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A floating multiparticulate system for ofloxacin based on a multilayer structure: In vitro and in vivo evaluation

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ABSTRACT

The purpose of this research was to develop a novel gastroretentive multiparticulate system with floating ability. This system was designed to provide drug-loaded pellets coated with three successive coatings—the retarding film (ethyl cellulose), the effervescent layer (sodium bicarbonate) and the gas-entrapped polymeric membrane (Eudragit® RL 30D). The floating pellets were evaluated for SEM, floating characteristic parameters, in vitro release and bioavailability in New Zealand rabbits. The zero-order release theory model is designed to interpret the release processes. Due to the swelling property, high flexibility and high water permeability, Eudragit® RL 30D was used as a gas-entrapped polymeric membrane. The obtained pellets exhibit excellent floating ability and release characteristics. Analysis of the release mechanism showed a zero-order release for the first 8 h because of the osmotic pressure of the saturated solution inside of the membrane, which was in accordance with that predicted. Abdominal X-ray images showed that the gastroretention period of the floating barium sulfate-labeled pellets was no less than 6 h. The relative bioavailability of the floating pellets compared with reference tablets was 113.06 ± 23.83%. All these results showed that the floating pellets are a feasible approach for the gastroretentive drug delivery system.

1. Introduction

Oral sustained release delivery systems are a common and popular route for drug administration, due to the excellent patient compliance and dose reliability. However, good drug bioavailability is a major problem in the development of oral sustained release drug delivery systems. The drug bioavailability of pharmaceutical dosage forms is influenced by various factors (Sungthongjeen et al., 2008). One of the most important factors is the gastric residence time (GRT) of these dosage forms (Desai and Bolton, 1993). A short gastric emptying time results in an incomplete release of the drug from the drug delivery system leading to reduce efficacy of the administered dose (Chueh et al., 1995). Among the various drug products of prolonging the gastric residence time, floating sustained release systems have been studied to obtain a well gastroretentive dosage form (Reddy and Murthy, 2002; Strusi et al., 2008). The system has a bulk density lower than that of the gastric fluid (Moes, 1993). The floating systems consist of two types: effervescent systems, and non-effervescent systems (Tadros, 2010). The former is divided into two sub types, including a single unit floating system and a multiple unit floating system (Tadros, 2010; Chavanpatil et al., 2006; Strubing et al., 2008; Jain et al., 2005; Arora et al., 2005). The multiple unit floating system has the advantages of reducing the chance of dumping and reducing any variation in drug release and absorption.

As reported, floating drug delivery is of particular interest for drugs which: (a) act locally in the stomach; (b) are absorbed primarily in the stomach; (c) exhibit poor solubility at an alkaline pH; (d) have a narrow window of absorption (Tadros, 2010; Singh and Kim, 2000).

Ofloxacin is a fluoroquinolone antibacterial agent, which has a broad antimicrobial spectrum against both Gram-positive and Gram-negative bacteria. It is approved for use in the treatment of gastrointestinal infections, respiratory tract infections and urinary tract infections (Zivanovic et al., 2006). The higher bioavailability and only minor biotransformation ensure a high therapeutic effect (Marier et al., 2006; Yue et al., 2008). Ofloxacin exhibits pH-dependent solubility (Chavanpatil et al., 2006). The drug is freely soluble in acid medium but poorly soluble at an alkaline pH. The absorption site is in the upper part of the gastrointestinal tract. Due to the poor solubility in the intestinal tract and the narrower absorption window, ofloxacin gastroretentive sustained release

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tablets have been developed by a research group (Chavanpatil et al., 2006). The aim of the present investigation was to design and develop novel ofloxacin floating sustained release pellets. Two-layer coated pellets have been reported in the literature, but these met only the demand of the drug that was poorly soluble in acid medium (Sungthongjeen et al., 2006). However, there are no relevant studies about floating sustained release pellets for drugs that are freely soluble in acid medium. In this paper, a new form involving three successive coatings of pellets based on the gas formation technique was described to prepare ofloxacin floating sustained release pellets with improved oral bioavailability.

The drug loaded pellets were prepared by the extrusion–spheronization technique followed by coating with a release retarding film (EC), an effervescent layer ($NaHCO_3$) and a gas-entrapped polymeric membrane (Eudragit® RL 30D), respectively. The amount of coating, in vitro and in vivo buoyancy, pellet characteristics, release properties and in vivo pharmacokinetics were investigated. A new theory for the evaluation of the release mechanism was designed. Pellets were subjected to in vivo X-ray analysis and a pharmacokinetics study was investigated by using UPLC/MS/MS. Until now, there have been no relevant literatures reports about the three mentioned above.

2. Materials and methods

2.1. Materials

Ofloxacin was purchased from Zhejiang Kangyu Pharmaceutical Co. Ltd. (Zhejiang, China). MCC (AVICEL® PH-105, USA) was used to prepare the core pellets. EC 10cp was a gift from Colorcon and PVP_{K30} was purchased from Boai Xinkaiyuan, Henan, China. HPMC E5 (Huzhou Zhanwang, ZheJiang, China) plasticized with PEG 6000 (Bodi, Tianjin, China) was used as a binder for $NaHCO_3$ (Bodi, Tianjin, China). Eudragit® RL 30D was a gift from Röhm Pharma, Darmstadt, Germany. Triethyl citrate and barium sulfate were China Pharmacopoeia grade. Methanol and acetonitrile were HPLC grade and dichloromethane was analytical grade. Ciprofloxacin (internal standard) was purchased from Zhejiang Jiangnan Pharmaceutical Co. Ltd. (Zhejiang, China). Ofloxacin tablets (0.1 g, Shuanghe, Beijing, China) were chosen as the reference preparation.

2.2. Theory methods

The pellets coated with a semi-permeable membrane were analyzed using the following theoretical methods. The two parallel transport processes through the coating: transport through the intact coating and transport through pores, cracks or a drilled hole (Lindstedt et al., 1991). For pellets coated with such a semi-permeable membrane, the main processes that occur are: influx of the dissolution medium, dissolution of the drug, build-up of osmotic pressure and hydrostatic pressure, transport of the drug from inside the membrane to the medium. The release pores are the main route of drug release. To facilitate and enable an evaluation of the osmotic pressure release mechanism, three assumptions were made as follows:

1) The core pellets are spheriform and the drug is uniformly distributed in the pellets.
2) The coating film is rigid and not deformed, and the thickness of the film is uniform.
3) To minimize the drug diffusion transport and avoid a build-up of hydrostatic pressure, the membrane release pores are the right size.

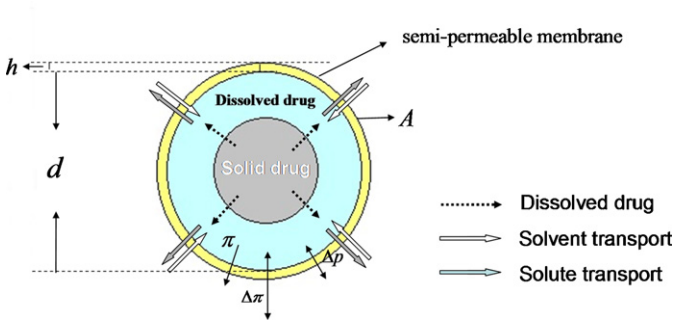

Fig. 1. Schematic illustration of the release process of the pellets.

A schematic illustration of the release process of the pellets is shown in Fig. 1.

The Theeuwes elementary osmotic pump equation (Theeuwes, 1975) was used in the semi-permeable membrane release system to describe the dissolution process:

$$\frac{dV}{dt} = \frac{A}{h} L_p \times (\Delta\pi - \Delta p) \tag{1}$$

where A is the surface area, h is the coating thickness, L_p is the mechanical permeability, $\Delta\pi$ is the osmotic pressure difference across the coating, Δp is the difference in the hydrostatic pressure across the coating.

As $\Delta\pi \gg \Delta p$ and the hydrostatic pressure is minimized, Δp can be neglected compared with the $\Delta\pi$. The drug concentration in the dissolution medium was minimized compared with that inside the pellet, so π can be substituted for $\Delta\pi$, the equation can be written as:

$$\frac{dV}{dt} = \frac{A}{h} L_p \times \pi \tag{2}$$

In Eq. (2) π is the osmotic pressure inside the pellet.

The osmotic pressure equation (Martin et al., 1994) was as follows:

$$V\pi = nRT \tag{3}$$

where V is the solution volume, n is the number of moles, R is the gas constant, and T is the thermodynamic temperature. Eq. (3) can be changed to Eq. (4):

$$\pi = \frac{c}{M} RT \tag{4}$$

where c is the concentration of the drug in the dissolved phase within the pellet. Eqs. (3) and (4) to give Eq. (5):

$$\frac{dm}{dt} = \frac{A}{hM} L_p RT \times c^2 \tag{5}$$

During the release process, if the solid drug inside the coating pellet is not completely dissolved, c is the drug concentration at saturation (c_{sat}) and the release rate is held constant. After a time point, c is not c_{sat} due to the fact that the solid drug inside the coated pellet was completely dissolved. c was reduced and release rate became slower. So, the change point is the critical point. Before the critical point, the drug release was kept zero order under steady state conditions. Thus, the release driven by the osmotic pressure was the main release mechanism. The critical point value can be calculated according to the following equation (McClelland et al., 1991; Zentner et al., 1991; Verma et al., 2002):

$$\frac{M_\tau}{M_0} = 1 - \frac{c_{sat}}{\rho} \tag{6}$$

Here, M_τ is the drug amount released at zero-order release, M_0 is the total amount of drug, and ρ is the density of solid drug. The critical point value can be calculated. By comparison with the

practical value, this method can be confirmed later. This method may, if shown to be valid, allow estimation of the critical value at zero-order release for this type of coating pellets.

2.3. Preparation of floating sustained-release pellets

2.3.1. Preparation of drug-loaded pellets

The drug-loaded pellets were prepared using the extrusion–spheronization device (WL350, Wenzhou Pharmacy Equipment Factory, China). 60% (w/w) ofloxacin and 40% (w/w) MCC were mixed uniformly through a 80 mesh sieve. A modest amount of distilled water was added and then the damp mass was extruded from a 1.2 mm screen at 100 rpm. The extrudates were spheronized and the rotation speed was held at 500 rpm for about 20 min. The damp drug-loaded pellets were dried at 40 °C for 12 h. The 16–24 mesh pellets was collected for the next coating.

2.3.2. Coating of drug-loaded pellets

The drug-loaded pellets were coated with three successive coatings. EC was chosen as the release retarding film. EC and PVP_{K30} (in a ratio of 4:1, w/w) were dissolved in 95% alcohol with an EC concentration of 3% (w/v). PVP_{K30} was added as the pore-forming and plasticizing agent. Then, 300 g drug-loaded pellets was coated in a fluidized bed coater (FD-MP-01, Powrex, Japan). The coating parameters were as follows: temperature = 30 ± 2 °C, spray rate = 2.5–3 ml/min, atomization pressure = 1.2 bar. After coating, the 16–24 mesh pellets was collected again for the next process step.

An effervescent layer and gas-entrapped polymeric membrane were the second and third coatings for the floating pellets, respectively. $NaHCO_3$ was dissolved in HPMC solution and the ratios of $NaHCO_3$ to HPMC were 1:4, 1:2, 1:1, 2:1 and 4:1 (w/w). The PEG6000 (10%, w/w of HPMC) was incorporated as the plasticizing agent. The weight gain was fixed at 12% (w/w). The coating process was conducted in a fluidized bed coater (FD-MP-01, Powrex, Japan). The conditions were given as follows: the temperature was 38–40 °C, the spray rate was 9–10 ml/min, and the atomization pressure was 1.2 bar. After coating, the pellets was further fluidized for 45 min at 40 °C to remove the residual moisture for further experiment.

The gas-entrapped polymeric membrane was sprayed to give $NaHCO_3$-layered pellets. Eudragit® RL 30D was used as the plasticizer with 20% DEP (w/w based on the polymer solids). The aqueous polymer dispersions were diluted with purified water to give a 15% (w/w) solid content of the coating dispersions. The process parameters were as follows: the temperature = 40 ± 2 °C, the spray rate = 4–5 ml/min, and the atomization pressure = 1.2 bar. The main formulations are listed in Table 1.

Table 1
The main formulations at different research stages.

Formulation/ composition	EC (w/w)	$NaHCO_3$:HPMC 12% (w/w)	Eudragit® RL 30D (w/w)
F1	–	1:4	5%
F2	–	1:2	5%
F3	–	1:1	5%
F4	–	2:1	5%
F5	–	4:1	5%
F6	–	1:4	10%
F7	–	1:2	10%
F8	–	1:1	10%
F9	–	2:1	10%
F10	–	4:1	10%
F11	–	1:4	15%
F12	–	1:2	15%
F13	–	1:1	15%
F14	–	2:1	15%
F15	–	4:1	15%
F16	1.7%	4:1	15%
F17	2.0%	4:1	15%
F18	2.3%	4:1	15%

2.4. In vitro release

In vitro release studies were carried out using an USP 29 dissolution apparatus 1. The rotation speed was 50 rpm. Studies were conducted at 37 ± 0.5 °C in 900 ml 0.1 N HCl. All tests were carried out in triplicate. Measurements were performed on the pellets (containing about 200 mg drug) filled into capsules. The capsules was placed in release medium. At predetermined time intervals, samples were analyzed in a UV spectrometer method at 293 nm.

2.5. Evaluation of the floating pellets

2.5.1. Scanning electron microscopy (SEM) studies

To study the micromorphology of the floating pellets, the surface and internal morphologies of the pellets were examined and photographed by SEM. After dissolving, the pellets were dried at 40 °C. The internal morphology of the pellets was examined by cutting them into hemisphere. Then samples for SEM were coated with gold under a high vacuum. The images were observed by scanning electron microscopy.

2.5.2. In vitro floating ability study

Floating behavior of the floating pellets was examined by using a USP 29 dissolution apparatus 1 (50 rpm, 37 ± 0.5 °C, 900 ml, 0.1 N HCl). For this, 100 pellets were placed in the medium and the time to float and total floating time were measured by visual observation. The percentage of floating pellets was determined by the following formula:

$$\text{Floating pellets } (\%) = \frac{W_i - W_f}{W_i} \times 100$$

where W_i and W_f represent the total number of pellets and the final number of settled pellets, respectively. The tests were carried out in triplicate.

2.6. In vivo X-ray study

To view and record the gastroretentive behavior of the optimized formulation in vivo, incorporation of Barium sulfate was necessary to make the pellets visible under X-ray. Half of the drug was replaced with barium sulfate and other ingredients were kept constant. The 16–24 mesh pellets were chosen as candidates for X-ray examination. The reference pellets were prepared in the same way without $NaHCO_3$.

After overnight fasting, three healthy New Zealand rabbits weighing 1.5–2 kg were fed with a little low calorie food given some water. One hour later, 100 barium sulfate-labeled pellets were administered orally with 15 ml water to every rabbit. The animals were held on a board. Radiographs were obtained at 1, 2, 3 h, and up to 6 h. Rabbits were given 15 ml water every hour. The X-ray parameters were kept constant throughout. The movements of the pellets were easily identified and observed. Permission was obtained from the University Ethics Committee for the use of experimental animals prior to the experiment.

2.7. In vivo evaluation of the floating pellets in New Zealand rabbits

2.7.1. Protocol

The experiment was approved by the University Ethics Committee for the experimental animals and operated according to the

principles of Laboratory Animal Care. Six healthy New Zealand rabbits weighing 1.5–2 kg were divided into 2 groups. A randomized, two-period crossover single-dose study was conducted and the washout period was one week. The rabbits were fasted overnight with free access to water. A little low calorie food was given before drug administration. One group was given the floating pellets containing 100 mg ofloxacin once a day and the other group was given reference preparation (commercial ofloxacin tablets) containing 50 mg ofloxacin every 12 h. In addition, 15 ml water was given to every rabbit every hour until 8 h after drug administration. For the test group, 1 ml blood samples were collected from the ear vein and placed in heparinized centrifuge tubes before and then 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, and 24 h after dosing. For the reference rabbits, the similar blood samples were taken before and then 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 h after every dosing. Plasma samples were obtained following centrifugation (4000 rpm) for 10 min and stored at −20 °C until determination.

2.7.2. Sample determination by UPLC –MS/MS

For this, 20 μl plasma was mixed with 20 μl internal standard solution (2000 ng/ml ciprofloxacin methanol solution) and 20 μl methanol. After 1 min vortex mixing, 800 μl CH_2Cl_2 was added and then vortex-mixed for 10 min. After centrifugation at 4000 rpm for 10 min, 600 μl of the organic layer was removed and evaporated to dryness under nitrogen at 45 °C. The residue was reconstituted in 400 μl water by vortex-mixing for 10 min. Following centrifugation at 12,000 rpm for 10 min, a 5 μl aliquot was injected.

Chromatography was carried out using an ACQUITY^{TM} UPLC system (Waters Corp., Milford, MA, USA). Separation was obtained using an ACQUITY^{TM} UPLC BEH C18 column (50 mm × 2.1 mm i.d., 1.7 μm; Waters Corp., Milford, MA, USA). The chromatographic conditions were as follows: isocratic elution (0.1%, v/v) formic acid:acetonitrile (80:20, v/v); flow rate 0.3 ml/min. The analytical run time was 1.2 min.

A Waters ACQUITY^{TM} TQD triple-quadrupole tandem mass spectrometer (Waters Corp., Manchester, UK) was used as a mass detector system with an electrospray ionization (ESI) interface. The samples were determined in positive ionization mode with optimal operation parameters as follows: ion source temperature 100 °C; desolvation temperature 500 °C; capillary voltage 3 kV; cone voltage 32 kV. The desolvation and cone gas flow rates were 600 l h^{-1} and 50 l h^{-1}, respectively. Multiple reaction monitoring (MRM) mode was employed for quantification: m/z 362 → 261 for ofloxacin and m/z 332 → 231 for ciprofloxacin (I.S.), respectively. All data were collected and processed by using MassLynx^{TM} NT 4.1 software with a QuanLynx^{TM} program (Waters Corp., Milford, MA, USA).

The calibration curve for ofloxacin was found to be linear over the range 10–25,000 ng/ml with a correlation coefficient value of more than 0.99. The LLOQ was 10 ng/ml. The R.S.D of precision at three concentrations (20, 500 and 10,000 ng/ml) was 5.87%, 8.38% and 6.61% for intra-day analysis, and the inter-day precision was less than 8%. The recoveries were 89.11%, 84.04% and 85.14%, respectively.

2.7.3. Statistical analysis

Pharmacokinetic data analysis was carried out using DAS 2.0. software (Mathematical Pharmacology Professional Committee of China, Shanghai, China). The area under the plasma concentration–time curve $AUC_{(0\rightarrow t)}$, C_{max} and T_{max} was calculated. C_{max} and T_{max} of the test preparation were actual observations, and the values of C_{max} and T_{max} of the reference preparation were the mean values of the two doses. The student's test was performed to determine the significance of difference between the pharmacokinetic parameters. The level of significance was defined as p value <0.05 using the statistical package for social science (SPSS, version 12.0).

3. Results and discussion

3.1. Design and optimizing of the preparation

3.1.1. Design of the preparation

The floating pellets were obtained by a gas formation technique. $NaHCO_3$ underwent neutralization to liberate CO_2 and the gas entrapped around the core pellets by a polymeric membrane could generate enough buoyancy to make the pellets float. The design of the floating sustained release pellets is shown in Fig. 2. The floating sustained release pellets consisted of a drug-loaded core pellet coated with a release retarding film, an effervescent layer and a gas-entrapped membrane, respectively. Since $NaHCO_3$ itself is not sticky, HPMC was chosen as the binder. The generated CO_2 gas was entrapped by the gas-entrapped membrane. The idea material of entrapped membrane should be highly water permeable to start the neutralization reaction and the floating process. The wet or hydrated coatings should also be impermeable to the generated gas CO_2 so as to promote and maintain floatation (Krogel and Bodmeier, 1999). Regarding their mechanical properties, the polymeric coatings should be sufficiently flexible in the wet state to be able to withstand the pressure of the generated gas and to avoid rupturing (Krogel and Bodmeier, 1999). It is reported that cellulose membranes are not suitable candidates (Krogel and Bodmeier, 1999). Cellulose polymer (cellulose acetate or ethyl cellulose) was rigid and not flexible. It can be ruptured easily under the pressure of the generated gas. Thus a high flexibility, high water permeable, gas impermeable and solid polymer was necessary. For these reasons, Eudragit® RL 30D, RS 30D and NE 30D were chosen as the gas-entrapped membrane. The systems coated with Eudragit® RS 30D and NE 30D as the gas-entrapped membrane did not float even when high amount of gas forming agent (12% weight gain of HPMC:$NaHCO_3$, 2:8, w/w) and low coating level (5% weight gain) were used (Sungthongjeen et al., 2006). Eudragit® RS 30D and NE 30D were only slightly water permeable which permit low amount of dissolution medium to induce the effervescent reaction and did not generate enough CO_2 to make the systems float (Sungthongjeen et al., 2008). Eudragit® RL 30D is highly water permeable to allow floatation. Eudragit® RL 30D was composed of a aqueous dispersion with 30% copolymer of ethyl methacrylate, methylmethacrylate and trimethylammonioethyl methacrylate chloride in a ratio of 1:2:0.2 (quaternary PMMA). Eudragit® RS 30D was the same copolymer as Eudragit® RL 30D but in a ratio of 1:2:0.1. The amount of quaternary ammonium groups decided the permeability, the water absorption and swelling property. Thus, Eudragit® RL 30D was more permeable, exhibited more water absorption and had a great swelling volume than Eudragit® RS 30D. Water can permeate more freely through Eudragit® RL 30D due to its hydrophilicity, while the relative

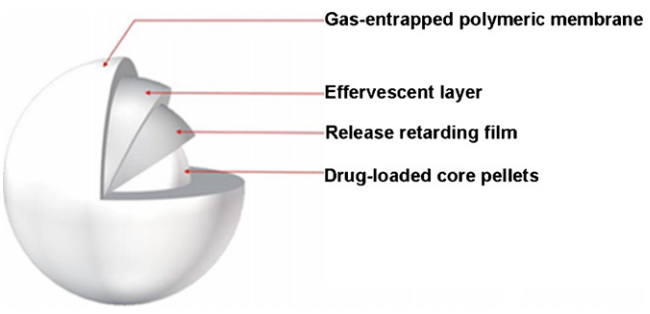

Fig. 2. Design of the floating sustained release pellets.

hydrophobicity of Eudragit® RS 30D makes it less permeable (Lin et al., 2000). The water absorption of Eudragit® RL 30D with 16% (w/w) diethyl phthalate was about 300 times that of Eudragit® RS 30D (Lin et al., 2000). This means Eudragit® RL 30D has higher swelling volume in the medium. The pore diameter of Eudragit® RS 30D is about 0.1–0.6 μm, but the pore diameter of Eudragit® RL 30D is 1–5 μm. Due to the difference in pore diameter, the water permeation rate of Eudragit® RL 30D is faster than that of Eudragit® RS 30D. Therefore, Eudragit® RL 30D was investigated as the gas-entrapped membrane. A release retarding film was necessary. Ofloxacin has a high solubility in acid medium. It is difficult to control the release of ofloxacin with Eudragit® RL30 and EC was used as the release retarding film. To prepare an optimal preparation, the parameters such as the ratio of $NaHCO_3$ and HPMC, the amount of gas-entrapped polymer and the level of EC were evaluated.

The whole process involved: the HCl medium permeating into pellets; the generated gas CO_2 was entrapped in the Eudragit® RL 30D membrane; then it can started to float and maintained floating for a long time; the drug was then released from the core pellets.

3.1.2. Formulation optimization

3.1.2.1. Optimizing of the effervescent layer and gas-entrapped polymeric membrane. In terms of the floating ability and release, the effervescent layer and the gas-entrapped polymeric membrane were correlated. The ratio of $NaHCO_3$ to HPMC and the amount of Eudragit® RL 30D was investigated in this study. The system should float in a few minutes after contact with gastric fluid to prevent the dosage form from transiting into the small intestine together with food (Iannuccelli et al., 1998). When the weight gain of the effervescent layer was fixed at 12% (w/w), the ratio of $NaHCO_3$ and HPMC and the level of the gas-entrapped polymeric membrane played an important role in the floating ability. $NaHCO_3$ was dissolved in HPMC solution in ratios of 1:4, 1:2, 1:1, 2:1 and 4:1 (w/w) ($NaHCO_3$ to HPMC). The amount of Eudragit® RL 30D was 5%, 10% and 15%. The pellets were only coated with the effervescent layer and the gas-entrapped polymeric membrane except for the release retarding film. The time to float decreased as the amount of $NaHCO_3$ increased. The reduced amount of Eudragit® RL 30D also cut down the time to float due to the faster water permeation through the coating. Also, Fig. 3 shows that most of the formulation starts to float within 5 min. When

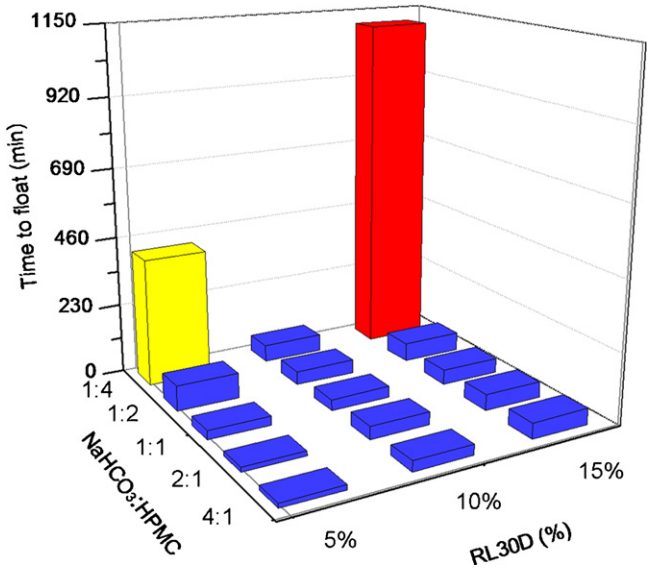

Fig. 3. Effect of the amount of $NaHCO_3$ of the effervescent layer and the level of Eudragit® RL 30D on time to float of the coated effervescent-layered pellets.

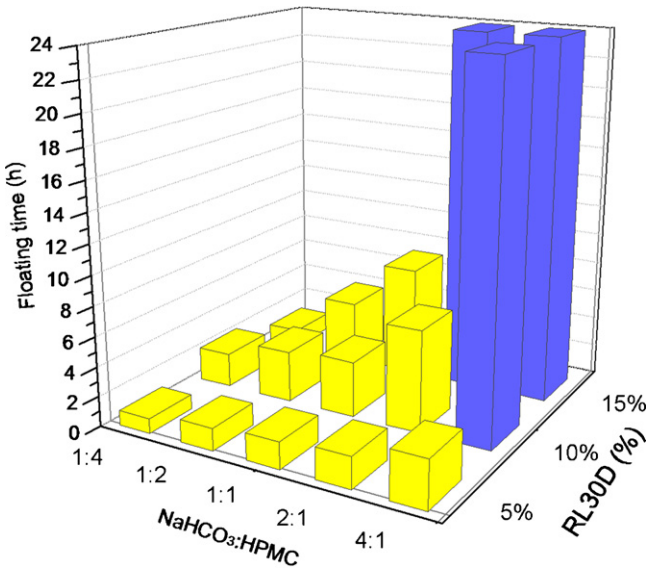

Fig. 4. Effect of amount of $NaHCO_3$ of the effervescent layer and the level of Eudragit® RL 30D on the floating time of the coated effervescent-layered pellets.

the ratio of $NaHCO_3$ and HPMC was 1:4 and Eudragit® RL 30D was 5%, the time to float was longer than for the others that Eudragit® RL 30D was 5%. That was because the amount of generated CO_2 was too little and the amount of Eudragit® RL 30D was too small to entrap the gaseous CO_2. As reported, the duration of floating should be longer than 24 h (Sungthongjeen et al., 2006; Iannuccelli et al., 1998). Therefore the floating time is an essential condition. The floating time increased with the increasing amount of $NaHCO_3$ and Eudragit® RL 30D. Fig. 4 shows that only three groups ($NaHCO_3$:HPMC = 4:1, 10% Eudragit® RL 30D and $NaHCO_3$:HPMC = 2:1, 4:1, 15% Eudragit® RL 30D) floated for 24 h. The results demonstrated that the Eudragit® RL 30D membrane was impermeable to the generated gaseous CO_2. Thus, the high water permeability, high swelling volume and gaseous CO_2 impermeability had beneficial effects on the floating properties. The drug release decreased with the thickness of the gas entrapped membrane increasing early release. Fig. 5 shows that the greater thickness of the membrane resulted in delayed water permeability and slightly reduced drug release in the first few minutes. After that the 15 formulations exhibited no differences in drug release. The pellets released all the ofloxacin in 60 min without any sustained release effect. Therefore, the release retarding film (EC) was chosen and investigated to obtain floating sustained release pellets. An interesting discovery was found that the EC coating was added to the three groups ($NaHCO_3$:HPMC = 4:1, 10% Eudragit® RL 30D and $NaHCO_3$:HPMC = 2:1, 4:1, 15% Eudragit® RL 30D) above mentioned as the first coating, but only one group ($NaHCO_3$:HPMC = 4:1, 15% Eudragit® RL 30D) floated stably for 24 h. One possible reason for this result is that the EC coating retarded the water penetration and the core pellets could not swell quickly to lower the density. Another reason is that less generated CO_2 and 10% Eudragit® RL 30D could not form an optimal floating system to maintain floatation. The optimal effervescent layer and gas-entrapped polymeric membrane was that: $NaHCO_3$:HPMC = 4:1, with a weight gain of 12% (w/w) and Eudragit® RL 30D with a weight gain of 15% (w/w).

3.1.2.2. Release of ofloxacin sustained release pellets with different ethyl cellulose coating levels. The optimized effervescent layer and gas-entrapped polymeric membrane ($NaHCO_3$:HPMC = 4:1, 12%, w/w and Eudragit® RL 30D 15%, w/w) were fixed. Therefore, different levels of EC coating were investigated in the study. The release profiles of ofloxacin pellets with 1.7%, 2.0% and 2.3% EC coatings in

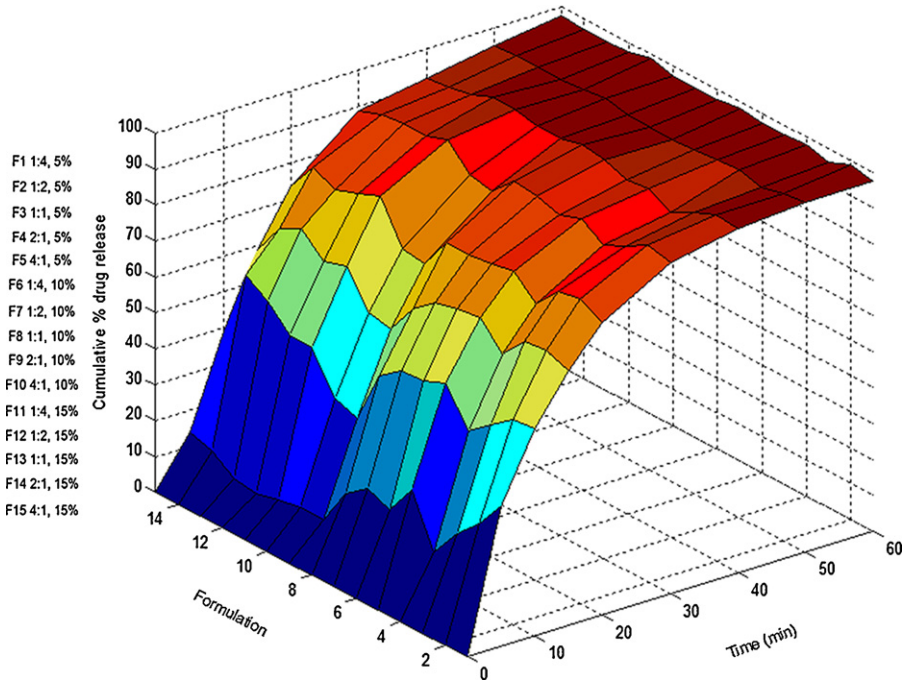

Fig. 5. Effect of the amount of $NaHCO_3$ on the effervescent layer and the level of Eudragit® RL 30D on the drug release of the coated effervescent-layered pellets.

0.1 N HCl are shown in Fig. 6. The drug release tended to decrease with the increasing amount of EC coating. The full release profiles reached about 100% at 24 h. According to the literature, an Indian company has prepared a sustained release ofloxacin tablet which was bioadhesive in the gastrointestinal tract for a longer time. The release of the tablet in 0.1 N HCl at 2, 4, 8 and 12 h was approximately 32%, 49%, 68%, and 78%, respectively (Sungthongjeen et al., 2006). Compared with this tablet, the profiles of the 2% EC coating indicated the same curve for the first 6 h. However, after 6 h, the pellets released more ofloxacin than the tablet. This trend allows the full dose of drug absorbed into the blood. Thus, a high bioavailability could be obtained.

Fig. 7 shows the release of F17 and the pellets coated with EC 2% (w/w) without the effervescent layer and the gas-entrapped polymeric membrane. Due to the Eudragit® RL 30D coating, the release of F17 became lower than that of the pellets with only the EC coating without any other coating. One possible reason is that Eudragit® RL 30D coating delayed the water penetration into the core drug.

3.2. Characterization of the release mechanism

The release mechanism of the pellets was osmotic pressure driven drug release through the ethyl cellulose membrane. It was found that the drug delivery rate remained constant for the first 8 h and the release data fitted a straight line very well ($R^2 = 0.9878$) (Fig. 8A). After 8 h, the release rate was reduced (Fig. 8B). The release process followed zero-order kinetics for the first 8 h. An interesting and significant discovery was that when the stirring rate was 50 rpm and 100 rpm and other dissolution parameters remained unchanged, the release curves of F17 were almost the same as Fig. 9. This characterization of the release agreed with the osmotic pump mechanism.

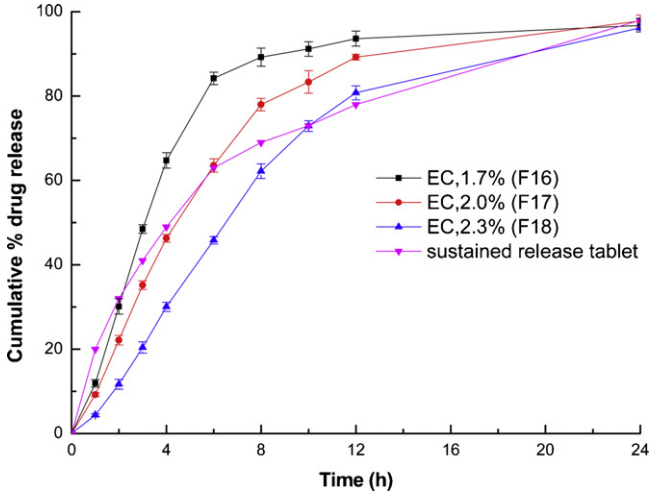

Fig. 6. Release profiles of the ofloxacin pellets with different EC coating levels in 0.1 N HCl (the effervescent layer: $NaHCO_3$:HPMC = 4:1, 12%, w/w and the gas-entrapped polymeric membrane: Eudragit® RL 30D 15%, w/w).

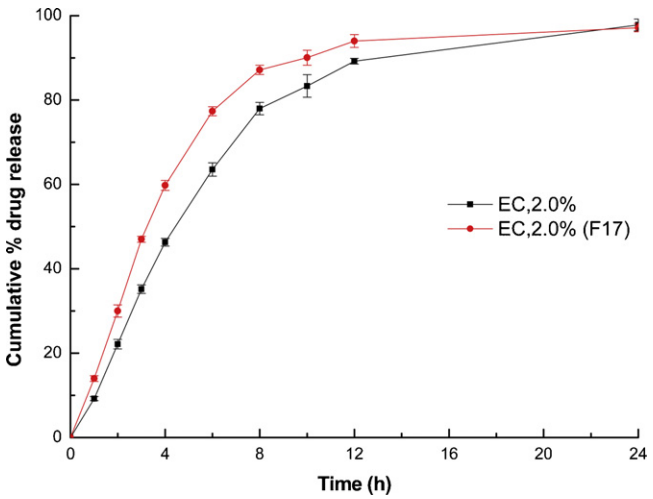

Fig. 7. Effect of Eudragit® RL 30D as the gas-entrapped polymeric membrane on the drug release of ofloxacin sustained release pellets.

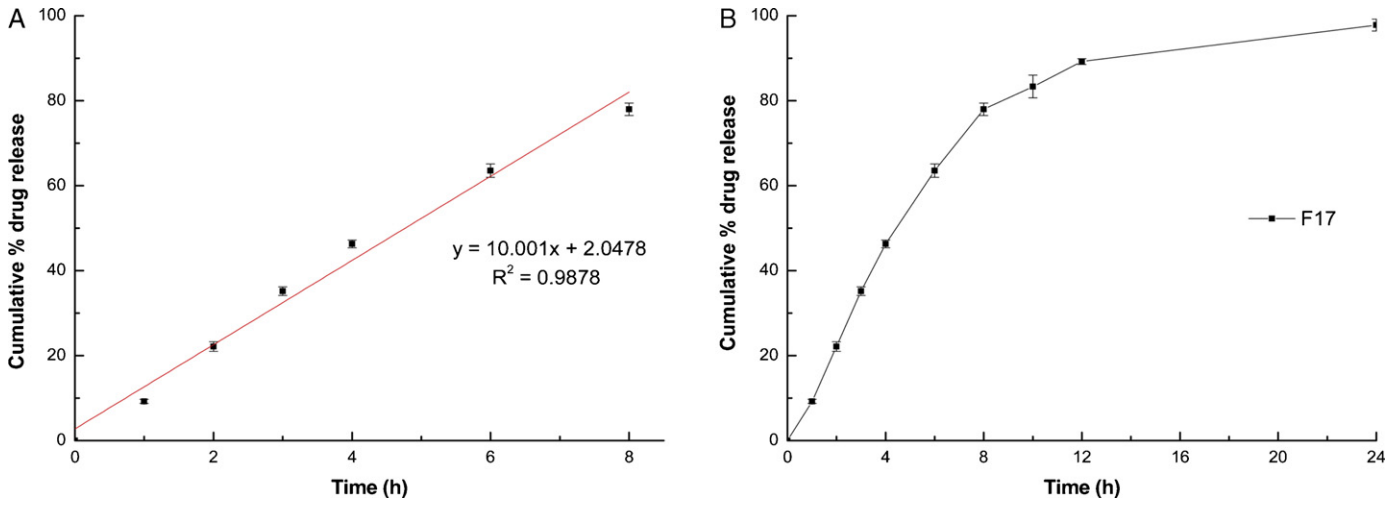

Fig. 8. Release of F17 in the 0.1 N HCl medium. (A) The release of F17 over the first 8 h. (B) The release of F17 over the whole 24 h.

Table 2
The reference parameters and results of theoretical calculated value.

Parameter/result	Symbol	Value
Diameter	d	1190–710 μm
Total volume	V	0.529–0.112 cm^3
Drug content	m	200 mg
Saturation solubility (0.1 N HCl medium 37 °C)	c_{sat}	92.56 mg/ml
Theoretical values	M_τ/M_0	75.51–94.80%

To study the release mechanism of the pellets, the derived equation Eq. (5) in the theoretical method was applied. The ofloxacin release rate was steady state for the first 8 h. The osmotic pressure and release rate changed after 8 h due to the decreased concentration. Thus, in this case, the eighth hour point is the critical time point. The cumulative of ofloxacin release at the critical time point (8 h) was found to be 77.97% in Fig. 6. The critical value can be calculated according to Eq. (6).

As can be seen in Table 2, the critical value of cumulative of drug release (%) calculated from Eq. (6) is between 75.51% and 94.80% and 77.98% was within the range of the theoretical values. Due to ignoring the volume of ethyl cellulose membrane swelling, the theoretical values are a little larger than the true value. Thus, the good agreement between the experimental and theoretical calculated critical cumulative of drug release confirms the validity of the assumption.

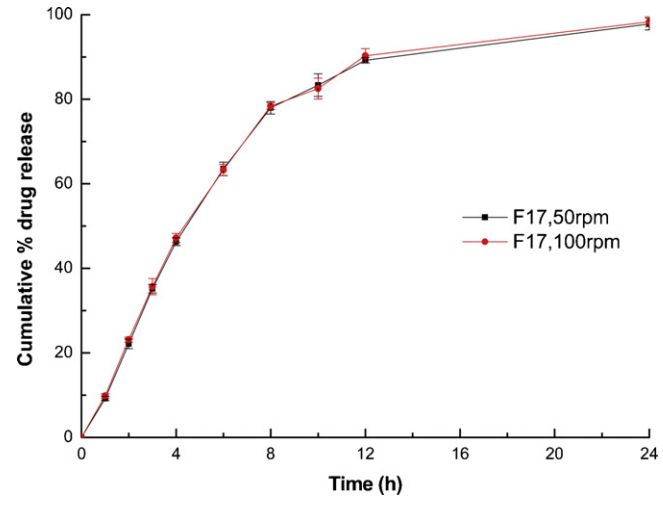

Fig. 9. Release of F17 in 0.1 N HCl medium with a stirring rate of 50 rpm and 100 rpm.

3.3. In vitro floating ability study

The best formulation (F17) showed that the time to float was approximately 1.1 min and floating duration was no less than 24 h during the release test. The percentage of floating pellets (F17) equalled to 97 ± 1.6%. The higher percentage buoyancy provided a sound basis for gastroretentive floatation. The floating sequence of the floating pellets at different times was shown in Fig. 10.

3.4. Characterization of pellets in SEM

The surface and cross-sectional morphology of the pellets were examined and analyzed using SEM. Fig. 11A shows the surface morphology of the core pellets under SEM. The core pellets had a slightly rough surface. The surface of the pellets coated with ethyl cellulose was slightly smoother (Fig. 11B), but the surface of the effervescent-layered pellets was rougher. The smoothest was the surface of pellets with all three coating membranes. Fig. 11E shows the cross-sectional morphology of the pellets with all three coatings. The three successive coating membranes of the floating sustained release pellets were seen from the image. The most inside layer was the EC coating. The interlayer and the most outside were $NaHCO_3$ and Eudragit® RL 30D, respectively. The surface morphology of the pellets before and after the dissolution test is shown in Fig. 11D and F. After dissolution in HCl medium, the polymeric membrane of the coated pellets swelled due to the swelling capacity and the inner pressure of the generated gas. A larger swelling volume could entrap the generated CO_2 just like a balloon. The broken hole in the membrane was made according to the vacuum process before the SEM observations.

3.5. In vivo X-ray

The in vitro buoyancy time of the barium sulfate-loaded pellets was more than 24 h. The time to float increased compared with the original formulation (F17), as expected. This is because $BaSO_4$ has a high relative density of about 4.5 g/mg^3. Fig. 12 shows the X-ray images of the reference barium sulfate-labeled pellets (non-floating pellets) at different times in New Zealand rabbits. It is clear that a few pellets are transported into the small intestine during the

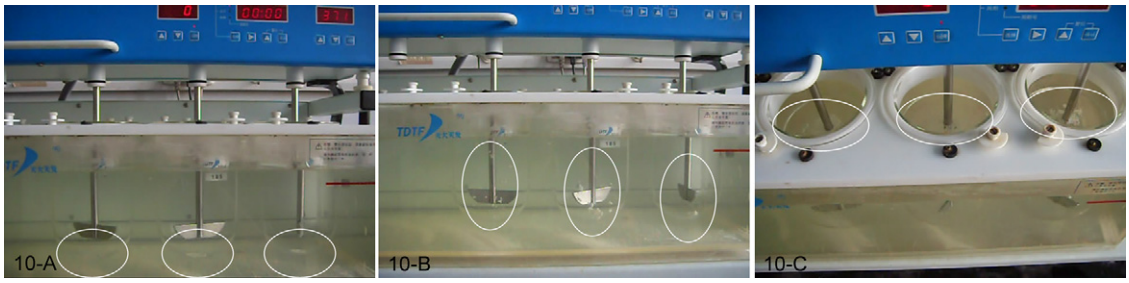

Fig. 10. Floating sequence in 0.1 N HCl of ofloxacin floating sustained release pellets: (A) 0 min, (B) 1.1 min, and (C) after 24 h.

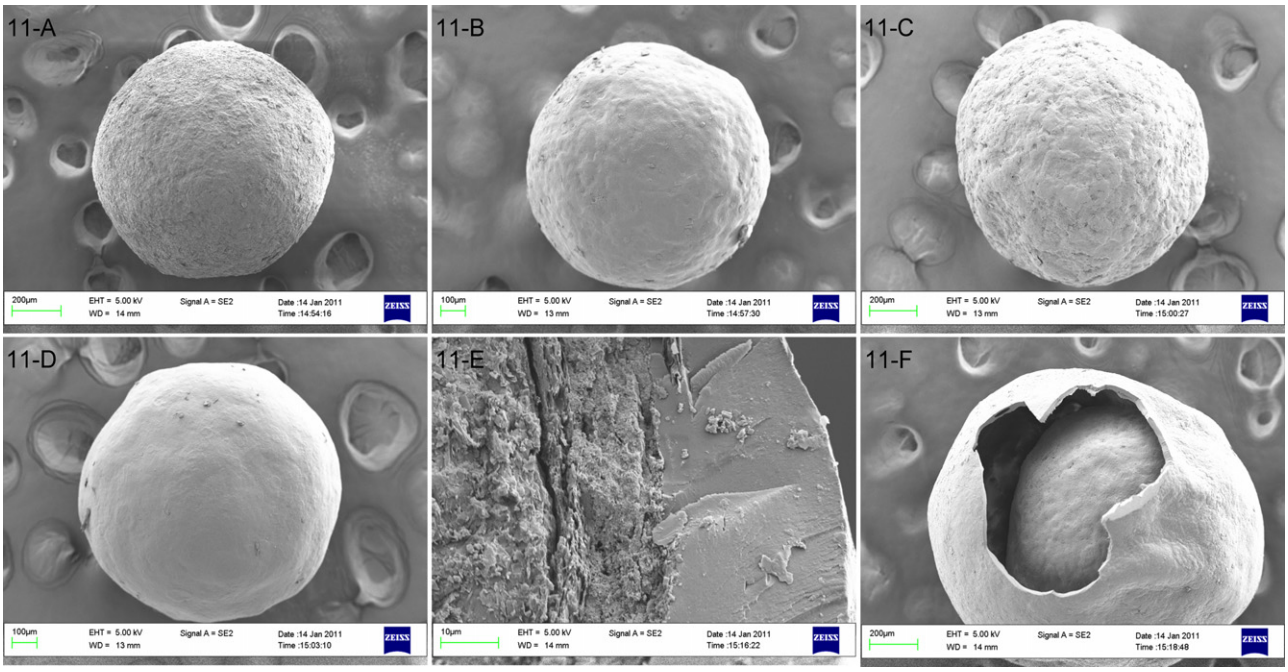

Fig. 11. SEM photomicrographs of: (A) core pellets, (B) pellet coated with 2% ethyl cellulose, (C) pellets coated with 2% ethyl cellulose and 12% effervescent-layer ($NaHCO_3$:HPMC = 4:1), (D) the surface morphology of optimized formulation, (E) the cross-section morphology of the optimized formulation, and (F) the surface of the optimized formulation after exposure to 0.1 N HCl.

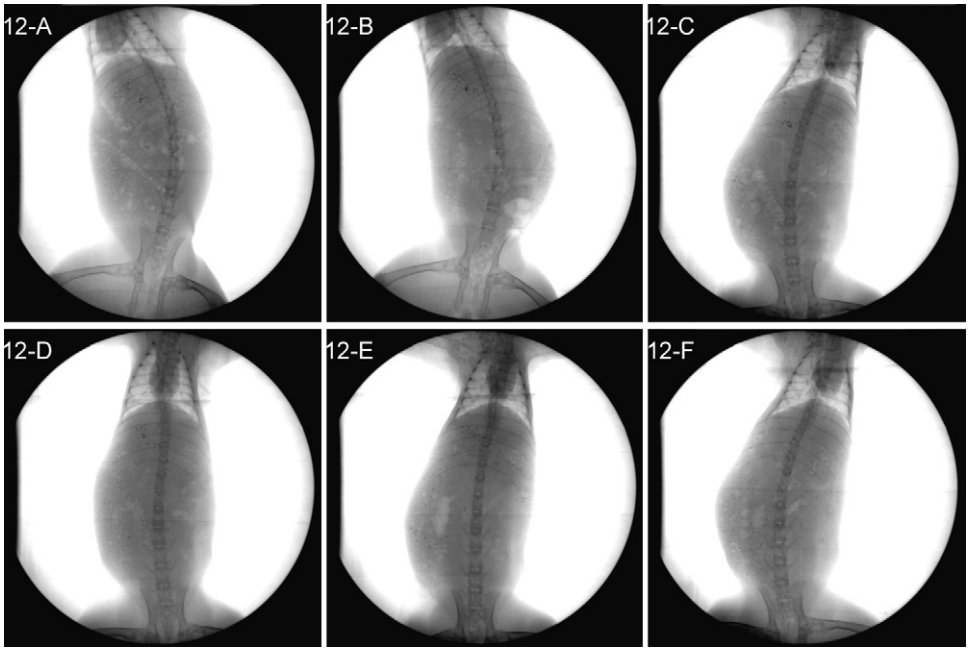

Fig. 12. (A–F) X-rays indicating the position of the reference barium sulfate-labeled pellets (non-floating pellets) in the gastrointestinal tract of New Zealand rabbits at different time periods X-ray taken at (A) 1 h, (B) 2 h, (C) 3 h, (D) 4 h, (E) 5 h, and (F) 6 h.

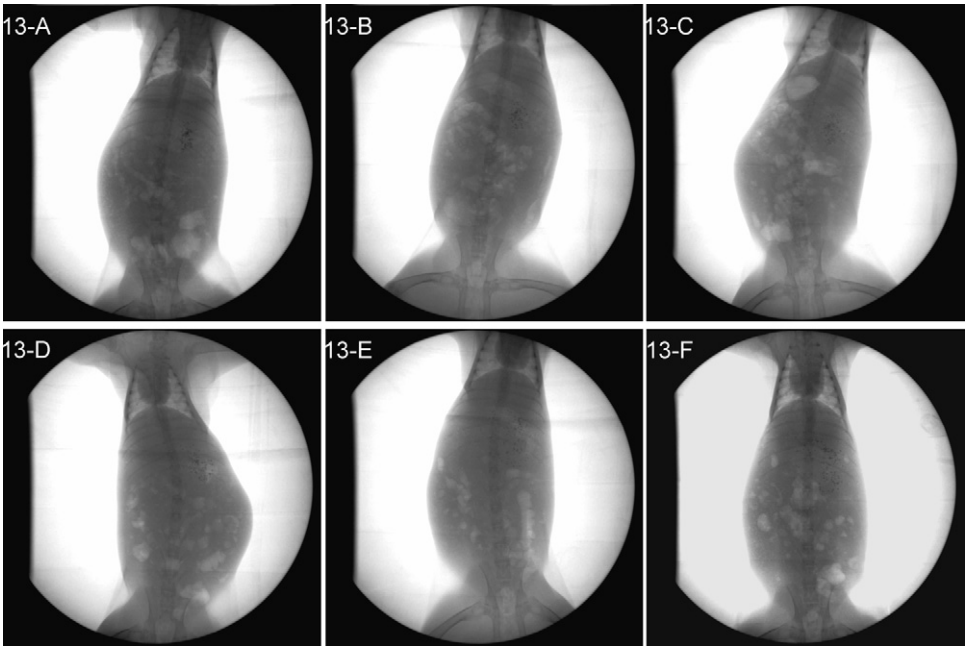

Fig. 13. (A–F) X-rays indicating the position of the floating barium sulfate-labeled pellets in the gastrointestinal tract of New Zealand rabbits at different time periods X-ray taken at (A) 1 h, (B) 2 h, (C) 3 h, (D) 4 h, (E) 5 h, and (F) 6 h.

first 2 h and most of the pellets are transferred to the small intestine at 6 h. Fig. 13 shows the X-ray images of the floating barium sulfate-labeled pellets in New Zealand rabbits. It was seen that all the floating barium sulfate-labeled pellets remain buoyant in the stomach at 6 h compared with the reference pellets. These results show the good gastroretentive behavior in vivo.

3.6. The pharmacokinetics of ofloxacin floating sustained release pellets in New Zealand rabbits

Although the floating pellets exhibit ideal in vitro dissolution and floating behavior, the bioavailability was also studied to confirm the pharmacokinetic parameters. No pharmacokinetic studies of ofloxacin floating sustained release pellets have been reported until now. The mean plasma concentrations of ofloxacin for the test and reference preparation in six New Zealand rabbits at steady-state are shown in Fig. 14 and the main pharmacokinetic parameters are presented in Table 3. The mean relative bioavailability of ofloxacin floating sustained release pellets to the ofloxacin tablet was calculated from the $AUC_{(0-t)}$ was 113.06 ± 23.83%. The main pharmacokinetic parameters were performed by analysis of variance. The results indicated that the AUC and C_{max} of ofloxacin for test and reference preparations exhibited no difference in periods, formulations and subjects. Further more, all the data were analyzed by a two-sided *t*-test and the (1–2α) confidence interval method, and the 90% confidence interval of the $AUC_{(0-t)}$ of the test preparation was 90.0–136.8% of reference preparation. The $AUC_{(0-t)}$ values of the test and reference preparation were 136,585.03 ± 29,533.97 ng/ml h and 121,548.86 ± 15,642.86 ng/ml h, respectively. The higher $AUC_{(0-t)}$ of the test formulation was due to the slow release and sustained absorption, or the prolonged gastric residence time (Hu et al., 2011). Table 3 shows that there was a significant difference between the values of $t_{1/2}$, T_{max} and C_{max}. It can be seen from the curve that there were two peaks at 2 h and 14 h in the ofloxacin tablet drug concentration–time profile. After oral administration of the first dose of the reference tablet, C_{max} was 12,549.74 ± 3656.74 ng/ml for the 50 mg reference dose, while, the C_{max} in the test preparation was 13,368.73 ± 2190.65 ng/ml for 100 mg. The C_{max} for the reference increased to 14,795.56 ± 3234.22 ng/ml after the second administration. The C_{max} of the test was similar with that of the reference. Also the drug concentration of the test preparation maintained a higher level than that of the reference. It

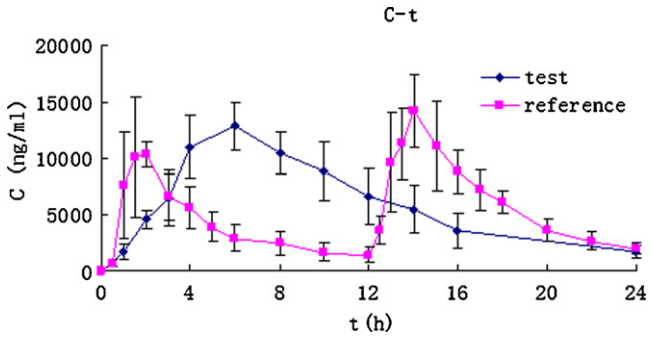

Fig. 14. Plasma concentration–time profile of ofloxacin floating sustained release pellets and conventional ofloxacin tablets. Results are expressed as the mean ± SD.

Table 3
Pharmacokinetic parameters of test and reference preparations.

Parameters	Unit	Test	Reference
$AUC_{(0-t)}$	ng/ml h	136,585.03 ± 29,533.97	121,548.86 ± 15,642.86
$AUC_{(0-\infty)}$	ng/ml h	153,683.49 ± 33,093.21	128,912.84 ± 15,420.15
$t_{1/2}$	h	6.32 ± 2.31	3.14 ± 0.39*
			1.88 ± 0.14*
T_{max}	h	5.67 ± 0.82	(1.83 ± 0.26)[a]
			(13.92 ± 0.20)[b]
			13,672.65 ± 3274.23
C_{max}	ng/ml	13,368.73 ± 2190.65	(12,549.74 ± 3656.74)[a]
			(14,795.56 ± 3234.22)[b]
C_{max}/dose	ml^{-1}	0.14 ± 0.02	0.27 ± 0.07

All the data are presented in the form of mean ± SD.
[a] The first dose.
[b] The second dose.
* $p < 0.05$.

has been reported that ofloxacin exhibits concentration-dependent antibacterial activity (Marier et al., 2006). So it appears that the test pellets are more effective than the conventional tablet. However the C_{max} of the test preparation which was administrated as a double dose was not twice as high as that of the reference. Thus it improved the safety. As found, the test preparation prolonged the T_{max} from 1.88 ± 0.14 h to 5.67 ± 0.82 h and the T_{max} of the floating pellets was significantly delayed ($p < 0.05$) compared with the commercial tablet. The C_{max} and T_{max} indicated that the test preparation had better sustained-release characteristics than the conventional preparation. It was also shown that the test preparation had $t_{1/2}$ was 6.32 ± 2.31 h; however, the reference tablets had a $t_{1/2}$ of 3.14 ± 0.39 h. This indicated that the delayed T_{max} and prolonged $t_{1/2}$ represented a slow release of the drug from three-layer floating pellets in comparison with the reference tablet. As a result, the floating sustained release system could provide constant drug delivery, in which the plasma concentration exhibited a small variation in vivo. All the results of the test preparation (F17) of the pharmacokinetics in New Zealand rabbits indicated good floating and sustained release. The data showing the prolonged T_{max} and $t_{1/2}$ of the test preparation (F17) also confirmed a longer gastroretention time. These results were in accordance with the results of the X-ray study in New Zealand rabbits. In the present study, it could be concluded that floating sustained release pellets were an efficient preparation for improving the oral bioavailability and prolonging the gastroretention time.

4. Conclusion

In conclusion, taking all the findings into consideration, the floating pellets investigated in this paper, are excellent for further research. The ofloxacin floating sustained release pellets were successfully prepared and studied. The addition of the release retarding film, the gas-generating agent $NaHCO_3$ and the gas-entrapped polymeric membrane (Eudragit® RL 30D) were essential for optimum buoyancy and release. Based on a series of investigations, the F17 (EC 2%, w/w, $NaHCO_3$:HPMC = 4:1, 12%, w/w, Eudragit® RL 30D 15%, w/w) was found to be offer the optimum release and buoyancy. The prepared pellets started floating at about 1.1 min and this was maintained for 24 h. The analysis of the release mechanism showed a zero-order release for the first 8 h. According to the results of in vivo study of the X-ray and pharmacokinetic data in New Zealand rabbits, the floating preparation (F17) could prolonged the T_{max} and improved the bioavailability without increasing the fluctuation of plasma concentrations. Therefore, the ofloxacin floating pellets are a feasible approach for a sustained release preparation drugs, which have a narrow window of absorption in the stomach or in the upper part of the gastrointestinal tract.

Acknowledgments

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