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Preparation and evaluation of a novel gastric floating alginate/poloxamer inner-porous beads using foam solution

Huimin Yao[a,*], Huijuan Yao[b], Junyi Zhu[a], Junlin Yu[a], Lifan Zhang[a]

[a] Department of Pharmacy and Food Engineering, Tonghua Normal University, Tonghua 134002, People's Republic of China
[b] Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai 202150, People's Republic of China

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ABSTRACT

In the present study, a simple and rapid method was developed to prepare a novel kind of inner-porous floating beads. The beads were prepared by dripping the foam solution into $CaCl_2$ solution using disposable syringe needle, where the foam solution consisting numerous of microbubbles with poloxamer 188 as foaming agents, alginate as foaming stablizer. Foamability and foam stability of different polymer ratios were evaluated. The SEM cross-section pictures of the beads showed that the beads were inner-porous and composed of bubbles with very thin wall bubbles stacked together. The visual observation result and the resultant-weight method confirmed that the floating beads showed good buoyancy, most beads could float in the stomach for more than 6 h. The floating beads release behavior *in vitro* showed that drug release from the beads in a sustained-release fashion for 10 h. Gamma scintigraphic images and pharmacokinetic studies *in vivo* showed that the beads can retained in the stomach for over 6 h and can improve the bioavailability of drug with narrow absorption window.

1. Introduction

Oral route is the most convenient and commonly employed form of drug delivery. Many oral drug delivery systems by means of controlled release have been developed to improve drug bioavailability. However, some of these systems do not work as planned as oral sustained release formulation is subjected to frequently changing environments in the gastrointestinal (GI) tract and variation of the stomach emptying time. Therefore, the design of a kind of delivery system with prolongation of gastrointestinal transit and controlled release is required. Several gastrointestinal targeting dosage forms, including intra gastric flotation systems, mucoadhesive systems, adhesion to the gastric mucosal surface in order to extend gastric residence time, magnetic systems, unfoldable, extendible, or swellable systems and super porous hydrogel systems, have been developed. It seems that floating dosage forms offer the most effective and rational protection against early and random times of gastric emptying. Floating has been achieved with the preparation of low-density dry solid systems e.g. inclusion of sponges, highly porous systems (Muller and Anders, 1989; Nakamichi et al., 1996) or with systems, which decrease in density upon contact with gastric fluids based on the expansion of swelling agents (Bolton et al., 1989) or CO_2 generation (Ichikawa et al., 1987). In comparison to the single-unit systems, which are characterized by an all-or-none process, the multiple-unit dosage forms have been shown to reduce inter- and intra-subject variability. Recently, hollow beads with a lower density than that of the GI fluids were adopted.

The floating beads were prepared using some polymers such as alginates (Teerawat et al., 2010), polycarbonate/dichloromethane (Thanoo et al., 1993), silicate (Yousef et al., 2010), cellulose acetate butyrate/Eudragit RL100 mixture in acetone (Stithit et al., 1998) and Eudragit S100/isopropanol. The floating beads can be made by solvent evaporation technique, or by incorporating of gasforming agent such $CaCO_3$ (Ma et al., 2008) or porous structural element (Muller and Anders, 1989). However, organic solvent is introduced which is the drawback of the solvent evaporation technique. While glacial acetic acid which is irritating solvent is used in the technology of incorporating of gasforming agent such $CaCO_3$.

So we developed a new foam technology to prepare the floating beads by dripping method using poloxamer 188 as foaming agents and alginate as foaming stablizer. Alginates are non-toxic, biodegradable, linear co-polymers composed of L-glucuronic and D-mannuronic acid residues. They are widely used in food and pharmaceutical industries. 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). Foam is defined as a dispersion of gas in a liquid or a

* Corresponding author. Tel.: +86 0435 3209377; fax: +86 0435 3208019.
E-mail address: huiminyao@yahoo.cn (H. Yao).

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solid. The presence of a foaming agent is essential for foam generation and stabilisation. Foaming agents are amphiphilic substances: the hydrophilic part of a molecule is responsible for their solubility in water. When a foaming agent is added to water the hydrophobic parts of the molecule arrange themselves in a way to minimize the area of contact with water. This leads to their orientation at the air–water interface and to formation of micelles in the bulk of liquid phase. When a foaming agent is adsorbed into the air–water interface, the surface tension of water is lowered and the surface pressure is increased. Addition of some polymers which leads to the formation of a surfactant–polymer complex through interactions between polymer and surfactant, which contributes to the foam stability (Zhukov et al., 1987) to foamable formulations can lead to an increase of foam stability.

In this article, a simple and rapid method was developed to prepare a novel kind of inner-porous floating beads. The beads were prepared with foam solution using poloxamer 188 as foaming agents, alginate as foaming stablizer. Poloxamer 188 is an effective amphiphilic surfactant and can lower the water surface tension significantly. Foam solution can be formed by stirring in the presence of poloxamer 188, the alginate can winding in microbubbles and stabilised the foam solution. Then the foam solution was dripped into $CaCl_2$ solution through a syringe, the porous beads were formed. The water insoluble Ca–Alg was rapidly formed by gelation of alginic acid in the presence of calcium ions. Poloxamer 188 has also already used in nimodipine sustained-release tablet capable of floating on gastric fluid with prolonged gastric resident time (Wu et al., 1997). The model drug we used, riboflavin (VB_2), is a water-soluble vitamin. It has a recognized narrow absorption window in the upper part of the intestine and a saturable absorption mechanism (Jusko and Levy, 1967). Gamma-scintigraphy was employed to investigate the *in vivo* floating behaviors of the floating beads in volunteers. The pharmacokinetic profile of riboflavin via analysis of urinary excretion of riboflavin on man was used in evaluating the possible gastro-mucoadhesive behavior of floating beads.

2. Materials and methods

2.1. Materials

Sodium alginate (high viscosity grade) was purchased from Yuanhang Chemical (Tianjin, China). Poloxmaer 188 was kindly provided by Beijing Fengli Qingqiu Technology Limited (Shanghai, China). Riboflavin was purchased from Beijing Aoboxing Universeen Bio-Tech Co., Ltd. (Beijing, China). The double distilled water was used throughout the investigations. All other chemicals and reagents were standard pharmaceutical grade.

2.2. Preparation of alginate/poloxamer floating beads

Sodium alginate (Alg) was dissolved in distilled water at a concentration of 1.5% (w/v), poloxamer 188 (poloxamer/Alg = 1/6, w/w) was then added into the sodium alginate solution and agitated vigorously for 20 min. VB_2(VB_2/Alg = 1/6, w/w) was added into the foam solution under vigorous stirring condition continuously. The foam solution was pumped using a pipe with diameter of 2 mm into the $CaCl_2$ solution under gentle stirring condition using a peristaltic pump at a flow rate of 3 ml min^{-1}. The distance between the edge of the needle and the surface of the $CaCl_2$ medium was about 10 cm. The beads formed were left in the solution with gentle stirring for 10 min at room temperature to be cured. The beads were collected, washed with distilled water twice and oven-dried subsequently (40 °C).

2.3. Preparation of alginate non-floating beads

Sodium alginate (Alg) was dissolved in distilled water at a concentration of 1.5% (w/v), VB_2 (VB_2/Alg = 1/6, w/w) was added into the foam solution under vigorous stirring condition continuously. And the prepared method was the same as alginate/poloxamer floating beads, see Section 2.2.

2.4. Foamability and foam stability

Foamability refers to the "ability" of the system to form foam. Foam stability is a parameter describing variations of the foam properties (mostly as changes of height or volume) with time, immediately after the foam was generated (Malysa and Lunkenheimer, 2008). Foams were prepared using magnetic stirring. Different amount of Alg, poloxamer and VB_2 was added into water and agitated for 20 min at 2600 rpm, foams were immediately transferred into a graduated cylinder for continued observation. The initial foam volume after preparation is used to evaluate the foamability. Foamability (FD) was characterized as the physical density of the foam (ratio of volume of foam/volume of liquid used).

$$\mathrm{FD} = \frac{V(\mathrm{foam})}{V(\mathrm{liquid})} \quad (1)$$

Foam stability is characterized as the time interval after which 10 percent of the original amount of liquid has drained from the foam.

2.5. Drug loading and encapsulation efficiency

The riboflavin content in the beads was determined by pulverizing the riboflavin-loaded beads (50 mg) followed by immersing them in 100 ml simulated gastric fluid (SGF, pH 1.2) with agitating at 37 °C for 12 h. After filtration through a 0.45 μm membrane filter (Millipore), the drug concentration was determined spectrophotometrically at the wavelength of 267 nm. The filtered solution from the empty beads (without riboflavin) 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:

$$\mathrm{DL}\ (\%) = \frac{W_D}{W_T} \times 100 \quad (2)$$

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

$$\mathrm{EE}\,(\%) = \frac{W_A}{W_T} \times 100 \quad (3)$$

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

2.6. Morphology

Morphological examination of the wet beads was carried out using vertical microscope (SMZ168-TL, Shanghai, China). The surface characteristic (morphology) and the dimension of the dried beads were carried out using SEM (N-3000, HITACHI, Japan).

2.7. In vitro evaluation of floating ability

2.7.1. Visual observation method

The obtained beads were studied for floating time using ChP XC paddle type dissolution apparatus. Beads (one hundred) of each batch were placed in 100 ml of 0.1 N HCl agitated at 100 rpm, temperature was maintained at 37 °C ± 2. The number of sinking beads

was observed visually. The percentage of floating beads was calculated according to the following equation:

$$F\ (\%) = \frac{N_F}{N_T} \times 100 \quad (4)$$

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

2.7.2. Resultant-weight method

The buoyancy properties of the beads were determined using the resultant weight method. The theoretical background is given by Timmermans and Moes (1989, 1990a,b) and Timmermans (1991). We used the apparatus which is described by Ma et al. (2008). It is composed of an electronic balance (accuracy 1.0 mg), a support, a linear force transmitter, a specially designed basket sample holder and fluid medium. The beads were placed in a basket sample holder, which was immersed in 1000 ml of preheated simulated gastric fluid. One side of the linear force transmitter was fixed on the support, another side was connected to the basket. Thus, the upward and downward forces of the immersed beads were transmitted by the linear force transmitter and support to the balance.

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.

$$F = F_{buoy} - F_{grav} = d_f Vg - d_s Vg = (d_f - d_s)Vg = \left(d_f - \frac{M}{V}\right) Vg \quad (5)$$

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.

2.8. In vitro release studies

The *in vitro* release of riboflavin from the beads was examined using ChP XC paddle dissolution apparatus, that have been modified by placing a wire and mesh assembly in the device with aim to make sure all of the beads immersed in the dissolution media. 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 267 nm after filtration through a 0.45 μm membrane filter (Millipore). All experiments were performed in triplicate.

2.9. In vivo evaluation of floating ability of beads (gamma scintigraphy)

2.9.1. Radiolabeling of beads

Technetium (^{99m}Tc) was selected to radiolabel the beads for its short half-life of 6 h and very less amount of electron emission. Floating beads (FB) and non-floating beads (NFB) prepared using above mentioned preparation method with poloxamer and without poloxamer respectively were placed separately into the screw cap tube. An aliquot of sodium pertechnetate in saline equivalent to radioactivity of 15 mci eluted from the technetium generator was added to each tube. The screw cap tubes were shook to ensure that the beads were soaked sufficiently by the labeling solution. The labeled beads were recovered by filtration through a filter paper and dried in oven at 40 °C for 2 h.

2.9.2. Stability of radiolabelled beads

Stability tests of ^{99m}Tc-labeled FB and NFB were carried out to confirm that the sodium pertechnetate remained bound to the beads for the duration of the study. Tests were carried out according to Atyabi et al. (1996) and Jain et al. (2006) 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. Radiolabelled beads (1 g) were placed in these test tubes and kept stirring. At predetermined time intervals 0.2 ml of samples were taken using a pipette with a glass wool filter tip and at the end of the experiment the beads were recovered, washed, dried. The radioactivities of the samples, beads and the filtrate were counted in an auto gamma counter (CRC-15R, USA). The sum of radioactivity of beads, the filtrate and the extreme samples was expressed as the total radioactivity.

2.9.3. Gamma imaging in volunteers

Two healthy males, the age, height, weight were 23 and 26 years old, 1.76 and 1.74 m, 68 and 65 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 consumed a breakfast consist of bread and milk. The radiolabelled FB and NFB were filled separately in hard gelatin capsules and were administered to volunteers 1 and 2, respectively. The 140 keV gamma rays emitted by ^{99m}Tc 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.

2.10. Pharmacokinetic studies of floating beads

2.10.1. Administration regimen of floating beads

The study was performed on volunteers to evaluate the floating ability of the beads. Six healthy male volunteers, 23–26 years old, gave informed written consent before participation. All subjects were judged to be healthy on the basis of medical history and were not taking any medication including multiple vitamins or riboflavin. Subjects were also asked to avoid eating certain foods known to contain appreciable amounts of riboflavin (such as liver, milk, eggs, and riboflavin-enriched food such as cereals, corn products and noodle products) for at least 48 h prior to and during the study. Each of them ingested FB (containing VB_2 50 mg) and NFB (containing VB_2 50 mg) in hard gelatin capsules with 100 ml of water in a randomized crossover design with a washout period of at least 1 week. Between the study, volunteers were permitted to sit or stand or carry out normal activities.

2.10.2. Collection of urine sample

Subjects emptied their bladder and provided a zero time urine sample prior to dosing, then ingested a formulation. Subjects collected the contents of their urine in volumetric cylinder at 1, 2, 3, 4, 6, 8, 10, 12, and 24 h post dosing. Volume and time elapsed since vitamin ingestion was recorded directly after voiding for each urine sample. Aliquots were frozen at −20 °C until analyzed for riboflavin. Urine samples were protected against exposure to light because of light sensitivity of riboflavin.

2.10.3. Analyses of samples

Approximately 10 ml of urine was centrifuged at 4000 rpm for 10 min. The supernatant was injected onto the HPLC column. The amounts of riboflavin in urine were determined by high performance liquid chromatography (HPLC) (Aglient 1100, USA) with flurorimetric detection (λ_{ex} 450 nm and λ_{em} 530 nm) with the mobile phase of 60:40 orthophosphoric acid (0.5%)–methanol

adjusted to pH 3.0 using triethylamine. The column was a reverse-phase micro-particulate C_{18} (5 μm, 4.6 × 200 mm). Measurements were performed in triplicate. Endogenous riboflavin was taken into account by subtracting the area obtained from analysis of zero time urine sample from standards and samples.

2.10.4. Pharmacokinetic analysis

The different treatments were compared in terms of urinary recovery of riboflavin during the first 24 h after administration, $Recovery_{0-24h}$, maximum urinary excretion rate (R_{max}), and the time (T_{max}) required to reach R_{max}. All parameters were determined from the individual urinary excretion rate–time curves, a plot of urinary excretion rate against the mid-point of a urine collection interval. Urinary excretion rate for each time point of urine collection was calculated by multiplying the concentration of drug in urine for each time point (as determined from the standard curve) by the volume of urine collected to get the amount of unchanged drug excreted in urine during this time interval (Du). This amount was then divided by the time interval for collection of urine sample to obtain the urinary excretion rate (Du/t). Graphs were constructed by plotting (Du/t) versus the midpoint of collection period (t^*). $Recovery_{0-24h}$ was determined from the individual cumulative urinary drug excretion–time curves, a plot relating the cumulative unchanged drug excreted (Du) to the collection time.

3. Results and discussion

3.1. Foamability and foam stability

The process of dispersing the gas phase into bubbles is hard to control because there are various multi-body interactions between the bubble streams generated under dynamic conditions. Thus, the degree of adsorption at the bubbles' interfaces formed depends on the adsorption kinetics and on the velocity of generating the "fresh" solution/gas interface. Pure liquids do not foam (Bikerman, 1973). The presence of a foaming agent is a necessity for foam generation and stabilisation. The foaming agent can lower the surface tension of water and increase the surface pressure as it is adsorbed into the air–water interface. The surface pressure indicates the activity of a foaming agent. Nevertheless, higher values for surface pressure and low values for surface tension do not always lead to an increase of foam stability. For foam stability, the concentration of foaming agent in an adsorbed layer is more important (surface concentration of a foaming agent). During foam formation a rapid adsorption of the foaming agent is desirable. The rate of foaming agent adsorption depends on its diffusion rate, concentration and agitation in the bulk of liquid.

In some homologous series of foaming agents the maximum of foaming ability is observed at a concentration equal to, or near to the critical micelle concentration (Bikerman, 1973). Addition of some polymers to foamable formulations can lead to an increase of foam stability. The similar result was also observed in our study, only a little foam was formed in the surface of the simple poloxamer 188 solution even the concentration reached to 8%, i.e. simple poloxamer 188 is not a kind of good foaming agents. But foam solution was formed even when only 0.025% poloxamer 188 was added into Alg solution. The presence of Alg can increase the foamability of poloxamer 188 significantly. The results of foamability and foam stability were listed in Table 1. It was shown that addition of non-ionic surfactants poloxamer 188 leads to the formation of a surfactant–polymer complex through interactions between polymer and surfactant, which contributes to foamability and foam stability. The higher concentration of the poloxamer 188 can increase the foamability and foam stability of the mixed solution.

Table 1
The foamability and foam stability under different concentration of poloxamer ($n = 3$).

Alg (%)	Poloxamer 188 (%)	Foamability (%)	Foam stability (min)
–	8.0	–	–
1.5	0.4	4.20 ± 0.11	100 ± 4
1.5	0.25	5.63 ± 0.08	75 ± 2
1.5	0.125	6.25 ± 0.14	60 ± 2
1.5	0.05	8.36 ± 0.21	50 ± 3
1.5	0.025	10.71 ± 0.25	37 ± 2
1.5	0	–	–

The concentration of Alg was also evaluated so that it can form beads by dripping it through a syringe. When it is too dilute, no beads were formed when dripping the solution to coagulation solution. On the other hand, when it is too sticky the beads will have a tail or even cannot be dripped out from the syringe. With 1.5% Alg and poloxamer 188 range from 0.025 to 0.4%, beads can form by dripping the solution into coagulation solution. The foam is stable, and can meet our need to prepare floating beads.

3.2. Determination of drug loading capacity and encapsulation efficiency

The maximum drug loading capacity and corresponding encapsulation efficiency of the beads with different polymer ratio were listed in Table 2. From Table 2 we can see that increased poloxamer 188 ratio can improve the maximum loading capacity, and its corresponding encapsulation efficiency can also be improved perhaps the lower efficiency can be attribute to the leak of VB_2 into $CaCl_2$ solution. As the volume of $CaCl_2$ solution and the solubility of VB_2 were fixed, the encapsulation efficiency then can be increased correspondingly.

Another interesting phenomenon was observed. The adding of drug procedure can affect the maximum drug loading capacity significantly. When the Alg, poloxamer 188 and VB_2 were added simultaneously, the stable foam solution cannot be formed even with low VB_2 amount perhaps due to the poloxamer 188 emulsification of VB_2 and then reduce the foamability. Higher drug loading capacity can be obtained by adding VB_2 into formed foam solution. We can conclude that the presence of VB_2 can reduce the foamability, the VB_2 molecular orientate themselves at the surface of foaming solution, cause an increase of the surface pressure and a reduction of the elasticity of the surface film, leading to the rupture of foam.

3.3. Morphology of beads

The vertical microscope pictures of the wet beads were shown in Fig. 1. From Fig. 1 we can see that the inner-porous floating beads were composed of uniform bubbles superimposed together, while the beads prepared with $CaCO_3$ as gas-forming agent contained several large bubbles. The beads with $CaCO_3$ as gas-forming agent were prepared according to Ma et al. (2008). The scanning electron microscopy (SEM) pictures were shown in Fig. 2. The mean

Table 2
Maximum drug loading capacity, corresponding encapsulation efficiencies and density of the beads with different polymer ratio ($n = 3$).

Alg/poloxamer 188	Maximum drug loading (%)	Encapsulation efficiency (%)	Density (g/cm^3)
3.75	23.25 ± 0.31	91.39 ± 0.41	0.23 ± 0.02
6	22.75 ± 0.28	90.27 ± 0.36	0.19 ± 0.01
12	18.38 ± 0.32	85.77 ± 0.34	0.16 ± 0.02
30	13.12 ± 0.23	80.44 ± 0.43	0.12 ± 0.01
60	10.79 ± 0.18	76.53 ± 0.28	0.10 ± 0.01

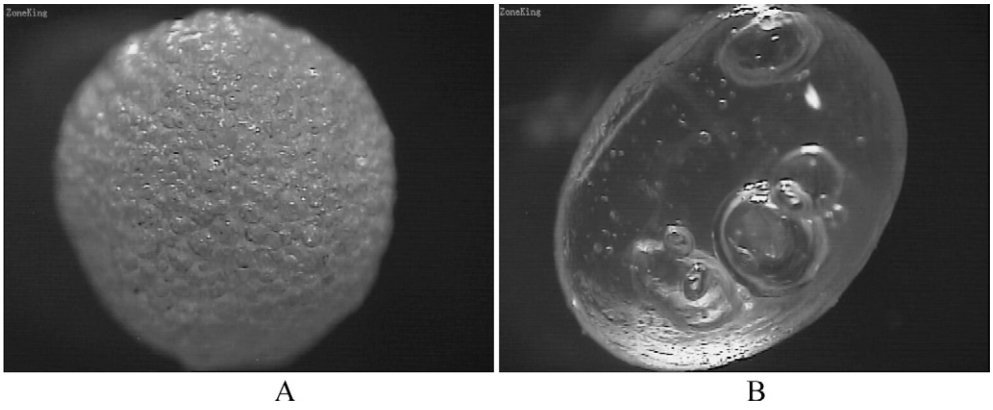

Fig. 1. The surface characteristic (morphology) of the wet beads was evaluated by vertical microscope picture.

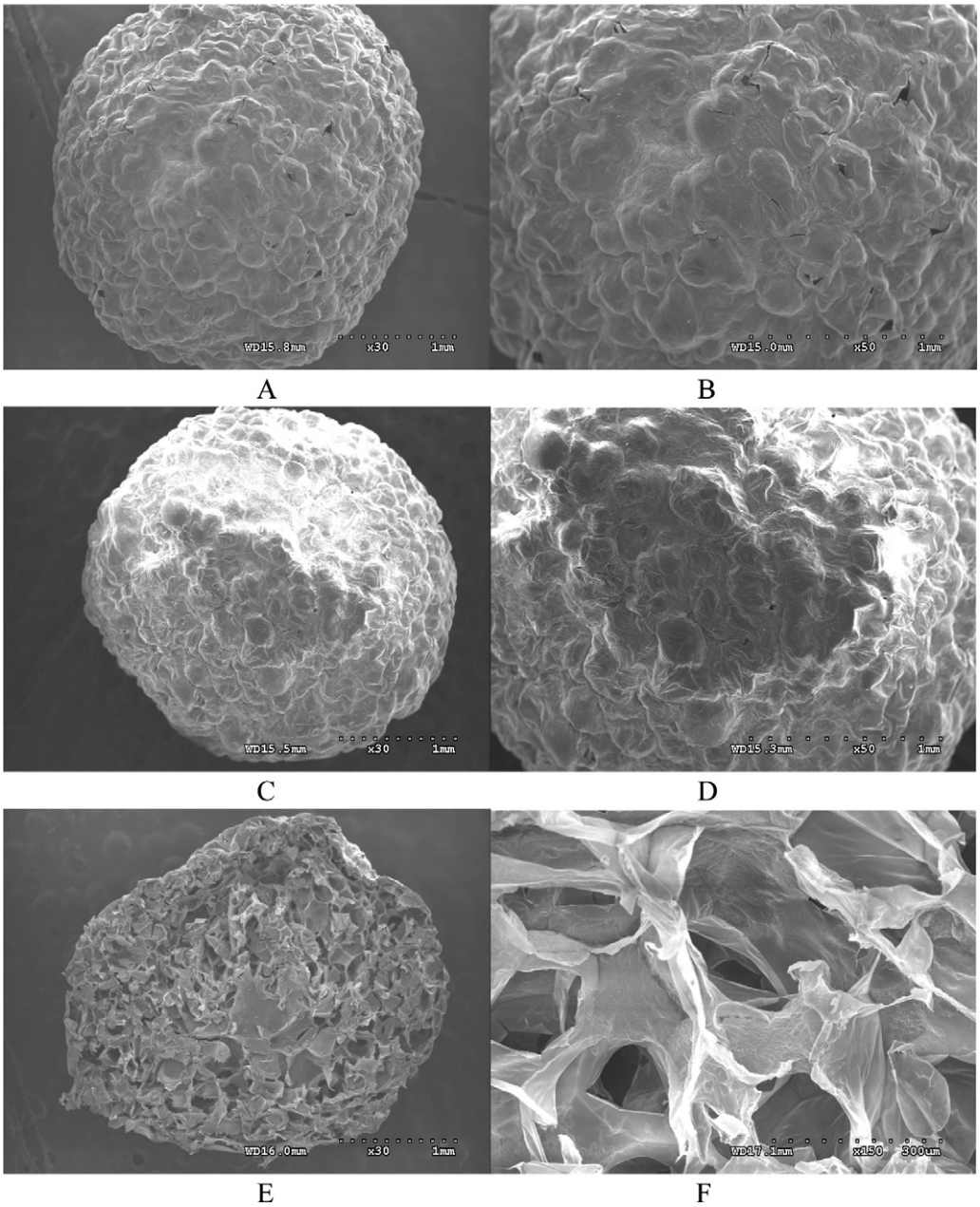

Fig. 2. The surface characteristic (morphology) and the dimension of the dried beads were evaluated by scanning electron microscopy (SEM) picture.

Table 3
The visual observation result of beads with different VB_2 drug loading prepared using Alg/poloxamer 188 (6:1).

Time (h)	0	0.5	1	2	4	6	8	10
Drug loading (%)								
22.75	100	100	96	81	78	73	70	64
18.60	100	100	98	95	93	91	88	85
12.5	100	100	100	100	100	94	86	75
5.4	100	100	100	100	100	95	89	79

diameter of dried inner-porous floating beads was 3.21 ± 0.14 mm, the surface of the beads is smooth and bubble-like. There are some break bubbles in the higher VB_2 loading beads, while the lower VB_2 loading beads are more intact. This is corresponding to the drug releasing results, the burst release phenomenon of with higher drug loading amount was more obvious due to the break bubbles. It can be seen that the presence of too many VB_2 molecules can reduce the foam stability and lead to the break of some bubbles. The cross-section pictures of the beads showed that the beads were inner-porous and composed of bubbles with very thin wall bubbles stacked together.

3.4. In vitro evaluation of floating ability of beads

The *in vitro* buoyancy ability of beads was evaluated by two methods: visual observation method and resultant-weight method.

The beads can float on the surface of SGF at the beginning of the experiment. As time passed on, the beads could absorb water and sink in water or disintegrated. It is the most important factors challenging the floating ability of these beads. The beads are floating because of their low density than SGF. When water penetrating into the beads and take the place of gas, the density of the beads became bigger than that of SGF, then the beads will sink. If the beads were disintegrated, the fragment will disperse in the SGF.

The visual observation result of beads with different VB_2 drug loading prepared using Alg/poloxamer 188 (6:1) was shown in Table 3. From the Table 3, we can see that the beads showed good buoyancy, most beads could float for more than 6 h. The reduce of VB_2 drug loading could prolong the floating time of the beads, perhaps due to the break bubbles in the beads surface (Fig. 2). The beads with VB_2 drug loading of 12.5% already showed good buoyancy, and was chosen for the premium formulation.

Although the method verified that the beads are buoyant, it is inadequate. As such a system provides no information about the kinetics of the floating dosage form. The resultant-weight method was performed using the apparatus designed by Ma et al. (2008).

The resultant-weight method results of beads with different VB_2 drug loading prepared using Alg/poloxamer 188 (6:1) was shown in Fig. 3. The floating ability of the beads reduced as the increase of the drug loading amount. It possessed good floating ability when the drug loading ration decreased to 12.5%. We can conclude that floating ability of beads is directly affected by the foamability and foam stability of the foam solution.

3.5. In vitro release studies

Dissolution testing is frequently used to assess drug release from oral dosage forms and is essential to estimate how the release of the model drug would occur *in vivo*. The dissolution of a drug from a dosage form is affected by agitation intensity, pH, the type of medium, amount of aeration of the medium and the area of exposure of the drug delivery system to the medium. Traditionally baskets have been used to enclose multi-particulate formulations, but using such a method to assess drug release from the dosage from results in a reduced exposure of some of the calcium alginate

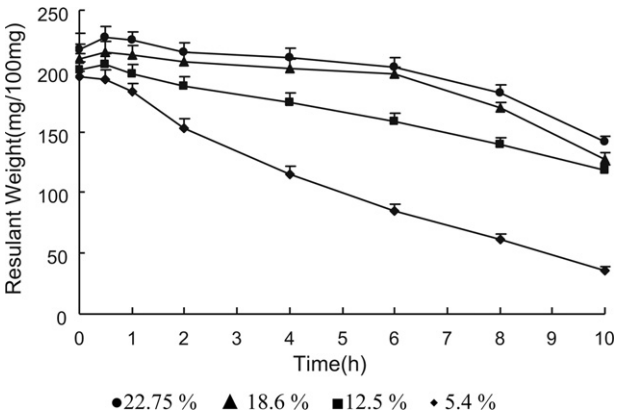

Fig. 3. The resultant-weight values of beads with different VB_2 drug loading prepared using Alg/poloxamer 188 (6:1).

beads to the medium, due to the volume of the basket being taken up by a comparatively large volume of beads.

Performing dissolution studies using the paddle method only is equally unacceptable for floating dosage forms because only a proportion of the dosage form is exposed to the media. In addition, the placement of the beads is not consistent and hence results are not reproducible (Fassihi, 1995). The presence of the mesh, as used for the current work, ensured that the full surface area of the beads was exposed to the dissolution medium (Pillay and Fassihi, 1998) and the paddle ensured sufficient agitation of the medium for drug dispersion. Previous studies have shown that the mesh and paddle method provides more reproducible and reliable dissolution profiles compared to other conventional methods (Fassihi, 1995).

The *in vitro* VB_2 release from the beads in SGF is shown in Fig. 4. From Fig. 4, we can see that drug release from the beads in a sustained-release fashion for 10 h. There exists burst release phenomenon during the first initial release stage for 1 h. The burst release phenomenon of with higher drug loading amount was more obvious. Reducing the drug loading amount can avoid the burst release phenomenon effectively. The release kinetics behavior of the inner-porous floating beads was modeled using zero-order kinetics model. The release data in 1 h were omitted to minimize the effect of the burst release phenomenon. The release kinetics of the inner-porous floating beads after 1 h fitted the zero-order kinetics model well. The correlation coefficients were larger than 0.96. The sustained release behavior of the floating inner-porous beads might due to the inner-porous structure. The beads consisted of numerous of bubbles, drug could only be released when the water penetrate into the inner of the beads. While the bubbles were intact and filled with air, so it would need time for water to wet the beads

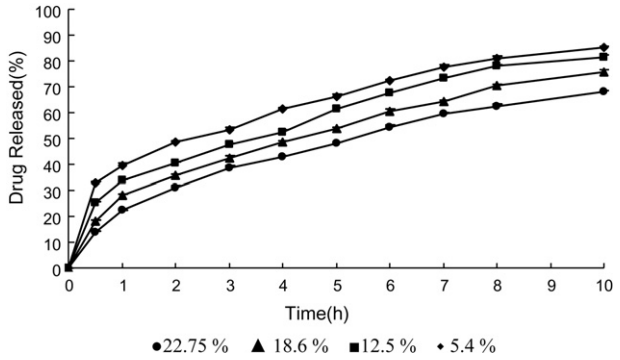

Fig. 4. The release profiles of beads with different VB_2 drug loading prepared using Alg/poloxamer 188 (6:1).

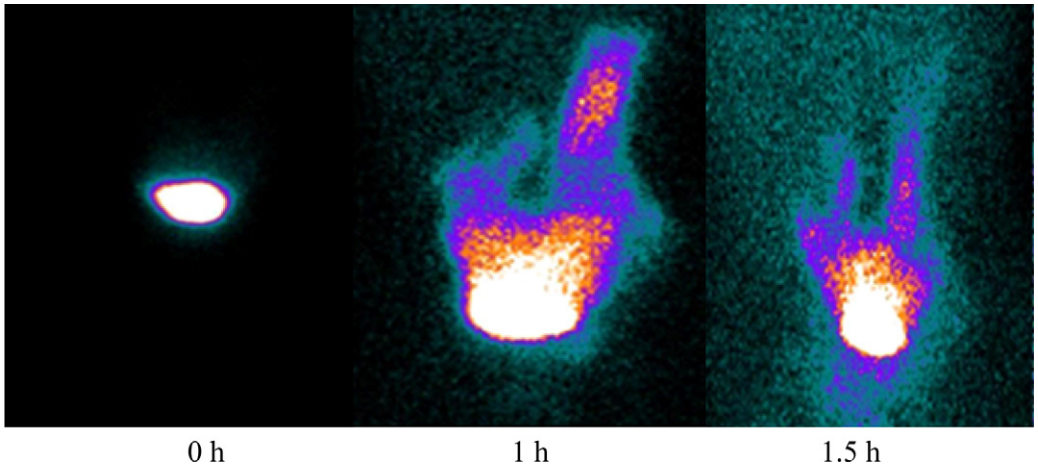

Fig. 5. Gamma scintigraphic images of NFB in volunteer 1 after 0, 1, 1.5 h respectively.

core. However the release mechanism and kinetics of the floating inner-porous beads should be further studied.

3.6. Gamma scintigraphy studies

The gamma scintigraphy was applied in order to assess gastro-retentive behavior of the floating beads in healthy human volunteer with non-floating beads as a comparison. The mean diameter of dried non-floating beads was 3.12 ± 0.16 mm, the drug loading was 12.3%. The stability of ^{99m}Tc-labeled floating beads (FB) and non-floating beads (NFB) was tested in standard buffer solutions of pH 1.2, 6.8 and 7.4 in order to confirm that the activity would not leached out from the beads during transit time of the formulation through GI tract. The activity released from ^{99m}Tc-labeled floating beads (FB) and non-floating beads (NFB) was about 0.21% and 0.22% in pH 1.2, 0.22% and 0.20% in pH 6.8, 0.22% and 0.21% in pH 7.4, respectively in the study period of 4 h. Sufficient stability allowed successive gamma imaging for the duration of the study.

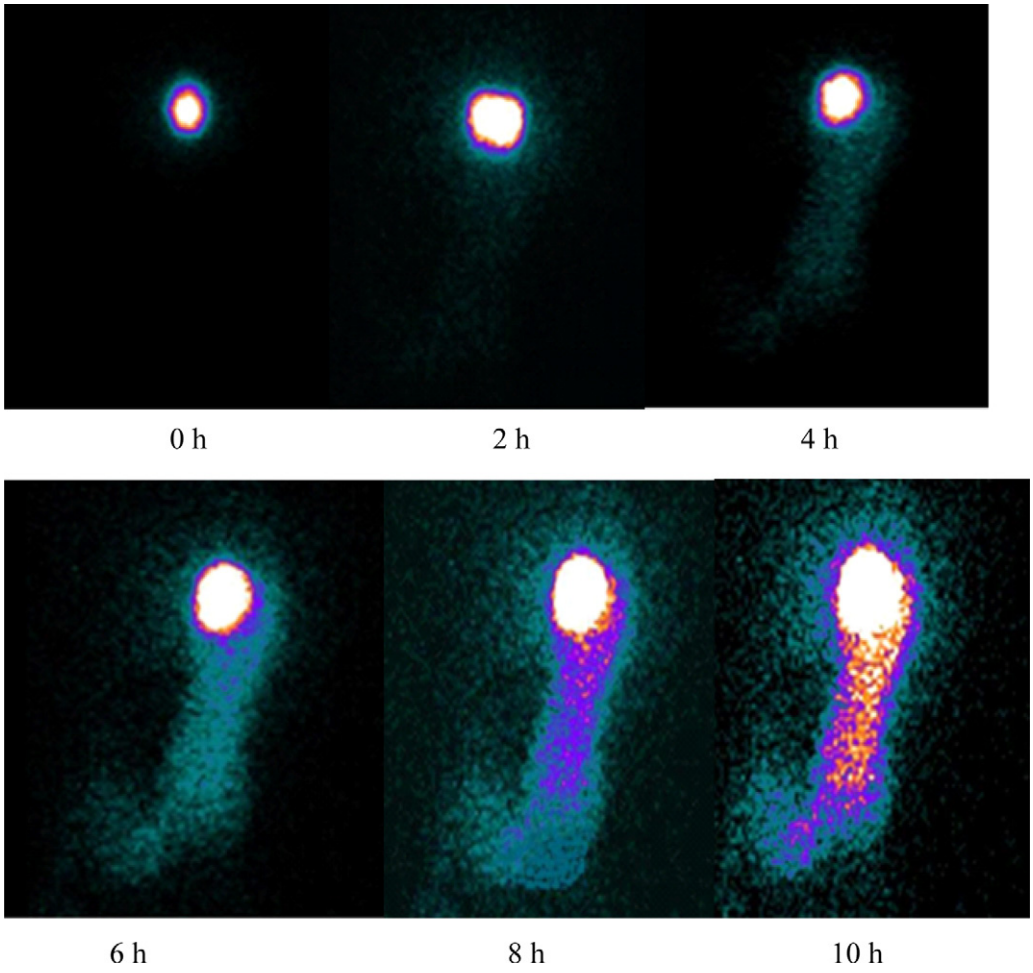

Fig. 6. Gamma scintigraphic images of FB in volunteer 2 after 0, 2, 4, 6, 8, 10 h respectively.

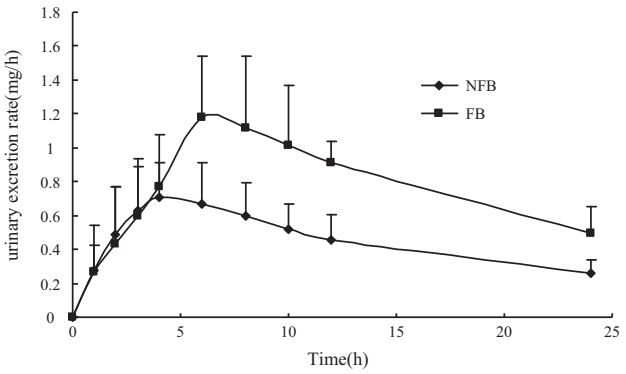

Fig. 7. Average urinary excretion rate of riboflavin for fasted volunteers after administration of NFB and FB (data are mean values ($n = 6$) ± S.E.).

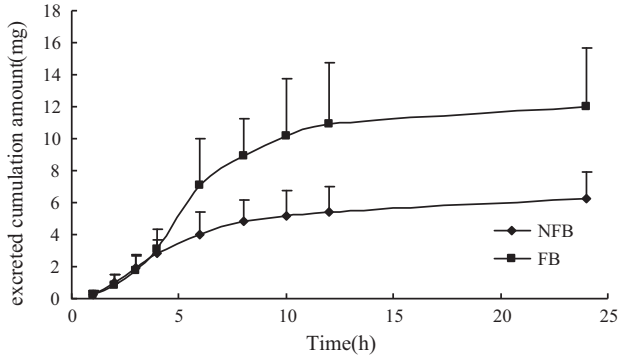

Fig. 8. Average cumulative amount of riboflavin excreted in urine for fasted volunteers after administration of NFB and FB (data are mean values ($n = 6$) ± S.E.).

Gamma scintigraphic images of volunteers 1 (NFB) and 2 (FB) were shown in Figs. 5 and 6. It can be seen from sequential gamma scintigraphic images that the NFB sank rapidly to the base of the stomach after administration, where they gave a discrete bright spot and then been emptied. There were only about 30% microspheres retained in the stomach. In contrast, most of the FB floating on the top of stomach content for about 6 h. There were still about 70% microspheres floating in the stomach.

3.7. Pharmacokinetic studies

The adsorption of riboflavin was evaluated from urinary excretion data (Fig. 7). The cumulative unchanged drug excreted (*D*u) to the collection time was shown in Fig. 8. Statistical comparison of $Recovery_{0-24h}$ parameters indicated a significant difference ($P < 0.05$) between results obtained from NFB and FB, i.e. $Recovery_{0-24h}$ for FB was 11.97 ± 2.60 mg, whereas that for NFB was 6.21 ± 0.38 mg. Statistical comparison of T_{max} parameters also indicated a significant difference ($P < 0.05$) between results obtained from NFB and FB. The improved bioavailability of riboflavin from FB (urinary recovery was about twice than that measured after administration of NFB) obtained in this study, suggests that the FB could retain in the stomach and release drug slowly. The FB stayed in the stomach for enough time to slowly release drug and consequently the released riboflavin passed gradually through the absorption window and was absorbed more efficiently.

4. Conclusion

In the present study, a simple and rapid method was developed to prepare a novel kind of inner-porous floating beads. The beads were prepared using foam solution consisting numerous of microbubbles with poloxamer 188 as foaming agents, alginate as foaming stablizer. Foamability and foam stability of the foam were investigated. The addition of non-ionic surfactants poloxamer 188 could lead to the formation of a surfactant–polymer complex through interactions between polymer and surfactant, which contributes to foamability and foam stability. The higher concentration of the poloxamer 188 can increase the foamability and foam stability of the mixed solution. The SEM cross-section pictures of the beads showed that the beads were inner-porous and composed of bubbles with very thin wall bubbles stacked together.

The maximum drug loading capacity and corresponding encapsulation efficiency of the beads can be improved by increasing poloxamer 188 ratio. The adding of drug procedure can affect the maximum drug loading capacity significantly, the floating beads could only be formed by incorporating VB_2 into the already prepared foam solution. The visual observation result and the resultant-weight method confirmed that the floating beads showed good buoyancy, most beads could float in the stomach for more than 6 h. The floating beads release behavior *in vitro* showed that drug release from the beads in a sustained-release fashion for 10 h.

Gamma scintigraphic images and pharmacokinetic studies *in vivo* showed that the beads can retain in the stomach for over 6 h and can improve the bioavailability of drug with narrow absorption window.

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