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Preparation and evaluation of glyceryl monooleate-coated hollow-bioadhesive microspheres for gastroretentive drug delivery

Yuanfen Liu[a,1], Jianjun Zhang[a,1], Yuan Gao[b,**], Jiabi Zhu[a,*]

[a] Department of Pharmaceutics, China Pharmaceutical University, Tongjiaxiang 24, Nanjing 210009, China
[b] School of Traditional Chinese Medicine, China Pharmaceutical University, Tongjiaxiang 24, Nanjing 210009, China

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ABSTRACT

The purpose of this study was to produce hollow and bioadhesive microspheres to lengthen drug retention time in the stomach. In these microspheres, ethylcellulose was used as the matrix, Eudragit® EPO was employed to modulate the release rate, and glyceryl monooleate (GMO) was the bioadhesive polymer *in situ*. The morphological characteristics of the microspheres were defined using scanning electron microscopy. The *in vitro* release test showed that the release rate of drug from the microspheres was pH-dependent, and was not influenced by the GMO coating film. The prepared microspheres demonstrated strong mucoadhesive properties with good buoyancy both *in vitro* and *in vivo*. Pharmacokinetic analysis indicated that the elimination half-life time of the hollow-bioadhesive microspheres was prolonged, and that the elimination rate was decreased. In conclusion, the hollow-bioadhesive synergic drug delivery system may be advantageous in the treatment of stomach diseases.

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1. Introduction

Recently, research efforts have increasingly been focused on controlling drug release in a particular region for site-specific drug delivery. A gastric-retention delivery system can lengthen drug retention time, thereby increasing the concentration in the stomach of drugs used to treat gastric disease. Proposed gastric-retentive delivery systems for the enhancement of local drug delivery mainly employ either floating or bioadhesive systems. Hollow microspheres, a type of floating drug delivery system, have attracted a great deal of attention because of their properties of low density, high specific surface area, and good flowability. Among the preparation methods of hollow microspheres, the emulsion solvent diffusion method is easy and feasible (Wang et al., 2007). In 1991, Kawashima et al. (1991) were the first group to report the preparation of hollow microspheres by emulsion solvent diffusion as a drug delivery system and explained the mechanism of cavity formation inside the microspheres. Subsequently, many drugs have been reportedly delivered by hollow microspheres including riboflavin, aspirin, nifedipine, ibuprofen and many others (Jain et al., 2008).

The mucoadhesive drug delivery system uses adherence to mucus surfaces to enhance gastric retention and control drug release (Vasir et al., 2003). The bioadhesive polymer, glyceryl monooleate (GMO), is a polar amphiphilic lipid that, when placed in water, can be organized into lipid bilayers, forming a reversed micellar phase (L) and three types of liquid crystalline phases (lamellar, reversed hexagonal and the cubic phase). The structure of the cubic phase is unique and consists of a curved bicontinuous lipid bilayer extending in three dimensions, separating two congruent networks of water channels. When GMO absorbs excess water from body fluids such as the gastrointestinal fluids, it forms a stiff viscous cubic phase *in situ* (Sallam et al., 2002). The high viscosity of the *in situ*-formed liquid crystal phase makes GMO bioadhesive *in vivo*. Moreover, GMO is an FDA-approved food additive that is non-toxic, biodegradable, and biocompatible in pharmaceutical applications (Ganem-Quintanar et al., 2000) and, in contrast with other water-soluble bioadhesive materials such as carbopol and chitosan, GMO blends and coats easily (Nielsen et al., 1998). GMO has therefore been widely used in drug delivery through mucosal, vaginal, periodontal and topical routes, among others (Shah et al., 2001).

However, both systems have limitations. Floating systems are unable to retain drug in the gastric mucous layer, whereas in bioadhesive systems, the mucoadhesive polymers interact non-specifically with the mucus. Therefore, a synergic drug delivery system combining buoyancy and mucoadhesion may overcome these problems and prove more effective in treating gastric disease (Chitnis et al., 1991; Jiménez-Castellanos et al., 1994;

* Corresponding author. Tel.: +86 25 83271316.
** Co-corresponding author. Tel.: +86 25 86185144.
E-mail addresses: newgaoyuan@163.com (Y. Gao), zhujiabi1015@gmail.com (J. Zhu).
[1] Both authors contributed equally to this work.

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Umamaheswari et al., 2002; Zheng et al., 2006; Varshosaz et al., 2008).

The aim of this study was to develop a floating and bioadhesive system for gastric-specific drug delivery, and to evaluate the *in vitro* and *in vivo* properties of these hollow-bioadhesive microspheres. GMO was initially used for oral bioadhesive microspheres in the stomach because the liquid crystal structure formed by GMO would not be influenced by the drug during the coating process, in contrast to conventional methods such as blending and melting the coating substance with the drug. Psoralen, a linear furanocoumarin compound, was utilized in the delivery system as a model drug, due to its hydrophobic behavior and its potential effect as a treatment for gastric *Helicobacter pylori* infection (Zaidi et al., 2009).

2. Materials and methods

2.1. Materials

Ethylcellulose (EC) was obtained from Shanghai Colorcon Coating Technology, Ltd. (Shanghai, China). Eudragit® EPO was from Röhm Pharma GmbH (Germany). Psoralen was purchased from Nanjing Zelang Medical Technological Co., Ltd. (Najing, China) GMO (Rylo™ MG Pharma19) was provided by Danisco Ingredients (Denmark). The polyvinyl alcohol 124 (PVA 124) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) All other chemicals and reagents were of the highest purity available from local sources.

2.2. Preparation of psoralen hollow-bioadhesive microspheres

Psoralen-containing hollow microspheres were prepared using the emulsion solvent diffusion method. The polymer was composed of EC (0.4 g) and Eudragit® EPO (0.2 g) (2:1). Briefly, 0.06 g of drug and 0.6 g of the polymer (1:10) were dissolved in a mixture of 2 ml dichloromethane and 2 ml ethanol. Then, the dispersion solution was added drop-by-drop into 30 ml 1.5% PVA solution containing 0.3% Tween-80. The resultant emulsion was stirred at 350 rpm using a propeller-type agitator for 2 h. The system temperature was kept at 15 °C throughout the process. The hollow microspheres were separated by filtration, washed with water and vacuum-dried at room temperature for 24 h (Kawashima et al., 1991; Sato et al., 2003, 2004a). The 500–1000 μm microspheres were selected for subsequent preparation steps.

The hollow-bioadhesive microspheres were prepared by the coating method described by Maharaj et al. (1984). Hollow microspheres (0.1 g) were introduced into a coating solution (optimized in our previous study) consisting of GMO (0.25 g) dissolved in 10 ml petroleum ether at a concentration of 25 mg/ml. After 3 min of dispersion by magnetic agitation at room temperature, the microspheres were filtered using a Büchner flask under agitation. The hollow microspheres were then coated with the retained GMO from the solution. Subsequently, the prepared hollow-bioadhesive microspheres were dried in a vacuum for 24 h at room temperature for further analysis.

2.3. Morphological characterization and drug crystallinity of psoralen within microspheres

The dried samples were coated with gold film under a vacuum using a sputter coater. The surface and inner part of the microspheres were observed under scanning electron microscopy (SEM; Hitachi S-3000, Tokyo, Japan) at an accelerating voltage of 20 kV.

The X-ray diffraction patterns of the drug in the microspheres were obtained using an X-ray diffractometer (TETA ARL X'TRA, Thermo Electro Corporation, USA/Switzerland) using Cu Kα radiation in the 2θ angle range of 2°–50°.

2.4. Drug and GMO content

To assess drug and GMO content, 10 mg of the hollow and hollow-bioadhesive microspheres were weighed and each dissolved in 10 ml methanol under ultrasonication. This solution was used for the detection of both drug and GMO content. To assess drug content, 0.3 ml of the solution was further diluted with methanol to 10 ml total volume. After filtration through a 0.45 μm membrane, the drug content in the methanol phase was determined with a 752 UV spectrophotometer (Shanghai Technology Instrument Co., Ltd., China) at 245 nm. The filtered solution from the empty microspheres (without psoralen) was taken as blank. In the concentration range of 1–8 μg/ml, the absorbance of psoralen (Y) correlated well with its concentration (X): $Y = 0.1411X + 0.0115$ ($r^2 = 0.9997$, $n = 6$).

The GMO content in the hollow-bioadhesive microspheres was determined by high-performance liquid chromatography (HPLC) with UV detection at 231 nm (liquid chromatograph with an LC-20 AT pump, SPD-20 A detector; Shimadzu Corp.). The column used was an Agilent ZORBAX SB-Aq C_{18} (4.6 mm × 250 mm, 5 μm). The mobile phase was methanol, water and acetate buffer (pH 3.6) at a ratio of 420:60:20, and the flow rate was 1.0 ml/min (Nielsen et al., 1998). In the concentration range of 0.5–4 mg/ml, the peak area of GMO (Y) correlated well with its concentration (X): $Y = 104559X - 3706.9$ ($r^2 = 0.9991$, $n = 6$).

2.5. Tapped density

The tapped density was determined using a tapping method described previously (Rawat et al., 2008). Tapped density of the microspheres was calculated as the ratio between the mass of the microsphere sample (g) and its volume (ml) after 100 tappings.

2.6. Determination of microsphere buoyancy

The buoyancy of the microspheres was determined using the paddle method in a dissolution tester (Model ZRS-4, Tianjin University Radio Factory, China) (Kawashima et al., 1991; Sato et al., 2003, 2004a). One hundred microspheres were introduced into the vessels and the paddles were rotated at 50 rpm in 150 ml of hydrochloric acid (pH 1.2) at 37 ± 0.5 °C. After 10 h, the floating microspheres were counted. The buoyancy was determined by the ratio of the number of floating microspheres to the total number of microspheres.

2.7. Drug release study

The drug release rate from microspheres was determined using a dissolution tester (Model ZRS-4, Tianjin University Radio Factory) by a basket-type apparatus specified in the Chinese Pharmacopoeia (2005 Edition). A weighted amount of microspheres (equivalent to 6 mg of psoralen) was placed in the basket, and then put into the dissolution medium (900 ml simulated gastric fluid (SGF, pH 1.2, HCl)) at 37 ± 0.5 °C with a paddle rotation speed of 50 rpm. At 1, 2, 3, 4, 6, 8 and 10 h, 5 ml samples were withdrawn, passed through a 0.45 μm membrane filter, and analyzed using a 752 UV spectrophotometer at 245 nm to determine the concentration of psoralen. Simultaneously, 5 ml of fresh dissolution fluid was added to the dissolution medium after each withdrawal. The release study was repeated using other dissolution media (pH 3.0, 6.8 and 7.4). All experiments were conducted in triplicate.

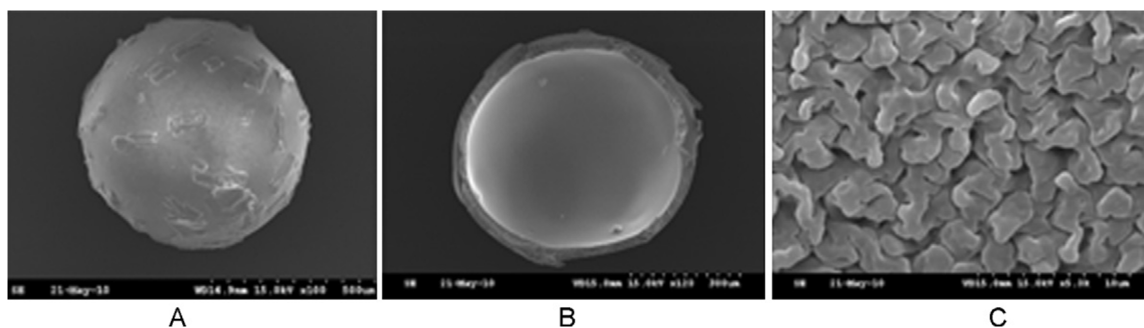

Fig. 1. Scanning electron microphotographs of hollow microspheres showing (A) general appearance (100×); (B) hollow structure (120×); and (C) surface morphology (5000×).

2.8. Determination of microsphere bioadhesion in vitro and in vivo

2.8.1. In vitro evaluation of bioadhesive of microspheres

The mucoadhesion of microspheres was tested according to methods described previously (Rao and Buri, 1989; Liu et al., 2005). The stomachs of male Sprague-Dawley (SD) rats (220 ± 10 g) were removed and washed using a small amount of physiological saline, and mounted on a glass slide. One hundred microspheres were then uniformly introduced onto the inner surface of the stomachs. Subsequently, the slide was placed in a closed container with 93% humidity for 30 min. The slide was then removed and placed on a support at an angle of 45°. These microspheres were washed with a mixture of HCl and physiological saline (pH 1.2) at a rate of 22 ml/min for 5 min. Microspheres remaining on the inner surface of the stomachs after this period were counted and data were statistically analyzed using the ANOVA test. All data are the average of at least three determinations. All animal experiments in this research complied with the regulations of the Committee on Ethics in the Care and Use of Laboratory Animals of China Pharmaceutical University.

2.8.2. In vivo evaluation of microspheres

After being fasted for 24 h, SD rats (220 ± 10 g) were randomly divided into three groups ("HM", "solid" and "GHM") with 12 rats in each group. Rats in these groups were administered orally with 100 microspheres of the hollow, solid, or hollow-bioadhesive varieties, respectively. Four rats from each group were killed at 2, 4 and 6 h after administration. Stomach-retained microspheres were counted, and the percentage of remaining microspheres calculated and statistically analyzed using the ANOVA test (Liu et al., 2005).

2.9. Pharmacokinetics studies

Ten male SD rats (220 ± 10 g) were fasted for 24 h (but with free access to water) before being randomly assigned into two groups with five rats in each group. Hollow-bioadhesive microspheres and raw psoralen suspension were orally administered to these rats at a drug dose of 25 mg/kg. Blood samples (0.5 ml) were collected from the fossa orbitalis vein at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 h. The blood samples were introduced into heparinized microcentrifuge tubes, and then separated by centrifugation (Sigma 3K30 centrifuge; Sigma Laborzentrifugen GmbH, Postfach, Germany). The resulting plasma samples (200 μl) were stored at −20 °C until analysis. The drug was extracted with N-hexane:dichloromethane (2:1, v/v) twice, dried with nitrogen gas on a water bath at 37 °C, and then dissolved in methanol. The plasma concentration of psoralen was determined by HPLC with a UV detector at 245 nm. The mobile phase was methanol: water (50:50, v/v) at a flow rate of 1 ml/min (Stolk et al., 1987; Stolk and Siddiqui, 1988).

The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were directly obtained from the experimental data. The elimination rate constant (Ke) was calculated through linear regression of the terminal semi-log plot of plasma concentration vs. time. Half-life time ($T_{1/2}$) was calculated as $0.693/Ke$. The $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C_t/Ke$, with C_t as the last detectable plasma concentration. Differences in the parameters were tested for statistical significance using the *t*-test, and were considered to be significant at $P < 0.05$.

3. Results and discussion

3.1. Microsphere shape and drug crystallinity

Under the scanning electron microscope, hollow microspheres were characterized by a spherical cavity enclosed within a hard polymer shell, and loaded with drug in the shell (Fig. 1A and B). Many small scale-like pores were present in the microsphere surface (Fig. 1C). The amorphous form of the hollow structure resulted from the emulsion solvent diffusion technique and has been studied previously in detail (Kawashima et al., 1991). According to the study, the central cavity was formed by dichloromethane volatilization. When the drug and polymer solution in the dichloromethane and ethanol mixture was dropped into PVA solution, the ethanol diffused into the aqueous solution, leaving the polymer and drug to dissolve out and form a shell covering the dichloromethane core. The hydrophobic dichloromethane was then volatilized and the hollow structure was formed, allowing the microspheres to become buoyant and therefore float on the gastric fluids.

After the hollow microspheres were coated with GMO employing organic solvent diffusion methods, the coating film was uniform and smooth, and the microsphere was still spherical in shape (Fig. 2A). A hollow cavity was a common feature of these hollow-bioadhesive microspheres (Fig. 2B). The particle size of hollow and hollow-bioadhesive microspheres was almost identical. On the surface, the small pores visible in the hollow microspheres could not be observed in hollow-bioadhesive microsphere surface due to the extensive formation of the coating film (Fig. 2C).

The X-ray diffraction patterns of the microspheres are shown in Fig. 3. In the X-ray analysis, the crystalline peaks of the psoralen in hollow microspheres and the hollow-bioadhesive microspheres identical to that of the raw drug. These findings suggest that the crystallinity of the drug in the microspheres does not change during the preparation.

3.2. Drug and GMO content in microspheres

The drug content, remaining GMO content and tapped density of the hollow microspheres and hollow-bioadhesive microspheres (diameter 500–1000 μm) are given in Table 1. The drug con-

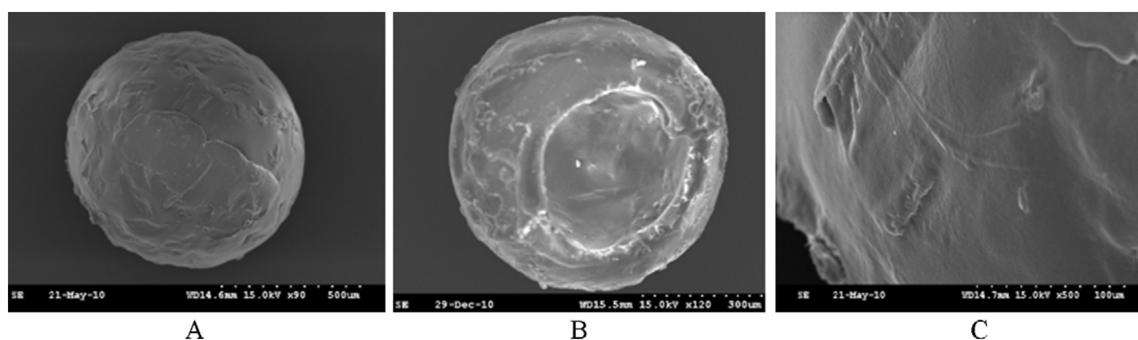

Fig. 2. Scanning electron microphotographs of the hollow-bioadhesive microspheres showing (A) general appearance (90×); (B) hollow structure (120×); and (C) surface morphology (500×).

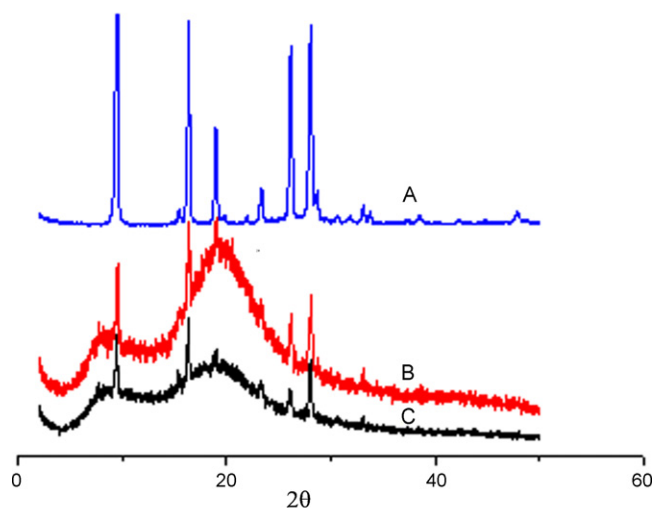

Fig. 3. X-ray powder diffractograms. A: Original crystals of psoralen. B: Psoralen crystals in the hollow microspheres. C: Psoralen crystals in the hollow-bioadhesive microspheres.

tent was 8.70% in hollow microspheres, compared to 6.82% in hollow-bioadhesive microspheres. The GMO content quantified by reversed phase HPLC procedures in the hollow-bioadhesive microspheres was about 11.5%. The tapped densities of the hollow microspheres and the hollow-bioadhesive microspheres were low, only about 0.202 g/ml and 0.348 g/ml, respectively, which may be due to their common hollow structure.

3.3. Drug release behavior

The release of psoralen from the microspheres was evaluated in SGF (pH 1.2) and the results are shown in Fig. 4. Approximately 80% of the drug was released into the SGF solution over a period of 6 h. In the SGF solution, increasing the concentration of the GMO coating solution (5, 15 and 25 mg/ml) had no apparent effect on the drug release rate (Fig. 4). There was also no apparent change

Table 1
Physicochemical properties and buoyancy of hollow microspheres and hollow-bioadhesive microspheres.

	Drug content (%)	GMO content (%)	Tapped density	Buoyancy (10 h)
HM	8.70	0	0.202 g/ml	98.70%
GHM	6.82	11.5	0.348 g/ml	82.00%

Buoyancy: % of microspheres remaining on the surface of SGF solution in 10 h.
HM: Hollow microspheres; GHM: Hollow-bioadhesive microspheres.

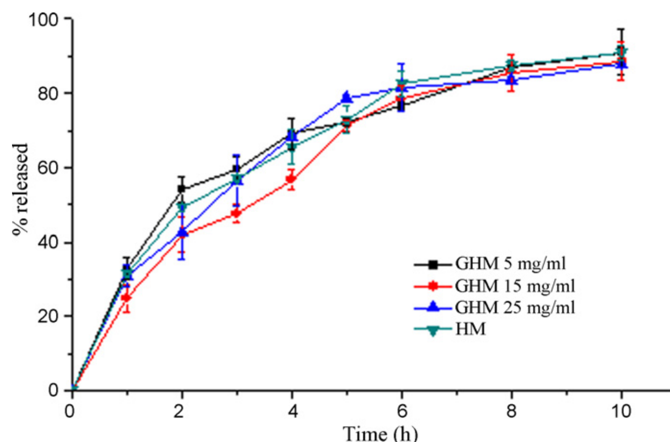

Fig. 4. *In vitro* drug release from microspheres in SGF (pH 1.2) at different GMO concentrations (5, 15, and 25 mg/ml).

in the drug release between hollow microspheres and hollow-bioadhesive microspheres (Fig. 4).

In order to understand the influence of the pH on the drug release, the dissolution test was carried out at variable pH values (pH 3.0, 6.8 and 7.4). As shown in Fig. 5, the release rates from the microspheres at pH 6.8 and pH 7.4 were significantly lower than those in the SGF and at pH 3.0. In media at pH 6.8 and pH 7.4, no more than 50% of drug was released. Given that the Eudragit® EPO polymer was only soluble in media of low pH, the drug release rate

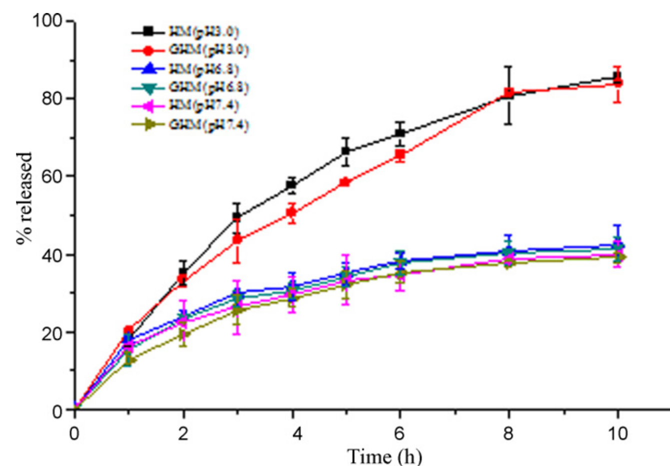

Fig. 5. *In vitro* drug release from microspheres at varying pH (3.0, 6.8 and 7.4). HM: hollow microspheres; GHM: hollow-bioadhesive microspheres coated with GMO (25 mg/ml).

from the microspheres decreased as pH increased. The drug release from different pH media followed the order: pH 1.2 > pH 3.0 > pH 6.8 > pH 7.4. The results clearly showed that drug release from the microspheres is a pH-dependent behavior.

From the same results, it can also be seen that there was no such variation between release rates from hollow and hollow-bioadhesive microspheres, indicating that the bioadhesive coating film of GMO did not delay the release of drug. This phenomenon may be related to the liquid crystal structure formed from the GMO/water mixture. It was reported that when GMO absorbed water it assumed a cubic liquid crystalline form, the structure of which consists of a curved bicontinuous lipid bilayer extending in three dimensions and containing pores of about 5 nm in diameter. Moreover, a number of studies have reported that when drug is dispersed or dissolved in a matrix formed by the GMO, the release of drug depends on its location in the cubic phase (i.e. in the lipid bilayer or the aqueous channels) (Shah et al., 2001). In our study, the drug release rate of hollow-bioadhesive microspheres was apparently not altered by the GMO coating film or increasing coating solution concentration, perhaps because the drug has a low molecular weight and did not locate in the GMO phase. Considering the results of our drug release experiments together, it seems reasonable to conclude that, over a pH range of 1.2–7.4, drug dissolved from the EC matrix may diffuse freely into the SGF through the pores of the liquid crystalline structure.

3.4. Buoyancy and bioadhesion of the microspheres

3.4.1. In vitro test

The floating test was carried out to investigate the buoyancy of the microspheres. The percentages of the microspheres floating at the surface of SGF at 37 ± 0.5 °C are shown in Table 1. More than 80% of the hollow microspheres and the hollow-bioadhesive microspheres continued to float for at least 10 h *in vitro*, which may be considered as satisfactory when testing *in vivo*. After the hollow microspheres were coated with GMO, the percentage floating at the SGF surface decreased slightly because of some microsphere aggregation, but nevertheless remained high at 82%. Our experiments show that the density of both types of microsphere was considerably lower than that of SGF, which provides them with buoyancy. This buoyancy may be attributed to the common hollow structure, and there was little variation in the relative buoyancy of the hollow and hollow-bioadhesive microspheres.

In 1991, Kawashima et al. (1991) first reported the preparation of hollow microspheres by emulsion solvent diffusion for use as a floating drug delivery system. Subsequent studies have generally documented that microspheres prepared by similar methods with hollow cavities have good buoyancy *in vitro* and *in vivo*. In this study, from the results of the *in vitro* floating experiment, it can be also deduced that these hollow microspheres can float in full gastric fluid, retarding the passage of the spheres (and therefore the drug contained in them) into the intestinal region and prolonging their presence in the stomach.

The results of the *in vitro* bioadhesive test are shown in Fig. 6. The percentage of the GMO-coated microspheres that remained on the gastric mucosa of the rats was 87.7 ± 1.5%, much higher than those of the hollow microspheres (26.7 ± 4.6%) and solid microspheres (57.3 ± 4.2%). The high percentage of adhesion to gastric mucosal tissue *in vitro* indicates that microspheres are likely to have excellent mucoadhesion to stomach tissue *in vivo*. Notable differences existed between hollow-bioadhesive microspheres and other groups (ANOVA, $P < 0.01$).

There are six general theories of adhesion including electronic, wetting and adsorption theories (Smart, 2005). The most compatible of these in describing the adhesion between GMO and mucin is the dehydration theory (Nielsen et al., 1998). In the current study, the coating of GMO bioadhesive polymer was utilized as a precursor. When GMO comes into contact with mucus, it takes up excess water to form a liquid crystal gel *in situ*, forcing the dehydration and intermixing of the mucus joint.

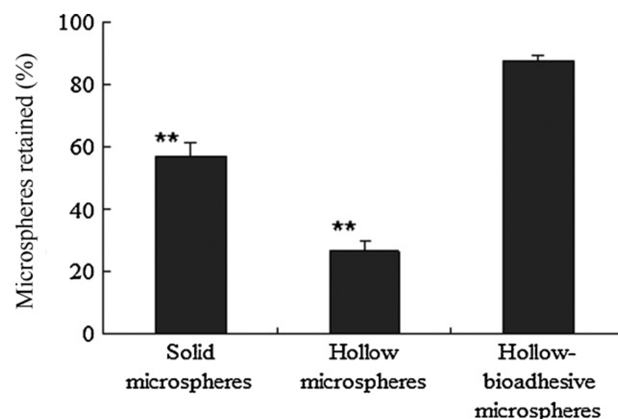

Fig. 6. Percentage of microspheres retained on the gastric mucus. **$P < 0.01$ (when compared with Hollow-bioadhesive microspheres using one-way analysis of variance (ANOVA) test).

3.4.2. In vivo remaining test

The results of the *in vivo* remaining test under fasting conditions are given in Table 2. After 2 h, the percentage of hollow-bioadhesive microspheres remaining in the stomach of rats was higher than that in both the solid and the hollow microsphere groups. Moreover, significant differences were observed among the three groups (ANOVA, $P < 0.05$). The values for the hollow-bioadhesive and solid groups were notably different at 4 h (ANOVA, $P < 0.05$). After 6 h, most microspheres from each group had already passed the stomach of the rats. However, although there were no significant differences among the three groups at this time point, the values in the solid group were appreciably lower than the other groups. The differences in the values between the "HM" and the "GHM" groups, with a higher percentage of hollow-bioadhesive microspheres retained in the stomach, suggest that the combined effects of buoyancy and mucoadhesion provide better retention than buoyancy alone.

At present, most relevant studies have shown that floating or bioadhesive formulations can prolong the gastric retention time, and are more effective in treating gastric disease (for *H. pylori* clearance) *in vitro* and *in vivo* (Umamaheswari et al., 2002; Zheng et al., 2006). In our *in vitro* study, the floating and bioadhesive tests clearly indicate that the prepared microspheres possess both buoyancy and mucoadhesion properties, which enable them to remain longer in the stomach *in vivo*. These results confirm the great potential of synergistic hollow-bioadhesive microspheres to be retained for longer periods in the stomach, to achieve an effective and sustained drug concentration, and to therefore enhance our ability to treat gastric disease. However, further studies of drug efficiency will be necessary to prove this potential before full adoption of this technology in the clinic.

Table 2
Percentage of microspheres remaining in rat stomach (Mean ± S.D.).

Time (h)	Solid microspheres (%)	HM (%)	GHM (%)
2	51 ± 11.0*	59 ± 6.9*	78.7 ± 4.2
4	18.3 ± 8.0**	49.3 ± 11.2*	77 ± 10.4
6	8 ± 13.9	12.3 ± 10.9	18.3 ± 18.9

HM: Hollow microspheres; GHM: Hollow-bioadhesive microspheres.
* $P < 0.05$ when compared with GHM using ANOVA test.
** $P < 0.01$ when compared with GHM using ANOVA test.

Table 3
Pharmacokinetic parameters of raw psoralen suspension and hollow-bioadhesive microspheres after oral administration to rats at a dose of 25 mg/kg (Mean ± S.D.).

	Ke (h^{-1})	$AUC_{0-\infty}$ (ng h/ml)	$T_{1/2}$ (h)	C_{max} (ng/ml)	T_{max} (h)
Psoralen suspension	0.26 ± 0.1	6367.5 ± 1542.3	2.61 ± 0.91*	1190.3 ± 567.2	2.67 ± 0.3*
GHM	0.11 ± 0.015	5849.3 ± 574.6	6.65 ± 0.88	565.9 ± 156.1	5.33 ± 1.2

GHM: Hollow-bioadhesive microspheres.
* $P < 0.05$ when compared with GHM using t-test.

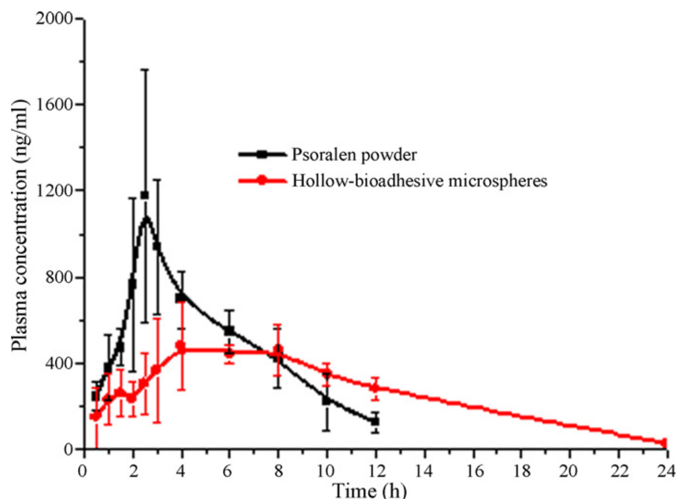

Fig. 7. Mean plasma concentration of psoralen after oral administration of raw psoralen suspension and hollow-bioadhesive microspheres.

3.5. Pharmacokinetics analysis

Fig. 7 summarizes the mean plasma concentration of psoralen at various time points following a single dose (25 mg/kg) administered orally to rats in the fasted state. The pharmacokinetics parameters of Ke (h^{-1}), $AUC_{0-\infty}$, C_{max}, $T_{1/2}$ and T_{max} of the raw psoralen suspension and the hollow-bioadhesive microspheres are listed in Table 3. The $AUC_{0-\infty}$ values of the raw psoralen suspension and the psoralen hollow-bioadhesive microspheres were 6367 ± 1542 ng h/ml and 5849 ± 574 ng h/ml, respectively, and were not significantly different ($P > 0.05$). This result shows that gastroretentive formulations may not increase the bioavailability of a poorly absorbed drug such as psoralen to the same extent as has been reported for other drugs (Crevoisier et al., 1987; Akiyama et al., 1994; Rouge et al., 1998). However, the $T_{1/2}$ (6.65 h) and T_{max} (5.33 h) of the hollow-bioadhesive microspheres were significantly longer than that of the psoralen suspension ($T_{1/2}$ 2.61 h, T_{max} 2.67 h) ($P < 0.05$), and both the elimination rate constant and the peak concentration of drug (C_{max}) were evidently lower than that of the psoralen suspension. These findings are convincing evidence that the hollow-bioadhesive microspheres effectively sustain the drug release compared with the psoralen suspension. For the treatment of stomach disease, prolonged residence of the drug in the stomach may be advantageous. The pharmacokinetic properties of the microspheres would present advantages for the treatment of stomach disease.

4. Conclusions

In this study, we successfully prepared hollow microspheres (using the emulsion solvent diffusion technique) and initially coated these with the bioadhesive polymer, GMO, to improve bioadhesion for use as a gastroretentive delivery system. The preparation process was simple, reliable, and inexpensive. The prepared hollow-bioadhesive microspheres were spherical with a smooth surface. The *in vitro* release test indicated the hollow microspheres and the hollow-bioadhesive microspheres possessed almost similar drug release profiles, and the drug release was not constrained by the GMO coating film. Moreover, drug release from the microspheres was pH-dependent because of the Eudragit® EPO polymer. The good buoyancy and bioadhesion properties of the microspheres were demonstrable both *in vitro* and *in vivo*. The *in vivo* study showed that the microspheres were retained in the stomach and resulted in prolonged half-life time within the gastric chamber and decreased plasma drug concentration in the pharmacokinetic profile. In conclusion, our study demonstrates clearly that the synergic drug delivery system combining hollow structure with bioadhesive properties could increase drug retention time in the gastric chamber to improve the treatment of gastric disease. This novel system could play a potentially important role in pharmaceutical drug delivery for gastric therapeutics.

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