Preparation and Characterization of Freeze-dried Chitosan-Poly(Ethylene Oxide) Hydrogels for Site-Specific Antibiotic Delivery in the Stomach

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Purpose. The purpose of this study was to develop novel drug delivery systems with pH-sensitive swelling and drug release properties for localized antibiotic delivery in the stomach.

Methods. The drug delivery systems were synthesized by crosslinking chitosan and poly(ethylene oxide) (PEO) in a blend to form semi-interpenetrating polymer network (semi-IPN). Scanning electron microscopy was used to compare the surface and bulk morphology of the freeze-dried and air-dried chitosan-PEO semi-IPN. The hydrogels were allowed to swell and release the antibiotics—amoxicillin and metronidazole—in enzyme-free simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.2) at 37°C.

Results. Freeze-dried chitosan-PEO semi-IPN with a porous matrix had swollen extensively as compared to the air-dried hydrogel. The swelling ratio of freeze-dried and air-dried chitosan-PEO semi-IPN after 1 h in SGF was 16.1 and 2.30, respectively. More than 65% of the entrapped amoxicillin and 59% of metronidazole were released from the freeze-dried chitosan-PEO semi-IPN after 2 h in SGF.

Conclusions. The results of this study suggest that freeze-dried chitosan-PEO semi-IPN could be useful for localized delivery of antibiotics in the acidic environment of the gastric fluid.

KEY WORDS: hydrogels; site-specific; drug delivery; chitosan; poly(ethylene oxide).

INTRODUCTION

The gastrointestinal (GI) tract is the most common route of drug administration in the body. Ease of drug administration for compliant therapy, large surface area for systemic absorption, and the flexibility of the GI tract to accommodate many different formulations are some of the advantages of the GI tract in drug delivery (1). In addition to drug formulations that deliver the drug for a prolonged period of time, it is important to achieve spatial placement of the dosage form in the GI tract for efficient therapy (2). Site-specific drug delivery, using novel formulation designs, would improve local therapy in the GI tract, optimize systemic absorption, and would minimize premature drug degradation (3, 4). Drug delivery systems could be designed to deliver drugs locally in the oral cavity, stomach, small and large intestine, and the rectum. Stomach-specific antibiotic drug delivery, for instance, would be highly beneficial in the treatment of *Helicobacter pylori* infection in peptic ulcer disease (5).

Since the discovery by Warren and Marshall in 1983 (6, 7), *H. pylori* infection is considered to be the main pathogenic factor in the development peptic ulcer disease. The helical-shaped gram-negative bacilli has been isolated in almost 84% of the patients with gastric ulcer and 100% of the patients with duodenal ulcer (8). The organisms reside in the stomach and secrete urease which converts urea into ammonia and bicarbonate to allow safe passage through the gastric acid barrier until it can colonize the protective mucus layer (9). Amoxicillin and metronidazole, two common antibiotics that are clinically used to eradicate *H. pylori* infection, have a minimum inhibitory concentrations in 50% of the isolates (MIC_{50}) of <0.008 μg/ml and 2.0 μg/ml, respectively, in an *in vitro* assay (Table I). Unfortunately, when administered *in vivo,* no single antibiotic is effective in the treatment of *H. pylori* infection. The failure of single antibiotic therapy could be due to poor stability of the drug in the acidic pH of the stomach, poor permeability of the antibiotics across the mucus layer, or due to the availability of sub-therapeutic antibiotic concentrations at the site of infection after oral administration from a conventional capsule or tablet dosage form (10). To overcome some of these problems, a novel drug delivery system that localizes the antibiotic at the site of infection to achieve bactericidal concentrations would be desirable. Cationic hydrogels, with pH-sensitive swelling and drug release properties, are ideally suited for localized antibiotic delivery in the acidic environment of the stomach (11).

Previously, we have developed chitosan-poly(ethylene oxide) (PEO) semi-interpenetrating polymer network (semi-IPN) with pH-sensitive swelling and drug release properties (12, 13). Chitosan, a linear polymer of β(1→4)-linked 2-amino-2-deoxy-D-glucopyranose, is a natural cationic polysaccharide derived from N-deacetylation of chitin (14, 15). Chitin is isolated from the exoskeleton of crustaceans such as crabs, krill, and shrimps. Chitosan-PEO semi-IPN swelled almost ten times more in the low pH environment of the gastric fluid (pH 1.2) than in the intestinal fluid (pH 7.2) after 6 hours. More than 50% of the entrapped riboflavin was released from chitosan-PEO semi-IPN after 6 hours in gastric fluid. In the intestinal fluid, on the other hand, only 29% of the drug was released after 6 h from chitosan-PEO semi-IPN (13). Although the air-dried chitosan-PEO semi-IPN did exhibit pH-responsive swelling and drug release properties, due to the limitations of the gastric emptying time, a more rapid swelling and drug release system would be beneficial for stomach-specific delivery.

Shalaby *et al.* (16) showed that the release of dextromethorphan hydrobromide was much faster from the freeze-dried albumin-crosslinked poly(vinyl pyrrolidone) hydrogels than

Table I. Minimum Inhibitory Concentrations of Amoxicillin and Metronidazole Against *Helicobacter pylori*[a]

Antibiotic	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range
Amoxicillin	<0.008	0.06	0.008–0.06
Metronidazole	2.0	2.0	2.0–64

[a] Minimum inhibitory concentrations for 50% and 90% of the isolates in an *in vitro* assay. (From reference 5).

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from the air-dried hydrogels. In the present study, we have developed freeze-dried chitosan-PEO semi-IPN for pH-sensitive swelling and antibiotic release in simulated gastric fluid and simulated intestinal fluid at 37°C.

MATERIALS AND METHODS

Materials

Chitosan (Sea-Cure®-240, ≥80% deacetylated) was obtained from Pronova Biopolymers (Raymond, WA). PEO with an average molecular weight of 1,000,000 daltons, commercially available under the trade name of Polyox® N-12K water-soluble resin, was obtained from Union Carbide (Danbury, CT). The crosslinking agent, glyoxal, was purchased from Aldrich Chemicals (Milwaukee, WI). Amoxicillin and metronidazole were purchased from Sigma Chemical Company (St. Louis, MO). Deionized distilled water (DDW, NANOpure® II, Barnsted/Thermolyne, Dubuque, IO) was used exclusively to prepare all aqueous solution. All other reagents and chemicals were of analytical grade or better.

Hydrogel Synthesis and Characterization

Synthesis of Chitosan and Chitosan-PEO Hydrogels: Chitosan-PEO semi-IPN was synthesized by a method described previously (13). Briefly, 2% (w/v) of chitosan and PEO, each dissolved in 0.1 M acetic acid, were mixed to form a blend with a percent weight ratio of chitosan to PEO of 80:20. Glyoxal, at a final concentration of 8.0 mg/ml, was added to 50 ml of the feed mixture to form chitosan-PEO semi-IPN. Control chitosan hydrogels were prepared similarly without the addition of PEO. After crosslinking, the hydrogels were neutralized with 0.1 M sodium hydroxide and washed extensively with DDW to remove residual glyoxal and sodium hydroxide. Chitosan and chitosan-PEO hydrogels were then dried either by freeze-drying or air-drying technique. For freeze-drying, the swollen hydrogels were rapidly frozen in dry ice-acetone mixture followed by sublimation of the solvent using a Virtis Freeze Mobile® 5SL (Gardiner, NY) freeze-dryer. Air-dried hydrogels were prepared by allowing the solvent to evaporate at room temperature for approximately 48 h.

Scanning Electron Microscopy (SEM) Studies: Freeze-dried chitosan and chitosan-PEO hydrogels are expected to be highly porous. To confirm the porosity and to determine the pore size in these gels, the hydrogel surface and cross-sectional morphology were examined by SEM. Freeze-dried and air-dried samples were mounted on an aluminum sample mount. After coating with gold-palladium, the hydrogel samples were analyzed with an AMR-1000 (Amray Instruments, Bedford, MA) scanning electron microscope at a working distance of 10 mm and an accelerating voltage of 5.0 kV.

Swelling Studies

Freeze-dried and air-dried hydrogels were swollen in 50 ml of enzyme-free simulated gastric fluid (SGF, pH 1.2) or simulated intestinal fluid (SIF, pH 7.2) at 37°C. SGF and SIF were prepared according to the procedure described in the United States Pharmacopeia (17). Previously, we have found that pepsin and pancreatin, when added to SGF an SIF, respectively, do not have a significant effect on the swelling of the air-dried chitosan and chitosan-PEO hydrogels (13). At pre-determined time intervals, the samples were removed from the swelling medium and blotted on a piece of Kimwipe® tissue (Kimberly-Clark, Roswell, GA) to get rid of excess surface moisture. The swelling ratio (Q) of chitosan and chitosan-PEO hydrogels was determined according to the following expression:

$$Q = W_s/W_d$$

where W_s is the weight of the swollen hydrogel and W_d is the weight of the dried hydrogel. The data represents mean ± S.D. from four independent experiments.

Antibiotic Loading and Release Studies

Ten mg of amoxicillin or metronidazole were mixed with chitosan and chitosan-PEO solutions in 0.1 M acetic acid for 1 h at room temperature. Once the drug was completely dissolved, the feed mixture was crosslinked with glyoxal to form the hydrogels. Amoxicillin- and metronidazole-containing hydrogels were dried by freeze-drying or air-drying method as described above. For the release studies, chitosan and chitosan-PEO hydrogels, containing amoxicillin or metronidazole, were immersed in SGF and SIF at 37°C. At pre-determined time points, 3 ml of SGF or SIF was removed and assayed for the amoxicillin and metronidazole at 276 nm and 285 nm, respectively, using a Shimadzu UV-160U spectrophotometer (Columbia, MD). The cumulative amount of amoxicillin or metronidazole released from the hydrogel was determined from the appropriate calibration curves. The data represents mean ± S.D. from four independent experiments.

RESULTS AND DISCUSSION

SEM Characterization

Hydrogels prepared with highly porous matrix, such as freeze-dried hydrogels or foams, swell significantly faster than the conventional air-dried hydrogels (16, 18). Figure 1 shows the scanning electron micrographs of freeze-dried and air-dried chitosan-PEO semi-IPN. The top view of freeze-dried hydrogel (Fig. 1-A) shows a highly porous surface with an approximate pore size of 8–10 μm in diameter. It is important to note that since the SEM analysis was done with dry hydrogels, the pore size cannot absolutely reflect the magnitude of drug diffusion from the freeze-dried hydrogels in the swollen state. The effective pore size of the hydrogel in the swollen state would also depend on the hydrodynamic properties of the polymer chains. The surface of air-dried chitosan-PEO semi-IPN, on the other hand, was completely non-porous. In addition, the freeze-dried chitosan-PEO semi-IPN matrix had an open cell structure which was confirmed by examining the cross-section of the hydrogel as depicted in Fig. 1-C. These open cell structures, confirmed by SEM analysis, would have a significant influence on the rate and extent of hydrogel swelling and drug release in SGF and SIF. In addition to the ionization of glucosamine residues and the osmotic effect of high molecular weight PEO, the aqueous medium would also be taken up by the capillary action through the open channels in the hydrogel matrix. Faster rate of SGF and SIF uptake into the freeze-dried hydrogel would facilitate drug release from the hydrogel matrix. Air-dried

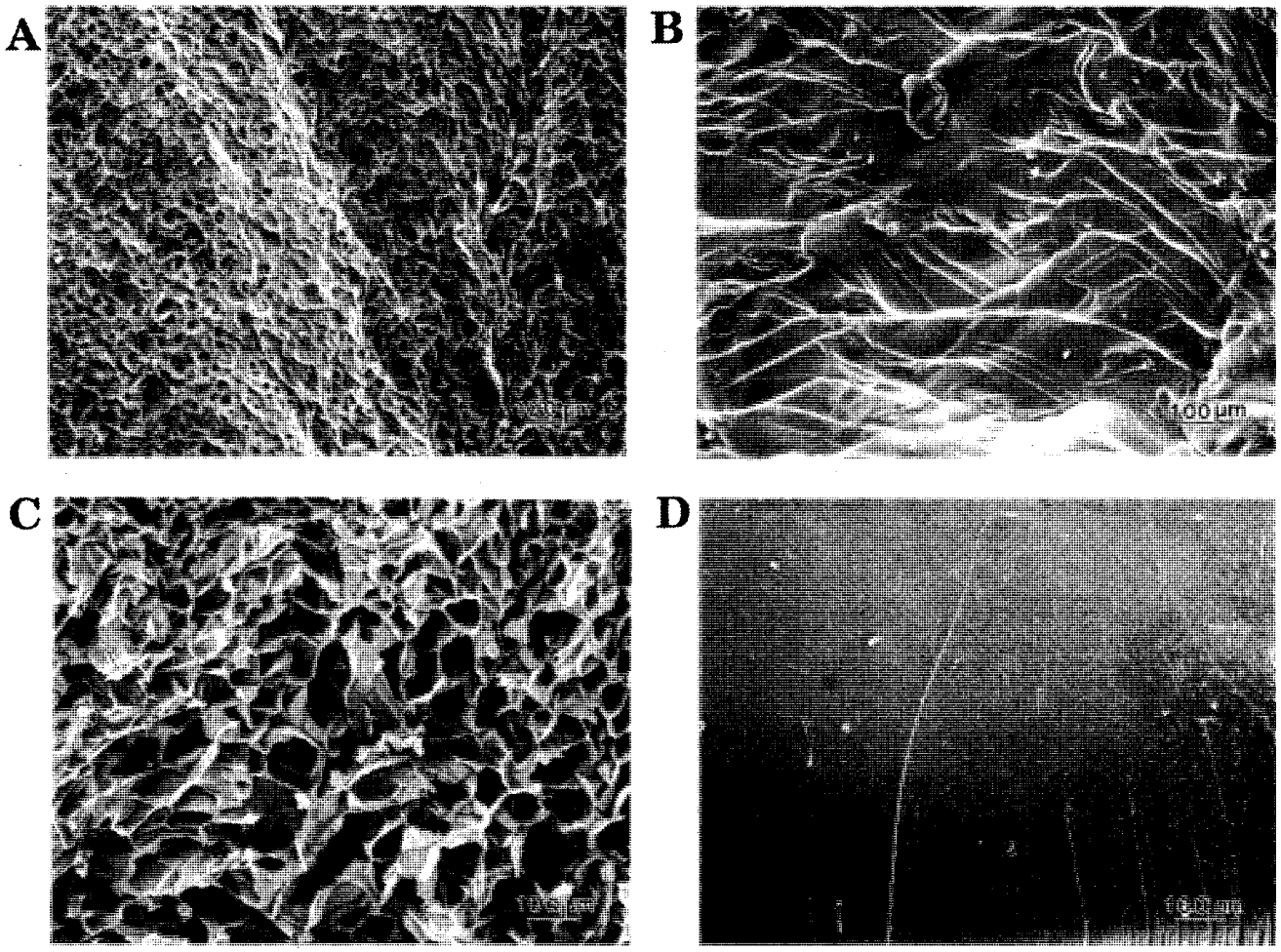

Fig. 1. Scanning electron micrographs of freeze-dried and air-dried chitosan-PEO semi-interpenetrating polymer network (semi-IPN). The micrographs depict top view of freeze-dried (A) and air-dried (B) chitosan-PEO semi-IPN and cross-sectional view of freeze-dried (C) and air-dried (D) chitosan-PEO semi-IPN. Original magnification was 100X and the scale bar is equal to 100 μm.

hydrogels, on the other hand, do not have the open channels. As such, equilibrium swelling of the air-dried hydrogel would require a longer duration for the polymer hydration and loss in the crystallinity of the matrix.

pH-Sensitive Swelling Studies

Chitosan and chitosan-PEO hydrogels were allowed to swell at 37°C to determine the effects of porosity, pH of the medium, and the presence of high molecular weight PEO on the rate and extent of swelling in SGF and SIF. Freeze-dried chitosan and chitosan-PEO hydrogel had swollen extensively, as shown in Figure 2-A in SGF. After 1 h, for instance, the swelling ratio of freeze-dried chitosan and chitosan-PEO in SGF was 13.1 and 16.1, respectively. In contrast, the swelling ratio of air-dried chitosan and chitosan-PEO after 1 h in SGF was only 1.40 and 2.30, respectively (Fig. 2-B). Rapid swelling of freeze-dried hydrogels in SGF is expected due to the porosity of the network. In addition to the porosity of the network, the freeze-dried chitosan and chitosan-PEO hydrogels did swell extensively in SGF due to the cationic properties of the polymer. pH-sensitivity in swelling of the freeze-dried chitosan and chitosan-PEO hydrogels was confirmed, since these hydrogels have a higher swelling ratio in SGF than in SIF. In SIF, after 1 h, the swelling ratio of freeze-dried chitosan and chitosan-PEO hydrogel was only 5.20 and 8.60, respectively. Unlike the air-dried hydrogels, where equilibrium swelling required 6 to 10 h in SGF (12, 13), the swelling of freeze-dried hydrogels is expected to reach equilibrium in a shorter time period. The faster rate and extent of swelling of freeze-dried chitosan-PEO semi-IPN, therefore, are dependent on the porosity of the matrix as well as the cationic properties of the polymer.

Using infra-red analysis, Yao *et al.* (19) showed that the conversion of the cationic amine functional group of glucosamine in acidic medium to the neutral from in alkaline medium was reversible in chitosan-poly(propylene oxide) (PPO) semi-IPN when the gel was transferred from a solution of pH 1.0 to 7.8. Being a water-insoluble polymer, PPO did not have much influence on the swelling of the hydrogels. The presence of high molecular weight PEO, a water soluble polymer, on the other hand, did significantly influence the initial swelling behavior of freeze-dried chitosan-PEO semi-IPN in this study. Compared to freeze-dried chitosan hydrogels, the chitosan-PEO semi-IPN had a higher swelling ratio in both SGF and SIF at lower time points. Addition of high molecular weight PEO

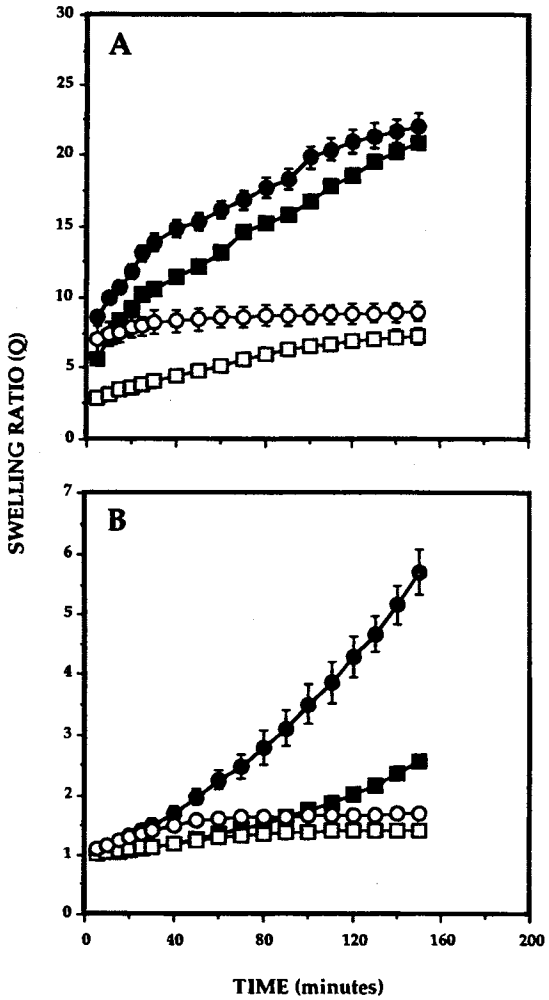

Fig. 2. Swelling kinetics of freeze-dried (A) and air-dried (B) chitosan and chitosan-PEO hydrogels in enzyme-free simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.2) at 37°C. The symbols represent chitosan (■) and chitosan-PEO (●) hydrogels in SGF and chitosan (□) and chitosan-PEO (○) hydrogels in SIF.

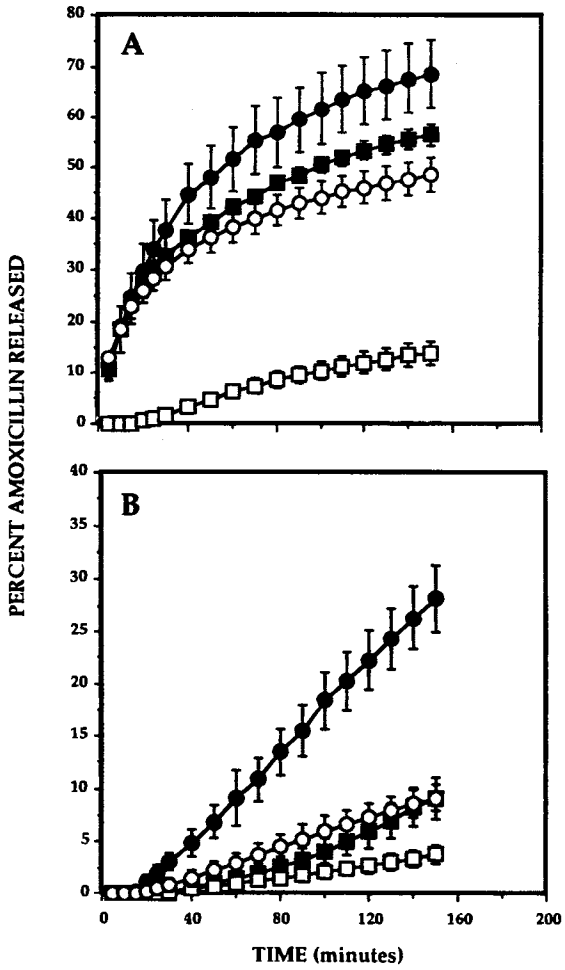

Fig. 3. Release of amoxicillin from freeze-dried (A) and air-dried (B) chitosan and chitosan-PEO hydrogels in enzyme-free simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.2) at 37°C. The symbols represent chitosan (■) and chitosan-PEO (●) hydrogels in SGF and chitosan (□) and chitosan-PEO (○) hydrogels in SIF.

could help in facilitating the initial hydration of the hydrogels by creating an osmotic gradient or could have decreased the crystallinity of the chitosan matrix.

Antibiotic Release Studies

Previous studies have shown that the rate of drug release from the air-dried chitosan matrix is significantly lower even in acidic medium (13, 20). Due to the limitations of the gastric emptying time, it is desirable to have a rapid swelling and drug release system for site-specific delivery into the stomach. The release of antibiotics from freeze-dried hydrogels, as a result of the highly porous matrix, was significantly higher than from the corresponding air-dried hydrogels. After 30 minutes, more than 33% and 38% of the entrapped amoxicillin was released from the freeze-dried chitosan and chitosan-PEO hydrogels, respectively, at 37°C in SGF (Fig. 3-A). From the air-dried chitosan and chitosan-PEO hydrogels, on the other hand, no drug was released at all for the first 30 minutes in SGF (Fig. 3-B). At longer duration, faster drug release occurred from the freeze-dried hydrogels and from the air-dried hydrogels. After 2 h in SGF, for instance, more than 54% and 65% of the entrapped amoxicillin was released from the freeze-dried chitosan and chitosan-PEO hydrogels, respectively. In comparison, only 6.0% and 22% of the entrapped amoxicillin was released from air-dried chitosan and chitosan-PEO hydrogels, respectively, after 2 h in SGF. It is important to note here that drug release from the freeze-dried chitosan-PEO semi-IPN was higher than from freeze-dried chitosan hydrogels in SGF. The osmotic effect of high molecular weight PEO on the swelling and drug release is apparent. In addition, the low pH environment of the SGF significantly influences drug release from a porous freeze-dried matrix. In SIF after 2 h, only 12% and 46% of the entrapped amoxicillin was released from freeze-dried chitosan and chitosan-PEO hydrogels, respectively. The air-dried systems released even lower percentages of the drug in SIF.

Fig. 4-A shows the release of metronidazole from freeze-dried chitosan and chitosan-PEO hydrogels in SGF and SIF at 37°C. Similar to amoxicillin release in SGF, more than 33% and 39% of entrapped metronidazole was released from chitosan

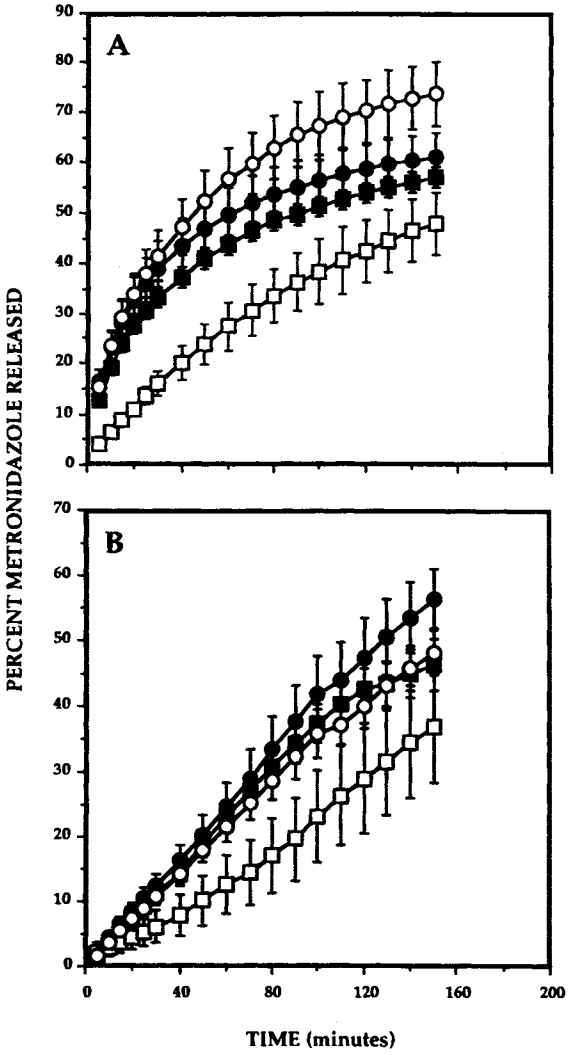

Fig. 4. Release of metronidazole from freeze-dried (A) and air-dried (B) chitosan and chitosan-PEO hydrogels in enzyme-free simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.2) at 37°C. The symbols represent chitosan (■) and chitosan-PEO (●) hydrogels in SGF and chitosan (□) and chitosan-PEO (○) hydrogels in SIF.

and chitosan-PEO hydrogels, respectively, after 30 minutes. From the air-dried chitosan and chitosan-PEO hydrogels, on the other hand, only 11% and 12% of the drug was released, respectively, after 30 minutes (Fig. 4-B). Clearly, the presence of open cell structures in the freeze-dried chitosan and chitosan-PEO matrices does profoundly influence the rate and extent of metronidazole release in SGF. In SIF, 16% and 42% of metronidazole was released after 30 minutes from freeze-dried chitosan and chitosan-PEO hydrogels, respectively. Contrary to our expectations, greater amount of drug release was observed in SIF than in SGF from freeze-dried chitosan-PEO semi-IPN. At the present time, we do not have a clear explanation for this observation. Even with the slight decrease in the release of metronidazole in SGF as compared to SIF, drug release from the freeze-dried hydrogels in SGF was much faster than from the corresponding air-dried hydrogels. Only 11% and 12% of the drug was released from air-dried chitosan and chitosan-PEO hydrogels, respectively, in SGF after 30 minutes. In SIF, only 6% and 11% of metronidazole was released after 30 minutes from air-dried chitosan and chitosan-PEO hydrogels, respectively. In addition to the porosity and the cationic property of the matrix, the effect of high molecular weight PEO on metronidazole release was also apparent in this case. In all instances, the release of metronidazole from chitosan-PEO semi-IPN was greater than from chitosan hydrogels.

CONCLUSIONS

Freeze-dried chitosan and chitosan-PEO hydrogels were fabricated for site-specific antibiotic delivery in the stomach. Compared to the air-dried hydrogels, freeze-drying in the swollen state did significantly influence the swelling and drug release properties. The porous matrix of the freeze-dried hydrogels, confirmed by SEM analysis, was able to swell faster than the air-dried hydrogels due to the rapid influx of SGF by capillary action. In addition to the porosity of the matrix, pH sensitivity due to the ionization of glucosamine residues in the acidic medium and the influence of high molecular weight of PEO in the freeze-dried chitosan-PEO semi-IPN were apparent on the swelling and drug release properties. After 1 h in SGF, the swelling ratio of freeze-dried chitosan-PEO semi-IPN was 16.1. In contrast, the swelling ratio in SIF after 1 h was only 8.60. Addition of high molecular weight PEO did influence the initial swelling and antibiotic release from the freeze-dried chitosan-PEO semi-IPN. More than 65% and 59% of the entrapped amoxicillin and metronidazole, respectively, were released from the freeze-dried chitosan-PEO semi-IPN after 2 h in SGF. The results clearly suggest that freeze-dried chitosan-PEO semi-IPN could be suitable for localized antibiotic delivery in the low pH environment of the gastric fluid for the treatment of *H. pylori* infection.

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