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Development and evaluation of new sustained-release floating microspheres

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ABSTRACT

A type of multi-unit floating alginate (Alg) microspheres was prepared by the ionotropic gelation method with calcium carbonate ($CaCO_3$) being used as gas-forming agent. Attempts were made to enhance the drug encapsulation efficiency and delay the drug release by adding chitosan (Cs) into the gelation medium and by coating with Eudragit, respectively. The gastrointestinal transit of optimized floating sustained-release microspheres was compared with that of the non-floating system manufactured from identical material using the technique of gamma-scintigraphy in healthy human volunteers. It was found that the drug encapsulation efficiency of Cs–Alg microspheres was much higher than that of the Ca–Alg microspheres, and coating the microspheres with Eudragit RS could extend the drug release significantly. Both uncoating and coating microspheres were able to continuously float over the simulated gastric fluid (SGF) for 24 h in vitro. Prolonged gastricretention time (GRT) of over 5 h was achieved in the volunteer for the optimized coating floating microspheres (FM). In contrast, non-floating system (NFM) could be emptied within 2.5 h. In the present study, a multi-unit system with excellent floating ability, optimum drug entrapment efficiency and suitable drug release pattern has been developed.

1. Introduction

Drugs that are easily absorbed from the gastrointestinal tract (GIT) and have a short half-life are eliminated quickly from the blood circulation, so they require frequent dosing. To avoid this drawback, the oral sustained-controlled release formulations have been developed in an attempt to release the drug slowly into the GIT and maintain an effective drug concentration in the serum for longer period of time. However, such oral drug delivery devices have a physiological limitation (Soppimath et al., 2001) of gastricretention time (GRT), Variable and short gastric emptying time can result in incomplete drug release from the drug delivery system (DDS) in the absorption zone (stomach or upper part of small intestine), leading to diminished efficacy of the administered dose (Chueh et al., 1995; Iannuccelli et al., 1998). To overcome these limitations, approaches being proposed to prolong the GRT include: floating drug dosage systems (FDDS) (Whitehead et al., 2000; Goole et al., 2007; Streubel et al., 2003; Sungthongjeen et al., 2006), swelling or expanding systems (Deshpande et al., 1996, 1997), mucoadhesive systems (Santus et al., 1997), high-density systems (Rouge et al., 1998), modified-shape systems (Kedzierewicz et al., 1999), and other delayed gastric emptying devices. However, many floating systems previously reported are single-unit systems such as hydrodynamically balanced systems (HBS), which are unreliable in prolonging the GRT owing to their 'all-or-nothing' emptying process and, thus, may result in high variability in bioavailability and local irritation due to a large amount of drug delivered at a particular site of GIT (Whitehead et al., 1998). In contrast, multiple-unit particulate dosage forms (e.g. microspheres) have the advantages of passing through the GIT uniformly, which not only avoid the vagaries of gastric emptying but also provide an adjustable release, and reduced inter-subject variability in absorption and risk of local irritation were achieved consequently (Kawashima et al., 1992; Stithit et al., 1998). Various multiple-unit floating systems have been developed in different forms and are based on various principles, such as air compartment multiple-unit system (Iannuccelli et al., 1998), microparticles based on porous carriers (Sharma and Pawar, 2006; Streubel et al., 2002, 2003), hollow microspheres (microballoons) (Sato et al., 2003, 2004), oil-entrapped gel beads prepared by gelation method (Sriamornsaka et al., 2005; Tang et al., 2007).

Alginates are non-toxic, biodegradable, linear co-polymers composed of L-glucuronic and D-man-nuronic acid residues. They are widely used in food and pharmaceutical industries. Ca–Alg is rapidly formed by gelation of alginic acid in the presence of calcium ions. Alginate beads had been developed as floating dosage forms to prolong the GRT as early as 1980s (Stochwell and Davis, 1986). In recent years, alginate gel beads have frequently been employed as a unique vehicle for FDDS (Iannuccelli et al., 1998; Whitehead et al., 1998, 2000; Choi et al., 2002; Murata et al., 2000). However, the encapsulation efficiency for the hydrophilic drug in the Ca–Alg gel beads is low, and the release of many hydrophilic drugs cannot

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sustain for more than 4 h even after further modification of Ca–Alg gel beads. The practical value is limited for the floating dosage form in the absence of sustained release.

The object of the present experiment was to develop a type of floating microspheres of alginate by the addition of $CaCO_3$ gas-forming agent, which combines sustained release and prolonged gastricretention time of the hydrophilic model drug. Diltiazem hydrochlorid (DTZ), an effective drug in the treatment of angina pectoris and hypertension, was selected as the model drug because its high frequency of drug administration resulted from relatively short biological half-life of 3–4 h (Chaffman and Brogden, 1985). Calcium carbonate ($CaCO_3$) as a gas-forming agent is reacted with acetic acid in the gelation medium to produce carbon dioxide. The evolving gas permeates through the alginate leaving gas bubbles or pores (Choi et al., 2002), and therefore results in microspheres that can afloat over the medium. The floating ability of a range of microspheres containing different weight ratio ($CaCO_3$/Alg) of $CaCO_3$ was investigated. Chitosan is a kind of biocompatibility, non-toxicity and biodegradability cationic biopolymer, which could form gels with sodium alginate (Aral and Akbˆuga, 1998; Anal et al., 2003) by ionic cross-linking. In the present study, the effects of chitosan in the gelation medium on drug encapsulation efficiency and drug release behavior were examined. The contribution of Eudragit coating on drug release was also studied. Gamma-scintigraphy was employed to investigate the in vivo transit behaviors of the optimized coating floating formulation (FM) and non-floating microspheres (NFM) prepared from the identical polymer.

2. Materials and methods

2.1. Materials

Sodium alginate (low viscosity grade) was purchased from Yuanhang Chemical (Tianjin, China). Chitosan ((MW 720 000 Da, DA > 90%) was obtained from Golden-shell Biochemical (Hangzhou, China). Diltiazem hydrochlorid was purchased from ECUST Biomedicine (Shanghai, China). Eudragit ®RS30D was a gift from Degussa Chemical (Shanghai, China). Technetium-99 m (as pertechnetate) ($^{99\,m}TcO_4^-$) was obtained from the Nuclear Medicine Department, The General Hospital of Shenyang Military Command (Shenyang, China). All other chemicals were standard pharmaceutical grade.

2.2. Methods

2.2.1. Preparation

2.2.1.1. Preparation of floating Ca–Alg microspheres. Alginate (Alg) was dissolved in distilled water at a concentration of 3% (w/v), the solution was stirred thoroughly after DTZ (D/Alg = 1/4, w/w) and calcium carbonate of different weight ratio ($CaCO_3$/Alg = 0, 1/4, 1/2 and 3/4, w/w) were added. The gelation medium was prepared by dissolving calcium chloride ($CaCl_2$) of different concentrations (0.5, 1, 2 and 3%, w/v) in 2% acetic acid glacial. The homogenous alginate solution was extruded using a 21 G syringe needle into the gelation medium. The distance between the edge of the needle and the surface of the gelation medium was about 10 cm. The gel microspheres formed were left in the solution with gentle stirring for different time (10, 20, 30 min) at room temperature to be cured. After microspheres were collected, washed with distilled water twice and oven-dried subsequently (40 °C).

2.2.1.2. Preparation of floating Cs–Alg microspheres. The floating Cs–Alg microspheres were prepared as described in Section 2.2.1.1 except that different concentrations of chitosan (0.2, 0.6, 1 and 1.4%, w/v) were dispersed in calcium chloride ($CaCl_2$) solution containing 2% acetic acid glacial before alginate solution was extruded into the gelation medium.

2.2.1.3. Coating of floating Cs–Alg microspheres. The coating on floating Cs–Alg microspheres was performed by fluidized bed coater (self-made) using aqueous colloidal polymethacrylate dispersion Eudragit RS30D. For the preparation of the coating solution, aqueous-based polymeric Eudragit RS30D was diluted with distilled water. The talc (30% based on solid polymeric content) and the plasticizers (TEC, 20% based on polymeric contents) were dispersed in distilled water and thereafter the suspension was added to the above polymeric dispersion and the total solid content was adjusted with distilled water to 15%. The coating solution was completely stirred for at least 2 h prior to filtering through 80-mesh sieve. During the coating operations, the aqueous dispersions were continuously stirred in order to prevent the sedimentation of the insoluble particles. The coating conditions were as follows: bead charge, 20 g; preheating temperature, 40 °C; preheating time, 10 min; inlet temperature, 35 °C; outlet temperature, 28–30 °C; atomizing air pressure, 0.2 MPa; spray rate, 1 ml/min. Coat application continued until the desired coating level was achieved. The microspheres were further dried in the coating chamber for 20 min after the coating was finished and then were collected and dried in a hot air oven at 40 °C for 12 h.

2.2.2. Determination of drug loading and encapsulation efficiency

The DTZ content in the microspheres was determined by pulverizing the DTZ-loaded microspheres (10 mg) followed by immersing them in 100 ml simulated gastric fluid (SGF, pH 1.2, without enzymes) with agitating at room temperature for 12 h. After filtration through a 0.45 μm membrane filter (Millipore), the drug concentration was determined spectrophotometrically at the wavelength of 236 nm. The filtered solution from the empty microspheres (without DTZ) was taken as blank. All samples were analyzed in triplicate and the drug loading (DL) and encapsulation efficiency (EE) was calculated according to the following equation:

$$\mathrm{DL}(\%) = \frac{W_\mathrm{D}}{W_\mathrm{T}} \times 100$$

DL: drug loading; W_D: the weight of the drug loaded in the microspheres; W_T: the total weight of the microspheres.

$$\mathrm{EE}(\%) = \frac{W_\mathrm{A}}{W_\mathrm{T}} \times 100$$

EE: encapsulation efficiency; W_A: actual drug content; W_T: theoretical drug content.

2.2.3. Study of particle size and morphology of microspheres

The mean diameter of 20 wet and dried microspheres was determined using the optical microscopy (DMBA450, Motic. China). The microscope eyepiece was fitted with a micrometer by which the size of the microsphere could be determined.

Morphological examination of the wet microspheres containing different weight ratio $CaCO_3$ was also carried out by using the optical microscopy (DMBA450, Motic. China).

2.2.4. In vitro evaluation of floating ability of microspheres

2.2.4.1. Visual observation method. For each sample of microspheres, 50 individual microspheres were placed into 500 ml of simulated gastric fluid (SGF, pH 1.2, without enzymes) filled in ChP XC basket type dissolution apparatus. Paddle rotation speed was at 100 rpm, temperature was maintained at 37 ± 0.5 °C. The number

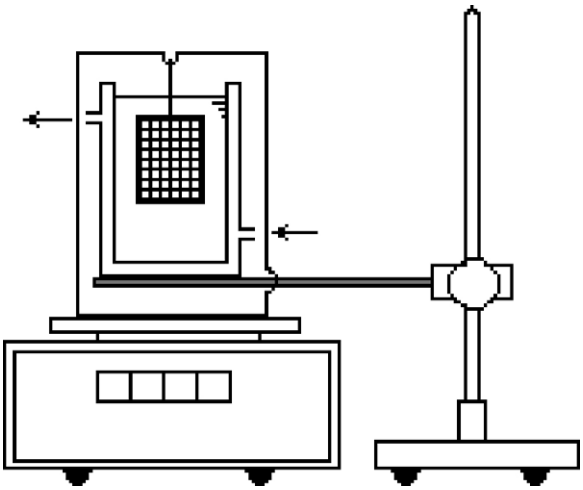

Fig. 1. The apparatus diagram of resultant-weight measuring system.

of floating microspheres was counted visually after 24 h. Experiments were performed in triplicate and the percentage of floating microspheres was calculated according to the following equation:

$$F(\%) = \frac{N_F}{N_T} \times 100$$

F: floating percent; N_F: number of floating microspheres; N_T: total number of the microspheres.

2.2.4.2. Resultant-weight method. The magnitude and the direction of total force F correspond to the vectorial sum of the buoyancy (F_{buoy}) and gravity (F_{grav}) forces acting on the object.

$$F = F_{buoy} - F_{grav} = d_f Vg - d_s Vg$$
$$= (d_f - d_s)Vg = (d_f - M/V)Vg$$

where F is the total vertical force (resultant weight force of the object), g the acceleration of gravity, d_f the fluid density, d_s the object density, M the object mass and V is the object volume.

The total force F acting vertically on an immersed object may be used to quantify the object floating or non-floating capabilities. A positive total force F signifies that the object is able to float, whereas a negative F means that the object sinks (Timmermans and Möes, 1990a). Moreover, the larger the total force F value of the object is, the more excellent its floating capabilities are. The total force F determines resultant-weight of the object in immersed conditions and therefore a special apparatus (Fig. 1) was designed to determine resultant-weight values of microspheres based on the mechanism described by Timmermans and Möes (1990a,b). The medium was 1000 ml preheated simulated gastric fluid (SGF, pH 1.2, without enzymes), the microspheres were placed in a basket which acted as a sample holder. Experiments were performed in triplicate at 37 °C.

2.2.5. In vitro release studies

The in vitro release of DTZ from the different formulations was examined using ChP XC basket type dissolution apparatus. The amount of floating microspheres equivalent to 20 mg drug was placed in the basket. Simulated gastric fluid (pH 1.2, without enzymes) (900 ml) was used as the dissolution medium and maintained at 37 ± 0.5 °C at a rotation speed of 100 rpm. An aliquot of 5 ml of the solution was withdrawn at predetermined time intervals and replaced by 5 ml of fresh dissolution medium. Samples were assayed spectrophotometrically at 236 nm after filtration through a 0.45 μm membrane filter (Millipore). All experiments were performed in triplicate.

2.2.6. In vivo evaluation of floating ability of microspheres (gamma scintigraphy)

2.2.6.1. Radiolabeling of microspheres. Technetium (^{99m}Tc) was selected to radiolabel the microspheres because of its short half-life of 6h and very less amount of electron emission. Floating Cs–Alg microspheres (FM) and non-floating Cs–Alg microspheres (NFM) prepared using above mentioned preparation method containing 1/2($CaCO_3$/Alg, w/w) and 0 $CaCO_3$ respectively were placed separately into the screw cap tube. An aliquot of sodium pertechnetate in saline equivalent to radioactivity of 20 mCi eluted from the technetium generator was added to each tube. The screw cap tubes were shaken to ensure that the microspheres were soaked sufficiently by the labeling solution. The labeled microspheres were recovered by filtration through a filter paper and dried in oven at 40 °C for 0.5 h followed by coating according to above coating operation (coating weight gain = 17%, w/w).

2.2.6.2. Stability of radiolabeled microspheres. Stability tests of ^{99m}Tc-labeled FM and NFM were carried out to confirm that the sodium pertechnetate remained bound to the microspheres for the duration of the study. Tests were carried out according to Atyabi et al. (1996) and Jain et al. (2006) as described: three different standard buffers solutions (pH 1.2, 6.8 and 7.4) were added to three tubes respectively and kept in a water bath maintained at 37 °C. Radiolabeled microspheres (1 g) were placed in these test tubes and kept stirring. At predetermined time intervals 0.2 ml of samples was taken using a pipette with a glass wool filter tip and at the end of the experiment the microspheres were recovered, washed and dried. The radioactivities of the samples, microspheres and the filtrate were counted in an auto gamma counter (CRC-15R, USA). The sum of radioactivity of microspheres, the filtrate and the extreme samples was expressed as the total radioactivity.

2.2.6.3. Gamma imaging in volunteers. Two healthy males, the age, height, weight were 21 and 22 years old, 1.73 and 1.76 m, 63 and 67 kg, respectively, were selected as volunteers and they had given their informed consent to participate in the study. No volunteer was taking any regular medication or had a history of gastro-intestinal disorders. The study was approved by Ethics Committee. After an overnight fast, two volunteers were given 100 ml water to which had been added 20 mCi $^{99m}TcO_4^-$ prior to breakfast for the purpose of outlining the gastrointestinal tract. Then, the volunteers were allowed to consume breakfast consisting of egg, bread and milk. At last, the radiolabeled NFM and FM were filled separately in hard gelatin capsules and were administered to volunteer 1 and 2, respectively. The 140 keV gamma rays emitted by 99 mTc were imaged. The gamma images were recorded using an online computer system (Millennium VG hawk-eye, USA) and static 10-s anterior images were acquired at suitable time intervals. Between the images, volunteers were permitted to sit or stand or carry out normal activities but they were not allowed to take any food for the duration of the study.

3. Results and discussion

3.1. Determination of drug loading and encapsulation efficiency

The drug loading and encapsulation efficiency for all Ca–Alg microspheres were found to be very low (Table 1). The phenomenon can be explained as follows: (1) Diltiazem hydrochlorid is a good hydrophilic drug. (2) In the absence of chitosan, the cross-linking between the sodium alginate and $CaCl_2$ is insufficient and therefore

Table 1
Drug loading and encapsulation efficiencies for floating Ca–Alg microspheres prepared with different concentrations of $CaCl_2$ solution

$CaCl_2$ concentration (%, w/v)	Drug loading (%)	Encapsulation efficiency (%)
0.5	3.57	19.74
1	3.49	19.49
2	3.22	20.12
3	3.07	19.83

the microspheres produced have high degree of porosity, which results in the diffusion of DTZ during and after gelation. The variation in the concentrations of $CaCl_2$ solution had little effect on the encapsulation efficiency of DTZ, while the drug loading decreased as the $CaCl_2$ concentration increased. This may be due to the more crosslinks between the Ca^{2+} and COO^- group of alginate increase the weight of microspheres, but it cannot improve pore size of the microspheres and makes the majority of DLZ diffuse out during gelation. Thus the 0.5% (w/v) $CaCl_2$ was chosen in the subsequent formulae.

To improve the drug loading and encapsulation efficiency of alginate gel microspheres, an attempt was made to prevent the diffusion of the DTZ by dissolving chitosan in the gelation medium. It is obvious that the addition of chitosan to the coagulation fluid significantly increased the drug loading and encapsulation efficiencies (Table 2). The reason for this phenomenon can be explained as what has been described by Bajpai and Tankhiwale (2006): when alginate solution is allowed to be dropped into the gelation medium which is composed of $CaCl_2$ and chitosan in acetic acid glacial solution, the Ca^{2+} ions diffuse into the interior of the drop of alginate and form the gel matrix through ionotropic gelation. At the same time, cationic polymer chitosan present in the gelation media also crosslinks alginate molecules through electrostatic interactions between negatively charged $-COO^-$ groups of alginate and positively charged $-NH_3^+$ groups of chitosan. Alginate–chitosan complex block up the large pore of Ca–Alg gel matrix and form a polyelectrolyte complex membrane on the surface of the microspheres and thereby reduce the permeability of the microspheres. Thus, the diffusion of DTZ is effectively prevented during the gelation. In the presence of more chitosan, this effect became more obviously owing to a denser membrane that can be formed. This effect can be confirmed with the results presented in Table 2: with the increasing of the chitosan concentration (0, 0.2, 0.6, 1.0, 1.4%, w/v), the DEE (19.74, 49.73, 61.25, 78.87, 79.98%) increased accordingly. Nevertheless, it was found unexpectedly that the drug loading increased from 3.57% to 13.58% as the chitosan concentration increases from 0 to 1.0%, while the value is down to 12.95% when the concentration of the chitosan reaches up to 1.4%. This is probably due to the fact that when the chitosan concentration exceeds certain level (1.0%), its effect on reducing the drug loss is less than the function in increasing the weight of microspheres. Thus, chitosan concentration was maintained at 1% (w/v) unless otherwise noted.

On the other hand, in preparation procedure, the different curing time also has an effect on DEE. With the increasing of curing time (10, 20, 30 min), DEE (78.87, 71.22, 63.74%) decreased accordingly. The reason can be explained as what Patel et al. (2006) had described: increased curing time leads to the increase in leaching of hydrophilic drug.

Table 2
Drug loading and encapsulation efficiencies for floating Cs–Alg microspheres prepared with different concentrations of chitosan solution containing 0.5% $CaCl_2$

Cs concentration (%, w/v)	Drug loading (%)	Encapsulation efficiency (%)
0	3.57	19.74
0.2	9.07	49.73
0.6	10.84	61.25
1.0	13.58	78.87
1.4	12.95	79.98

Table 3
The effect of different weight ratio ($CaCO_3$/Alg) $CaCO_3$ on bead size in wet and dry conditions

$CaCO_3$/Alg (w/w)	Mean size (mm) ± S.D., wet microspheres	Mean size (mm) ± S.D., dry microspheres
0	1.68 ± 0.01	0.72 ± 0.02
0.25	2.27 ± 0.05	1.05 ± 0.03
0.5	2.35 ± 0.02	1.17 ± 0.02
0.75	2.44 ± 0.06	1.30 ± 0.05

3.2. Particle size and morphology of microspheres

The optical microscope pictures of a range of wet microspheres prepared with different weight ratio of $CaCO_3$/Alg (0, 0.25, 0.5, 0.75, w/w) are illustrated in Fig. 2. The number of the gas bubbles observed appears to be directly related to the amount of incorporated gas-forming agent. It is clearly demonstrated that the more $CaCO_3$ was added, the more CO_2 was produced by reacting with acetic acid glacial in the gelation medium.

It was observed that the mean sizes of both wet microspheres and dry microspheres increased prominently with the increasing weight ratio of $CaCO_3$/Alg, this was probably due to the effect of more gas bubbles formed after the addition of more $CaCO_3$ (Table 3).

As shown in Table 4, by increasing the chitosan concentration from 0 to 1.4%, the mean sizes of the wet microspheres and dry microspheres were increased from 2.13 and 1.01 mm to 2.42 and 1.25 mm, respectively. It may be attributed to the forming of a thicker chitosan layer with the increase of concentration of chitosan in the gelation medium.

3.3. In vitro evaluation of buoyancy of microsphere

Figs. 3 and 4 described continuously measured resultant-weight values as a function of time for different formulations. As shown in Fig. 3, the microspheres containing gas-forming agents in proportions ranging from 0.25:1 to 0.75:1 presented positive resultant-weight values attributed to the floating capabilities which result from the production of air bubbles during preparation. Furthermore, the resultant-weight values were directly related to the gas content of the polymer matrix. In contrast, the negative resultant-weight value was shown for the gas-forming agent free microspheres. These results were corroborated by the visual observation results: all the gas-forming agent free microspheres sank in the SGF. In contrast, about 90% of the microspheres with $CaCO_3$/Alg ratio of 0.25:1 still floating after 24 h, while more excellent floating abilities of microspheres with $CaCO_3$/Alg ratio of 0.5:1 and 0.75:1 were observed with 100% of the microspheres floating for 24 h at least. The coating microspheres were prepared with the 0.5:1 $CaCO_3$: Alg microspheres and the floating capacities of the differ-

Table 4
The effect of different concentration chitosan on bead size in wet and dry conditions ($CaCO_3$/Alg = 0.5, w/w)

Cs concentration (%, w/v)	Mean size (mm) ± S.D., wet microspheres	Mean size (mm) ± S.D., dry microspheres
0	2.13 ± 0.02	1.01 ± 0.03
0.2	2.26 ± 0.07	1.08 ± 0.05
0.6	2.31 ± 0.01	1.13 ± 0.02
1.0	2.35 ± 0.02	1.17 ± 0.02
1.4	2.42 ± 0.05	1.25 ± 0.04

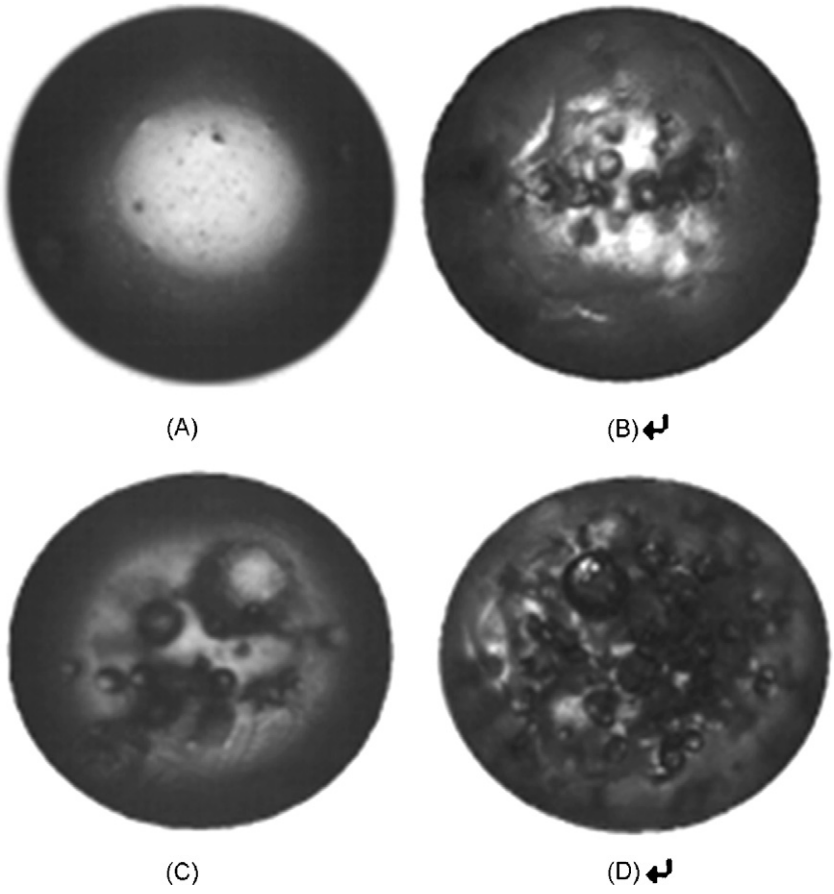

Fig. 2. The optical microscope pictures of different weight ratio of $CaCO_3$/Alg wet microspheres (0, 0.25, 0.5, 0.75, w/w).

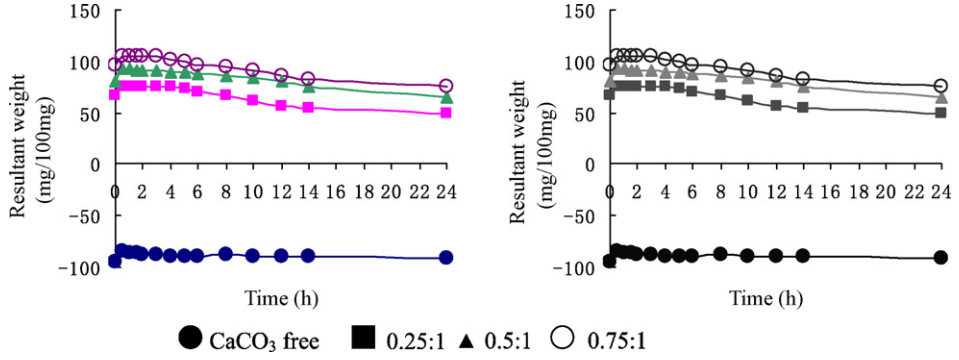

Fig. 3. The resultant-weight values for different $CaCO_3$/Alg weight ratio microspheres.

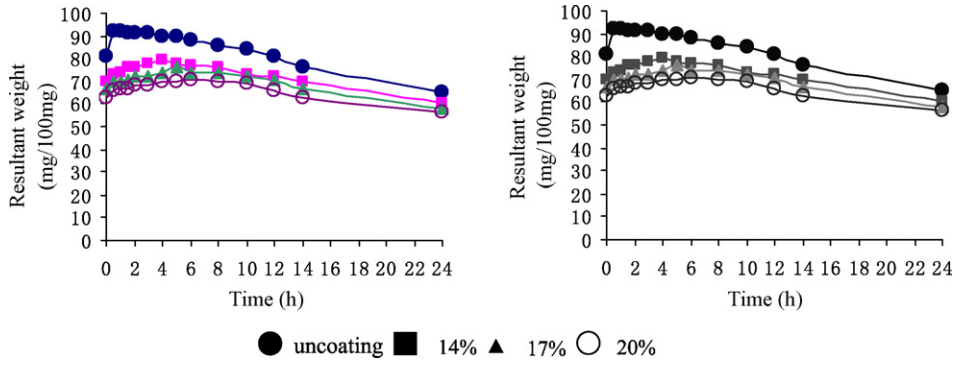

Fig. 4. The resultant-weight values for different coating weight gain microspheres prepared with 0.5:1 $CaCO_3$/Alg (w/w) Cs–Alg microspheres.

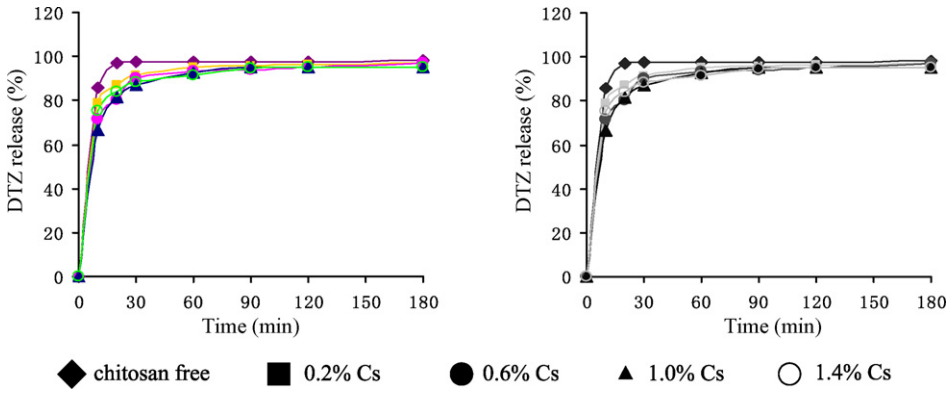

Fig. 5. Profiles of DTZ release from Ca–Alg and Cs–Alg microspheres (0.2–1.4% Cs, w/v).

ent coating weight gain microspheres (14, 17, 20%, w/w) were also evaluated using the above two methods. Fig. 4 showed that the resultant-weight values of the coating microspheres were still positive but decreased with the increasing of coating weight. This was in agreement with what Tang et al. (2007) have found: thick coatings compromised the bead buoyancy. Visual observation method also confirmed the coating microspheres could keep floating for a test cycle of 24 h.

3.4. In vitro release studies

Fig. 5 depicts that DTZ was released rapidly from Ca–Alg gel microspheres in HCl solution, with no more being released after 20 min. It may be attributed to the insufficient cross-linking between the sodium alginate and $CaCl_2$ and the good solubility of DTZ. It is of little value to prolong the gastricretention time (GRT) without sustaining the drug release. To overcome this drawback, two different approaches were adopted.

The first one aimed to make a stronger membrane by electrostatic interactions between alginate and chitosan. This was achieved by the addition of chitosan in the gelation medium. As previously described, the drug loading and encapsulation efficiencies increased significantly in this way. However, no significant change was seen on the drug release after different amounts of Cs were added (Fig. 5). It is most likely that the microspheres swell rapidly on contact with SGF as a result of the hydrophilic groups on the surface of Cs–Alg microspheres, therefore, the aqueous channels which were created owing to the water ingress are enough for hydrophilic micromolecule drug to permeate out in spite of the large pore of Ca–Alg gel matrix blocked by alginate–chitosan complex.

Furthermore, the gas bubbles in the microspheres are suspected to accelerate the DTZ release. The various weight ratio of $CaCO_3$ were used as gas-forming agent to investigate the effect of gas bubbles on the drug release. As shown in Fig. 6, the release of DTZ was not retarded significantly by the reduction of $CaCO_3$. This can be explained as what Choi et al. (2002) have found: $CaCO_3$ is present as an insoluble dispersion in neutral pH aqueous alginate solution; however, in acidic media, the $CaCO_3$ becomes water-soluble. $CaCO_3$ reacts with the acid to produce CO_2, at the same time, the ionized Ca^{2+} ions promote internal gelation by cross-linking with the alginate carboxyl group. Therefore, the addition of $CaCO_3$ did not significantly quicken the DTZ release even though it increased bead porosity and pore size.

It was apparent that the drug release was still rapid by the addition of chitosan and did not correspond with floating drug delivery system. Consequently, a different approach was applied for modifying the drug release by coating the Cs–Alg microspheres. Fig. 7 illustrated the effect of coating with Eudragit RS on DTZ release from the Cs–Alg microspheres. It was evident that the coating microspheres significantly prolonged the DTZ release compared with that of the uncoated Cs–Alg microspheres. The more extensively the microspheres were coated, the slower the drug was released. However, the different coating weight gain microspheres (14%, 17%, 20%, w/w) showed similar drug release patterns. The release of the DTZ contains generally three stages: after a slower release for the initial period, the release from the coated microspheres was approximately linear, followed by a sustained release till drug was exhausted. The drug release mechanism can be proposed as follows: Eudragit RS is water-insoluble, low permeability copolymers. At the incipient stage, the water penetrated through the coating membrane was little and limited amount of water

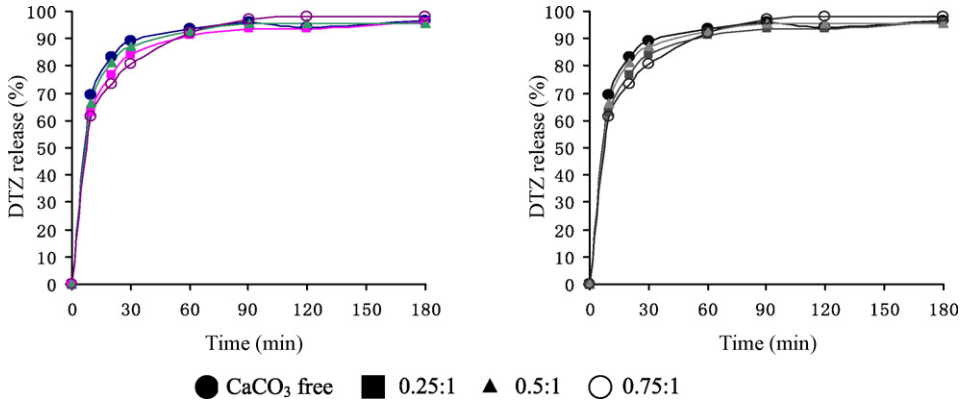

Fig. 6. Profiles of DTZ release from Cs–Alg microspheres prepared with different $CaCO_3$/Alg weight ratio.

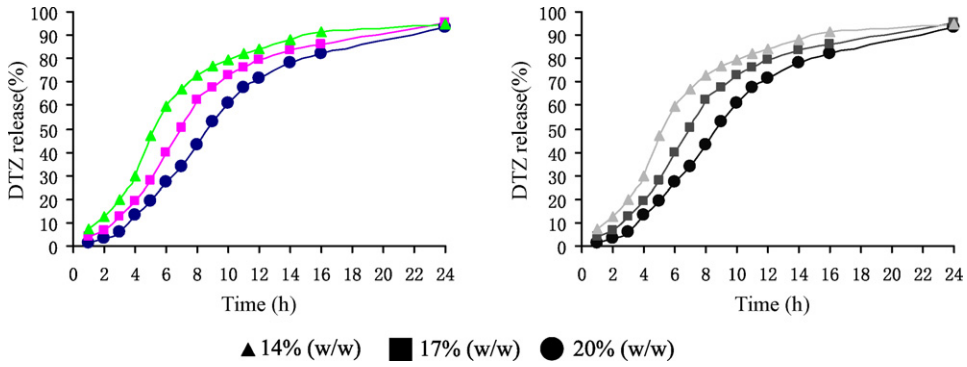

Fig. 7. Profiles of DTZ release from coating microspheres with different coating weight gain.

was diffused into the interior space of Cs–Alg microspheres, drug release become efficient only when the drug was water saturated and thereafter the transport processes through the membrane became diffusion controlled.

As shown in Fig. 7, the coating microspheres can prolong drug release for 24 h. At the incipient stage, the drug effective concentration can be obtained by mixing appropriate ratio uncoating microspheres.

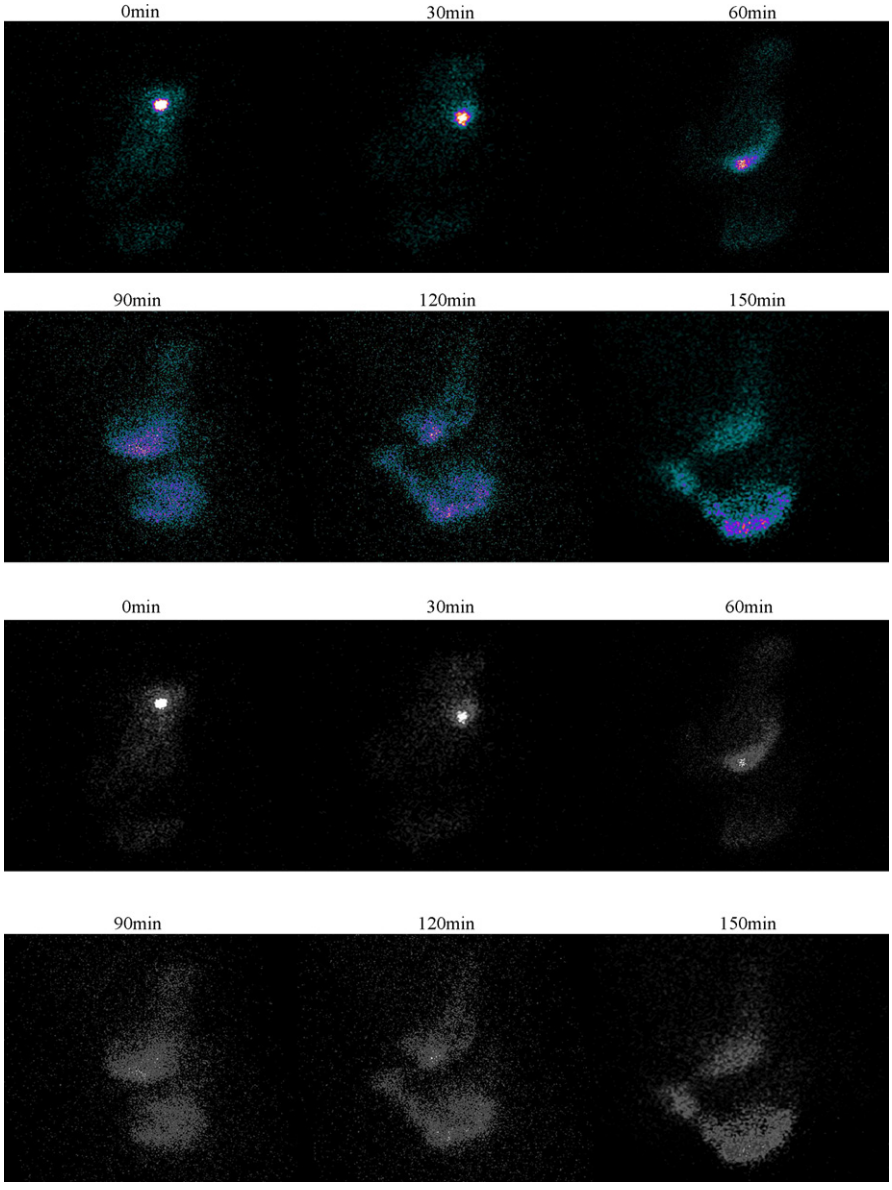

Fig. 8. Gamma scintigraphic images of NFM in volunteer 1.

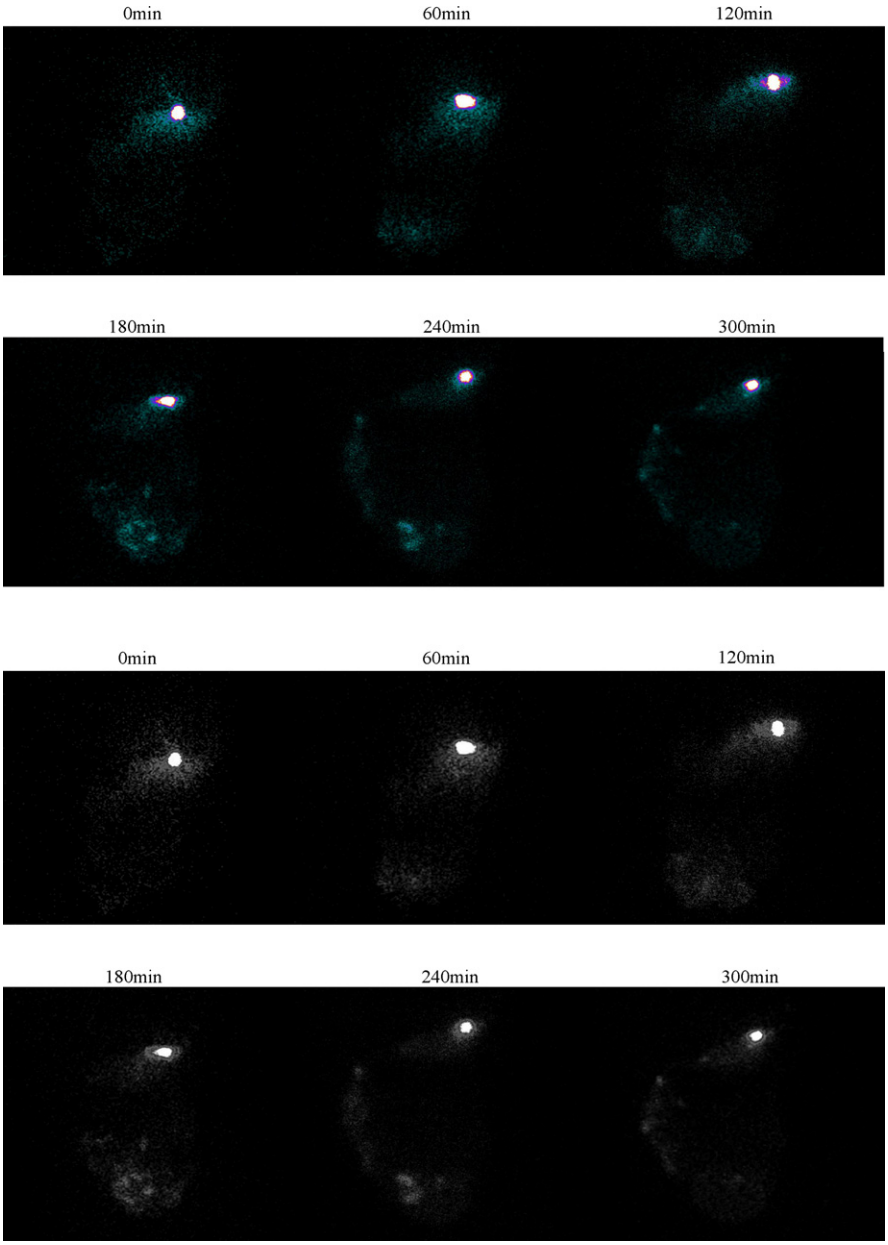

Fig. 9. Gamma scintigraphic images of FM in volunteer 2.

3.5. *Gamma scintigraphy studies*

The optimized coating floating formulation showed good in vitro floating ability and sustained-release behavior. The gamma scintigraphy was applied in order to assess gastro-retentive behavior of the optimized floating formulation in healthy human volunteers by comparison with non-floating microspheres.

The stability of ^{99m}Tc-labeled FM and NFM was tested in standard buffer solutions of pH 1.2, 6.8 and 7.4 in order to confirm that the activity would not leach out from the microspheress during transit time of the formulation through GI tract. The activity released from ^{99m}Tc-labeled FM and NFM was about 0.26% and 0.31% in pH 1.2, 0.33% and 0.26% in pH 6.8, 0.29% and 0.23% in pH 7.4, respectively in the study period of 4 h. Sufficient stability allowed successive gamma imaging for the duration of the study.

Gamma scintigraphic images of volunteer 1(NFM) and 2(FM) were shown in Figs. 8 and 9. It can be seen from sequential gamma scintigraphic images that after administration, the NMF sank rapidly to the base of the stomach, where they gave a discrete bright spot and then had been emptied, the GRT was less than 2.5 h. In contrast, the FM tended to adopt a floating location on the top of stomach content, where they remained and clumped together in one mass for 5 h until the end of the study period.

4. Conclusion

In this study, the alginate microspheres were designed and prepared by ionotropic gelation method for use in floating drug delivery systems. It was found that the drug encapsulation efficiencies of the Ca–Alg microspheres were low and drug release rate was rapid. The addition of the chitosan notably enhanced the drug encapsulation efficiencies but the effect on the drug release was slight and insignificant. The DTZ was released in a sustained manner for 24 h by coating the Cs–Alg microspheres with Eudragit RS.

The resultant weight value of the Cs–Alg microspheres increased with the augment of $CaCO_3$ weight ratio ($CaCO_3$/Alg); the resultant weight force value of the coating microspheres was still positive and inversely related to the coating level. The gamma scintigraphy study confirmed that the optimized floating sustained-release microspheres significantly prolonged GRT compared with non-floating microspheres in vivo. Therefore, such an alginate floating dosage form that is able to delay the release of the hydrophilic drugs within its extended gastric retention time appears to be a promising vehicle for enhancing bioavailability of some hydrophilic drugs.

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