preswollen in the simulated gastric fluid without pepsin before enzymatic digestion. (n = 4).
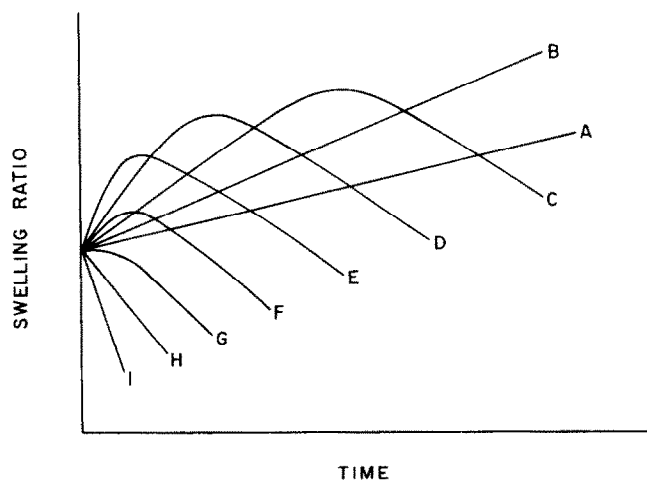

Figure 9   General trend in the enzyme-induced changes of the swelling ratio of albumin-cross-linked hydrogels. Changes in the swelling ratio of a particular hydrogel are determined by the relative concentrations of albumin and enzyme.

DISCUSSION

There has been a continuing interest in the development of enzyme-digestible hydrogels for a variety of reasons. Heller has made lysozyme-degradable chitin hydrogels for controlled drug release[25, 26]. Partially-de-acetylated chitin, which is a substrate for lysozyme[27], was cross-linked with glutaraldehyde. Thus, the backbone of the chitin hydrogels was degraded by enzymes. In contrast to the backbone degradation, hydrogels of which cross-linkers are degraded by enzymes have been synthesized by Kopecek and his associates. They have prepared hydrogels of *N*-(2-hydroxypropyl)methacrylamide cross-linked with oligopeptides which are cleavable by specific enzymes such as trypsin or chymotrypsin[28-30]. The rate of enzymatic cleavage was dependent on the length of the oligopeptide sequence as well as the cross-linker density. The oligopeptide chains require specific enzymes to be cleaved and the short chain length results in the steric hindrance for enzyme activity[31]. Degradation of the gels was characterized by the time required for complete dissolution of the gels and the change in molecular weight of dissolved polymers. In addition to these parameters, the swelling ratio is useful to monitor the dynamic changes in the gel structure by enzymes as shown in our study.

There are a few advantages in using proteins as cross-linkers. Since the size of proteins used as cross-linkers (e.g. albumin) is larger than that of enzymes, the diffusion of enzymes inside the gels, at least around the substrate, is expected to be less inhibited than gels cross-linked with short oligopeptides. The transient swelling of the enzyme-treated gels is thought to be due to the incomplete cleavage of most albumin molecules and/or the complete cleavage of a certain number of albumin molecules whilst others remain intact. It is possible that the partial cleavage of an albumin molecule results in the increase of the length of the cross-linker and subsequently more swelling. It is also possible that the complete cleavage of a portion of cross-linkers results in the lowering of the cross-linking density and subsequent further swelling. Distinction between these two effects may be useful in designing other enzyme-digestible hydrogels.

The protein cross-linkers can be cleaved by a variety of proteolytic enzymes, whilst oligopeptide cross-linkers require a specific enzyme. This non-specific enzymatic digestion ensures the complete digestion of the swelling hydrogels in the gastro-intestinal (GI) tract, if they are to be used as platforms for oral controlled drug delivery. Thus, the hydrogels will be completely dissolved in the GI tract and eliminate any possibility of GI tract blockage by undissolved gels. The resistance of a given protein molecule to proteolytic digestion can be varied by controlling the extent of chemical modification. Thus, the time for digestion of the hydrogels can be prolonged as necessary.

The diffusion rate of enzymes into the swelling hydrogels and fully swollen hydrogels may be reduced due to a number of factors, including the obstruction effect, hydrodynamic drag and the specific enzyme–polymer interactions[32]. The first two effects are expected to be minimal in our hydrogels which are cross-linked by albumin molecules, since the size of an albumin molecule is larger than that of the diffusing enzymes. The only significant effect might be the specific enzyme–polymer interactions, such as trypsin-poly(acrylic acid) interactions at neutral pH. Those interactions may selectively retain some enzymes, while allowing rapid passage for others. Obviously, further study is necessary to understand enzyme diffusion in gels better.

Many proteins other than albumin can be used as cross-linking agents. A variety of enzyme-digestible hydrogels can be made using different proteins and monomers. This versatility will satisfy specific requirements. Successful application of the hydrogels to the oral dosage forms requires more knowledge on the *in vivo* enzyme concentration and distribution. Little information is available in the literature on the concentrations of various enzymes in the GI tract. Currently our research is focussed on the characterization of proteolytic enzymes in the GI tract of animals.

REFERENCES

1   Bond, J.H. and Levitt, M.D., Investigation of small bowel transit time in man utilizing pulmonary hydrogen (H_2) measurements, *J. Lab. Clin. Med.* 1975, **85**, 546-555
2   Madan, P.L., Sustained-release drug delivery systems: Part II, preformulation considerations, *Pharmaceutical Manufacturing* 1985, **2**, 41-45
3   Shargel, L. and Yu, A.B.C., *Applied Biopharmaceutics and Pharmacokinetics*, 2nd Ed., Appleton-Century-Crofts, Norwalk, 1985, Chapter 7
4   Watnabe, S., Kayano, M., Ishino, Y. and Miyao, K., *Solid Therapeutic Preparation Remaining in Stomach*, US Patent 3 976 764, 1976
5   Sheth, P.R. and Tossounian, J., The hydrodynamically balanced system (HBSTM): a novel drug delivery system for oral use, *Drug Devel. Ind. Pharm.* 1984, **10**, 313-339
6   Mamajek, R.C. and Moyer, E.S., *Drug-Dispensing Device and Method*, US Patent 4 207 890, 1980
7   Bechgaard, H. and Ladefoged, K., Distribution of pellets in the gastrointestinal tract. The influence on transit time exerted by the density or diameter of pellets, *J. Pharm. Pharmac.* 1978, **30**, 690-694
8   Mitra, S.B., Oral sustained release drug delivery system using polymer film composites, *Polymer Preprints* 1983, **24**, 51-52
9   Muller-Lissner, S.A. and Blum, A.L., The effect of specific gravity and eating on gastric emptying of slow-release capsules, *N. Engl. J. Med.* 1981, **304**, 1365-1366
10   Michaels, A.S., Bashwa, J.D. and Zaffaroni, A., *Integrated Device for Administering Beneficial Drug at Programmed Rate*, US Patent 3 901 232, 1975
11   Park, H. and Robinson, J.R., Physico-chemical properties of water insoluble polymers important to mucin/epithelial adhesion, *J. Controlled Rel.* 1985, **2**, 47-57
12   Peppas, N.A. and Buri, P.A., Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues, *J. Controlled Rel.* 1985, **2**, 257-275
13   Davis, S.S., Stockwell, A.F., Taylor, M.J., Hardy, J.G., Whalley, D.R., Wilson, C.G., Bechgaard, H. and Christensen, F.N., The effect of density on the gastric emptying of single- and multiple-unit dosage forms, *Pharm. Res.* 1986, **3**, 208-213
14   Meyer, J.H., Ohashi, H., Jehn, D. and Thomson, J.B., Size of liver particles emptied from the human stomach, *Gastroenterology* 1981, **80**, 1489-1496
15   Fara, J.W., Gastrointestinal transit of solid dosage forms, in *Drug Delivery Systems*, (Ed. J. McCloskey), Aster Publishing Corp., Springfield, 1983, pp 23-26
16   Davenport, H.W., *The Digestive Tract. An Introductory Text*, Year Book Medical Publishers, Chicago, 1971, Ch 13
17   Cardinal, J.R., Controlled drug delivery: vetinary applications, *J. Controlled Rel.* 1985, **2**, 393-403
18   Jaworek, D., New methods for covalent binding of proteins to synthetic polymers, in *Insoluble Enzymes*, (Eds. M. Salmona, C. Saronio and S. Garattini), Raven Press, New York, 1974, pp 65-76
19   Torchilin, V.P., Maksimenko, A.V., Smirnov, V.N., Berezin, I.V., Klibanov, A.M. and Martinek, K., The principles of enzyme stabilization. IV. Modification of key functional groups in the tertiary structure of proteins, *Biochim. Biophys. Acta* 1979, **567**, 1-11
20   Brynda, E., Cepalova, N.A. and Stol, M., Equilibrium adsorption of human serum albumin and human fibrinogen on hydrophobic and hydrophilic surfaces, *J. Biomed. Mater. Res.* 1984, **18**, 685-693
21   Snyder, S.L. and Sobocinski, P.Z., An improved 2,4,6-trinitrobenzene-sulfonic acid method for the determination of amines, *Anal. Biochem.* 1975, **64**, 284-288

22   Keil-Dlouha, V., Zylber, N., Tong, N.T. and Keil, B., Cleavage of glucagon by α- and β-trypsin, *FEBS Lett.* 1971, **16**, 287–290

23   Dixon, M., Webb, E.C., Thorne, C.J.R. and Tipton, K.F., *Enzymes*, 3rd Edn, Academic Press, New York, 1979 pp 261–262

24   Cunningham, L.W., Molecular-kinetic properties of crystalline diisopropyl phosphoryl trypsin, *J. Biol. Chem.* 1954, **211**, 13–19

25   Heller, J. and Pangburn, S.H., A triggered bioerodible naltrexone delivery system, *Proc. Int. Symp. Contr. Rel. Bioact. Mater.* 1986, **13**, 35–36

26   Pangburn, S.H., Trescony, P.V. and Heller, J., Lysozyme degradation of partially deacetylated chitin, its films and hydrogels, *Biomaterials* 1982, **3**, 105–108

27   Heller, J., Bioerodible hydrogels, in *Hydrogels in Medicine and Pharmacy*, Vol. III, (Ed N.A. Peppas), CRC Press, Boca Raton, 1987, Chapter 7

28   Ulbrich, K., Strohalm, J. and Kopecek, J., Polymers containing enzymatically degradable bonds. 3. Poly(N-hydroxypropyl metha-crylamide) chains connected by oligopeptide sequences cleavable by trypsin, *Makromol. Chem.* 1981, **182**, 1917–1928

29   Ulbrich, K., Strohalm, J. and Kopecek, J., Polymers containing enzymatically degradable bonds. VI. Hhydrophilic gels cleavable by chymotrypsin, *Biomaterials* 1982, **3**, 150–154

30   Cartlidge, S.A., Duncan, R., Lloyd, J.B., Kopeckova-Rejmanova, P. and Kopecek, J., Soluble crosslinked N-(2-hydroxypropyl)methacrylamide copolymers as potential drug carriers, *J. Controlled Rel.* 1987, **4**, 253–264

31   Rejmanova, P., Obereigner, B. and Kopecek, J., Polymers containing enzymatically degradable bonds. 2. Poly(N-hydroxypropyl metha-crylamide) chains connected by oligopeptide sequences cleavable by chymotrypsin, *Makromol. Chem.* 1981, **182**, 1899–1915

32   Muhr, A.H. and Blanshard, J.M.V., Diffusion in gels, *Polymer* 1982, **23**, 1012–1026