Preparation and Evaluation of Floating-Bioadhesive Microparticles Containing Clarithromycin for the Eradication of *Helicobacter pylori*

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ABSTRACT: The development of a gastric floating-bioadhesive drug delivery system to increase the efficacy of clarithromycin against *Helicobacter pylori* is described. Floating-bioadhesive microparticles containing clarithromycin were prepared by a combined method of emulsification/evaporation and internal/ion gelation for the treatment of *H. pylori* infection. Ethylcellulose microspheres (EMs) were prepared by the dispersion of clarithromycin, ethylcellulose, and chitosan in dichloromethane and subsequent solvent evaporation. EMs were coated with alginate by the internal gelation process to obtain alginate–ethylcellulose microparticles (AEMs); then, AEMs were dispersed in a chitosan solution, and chitosan–alginate–ethylcellulose microparticles (CAEMs) were obtained by ion gelation to enhance the bioadhesive properties. The morphologies of EMs and CAEMs were investigated under optical and scanning electron microscopes. *In vitro* buoyancy and drug-release testing confirmed the good floating and sustained-release properties of CAEMs. About 74% of the CAEMs floated in an acetate buffer solution for 8 h, and 90% of the clarithromycin contained in the CAEMs was released within 8 h in a sustained manner. *In vivo* mucoadhesive testing showed that 61% of the CAEMs could be retained in the stomach for 4 h. Under a pretreatment with omeprazole, the clarithromycin concentration in gastric mucosa of the CAEM group was higher than that of the clarithromycin solution group. These results suggest that CAEMs might be a promising drug delivery system for the treatment of *H. pylori* infection. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 2226–2232, 2006

Key words: biological applications of polymers; biomaterials

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative and spiral-shaped organism that colonizes exclusively on the gastric mucosa of humans.[1] The presence of the bacterium is associated with chronic active gastritis, gastric ulcers, gastric carcinomas, peptic ulceration, and mucosa-associated lymphoid-tissue lymphomas.[2] Although *H. pylori* is highly susceptible to many antibacterial agents such as tetracycline, amoxicillin, and clarithromycin *in vitro*, no single antibiotic therapy has shown the capability for the clinical eradication of *H. pylori* infection.[3]

There could be several reasons for the failure of a single antibiotic therapy against *H. pylori*: First, many antibiotics are rapidly degraded in gastric acid. Second, conventional tablets or capsules do not remain in the stomach long. Therefore, antibiotics might not have enough time to diffuse into the mucosa layer, and the antibiotic concentrations in the gastric mucus cannot reach minimum inhibitory concentrations.[4,5] The first problem could be solved by a drug combination, namely, triple therapy with two antibiotics such as clarithromycin and amoxicillin and an acid-suppressing agent. For example, the clarithromycin concentrations in the gastric tissue and mucus are also increased with the concomitant administration of omeprazole, a proton pump inhibitor.[6] Omeprazole can quickly increase the gastric pH value and maintain a gastric pH level at about 4–6 without any serious side effect;[7] under these circumstances, the decomposition of clarithromycin can be avoided, and its antimicrobial activity is maintained. Unfortunately, frequent and high doses of antibiotics are still necessary and are usually accompanied by serious adverse effects. Thus, stomach-specific drug delivery that can prolong the residence time of antibiotics in the stomach and continuously release the contained antibiotics would provide better antimicrobial effects; at the same time, the amounts of antibiotics and adverse effects would be reduced.

Floating alginate gel beads and mucoadhesive chitosan microspheres have been made for gastroretention

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in attempts to improve the control over drug release or to achieve a stomach-specific delivery for *H. pylori* eradication.[8–10] Alginate gel beads are rapidly formed by the gelation of alginic acid in the presence of calcium ions and are able to incorporate some compounds such as drugs or polysaccharides into the gel matrix.[11,12] However, the contained drugs are released very quickly from the porous beads and microspheres.[13] Ethylcellulose, a water-insoluble polymer with a low density, has been selected as a matrix to enhance the floating and sustained drug release of alginate gel beads when suspended in an alginate gel matrix with drugs, but still about 85% of the contained drugs are released within 1 h.[14] Drugs entrapped in ethylcellulose microspheres (EMs) would have better sustained release. Liu et al.[15] prepared EMs consisting of amoxicillin that showed sustained-release performance and more complete *H. pylori* eradication. Chitosan, a natural linear biopolyaminosaccharide, is obtained by the alkaline deacetylation of chitin, the second most abundant polysaccharide after cellulose.[16] In the cationic form, the D-glucosamine residue of chitosan interacts with the *N*-acetyl neuraminic acid (sialic acid) residues of mucin by electrostatic forces. Thus, chitosan will improve the gastric residence time of a drug contained in microspheres.[17–20] Chitosan microspheres prepared by ionic crosslinking with sodium sulfate, which were easy to dissolve in the acidic environment of the stomach, failed to adhere to mucus and/or epithelial cells in an *in vivo* model.[21] Chitosan microspheres prepared by chemical crosslinking with glyoxal provided a longer residence time in a fasted gerbil stomach than microspheres prepared by ionic crosslinking with sodium sulfate.[22] In view of the toxicity of chemical crosslinking agents, better preparation is desirable.

The purpose of this study was to design floating-bioadhesive microparticles containing clarithromycin with ethylcellulose, sodium alginate, and chitosan. Clarithromycin was contained in EMs to form floating and sustained-release microspheres. Alginate-coated ethylcellulose microparticles were prepared to enhance the hydrophilicity of EMs, which favored drug release. Chitosan was chosen as a bioadhesive polymer to act with alginate by ion gelation and further improve the mucoadhesion of alginate-coated ethylcellulose microparticles to the gastric mucosa. Thus, chitosan avoided dissolution in the acid environment of the stomach without a chemical crosslinking agent after administration. Furthermore, the drug-release profile, *in vitro* floating and *in vivo* mucoadhesive properties of the microparticles, and clarithromycin concentration in the gastric mucosa were investigated to prove their applicability as a new type of drug delivery system for the eradication of *H. pylori*.

EXPERIMENTAL

Materials

Clarithromycin (powder) was purchased from Zhejiang Huayi Pharmaceutical Co., Ltd. (Zhejiang, China). Ethylcellulose (Ec-10 cp) was purchased from the China National Medicine Group of Shanghai Chemical Reagent Co (Shanghai, China). Sodium alginate [according to the supplier's specifications, the viscosity of a 2% (w/v) aqueous solution at 25°C was 100 cp] was obtained from Qingdao Jingyan Bio-Tech Development Co., Ltd. (Shandong, China). Chitosan with a viscosity-average molecular weight of 230 kDa and a degree of deacetylation of 97% was obtained from Yuhuan Ocean Biochemical Co., Ltd. (Zhejiang, China). Poly(vinyl alcohol) (PVA; PVA-124, China National Medicine Group of Shanghai Chemical Reagent) functioned as a dispersing agent. All other chemicals were reagent-grade.

Male Sprague–Dawley (SD) rats (200–250 g) were purchased from the Department of Laboratory Animals of Dalian Medical University (Liaoning, China).

Preparation of the microparticles

Simple EMs containing clarithromycin were prepared by an emulsification/evaporation method. Clarithromycin (0.5 g) and ethylcellulose (1.0 g) were dissolved in 25 mL of dichloromethane, and chitosan (0.15 g) was dispersed in the solution; then, the suspension was poured into 200 mL of a PVA (1% w/v) solution at 25°C. The resultant emulsion was continually stirred at 700 rpm with a propeller-type agitator for 1 h. Subsequently, the emulsion was heated to 30°C for 4 h to evaporate the dichloromethane. The microspheres were washed with distilled water three times and left to dry in an oven at 40°C for at least 8 h.

Alginate–ethylcellulose microparticles (AEMs) were prepared via the coating of EMs with sodium alginate as follows: EMs (0.2 g) and $CaCO_3$ (0.2 g) were dispersed in 10 mL of a sodium alginate (2% w/v) aqueous solution. The suspension was added dropwise to 100 mL of liquid paraffin containing Span 80 (2% w/v) and stirred at 400 rpm; 1 mL of acetic acid (40% v/v) was added to the suspension, which was stirred for a period of time. The microparticles were collected by filtration with a mesh (40-μm opening) and washed with *n*-hexane and distilled water three times. Alginate-containing clarithromycin microspheres (AMs) were prepared by the same method of ionic gelation as the control.

Chitosan–alginate–ethylcellulose microparticles (CAEMs) were prepared via the coating of AEMs with chitosan as follows: AEMs were dispersed in a chitosan (0.5% w/v, pH 6.0) solution formed by dissolution in an acetic buffer, gelled for a period of time, washed with water, and dried in a desiccator *in vacuo* at room temperature.

Morphological and dimensional analysis

The microsphere and microparticle morphologies were evaluated by optical and electron microscopy. The surface morphology of the samples was observed with scanning electron microscopy (SEM; XL 30, Philips, Amsterdam, The Netherlands). The samples for SEM were prepared by the light sprinkling of the microparticle powder on double-adhesive tape that was stuck to aluminum stubs. The stubs were vacuum-dried and coated with gold to a thickness of about 300°A with a sputter coater. The samples were then randomly scanned, and photographs were taken.

The volumetric size distribution of EMs was determined by laser light scattering with a particle analyzer (LS-100Q, Beckman Coulter, Beckman Coulter, America), with the mean diameter and standard deviation calculated from the cumulative distribution curve.

The diameters of AEMs and CAEMs were measured under an optical microscope (XDS-1 inverted biological microscope, Chongqing Optical Instrument Factory, Sichuan, China), with at least 100 microparticles calculated for each sample.

Drug content of the microparticles

The microparticles (20 mg) were ground to powder and dissolved in 15 mL of ethanol, which was then diluted to 25 mL with water (pH 6.5). After ultrasonication for 10 min, the suspension was filtered through a 0.45-μm filter, Then, the drug content was determined by a High-Performance Liquid Chromatography (HPLC) method with ultraviolet (UV) detection at 210 nm. The conditions for the HPLC assay were as follows: the HPLC apparatus consisted of a Waters 515 pump and Waters 2487 UV detector, the column was a Kromasil C18 (5 μm, 150 × 4.6 mm), the mobile phase was an acetonitrile/methanol/phosphate buffer (0.05*M*, pH 6.5, 44 : 6 : 50), the flow rate was 0.7 mL/min, and the drug concentration was 22.05%.

Clarithromycin stability

Clarithromycin (100 mg; powder) was dissolved in 200 mL of pH 1.2, 2.0, or 3.0 HCl and a pH 5.0 acetate buffer solution (ABS) and vibrated in a water bath maintained at 37°C. After clarithromycin was completely dissolved, an HPLC assay was carried out for 0, 1, 2, 4, and 8 h, and the conditions for the HPLC assay were the same as before. The main peak area of clarithromycin at 1, 2, 4, and 8 h was measured and compared with that at 0 h. The degradation percentages of clarithromycin at different times in the medium were calculated.

In vitro drug release

The release of clarithromycin from CAEMs was determined in 100 mL of pH 3.0 HCl, pH 5.0 ABS, and a pH 7.4 phosphate buffer solution at 37°C. The rotation speed was adjusted to 100 rpm for the homogeneous dispersion of the microparticles in the release medium. At appropriate time intervals, 4 mL of the preheated release medium was withdrawn, and the same amount of new medium (37°C) was added to maintain a constant volume. The release of clarithromycin from AMs was determined in pH 5.0 ABS at 37°C as the control. The clarithromycin concentration was estimated as described before. All dissolution tests were performed in triplicate. The data represent means plus or minus the standard deviation from three independent experiments.

In vitro buoyancy studies

Sample microparticles of CAEMs (100 granules) were spread over the surface of ABS, which was agitated at 100 rpm. After the agitation, the microparticles that floated over the surface of the medium and those that settled at the bottom of the flask were counted separately, and the percentage of floating microparticles was calculated with the following equation:

$$\text{Buoyancy}(\%) = Q_f/(Q_f + Q_s)$$

where Q_f and Q_s are the numbers of the floating and settled microparticles, respectively.

Animal studies

Measurement of the gastric pH

SD rats were fasted (water-fed) for 8 h and then divided into six groups before the experiments. Each group consisted of three animals. Three groups were used as the control for the measurement of the pH at 1, 4, and 8 h after fasting, and the other three groups were used as experimental groups for the measurement of the pH at 1, 4, and 8 h after the pretreatment by the intraperitoneal injection of omeprazole. The rats were sacrificed by cervical dislocation. The stomach was excised, and an incision was made through the greater curvature. A piece of universal indicator paper (SSS Reagent Co., Ltd., Shanghai, China) was placed inside the stomach to absorb the gastric juice. The distinct color that formed was compared with a color chart to estimate the pH. In the experimental groups, the animals were pretreated by an intraperitoneal injection of omeprazole at a 15 mg/kg dose for acid suppression. After 1, 4, and 8 h of the omeprazole pretreatment, the animals were sacrificed, and the pH was measured as described earlier. The gastric pH of the fasted rats was determined to be between 1.0 and 2.0, whereas the gastric pH of the

omeprazole-treated rats was measured to be between 4.0 and 6.0 within 8 h.

In vivo evaluation of the gastric residence time of the microparticles

SD rats were fasted (water-fed) for 8 h and then divided into three groups before the experiments. Each group consisted of three animals. After 1 h of the omeprazole treatment at a 15 mg/kg dose for acid suppression, the animals were given a single oral dose of a microparticle (500 granules) suspension with a gastric sonde. After 1, 2, and 4 h, the rats were sacrificed by cervical dislocation. The stomachs were removed. The microparticles that remained in the stomach were counted, and the percentage of the remaining microparticles was calculated.

Concentration of clarithromycin in gastric mucosa

SD rats were fasted (water-fed) for 8 h and then divided into six groups before the experiments. Each group consisted of three animals. Three groups were orally administered clarithromycin solutions, and the other three groups were orally administered CAEMs. The animals were pretreated by an intraperitoneal injection of omeprazole at a 15 mg/kg dose to suppress gastric acid secretion. After 1 h of the omeprazole treatment, a CAEM or clarithromycin solution was orally administered to SD rats that were fasted for 8 h at a clarithromycin dose of 50 mg/kg. The rats were sacrificed at 1, 2, or 4 h after administration. Then, the stomachs of the rats were removed and opened along the great curvature, the residue in the stomachs was removed carefully, and the stomachs

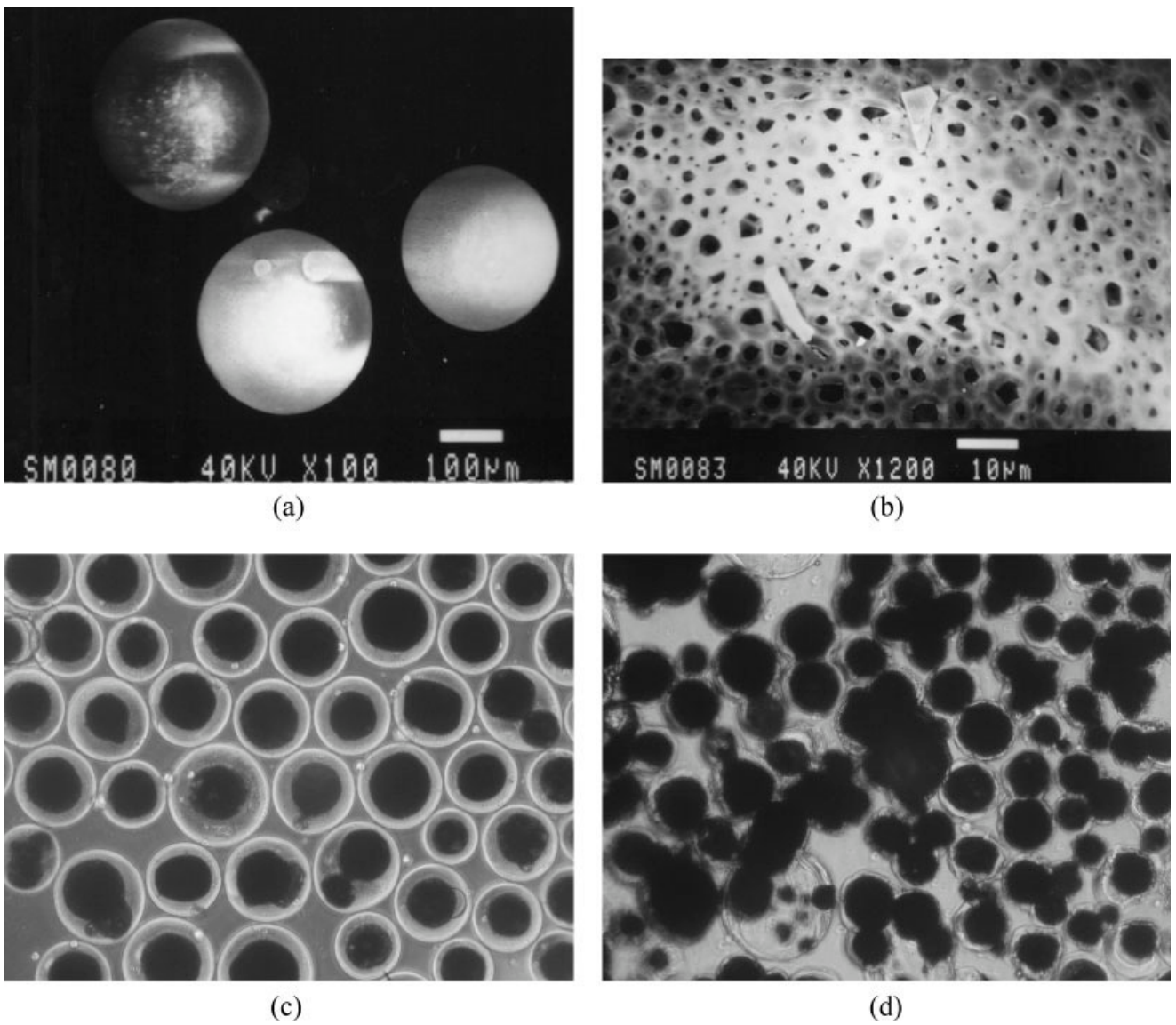

Figure 1 SEM and optical micrographs of EMs and CAEMs: (a) EMs, (b) surface of EMs, (c) CAEMs before being dried (40×), and (d) CAEMs after being dried (40×).

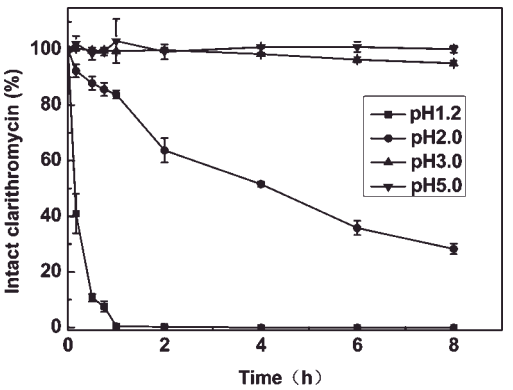

Figure 2 Stability of clarithromycin in solutions of different pHs.

were gently rinsed in 10 mL of distilled water and spread on a glass plate. The mucosal surface was scraped gently with a glass slide, and the top layer was separated from the muscular layers.[23] The removed mucosa was mixed with 3 mL of the mobile phase (acetonitrile/methanol/phosphate buffer) in a glass tissue grinder. After being ground, the homogenate was centrifuged in a refrigerated ultracentrifuge at 3500 rpm for 10 min. The supernatant was removed and filtered through a 0.45-μm filter. The amount of clarithromycin contained in the sample was then measured by HPLC.

Statistical analysis

Experimental results were expressed as the means plus or minus the standard deviation. A two-way analysis of variance was applied to check significant differences in the drug release and the concentration of clarithromycin in gastric mucosa from different formulations. Differences were considered to be statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Morphology of the microparticles

Figure 1 shows the scanning electron micrographs and optical micrographs of samples at different magnifications. EMs were rounded spheres with sizes ranging approximately from 100 to 400 μm [Fig. 1(a)]. SEM of the surfaces of EMs showed that many pores were present [Fig. 1(b)]. CAEMs before being dried, with sizes ranging approximately from 100 to 600 μm, showed a smooth surface, and one or more EMs were contained in one CAEM particle [Fig. 1(c)]. In contrast, the morphology of freeze-dried CAEMs became uneven because of water evaporation from the alginate gel matrix [Fig. 1(d)].

Clarithromycin stability

The stability of antibiotics in an acidic medium plays an important role in the eradication of *H. pylori*. In fact, lots of antibiotics have been reported with a strong *in vitro H. pylori* clearance effect but with poor *in vivo* results. One reason for the failure is their instability in an acidic medium. It is crucial to maintain the stability of antibiotics during the prolongation of the gastric retarding time of a stomach-specific drug delivery system.

As shown in Figure 2, the degradation profiles of clarithromycin solutions at pHs 1.2, 2.0, 3.0, and 5.0 were determined. At pH 1.2, the clarithromycin degradation was much quicker than the degradation at higher pHs, and about 95% of the drug was degraded within 45 min. At pH 2.0, the clarithromycin degradation became slower, and about 50% of the drug was degraded within 4 h. A different behavior was observed at pHs 3.0 and 5.0. At pH 3.0, the drug degradation was almost negligible in the considered time ranges. After 8 h, only 4% of the starting amount was degraded. At pH 5.0, clarithromycin was particularly stable. Under the pretreatment of omeprazole, the gastric pH of rats was measured to be between 4.0 and 6.0 within 8 h, so clarithromycin could keep its antimicrobial activity after administration.

In vitro drug release

Under the triple or double drug treatment of *H. pylori* associated peptic ulcers, the pretreatment of omeprazole enhanced the gastric pH to 4.0–6.0. To simulate the stomach environment, the release of clarithromycin from CAEMs was determined in different pHs. As shown in Figure 3, clarithromycin was released rapidly from AMs during the swelling process, and about 85% of the drug was released in 1 h in pH 5.0 ABS. Conversely, clarithromycin contained in EMs was released gradually into the solution, and about 90% of the clarithromycin contained in CAEMs was released

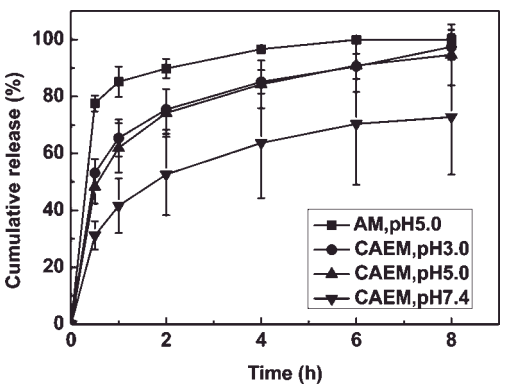

Figure 3 Release profiles of clarithromycin contained in AMs and CAEMs in solutions of different pHs.

in 4 h in the pH 3.0 HCl medium and pH 5.0 ABS. In a pH 7.4 phosphate buffer solution, the cumulative release of clarithromycin decreased with the increasing pH of the release medium; no more than 65% was released. No significant effect was observed for the *in vitro* release of clarithromycin at pHs 3.0, 5.0, and 7.4 ($p > 0.05$). Because chitosan and clarithromycin are acid-soluble, clarithromycin incorporated near chitosan is thought to be released quickly after the dissolution of chitosan at a low pH. The burst release of clarithromycin would help to achieve a quick and effective clarithromycin concentration in the gastric mucosa for the eradication of *H. pylori*.

In vitro buoyancy studies

To simulate the wetting action of gastric fluids under movement, the *in vitro* buoyancy behavior of CAEMs was investigated in ABS and agitated with a paddle at 100 rpm. The buoyancy of CAEMs was 91% at 10 min. As time passed, the buoyancy of CAEMs decreased to 74% at 8 h, whereas floating was not observed for AMs. This might be ascribed to the low apparent density of EMs, which formed porous structures for CAEMs. As water gradually penetrated CAEMs and subsequently changed the densities of CAEMs, the buoyancy of CAEMs decreased. During the floating test, no conglomeration or gelation of CAEMs was detected, so we concluded that CAEMs were dispersed individually in the stomach. CAEMs might release the drug gradually and then pass through the stomach individually, and this could lead to decreased variability of the drug action among patients in comparison with the conventional dosage form.

In vivo evaluation of the gastric residence time of the microparticles

A profile of the gastric residence time of CAEMs is shown in Figure 4. The remaining percentages of CAEMs in the stomach 1, 2, and 4 h after administration were 84, 74, and 61%, respectively. The muco/bioadhesive properties of chitosan might allow a prolonged interaction of CAEMs with the mucous layer. After administration, CAEMs floated in the stomach for some time and later adhered to the mucous layer, so both mechanisms enhanced the gastric residence time.

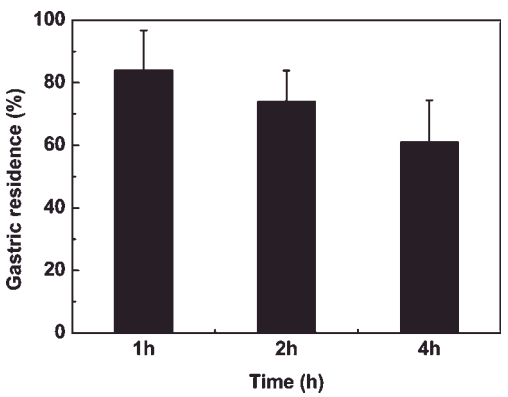

Figure 4 Percentage of CAEMs retained in the stomachs of rats *in vivo* ($n = 3$).

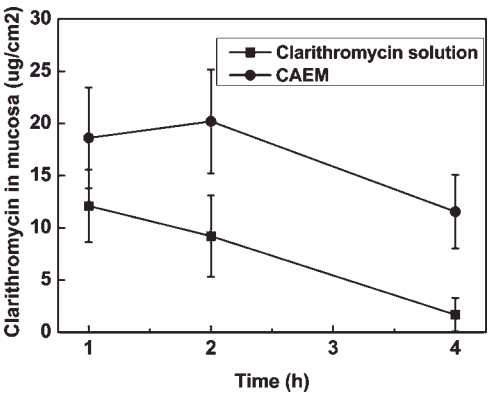

Figure 5 Clarithromycin concentration in the gastric mucosa after oral administration to rats ($n = 3$).

Concentration of clarithromycin in the gastric mucosa

When a clarithromycin solution was administered to rats, clarithromycin was immediately absorbed from the gastrointestinal tract. As shown in Figure 5, the concentration of clarithromycin in the gastric mucosa reached 12.1 μg/cm^2 at 1 h and then decreased gradually to 9.2 μg/cm^2 at 2 h and 1.7 μg/cm^2 at 4 h. After CAEMs were administered with the same clarithromycin dose, the amounts of clarithromycin reaching the gastric mucosa at 2 and 4 h were 20.2 and 11.5 μg/cm^2, respectively, so the concentration of clarithromycin in the gastric mucosa was greater than that of the solution, and the difference at 2 h was statistically significant ($p < 0.05$). CAEMs released the drug continuously in the stomach while floating on the gastric juice or adhering to the gastric mucosa, so the topical clarithromycin concentration in the rat stomach was enhanced. CAEMs may provide a therapeutic concentration at a much lower dose for a longer time, and this may significantly reduce the adverse effects.

CONCLUSIONS

A synergism between floating and bioadhesive systems containing clarithromycin was prepared in this study. Floating-bioadhesive microparticles, CAEMs, floated in the acid environment of the stomach. Clarithromycin contained in CAEMs was released in a sustained manner *in vitro*. At the same time, CAEMs could remain in the stomach for an expended period

of time *in vivo*, and the concentration of clarithromycin in the gastric mucosa from CAEMs was higher than that from a clarithromycin solution. These properties might be useful for facilitating and prolonging the penetration of clarithromycin into the gastric mucosa where *H. pylori* is localized. Thus, floating-bioadhesive microparticles have been proved to be a promising drug delivery system for the treatment of *H. pylori* infection.

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