Journal of Controlled Release, 19 (1992) 131–144

COREL 00648

In vitro and in vivo studies of enzyme-digestible hydrogels for oral drug delivery

Waleed S.W. Shalaby[1], William E. Blevins[2] and Kinam Park[1]

Purdue University, [1]School of Pharmacy, [2]School of Veterinary Medicine, West Lafayette, Indiana, U.S.A.

(Received February 24, 1991; accepted in revised form May 28, 1991)

Gastric retention of enzyme-digestible hydrogels in dogs was studied to develop a platform for long-term oral drug delivery. The movements of hydrogels in the canine stomach and the gastric tissue-hydrogel interactions were visualized in real-time using three imaging techniques: radiography, fluoroscopy, and 2-D ultrasonography. Hydrogels with appropriate size and integrity were retained in the stomach for more than 24 h even under fasted conditions. Gastric retention was prolonged up to 60 h when the 24 h-fasted state was followed by a once-a-day pelletized meal. In vitro drug release from enzyme-digestible hydrogels was examined. The hydrogels were loaded with flavin mononucleotide (FMN) and air-dried for 7 days. FMN was released from the gels in the simulated gastric juice for up to 454 h and 650 h in the presence and absence of pepsin, respectively. About 90% of the loaded FMN was released in 300 h. During this time, the presence of pepsin in the solution did not make any significant difference in the release profiles. When the FMN-containing hydrogels were administered to dogs, the blood concentration of FMN was maintained at the level of 0.75 μg/ml for 24 h under fasted conditions. In each experiment, the presence of a hydrogel in the stomach was confirmed using the three imaging techniques. When the 24-h-fasted state was followed by a once-a-day meal, appreciable FMN concentration was detected in the blood for up to 54 h. This study showed that once-a-day oral drug delivery is possible even with a drug which is absorbed only from the upper small intestine and has a short half-life.

Key words: Gastric retention; Enzyme-digestible hydrogels; Flavin mononucleotide; Once-a-day oral dosage forms

Introduction

Although significant advances have been made in controlled drug delivery [1–4], the application of controlled release technology to oral drug delivery has been limited. This is mainly due to the fact that the extent of drug absorption from the gastrointestinal (GI) tract is determined by the GI transit time of the dosage irrespective of the controlled release properties of the device. In general, the transit time from mouth to cecum can vary from 3 h to 16 h [5–8]. The transit time in the small intestine ranges from 3 h to 4 h under both fasted and fed conditions [7,9]. Thus, the time for absorption from the GI tract is limited for most drugs. If drug absorption is restricted to the upper small intestine, the duration of absorption is even further reduced. The gas-

Correspondence to: K. Park, Purdue University, School of Pharmacy, West Lafayette, Indiana 47907, U.S.A.

tric emptying time of drug delivery systems is usually within one to two hours in the fasted state [7,9–12]. The gastric emptying time, however, can be extended to over 14 h if fed state conditions are maintained [13]. Many attempts were made to control the gastric retention time by altering the size [6,12,14–17], shape [16,17], density [15,16,18–20], and surface properties [21–23] of the device. These attempts, however, have resulted in only limited success. Recently, Cargill et al. showed for the first time that objects can be retained in the stomach for 24 h under fasted conditions if they possess certain tetrahedral or ring-like geometries [24–25]. Their work demonstrated that the combined effects of size, shape, and flexibility were important in gastric retention. The mechanisms of gastric retention of those devices, however, are not understood. To develop a platform for long-term gastric retention, it is necessary to understand the physiological factors that control the gastric emptying. Thus, the physiology of gastric emptying will be discussed.

Gastric emptying of solid meals differs from emptying of liquid meals [26–29]. Although the mechanisms controlling solid/liquid partitioning are still unclear [26,30], it is generally agreed that the proximal stomach regulates the emptying of liquids while the distal stomach controls the emptying of solids [28,30–34]. The gastric emptying of liquids precedes the emptying of solids in a mixed meal [26,28–30]. The gastric emptying time of a complete meal depends on the type [27,35–38] and the nutritive density of the meal [39]. Dosage forms ranging from approximately 1 mm to 5 mm in diameter can be emptied from the stomach simultaneously with the meal [5,12,15,16]. Gastric emptying of nondigestible dosage forms with a diameter greater than 5 mm occurs once a significant fraction of the meal is emptied [12,14,15,40]. Since the rate of gastric emptying is dependent on the properties of the meal, the gastric emptying time of dosage forms will vary with different meals [6,15,40–42]. Mojaverian et al. [13] have shown that a Heidelberg capsule (7 mm in diameter by 20 mm in length) can be retained in the stomach for over 14 h with the frequent administration of food. Thus, the gastric emptying time of the device can be controlled as well as prolonged by maintaining fed conditions. Maintenance of fed conditions, however, is not a viable approach for long-term gastric retention of dosage forms since the same type of food needs to be consumed at regular intervals throughout the day.

In the fasted state, GI motility is characterized by a four-phase, cyclically recurring complex known as the interdigestive myoelectric motor complex (IMMC) [43,44]. In the canine stomach, pacesetter potentials originating in the greater curvature half of the orad corpus control the frequency of peristaltic contractions associated with the IMMC [45,46]. In man, the IMMC can originate in the esophagus [47]. When pacesetter potentials are coupled with action potentials, peristaltic contractions arise [48]. Phase I is a quiescent phase where less than 5% of the pacesetter potentials are associated with action potentials. Phase II is marked by random peristaltic activity associated with 10% of pacesetter potentials. Phase III activity is known as the activity front whereby 100% of the pacesetter potentials are coupled with action potentials [43]. Phase IV, which follows the activity front, is characterized by a rapid decline in peristaltic activity. It is the phase III activity that propels the residual stomach contents into the small intestine [10,49–51]. The gastric emptying time of conventional oral dosage forms is therefore determined mainly by the onset of phase III activity of the IMMC. Under maintained fasted conditions, phase III activity in the antrum occurs approximately every 100 min and usually lasts about 12 min [43]. Consequently, for once-a-day oral drug delivery to be feasible, controlled release devices must be constructed to overcome the propulsive efficiency of peristaltic contractions associated with phase III of the IMMC.

In our laboratory, albumin-crosslinked hydrogels have been used as a platform for long-term oral drug delivery [52]. The swelling and degradation properties of these hydrogels can be controlled by adjusting the degree of vinylic func-

tionality on the albumin crosslinker [53]. Swelling of those gels in simulated gastric fluids was found to depend on both the degree of functionality and the concentration of functionalized albumin. Hydrogel degradation by pepsin was characterized by a predominance of either bulk or surface degradation depending on the degree of albumin functionality. Transition from surface degradation to bulk degradation was observed when the degree of functionality exceeded 15%. We chose hydrogels undergoing bulk degradation in the presence of pepsin for in vivo studies, since the idea of our approach for the long-term gastric retention was initially based on the large swollen size of the gels. The focuses of this study were: (1) to characterize hydrogel behavior in the canine stomach, (2) to determine hydrogel properties necessary for gastric retention under fasted conditions, and (3) to achieve in vivo absorption of flavin mononucleotide (FMN) for more than 24 h under fasted conditions using selected hydrogels.

Experimental Methods

Hydrogel preparation

Human serum albumin was functionalized as previously described [52,53]. Albumin-crosslinked polyvinylpyrrolidone hydrogels were prepared by free radical polymerization at 60°C under nitrogen using 1-vinyl-2-pyrrolidinone (Aldrich) as monomer, 2,2-azobis(2-methylpropionitrile) (Eastman Kodak Co.) as initiator, and functionalized albumin (FA) as crosslinking agent. Synthesized hydrogels were washed over a 3-day period with distilled deionized water. The gels were then dried at room temperature for one week and then at 37°C for an additional week.

Imaging techniques

Healthy mongrel dogs weighing 16 kg were used throughout the study. In each experiment, an unanaesthetized animal was fasted for at least 18 h and a radiograph was made to ensure the absence of food in the stomach. Following the administration of a hydrogel, standard lateral and ventrodorsal radiographs of the abdomen were made using a 1200 Ma, 150 KVP X-ray generator (General Electric Corporation). The animal was positioned in right lateral and dorsal recumbency. The same radiographic technique (76 and 86 KVP, 12 MaS), X-ray cassettes, film and film processing were used so that the decreasing opacity of the hydrogel could be monitored. Fluoroscopy was accomplished with the animal in sternal recumbancy. This allowed the hydrogel to be positioned in the body and/or pyloric antrum so that observations of the hydrogel movements could be made and recorded on video tape.

The ultrasound examination of the stomach was performed with a real time mechanical sector scanner (Interspec) using a 5 mHz transducer. After clipping the hair on the ventral right side of the body just caudal to the costal arch, copious amounts of an ultrasound transmission gel (Lafayette Pharmacal Inc.) was applied to the skin. With the animal in a standing position, a transverse view of the cranial abdomen (sagittal view of the stomach) was obtained. The gastric tissue-gel interactions were observed and recorded on video tape. With a sagittal view of the hydrogel frozen on the monitor, the image of the gel was measured and recorded on a video format camera (Sony).

Gastric retention of hydrogels in the presence of water

Dried cylindrical gels (10 mm in diameter × 12 mm in length) were allowed to swell in a 4% (v/v) solution of Gastrografin (GG, diatrizoate meglumine/sodium diatrizoate, Squibb Diagnostics) for 32 h at 37°C. GG was loaded to make the gels radiopaque. The gels were then air dried for one week and oven dried at 37°C for at least one week. Just prior to administration of the gel to a dog, the dried hydrogel was partially swollen in a 4% (v/v) GG solution for 15 min to impart lubricity on the gel surface to ensure safe transit to the stomach. The partially swollen dimensions were 14 mm in diameter × 16 mm in length. In each experiment, 380

ml of water was instilled just prior to gel administration. The initial instillation of water was followed by an additional 380 ml of water every 30 min for up to 8 h to maintain gastric distention. Water was administered using a gastroesophageal tube and syringe.

Gastric retention of hydrogels under fasted conditions

The effects of the hydrogel integrity and size on gastric retention were studied under fasted conditions using the protocol for gel and animal preparation described above. In each experiment, 380 ml of water was instilled just prior to gel administration. The initial instillation of water was followed by an additional 380 ml of water every 30 min for 2.5 h for the larger dried hydrogels and 3 h for the smaller dried hydrogels (see below). Integrity of hydrogels was varied by controlling the size of the glassy core in the cylindrically shaped gels. The overall diameter and length of hydrogels in the stomach were kept the same. Only the size of the glassy core was varied. This was achieved by using hydrogels with different dimensions in the dried state and by controlling the time of hydrogel exposure to water in the stomach. Smaller dried gels (23 mm in diameter $\times$ 16 mm in length) swelled to a completely amorphous network in about 4 h. Larger dried gels (27 mm in diameter $\times$ 27 mm in length), however, contained a glassy core and an amorphous outer layer after the water had emptied from the stomach. The glassy core and amorphous outer layer of a partially swollen hydrogel were visualized by ultrasound imaging. Although the overall dimensions were approximately the same for both types of gels, the physical properties were distinctly different. The animal was fasted for 24 h after the gel administration. Once the hydrogel was retained in the stomach for 24 h, the animal was given a standard pelletized meal once-a-day. This allowed examination of the effects of food on hydrogel retention.

In vitro drug release

Hydrogels which were retained in the stomach for more than 24 h under fasted conditions were loaded with flavin mononucleotide (FMN, riboflavin-5′-phosphate, Eastman Kodak Co.). Hydrogels were swollen in a saturated solution of FMN in distilled deionized water containing 4% (v/v) GG for 48 h at 37°C. After loading, the gels were dried as described above. FMN was chosen as a model drug since its absorption is restricted to the upper small intestine [54] and its biological half-life is only about 70 min [55]. The release of FMN was examined using a USP II dissolution apparatus. The paddle speed was maintained at 50 rpm. Release studies were performed at 37°C using both pepsin-free and pepsin-containing (250 units/ml) simulated gastric fluids [56]. A pepsin concentration of 250 units/ml was chosen since the gel degradation was concentration-independent at pepsin levels greater than 125 units/ml [57]. In addition, pepsin concentrations under fasted conditions in man is estimated to be three times larger than the concentration used in the present study [50,58]. At timed intervals, 500 ml of simulated gastric fluid was removed from 1 liter vessels and replaced with 500 ml of fresh fluid. The collected samples were diluted with distilled deionized water and the FMN concentrations were measured spectrofluorometrically using a SLM 8000 spectrofluorometer (SLM Instruments, Inc.) at an excitation wavelength of 410 nm. FMN concentrations were calculated using a calibration curve. The calibration curve was constructed by determining the area under the curve (AUC) between 500 nm and 600 nm of the emission spectra for known concentrations of FMN in the simulated gastric fluid. The AUC was calculated using a computer program provided by SLM Instruments Inc. Appropriate control samples were prepared to correct for the effect of pepsin on the concentration of FMN. The relationship between the AUC and FMN concentration was found to be linear within the range of 0.1 μg/ml and 5 μg/ml.

In vivo drug release

Hydrogels which were used in the in vitro drug release study were also employed in the in vivo study. Hydrogels were loaded with FMN and GG as described above. GG was added to identify the location of hydrogels in the GI tract by radiography. At the beginning of each experiment, dried gels were partially swollen in a solution of saturated FMN and 4% (v/v) GG for 15 min to impart lubricity on the surface. Prior to gel administration, a blood sample was taken from the cephalic vein to measure the background concentration of total riboflavin which consists of FMN, flavin adenine dinucleotide, and free riboflavin. The gel was administered to the animal with 380 ml of water using a gastroesophageal tube and syringe. The same amount of water was given every 30 min for an hour. This was intended to promote gel swelling. Gastric retention was monitored using the imaging techniques described above. Venous blood samples were taken over time and stored at 4°C in sodium EDTA-coated vials (Vacutainer Systems). As a control in another experiment, a 7 cc gelatin capsule containing equivalent amounts of FMN (1200 mg) and GG (1 ml) was administered to the animal and the blood samples were obtained over time. After 24 h in the fasted state, a standard meal was given to the animal once-a-day. The concentration of FMN in the meal was 4 ppm.

The total riboflavin concentrations in the whole blood were measured by a spectrofluorometric procedure described by Baker and Frank [59]. The changes in the total riboflavin concentration in the whole blood following gel administration reflected the changes in the FMN concentration arising from FMN absorption in the small intestine. With the excitation wavelength of 410 nm, the AUC between 500 nm and 600 nm of the emission spectra was calculated. FMN concentration was calculated using a standard calibration curve which was constructed by adding known amounts of FMN to blood samples obtained from a dog fasted for 18 h. The relationship between the AUC and the total riboflavin concentration was found to be linear within the range of 0.01 μg/ml to 2.14 μg/ml.

Results

Gastric retention of hydrogels in the presence of water

The real-time movements of hydrogels in the stomach were visualized using both fluoroscopic and 2-D ultrasound imaging. Ultrasound imaging (Fig. 1) allowed visualization of the gel movements in relation to the gastric wall (g), the peristaltic contraction (p), and the degree of gastric distention. Hydrogel swelling was assessed by monitoring either the recession of the glassy core or the dimensional changes of the swelling gel. As swelling continued, the glassy core disappeared and the gel became sonolucent within 1 h (Fig. 1). If the gel was not completely swollen, the interface between the glassy core and the rubbery gel produced a bright echo and an acoustic shadow. When the stomach was distended by a large volume of water, the movements of the hydrogel in response to gastric contractions were minimal. The peristaltic contractions moving through the distended stomach appeared to be ineffective in propelling the hydrogel from the body to the pyloric antrum. Consequently, hydrogel movements were virtually independent of fluid movements and peristaltic activity. The hydrogel was retained in

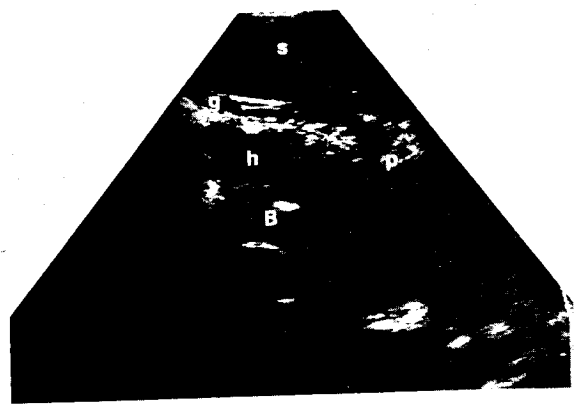

Fig. 1. A sagittal view of the stomach by ultrasound imaging. The sonolucent hydrogel (h) in the body of the stomach (B) can be easily observed in the near field. Spleen (s), gastric wall (g), and peristaltic contraction (p) are also shown in the figure.

the stomach as long as the stomach was distended with water. Gels were retained in the stomach during 8-h experiments if 380 ml of water was administered every 30 min. The size of the hydrogels at the end of 8 h was approximately 19 mm in diameter and 26 mm in length.

When the degree of gastric distention was not maintained (i.e. no additional water was administered after the emptying of previously administered water), the hydrogel moved into the pyloric antrum where it was propelled and retropelled repeatedly. The gel was in contact with the gastric tissues and propelled toward the pyloric sphincter during the migration of peristaltic contractions through the pyloric antrum. Retropulsion of the gels occurred as soon as the peristaltic contraction passed the most distal region of the gel. Once most of the residual water was emptied, the hydrogel became axially aligned with the pyloric sphincter and was emptied within minutes. The emptying of hydrogels resulted usually within 70 min to 120 min after the last administration of water.

Gastric retention of hydrogels under fasted conditions

Gastric retention of hydrogels with different rigidity was examined under fasted conditions. In the first set of experiments, hydrogels which were 23 mm in diameter × 16 mm in length in the dried state were used. After 2.5 h in the stomach, the gel increased in size to approximately 33 mm in diameter × 29 mm in length. The gels became completely amorphous after 4 h as detected by ultrasound imaging. In the fasted state, the gel was located in the pyloric antrum. Through fluoroscopy, when a peristaltic contraction moved through the pyloric antrum, the gel became compacted and partially deformed against the pyloric sphincter. As the contraction moved along the gel's surface, the hydrogel became significantly deformed (Fig. 2). Arrows in Fig. 2 indicate the location of the contraction. Once the contraction reached the distal end of the hydrogel, the gel was retropelled toward the body of the stomach. Fluoroscopic images taken after 5 h located the gel in the small intestine

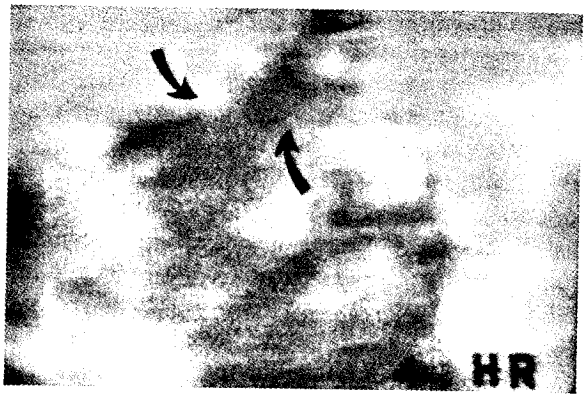

Fig. 2. Fluoroscopic image illustrating extensive deformation of the hydrogel at the pylorus in response to a peristaltic contraction. A peristaltic contraction moving toward the pyloric sphincter compresses the gel (arrows). The pyloric sphincter is on the right side of the gel. The gel was retropelled once the contraction passed the gel and reached the pyloric sphincter. The hydrogel was amorphous without a glassy core. The image was taken 4 h after gel administration.

where the gel transitted quite rapidly with fluid-like characteristics.

The second set of experiments used hydrogels whose size was 27 mm in diameter × 27 mm in length in the dried state. After 2.5 h in the stomach, the gel length became 36 mm. Ultrasound imaging at 3.5 h showed an acoustic shadow which was attributed to the glassy core. Once most of the fluids had emptied, the gel resided in the pyloric antrum. When a contraction moved through the pyloric antrum, the gel became compacted against the pyloric sphincter. As the wave migrated axially toward the most distal region of the gel, deformation occurred only at the gel edges (arrows in Fig. 3). The gel was subsequently retropelled toward the body of the stomach. Over the first 24 h under fasted conditions, the peristaltic contractions were ineffective in deforming the gel and pushing it through the pyloric sphincter. The gel integrity resulting from the presence of a glassy core was important for the gastric retention of the gel. When the size of the gel was 10 mm in diameter and 12 mm in length, gastric emptying occurred in spite of the presence of the glassy core [60]. Thus, it appears that the gel size and the gel integrity are two parameters critical to hydrogel retention under fasted conditions.

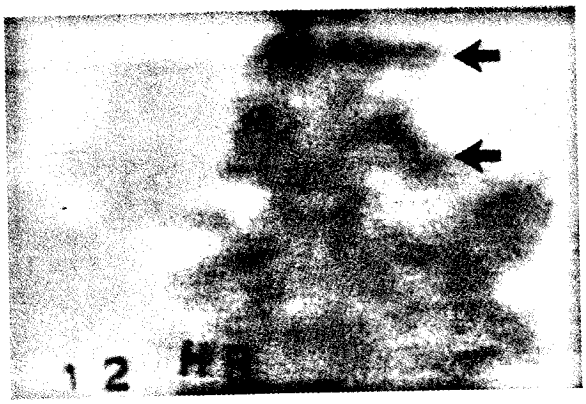

Fig. 3. Fluoroscopic image illustrating minimum deformation of the hydrogel in response to a peristaltic contraction. The deformation of the gel was limited to the edges of the gel (arrow) during peristalsis. The gel was only partially swollen and maintained a glassy core. The image was taken 12 h after the gel administration. The pyloric sphincter is on the right side of the gel.

Once most of the administered water is emptied from the stomach, swelling of the hydrogel is expected to be minimal since the gastric fluid content under fasted conditions is not significant [50]. Therefore, the gel integrity is expected to be maintained. In the absence of water, however, assessment of the glassy core with ultrasound was no longer effective due to the attenuation of sound arising from the presence of gas in the lumen. In this case, fluoroscopic imaging was used to indirectly monitor the presence of the glassy core by assessing the gel deformation.

On radiographic images made 24 h after the gel administration, significant quantities of GG were detected in the animal's colon. Since GG has fairly low solubility in gastric fluid, the release of GG from the hydrogel was most likely due to the attrition arising from contacts between the gastric tissue undergoing peristaltic contractions and the gel surface. The surface erosion of hydrogels was supported by the extensive fragmentation at the gel surface and the gradual decrease in gel size in the stomach (compare Fig. 4A and B). Furthermore, radiographic images of a GG-loaded hydrogel in the simulated gastric fluid showed that the loss of GG from the gel was insignificant. This indicates that the release of GG under fasted conditions may be due to a

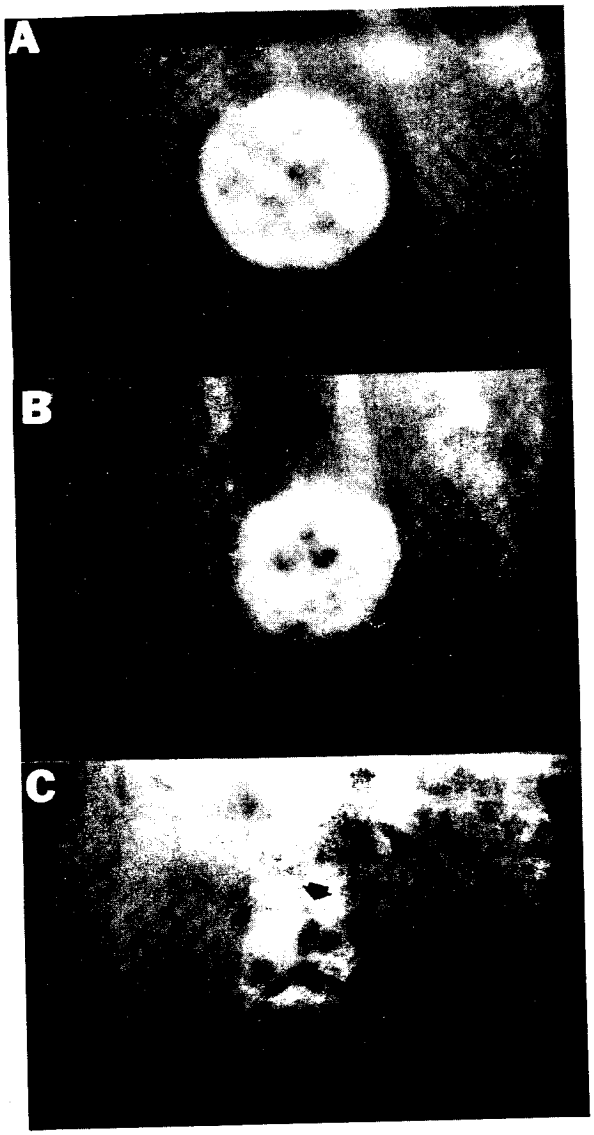

Fig. 4. Radiographic image of the GG-loaded gel made at 12 h (A), 24 h (B) and 48 h (C) after administration to a dog. Surface erosion (arrow) and size reduction were apparent in B. The reduction in the hydrogel size and the extent of surface erosion (arrows in C) were more significant in the presence of food.

mechanism other than simple diffusion. The presence of food, appeared to increase the rate of hydrogel surface erosion. As shown in Fig. 4C a reduction of the gel size was more prominent when the meal was given to the animal. In this case, the retention of the hydrogel in the stomach

was observed for up to 60 h. The gel was neither in the stomach nor in the intestine on radiographic images made at 72 h after the gel administration. This observation suggested that the hydrogel had disrupted in the stomach.

In vitro drug release from hydrogels

In the in vitro drug release and in vivo drug absorption studies, hydrogels which were retained in the stomach up to 60 h were used. The release of FMN from hydrogels in the simulated gastric fluid occurred for more than 400 h (Fig. 5). Only 17% of the total FMN content in the hydrogels was released after 24 h. Over a 60 h-period, less than 30% of the total FMN was released from the gels. The presence of pepsin did not significantly alter the release of FMN from the hydrogels. This suggested that the FMN release from the gels was independent of the fluctuations in the pepsin concentration during fasted and fed conditions. It has been shown that FMN binds to albumin in the plasma through electrostatic interactions [61]. The binding of FMN to the albumin molecules may have inhibited pepsin from cleaving the albumin molecules. In addition, it is possible that the presence of large quantities of FMN inside the gel can block pepsin from interacting with albumin. If this were the case, then hydrogel degradation would progress more slowly and the contribution of enzymatic digestion to FMN release would be minimized. The effects of FMN binding on hydrogel degradation, however, requires further investigation.

In vivo drug absorption

The FMN-containing hydrogels used in the in vitro drug release study were administered to dogs and the total riboflavin concentrations in the blood were measured over time. As shown in Fig. 6, the concentration of FMN in the blood was maintained for up to 54 h. It should be noted that the dogs were under fasted conditions for the first 24 h. The early rise in the FMN concentration was probably due to the burst release of FMN residing on the outer surface of the gel. The FMN concentration in the blood gradually declined to the steady value at approximately 2 h following the gel administration. Radiographic images made at each time point confirmed the retention of the hydrogel in the stomach for up to 36 h. The deformation of FMN-containing hydrogels in response to peristaltic contractions in the pyloric antrum (Fig. 7) was more pronounced than the gels without FMN shown in Fig. 3. Apparently, the presence of the FMN throughout the gel weakened the integrity of the gel to a certain extent. The integrity of the FMN-containing gels, however, was still high enough to withstand the propelling activity of the peristaltic contractions in the pyloric antrum.

In control experiments, FMN was adminis-

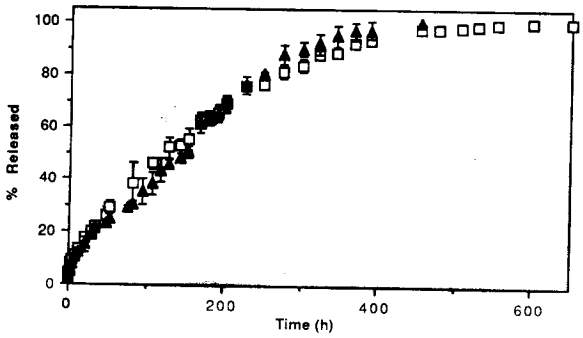

Fig. 5. FMN release from enzyme-digestible hydrogels in the simulated gastric fluid. The percentage of FMN released was observed in the presence of pepsin (250 units/ml) (▲), and in the absence of pepsin (□). Average ± SD, $n=3$.

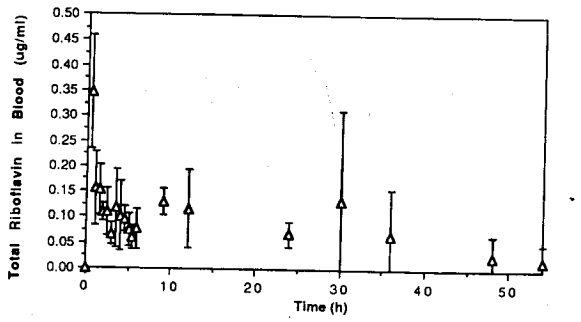

Fig. 6. The FMN concentration versus time profile in dogs following administration of FMN-containing hydrogels. Dogs were maintained under fasted conditions for the first 24 h and were given meals once-a-day afterwards. FMN was expressed as total riboflavin in the blood. Average ± SD, $n=4$.

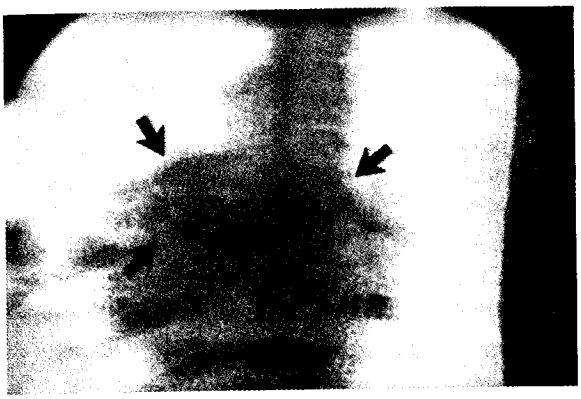

Fig. 7. Fluoroscopic image illustrating moderate deformation of the FMN-containing hydrogel in response to a peristaltic contraction. Gel deformation was pronounced only at the edges of the gel (arrows). The image was taken 12 h after the administration of the gel. The pyloric sphincter is on the right side of the gel.

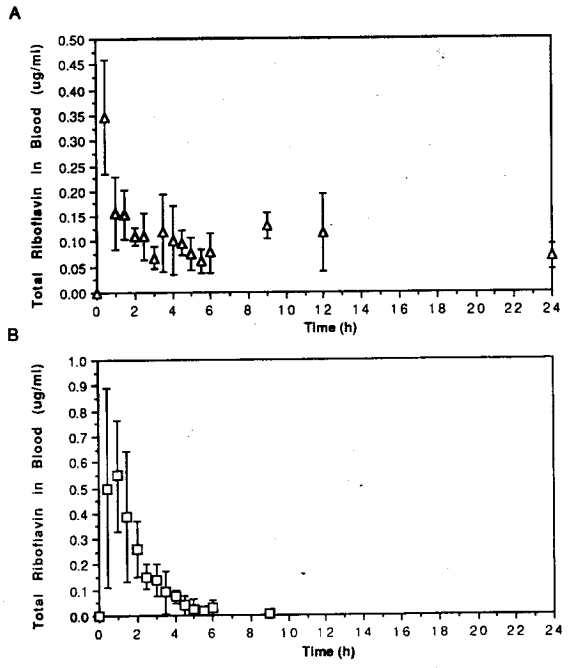

Fig. 8. The FMN concentration versus time profiles in dogs following administration of FMN-containing hydrogels (A) and gelatin capsules (B). Dogs were maintained under fasted conditions for the first 24 h. FMN was expressed as total riboflavin in the blood. Average ± SD, $n=4$ for A and $n=3$ for B.

tered using a gelatin capsule. The blood concentration-time profiles of FMN delivered by hydrogels and gelatin capsules were compared (Fig. 8). Fig. 8A was redrawn from Fig. 6 to compare the blood profiles obtained using gelatin capsules (Fig. 8B). The absorption of FMN from the gelatin capsule reached a peak within 60 min and then declined to an insignificant level within 6 h after the capsule was administered (Fig. 8B). The transient peak of the FMN concentration in the blood was expected since FMN is known to be absorbed only from the upper small intestine [54] and has a half-life of about 70 min. All the capsules separated within 30 min after administration as detected by radiographs. Some capsules, however, separated in less than 5 min.

In the presence of food, surface erosion of hydrogels was accelerated. The rate of reduction in gel size appeared to be more prominent in the FMN-containing gels than in the non-FMN containing gels. The hydrogel was observed in the stomach for only up to 36 h. It is believed that the gel surface erosion was accelerated as a result of continuous abrasive action between the gel, gastric tissue and food. Although the gel was disrupted, the presence of food was expected to retain the FMN in the upper GI tract and therefore prolong its absorption [62]. Bioavailability of FMN delivered by the hydrogel system was 3.7 times larger than that by the gelatin capsule. The important point here is that when FMN was delivered by hydrogels, relatively steady FMN concentrations in the blood were maintained even under the fasted conditions during the first 24 h.

Discussion

Both fluoroscopic and 2-D ultrasound imaging techniques were utilized in monitoring the real-time movements of a hydrogel in the stomach. Use of the two techniques allowed visualization of hydrogel swelling, hydrogel deformation, gastric tissue-gel interactions, and fluid movements. Ultrasound imaging was particularly useful in examining hydrogel movements and gastric motility simultaneously under varying degrees of gastric distention. Ultrasound dis-

tinguishes between different objects based on the acoustic impedance mismatch at the interface [63]. This technique, however, has limitations when used for human study. When images of the body/fundus region of the stomach are needed, ultrasound imaging may be time consuming due to anatomical variations and body weight differences [64]. Ultrasound imaging is more effective and less time consuming for obtaining images of the pyloric antrum [65].

The propulsive efficiency of peristaltic contractions varies depending on the viscosity of the meal [37]. Prove et al. have shown that the depth of indentations made by peristaltic contractions was deeper with lower viscosity meals than with higher viscosity meals. As a result, the low viscosity meals emptied more rapidly than the high viscosity meals. Thus, the efficiency of peristaltic contractions to empty the low viscosity meals was higher than the efficiency to empty the high viscosity meals. The efficiency of peristaltic contractions to propel smaller hydrogels (10 mm in diameter × 12 mm in length) into the intestine was found to be inversely proportional to the degree of gastric distention resulting from the administration of water. As the degree of gastric distention increased, both the orad (retropulsive) and caudad (propulsive) movements of a gel became less dependent on gastric motility. Under such conditions, the efficiency of peristaltic contractions to propel water was much higher than the efficiency to propel the hydrogel. Thus, the gastric emptying of water preceded the gastric emptying of the hydrogel. Conversely, as the stomach became less distended due to the emptying of water, the peristaltic contractions increased gastric tissue-gel interactions and resulted in a higher propulsive efficiency for the hydrogels. Once most of the water had emptied, the propulsive efficiency of peristaltic contractions reached a maximum. The degree of gastric distention was only qualitatively estimated since a sagittal view of the stomach was used in this study. It should be noted that the degree of gastric distention has been quantitatively determined through a transverse view of the stomach lumen [65–67].

In the fasted state, the gastric retention of hydrogels was dependent on the size and integrity of the gels. The contrasting physical properties of the two different types of hydrogels served as a useful comparison in understanding the effects of gel integrity on gastric retention. When hydrogels deformed minimally in response to peristalsis, the gastric contractions were ineffective in propelling the gel through the pyloric sphincter. Hydrogels undergoing only slight deformation apparently limited the propulsive efficiency of peristaltic contractions. High integrity of gels alone, however, does not guarantee the retention in the stomach. In previous studies on gels (10 mm in diameter × 12 mm in length) which had only a moderate degree of swelling, the gastric emptying occurred within 2 h after administration [60]. The gel emptied from the stomach even though its deformation in response to gastric contractions was not significant. Thus, the size of the gel must also be important to gastric retention. Under fasted conditions, a certain combination of the gel size and gel integrity served to minimize the efficiency of the peristaltic contractions to propel the gels through the pyloric sphincter. A third parameter that may influence gastric retention under fasted conditions may be the gel's surface properties. The "slippery" surface of the gel arising from hydration appeared to facilitate both the movements of contractions along the gel surface and hydrogel retropulsion. Since only one type of hydrogel made of PVP was used in this study, the effects of gel surface properties on gastric retention requires further investigation. In this regard, the gastric retention study with mucoadhesive hydrogels such as poly(acrylic acid) gels should be very interesting.

Maintenance of the FMN concentration in the blood for 24 h in the fasted state using our hydrogels is very encouraging. The solubility of FMN in the gastric fluid was approximately 0.8 mg/ml. It behaved more like a hydrophobic drug in the stomach and a hydrophilic drug in the small intestine. Therefore, the diffusional release of FMN from the hydrogel was expected to be limited since the content of gastric fluid under fasted

conditions is low. The in vitro release of FMN from the hydrogels in the simulated gastric fluid (Fig. 5) suggested that pure diffusional release of FMN was very slow and may not have accounted for the total amount of FMN released in vivo. The size reduction and surface fragmentation of gels in the fasted state indicated that surface erosion was significant and might also contribute to the release of FMN. Therefore, it appears that the release of FMN from the hydrogel in the fasted state was due to a combination of diffusion-controlled and erosion-controlled mechanisms. FMN is known to be absorbed only from the upper small intestine. Thus, to achieve drug absorption for 24 h under fasted conditions, the hydrogel needed to reside in the upper GI tract and release FMN at a rate large enough to overcome the short half-life of FMN. The concentration profiles of FMN in the fed state (between 30 h and 54 h in Fig. 6), however, did not reflect the accelerated surface erosion of the gel. This may be due to the rate of gastric emptying in the fed state which controlled both the amount of food and FMN released into the duodenum. The mechanism of drug release from the hydrogels in the fed state and subsequent drug absorption requires further investigation.

The deformation of FMN-containing gels in response to peristaltic contractions was more extensive than that of control gels without FMN. The reduced gel integrity was most likely due to the presence of FMN dispersed throughout the gels. The presence of large amounts of FMN may have altered the glassy structure of the network. It has been shown that the glass transition temperature of a glassy polymer was reduced as the drug content in the gel increased [68]. When water molecules penetrate through the glassy hydrogel, the presence of drug molecules will facilitate the mobility of polymer chains [68–70]. In effect, the FMN may have acted to plasticize the gel and increase the rate of solvent penetration [68]. The increased solvent uptake would, therefore, reduce the integrity of FMN-containing gels. As a result, the gastric retention time (36 h) was shorter than expected (60 h). Study on the rate of gastric fluid penetration into various hydrogels will be useful in understanding the effects of FMN on the hydrogel swelling.

Other studies have shown that FMN absorption from the upper GI tract occurs by a saturable mechanism [54,55]. The FMN concentration in human blood is known to reach a maximum value of 0.24 μg/ml if oral doses of FMN exceed 50 mg [55]. In our study, the amount of FMN loaded in the hydrogels and gelatin capsules was 1200 mg. This was substantially larger than the amount required to produce saturable absorption in man. Thus, the blood profile of FMN obtained in dogs using the gelatin capsule may likely represent saturated absorption kinetics. Since the upper GI residence time for the gelatin capsule was brief, only a small percentage of the total drug load in the capsule was absorbed from the GI tract. When FMN was delivered using our hydrogels, the FMN in the blood was maintained for up to 54 h including the first 24 h in the fasted state (Fig. 6). This clearly indicates that superior bioavailability can be achieved using hydrogels which can be retained in the stomach for more than 24 h.

Conclusions

In vivo study of enzyme-digestible hydrogels identified the size and the integrity of hydrogels as two parameters important to the long-term gastric retention in the fasted state. Using hydrogels with specific properties, we were able to maintain the blood concentration of FMN for more than 24 h under fasted conditions. These hydrogels may be used to release a variety of hydrophilic and hydrophobic drugs irrespective of the state of the stomach. This study clearly showed that a true once-a-day oral drug delivery is feasible if hydrogels with certain properties are used as a platform for drug delivery.

Acknowledgements

This study was supported in part by the ICI Pharmaceuticals Group. The authors wish to thank Dr. Garnet E. Peck for helping with in vitro drug release experiments.

References

1 N.A. Peppas, Hydrogels in Medicine and Pharmacy, Vol. III, CRC Press, Boca Raton, Florida, 1987.

2 R.S. Langer and D.L. Wise, Medical Applications of Controlled Release, Vol. I, CRC Press, Boca Raton, Florida, 1984.

3 R.S. Langer and D.L. Wise, Medical Applications of Controlled Release, Vol II, CRC Press, Boca Raton, Florida, 1984.

4 J.R. Robinson and V.H.L. Lee, Controlled Drug Delivery: Fundamentals and Applications, 2nd edit., Marcel Dekker Inc., New York, 1987.

5 L.C. Feely and S.S. Davis, Correlation of phenylpropanolamine bioavailability with GI transit by scintigraphic monitoring of 111 In-labeled hydroxypropylmethylcellulose matrices, Pharm. Res. 6(4) (1989) 274–278.

6 R. Khosla, L.C. Feely and S.S. Davis, Gastrointestinal transit on nondisintegrating tablets in fed subjects, Int. J. Pharm. 53 (1989) 107–117.

7 R. Khosla and S.S. Davis, Gastric emptying and small and large bowel transit of non-disintegrating tablets in fasted subjects, Int. J. Pharm. 52 (1989) 1–10.

8 S.S. Davis, R. Khosla, C.G. Wilson and N. Washington, Gastrointestinal transit of a controlled-release pellet formulation of tiaprofenic acid and the effect of food, Int. J. Pharm. 35 (1987) 253–258.

9 S.S. Davis, J.G. Hardy and J.W. Fara, Transit of pharmaceutical dosage forms through the small intestine, Gut 27 (1986) 886–892.

10 P. Mojaverian, J.C. Reynolds, A. Ouyang, F. Wirth, P.E. Kellner and P.H. Vlasses, Mechanism of gastric emptying of non-disintegrating radiotelemetry capsule in man, Pharm. Res. 8(1) (1991) 97–100.

11 P. Gruber, A. Rubenstein, V.H.K. Li, P. Bass and J.R. Robinson, Gastric emptying of nondigestible solids in the fasted dog, J. Pharm. Sci. 76(2) (1987) 117–122.

12 T. Itoh, T. Higuchi, C. Gardner and L. Caldwell, Effect of particle size and food on gastric residence time of nondisintegrating solids in beagle dogs, J. Pharm. Pharmacol. 38 (1986) 801–806.

13 P. Mojaverian, R. Ferguson, P. Vlasses, M. Rocci, A. Oren, J. Fix, L. Caldwell and C. Gardner, Estimation of gastric residence time of the Heidelberg capsule in humans: effect on varying food composition, Gastroenterology, 89 (1985) 392–397.

14 R. Khosla and S.S. Davis, The effect of tablet size on the gastric emptying of non-disintegrating tablets, Int. J. Pharm. 62 (1990) R9–R11.

15 P. Sirois, G. Amidon, J. Meyer, J. Doty and J. Dressman, Gastric emptying of nondigestible solids in dogs: a hydrodynamic correlation, Am. J. Physiol. 258 (1990) G65–G72.

16 J. Meyer, J. Dressman, A. Fink and G. Amidon, Effect of size and density on canine gastric emptying of nondigestible solids, Gastroenterology 89 (1985) 805–813.

17 H.M. Park, S.M. Chernish, B. Rosenek, R. Brunelle, B. Hargrove and H. Wellman, Gastric emptying of enteric-coated tablets, Dig. Dis. Sci. 29(3) (1984) 207–212.

18 H. Inganni, J. Timmermans and A. Moes, Conception and in vivo investigation of peroral sustained release floating dosage forms with enhanced gastrointestinal transit, Int. J. Pharm. 35 (1987) 157–164.

19 S.S. Davis, A. Stockwell, M. Taylor, J. Hardy, D. Whalley, C. Wilson, H. Bechgaard and F. Christensen, The effect of density on the gastric emptying of single-multiple-unit dosage forms, Pharm. Res. 3(4) (1986) 208–213.

20 H. Bechgaard, F. Christensen, S.S. Davis, J. Hardy, M. Taylor, D. Whalley and C. Wilson, Gastrointestinal transit of pellet systems in ileostomy subjects and the effect of density, J. Pharm. Pharmacol. 37 (1985) 718–721.

21 H.S. Ch'ng, H. Park, P. Kelly and J.R. Robinson, Bioadhesive polymers as platforms for oral controlled drug delivery II: synthesis and evaluation of some swelling, water-insoluble, bioadhesive polymers, J. Pharm. Sci. 74(4) (1985) 399–405.

22 D. Harris, J. Felt, H. Sharma and D. Taylor, GI transit of potential bioadhesive formulations in man: a scintigraphic study, J. Controlled Release 12 (1990) 45–53.

23 D. Harris, J. Fell, D. Taylor, J. Lynch and H. Sharma, GI transit of potential bioadhesive systems in the rat, J. Controlled Release 12 (1990) 55–65.

24 R. Cargill, K. Engle, C. Gardner, P. Porter, R. Sparer and J. Fix, Controlled gastric emptying II. In-vitro erosion and gastric residence times of an erodible device in beagle dogs, Pharm. Res. 6(6) (1989) 506–509.

25 R. Cargill, L. Caldwell, K. Engle, J. Fix, P. Porter and C. Gardner, Controlled gastric emptying. I. Effects of physical properties on gastric residence times of nondisintegrating geometric shapes in beagle dogs, Pharm. Res. 5(8) (1988) 533–536.

26 M. Horowitz, A. Maddox, M. Bochner, J. Wishart, R. Bratasvik, P. Collins and D. Shearman, Relationship between gastric emptying of solid and caloric liquid meals and alcohol absorption, Am. J. Physiol. 257 (1989) G291–G298.

27 J. Siegal, J. Urbain, L. Adler, N. Charkes, A. Maurer, B. Krevsky, L. Knight, R. Fisher and L. Malmud, Biphasic nature of gastric emptying. Gut 29 (1988) 85–89.

28 M. Camilleri, J. Malagelada, M. Brown, G. Becker and A. Zinsmeister, Relation between antral motility and gastric emptying of solids and liquids in humans, Am. J. Physiol. 249 (1985) G580–G585.

29 R. Hinder and K. Kelly, Canine gastric emptying of solids and liquids, Am. J. Physiol. 233(4) (1977) E335–E340.

30 L. Houghton, N. Read, R. Heddle, M. Horowitz, P. Collins, B. Chatterton and J. Dent, Relationship of the motor activity of the antrum, pylorus, and duodenum to gastric emptying of a solid-liquid mixed meal, Gastroenterology 94 (1988) 1285–1291.

31 K. Kelly, Gastric emptying of liquids and solids: roles of proximal and distal stomach, Am. J. Physiol. 239 (1980) G71–G76.

32 W. Rees, V. Go and J. Malagelada, Simultaneous measurement of antroduodenal motility, gastric emptying, and duodenogastric reflux in man, Gut 20 (1979) 963–970.

33 B. Wilbur and K. Kelly, Effect of proximal gastric, complete gastric, and truncal vagotomy on canine gastric electrical activity, motitily, and emptying. Ann. Surg. 178(3) (1973) 295–303.

34 R. Dozois, K. Kelly, C. Code, Effect of distal antrectomy on gastric emptying of liquids and solids, Gastroenterology 61(5) (1971) 675–681.

35 H.C. Lin, J. Doty, T. Reedy and J. Meyer, Inhibition of gastric emptying by sodium oleate depends on length of intestine exposed to nutrient, Am. J. Physiol., 259 (1990) G1031–G1036.

36 H.C. Lin, J. Doty, T. Reedy and J. Meyer, Inhibition of gastric emptying by glucose depends on length of intestine exposed to nutrient. Am. J. Physiol. 256 (1989) G404–G411.

37 J. Prove and H.-J. Ehrlein, Motor function of gastric antrum and pylorus for evacuation of low and high viscosity meals in dogs. Gut 23 (1982) 150–156.

38 K. Weiner, L. Graham, T. Reedy, J. Elashoff and J. Meyer, Simultaneous gastric emptying of two solid foods, Gastroenterology 81 (1981) 257–266.

39 J. Hunt and D. Stubbs, The volume and energy content of meal as determinants of gastric emptying. J. Physiol. 245 (1975) 209–225.

40 S.S. Davis, F. Christensen, R. Khosla and L. Feely, Gastric emptying of large single unit dosage forms, J. Pharm. Pharmacol. 40 (1988) 205–207.

41 M. Marvola, A.Kannikoski, H. Aito and S. Nyknen, The effects of food on gastrointestinal transit and drug absorption of multiparticular sustained-release verapimil formulation, Int. J. Pharm., 53 (1989) 145–155.

42 S.S. Davis, J. Hardy, M. Taylor, D. Whalley and C. Wilson, A comparative study of the gastrointestinal transit of a pellet and tablet formulation, Int. J. Pharm. 21 (1984) 167–177.

43 C. Code and J. Marlett, The interdigestive myo-electric complex of the stomach and small bowel of dogs, J. Physiol. 246 (1975) 289–309.

44 J. Szurszewski, A migrating electric complex of the canine small intestine, Am. J. Physiol. 6 (1969) 1757–1763.

45 K. Kelly and C. Code, Canine gastric pacemaker, Am. J. Physiol. 220(1) (1971) 112–118.

46 S.K. Sarna, E. Daniel and Y. Kingma, Simulation of the electric-control activity of the stomach by an array of relaxation oscillators, Dig. Dis. 17(4) (1972) 299–310.

47 J. Kellow, T. Borody, S. Phillips, R. Tucker and A. Haddad, Human interdigestive motility: variations in patterns from esophagus to colon, Gastroenterology 91 (1986) 386–295.

48 K. Kelly, C. Code and L. Elveback, Patterns of canine gastric electrical activity, Am. J. Physiol. 217(2) (1969) 461–470.

49 R. Oberle, T. Chien, C. Lloyd, J. Barnett, C. Owyang, J. Meyer and G. Amidon, The influence of the interdigestive migrating myoelectric complex on the gastric emptying of liquids, Gastroenterology 99 (1990) 1275–1282.

50 N. Schindlbeck, C. Heinrich and S. Muller-Lissner, Relation between fasting antroduodenal motility and transpyloric fluid movement, Am. J. Physiol. 257 (1989) G198–201.

51 C. Mroz and K. Kelly, The role of the extrinsic antral nerves in the regulation of gastric emptying, Surg. Gynec. Obstet. 145 (1977) 369–377.

52 K. Park, Enzyme-digestible swelling hydrogels as platforms for longterm oral drug delivery: synthesis and characterization, Biomaterials 9 (1988) 435–441.

53 W.S.W. Shalaby, K. Park, Biochemical and mechanical characterization of enzyme-digestible hydrogels, Pharm. Res. 7(8) (1990) 816–823.

54 W. Juskor and G. Levy, Absorption, metabolism, and excretion of riboflavin-5′-phosphate in man, J. Pharm. Sci. 56(1) (1967) 58–62.

55 B. Stripp, Intestinal absorption of riboflavin by man, Acta Pharmacol. Toxicol. 22 (1965) 353–362.

56 United States Pharmacopeia/National Formulary, USP XXI/NF XVI, U.S.P. Convention Inc., 1985, p. 1424.

57 C.K. Shim and K. Park, Examination of drug release from enzyme-digestible swelling hydrogels, Proc. Int. Symp. Control. Rel. Bioact. Mater. 16 (1989) 219–220.

58 J. Malagelada, G. Longstreth, W. Summerskill and V. Go, Measurement of gastric functions during digestion of ordinary solid meals in man, Gastroenterology 70 (1976) 203–210.

59 H. Baker and O. Frank, Riboflavin, R. Rivlin, (Ed.), Plenum Press, New York, 1975, pp. 49–79.

60 W.S.W. Shalaby, K. Park, W.E. Blevins, New analytical methods to study the gastric retention of albumin-crosslinked hydrogels in dogs, Proc. Int. Symp. Control. Rel. Bioact. Mater. 17 (1990) 132–133.

61 W. Jusko and G.Levy, Plasma protein binding of riboflavin and riboflavin-5′-phosphate in man, J. Pharm. Sci. 58(1) (1969) 58–62.

62 G. Levy and W. Jusko, Factors affecting the absorption of riboflavin in man, J. Pharm. Sci. 55(3) (1966) 285–289.

63 D. Herring, Physics, facts, and artifacts of diagnostic ultrasound, Vet. Clin. N. Am. Small Anim. Pract. 15(6) (1985) 1107–1122.

64 P. King, R. Adam, A. Pryde, W. McDicken and R. Heading, Relationships of human antroduodenal motility and transpyloric fluid movement: non-invasive observations with real-time ultrasound, Gut 25 (1984) 1384–1391.

65 L. Bolondi, M. Bortolotti, V. Santi, T. Calletti, S. Gaiani and G. Labo, Measurement of gastric emptying time by real-time ultrasonography, Gastroenterology 89 (1985) 752–759.

66 T. Hausken, S. Odegaard and A. Belstad, Antroduodenal motility studied by real-time ultrasonography: effect of enprostil, Gastroenterology 100 (1991) 59–63.
67 D. Bateman and T. Whittington, Measurement of gastric emptying by real-time ultrasound, Gut 23 (1982) 524–527.
68 C. Klech and X. Li, Consideration of drug load on the swelling kinetics of glassy gelatin matrices. J. Pharm. Sci. 79(11) (1990) 999–1004.
69 C. Robert, P. Buri and N.A. Peppas, Influence of the drug solubility and dissolution medium on the release from poly(2-hydroxyethylmethacrylate) microspheres. J. Controlled Release 5 (1987) 151–157.
70 P. Lee, Dimensional changes during drug release from a hydrogel matrix, Polym. Commun. 24 (1983) 45–47.