International Journal of Pharmaceutics 360 (2008) 47–52

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

In vitro and *in vivo* evaluation of ofloxacin sustained release pellets

Yue Cui, Yu Zhang, Xing Tang*

Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, PR China

ARTICLE INFO

Article history:
Received 23 December 2007
Received in revised form 6 April 2008
Accepted 10 April 2008
Available online 16 April 2008

Keywords:
Ofloxacin
Sustained release
Pellets
Centrifugal granulation
Bioavailability

ABSTRACT

Being a sustained release dosage form, pellets allow ofloxacin to exhibit improved release and absorption profiles. In this paper, the centrifugal granulation method was employed to prepare ofloxacin pellets. Then the pellets were subjected to a coating process with methacrylic acid copolymers to produce sustained release characteristics. The pellets with different coatings were investigated by release tests *in vitro*. Finally, pellets with the best coating suspension were subjected to a multiple doses pharmacokinetic study in beagle dogs. The *in vitro* release profiles showed that pellets coated with Eudragit® NE30D and Eudragit® L30D55, at a ratio of 1:8 (w/w) and a coating level of 8% with diethyl phthalate (DEP) as plasticizer equivalent to 10% of solid material in the coating suspension were suitable for sustained release. In the bioavailability study, the principal pharmacokinetic parameters showed there were differences between the sustained release pellets and the conventional ofloxacin capsules. The relative bioavailability of ofloxacin sustained release pellets compared with conventional ofloxacin capsules was 116.35 ± 33.31%. All the statistics indicate that the preparation has a sustained release effect with many advantages over conventional preparations.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Single drug-releasing-unit preparations such as tablets are vulnerable to the effects of food or fasting while multiple drug-releasing-unit preparations, like pellets, are superior (Hu et al., 2006). In a single dose of multiple drug-releasing-unit preparations, there are many units for drug release and, therefore, the overall effect is a cumulative one, taking the effects of each unit into account. So, the effect of the pellets is barely influenced by the condition of the stomach, taking stomach evacuation as an example. Meanwhile, pellets also exhibit the characteristics of steady absorption, and are capable of releasing the drug in a sustained manner (Dévay et al., 2006).

There are many methods of making pellets, including extrusion and spheronization (Tomer et al., 2002), fluid bed granulation (Hemati et al., 2003) and centrifugal granulation. It is known that centrifugal granulation allows the preparation of sustained release pellets (Rashid et al., 2001). This process uses blank beads as the starting material, and drug and/or excipients are layered onto the beads in the form of powder, increasing the diameter of the beads to the desired size. This technique is both time-saving and economic (Hu et al., 2006). The final products have excellent physical properties such as a high flowability and large surface area (Lyer et al., 2006). Usually, drug-loaded pellets need to undergo a coating process to fulfill the goal of prolonged release.

Ofloxacin is a fluoroquinolone antibacterial agent, which is highly sensitive to both Gram-positive and Gram-negative bacteria. It is also active against mycoplasma, chlamydia and legionella. While widely used to treat infections, such as those affecting the gastrointestinal and respiratory tracts, it is also used to treat urinary tract infections (Zivanovic et al., 2006). This is because ofloxacin can achieve high concentrations in the urinary tract in unchanged form after oral administration (Okhamafe and Akerele, 1989; Fünfstück et al., 1999). Approximately 90% of a dose was excreted in urine as unchanged drug within 48 h. Besides, ofloxacin was rapidly and almost completely absorbed from the upper small intestine, with a high bioavailability up to 95%. Biotransformation of ofloxacin was minimal, with less than 10% of the dose metabolized into desmethyl ofloxacin and ofloxacin N-oxide, both of which are pharmacologically inactive (Marier et al., 2006). For mild or moderate infections, administration of ofloxacin at a dose of 200 mg b.i.d is appropriate. For uncomplicated urinary infections, administration of ofloxacin 400 mg/d is normally adopted. Meanwhile, for the treatment of complicated urinary infections, a dose of 400 mg/d of ofloxacin has been shown to be effective (Mouton and Leroy, 1991). Except for the development of ofloxacin gastroretentive tablets which have a sustained release effect for 24 h in gastric fluid (Chavanpatil et al., 2005), almost all of the ofloxacin preparations commercially available are conventional and require to be taken twice a day to

* Corresponding author at: Mail Box 32, No. 103, Wenhua Road, Shenyang 110016, Liaoning Province, PR China. Tel.: +86 24 23986343; fax: +86 24 23911736.
E-mail addresses: tangpharm@sina.com, tangpharm@yahoo.com (X. Tang).

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.04.014

achieve therapeutic effects. Our aim was to develop a new preparation of ofloxacin to reduce the frequency of administration, thereby improving the patient compliance and increasing the therapeutic efficacy.

In this study, the core beads were made from microcrystalline cellulose (MCC), with ofloxacin powder layered onto the blank beads by a centrifugal granulator, followed by a coating process using methacrylic acid copolymers (Eudragit® L30D55 and Eudragit® NE30D) to achieve sustained release. The release tests and bioavailability study for multiple doses in beagle dogs were carried out to evaluate the final preparation.

2. Materials and methods

Methacrylic acid copolymers (Eudragit® L30D55, Eudragit® NE30D) were supplied by Röhm GmbH Chemische Fabrik, Darmstadt, Germany. Ofloxacin was purchased from Zhejiang Kangyu Pharmaceutical Co. Ltd. (Zhejiang, China). Ofloxacin standard substance was purchased from National Instituted for the Control of Pharmaceutical and Biological Product, China (Lot No. 130454-200604). Other excipients used to prepare the pellets, such as MCC, citric acid, lactose and DEP, were China Pharmacopoeia grade. Methanol and acetonitrile were HPLC grade and other reagents for ofloxacin determination were analytical grade. Double-distilled water was used. Ciprofloxacin purchased from Zhejiang Jiangnan Pharmaceutical Co. Ltd. (Zhejiang, China) was used in the *in vivo* assays as an internal standard. Commercially available ofloxacin capsules (0.1 g, Brand name: Jianglin, Xi'an Fangxing Pharmaceuticals Ltd., Xi'an, China) were chosen as the reference preparation in the multiple doses bioavailability study.

2.1. Preparation of ofloxacin sustained release pellets

2.1.1. Preparation of drug-loaded pellets

A laboratory-scale centrifugal granulator (Model BZJ-360M, Beijing Tianmin High Technology Development Co., China) was used to prepare drug-layered pellets. The formulation of the drug-loaded pellets was as follows: ofloxacin 2000 g; MCC blank beads 450 g; lactose 150 g; MCC 150 g and citric acid 150 g. MCC non-pareil pellets with a 40–60 mesh screen size (0.45–0.28 mm) were prepared by the same apparatus and used as the initial cores. The MCC pellets were put into the rotating chamber and a uniform mixture of drug and excipients (lactose, mixture of MCC and citric acid) were passed through a 120 mesh screen (0.15 mm) and added to the feeding chamber and then the machine was started. The rotating speed of the plate was 180 rpm, the rate of water spraying was 10×15 l/min, the air flow rate was 17 l/min, the pressure of the spray air was 0.5 MPa and the rotation rate of the powder feeder was 18 rpm. Ofloxacin with the excipients were layered on the core beads until the feeder was empty while simultaneously spraying water on the pellets. After addition of all the drug powder, the plate was kept rotating for an extra 4 min in order to polish the pellets. Finally, the drug-loaded products were discharged from the chamber and cured in a 40 °C oven for 24 h. The 18–24 mesh (1.00–0.80 mm) pellets were chosen as the candidates for coating.

2.1.2. Coating of drug-loaded pellets

A fluidized bed (FD-MP-01, Powrex, Japan) was used to modify pellets on a laboratory scale and 500 g drug-loaded pellets was the sample size. The different coating formulations used are listed in Table 1 and mainly involved Eudragit® L30D55, Eudragit® NE30D and DEP. Each substance was weighed accurately. Eudragit® NE30D was added dripwise to Eudragit® L30D55 the volume of which had been doubled with water and agitated by a magnetic bar at room temperature. Then, the plasticizer solution was also added dripwise to the copolymer suspension. The final suspension, stirred magnetically for at least 1 h, was sprayed onto the pellets under the following conditions: inlet temperature: 35 °C, outlet temperature: 28–30 °C, spray rate: 8 ml/min. Then the final pellets were cured for 24 h at 40 °C.

Table 1
Different formulations of coating suspensions

Formulation	Eudragit® NE30D:Eudragit® L30D55 (w/w)	DEP[a] (%)	Coating level (%)
F1	1:6	10	8
F2	1:8	10	8
F3	1:10	10	8
F4	1:8	10	3
F5	1:8	10	6
F6	1:8	10	10

[a] The weight of DEP was equal to that of 10% (w/w) of the dry polymer in the coating suspensions.

2.2. In vitro release test

The release of ofloxacin from pellets was investigated according to USP 29, using dissolution apparatus 1 and all the release tests were conducted in triplicate. The media used were 0.1 N hydrochloride acid with 0%, 0.9%, 2.0% and 5.0% sodium chloride, pH 4.5 and pH 7.4 phosphate buffers, separately, and the temperatures were all maintained at 37 ± 0.5 °C. The pellets of a single ofloxacin dose, i.e. pellets containing about 200 mg of ofloxacin, were placed in a rotating basket. The rotating speed of the basket was 50 rpm. At each predetermined time point, a 4 ml aliquot of the dissolution medium was taken from each vessel and replaced by the same volume of fresh medium. The total volume of medium was kept at 900 ml. The amount of ofloxacin released in the medium was determined by UV spectrometric method at 293 nm. The content of drug in ofloxacin sustained release pellets for calculating the percentages of drug released in the medium was assayed by UV spectrometric method, too.

2.3. Bioavailability study

2.3.1. Multiple dose bioavailability

The ofloxacin sustained release pellets were filled into hard gelatin capsules based on the dose for the investigation. Meanwhile, a commercially available ofloxacin capsule which was a conventional ofloxacin preparation was chosen as the reference preparation. The experimental protocol was approved by the University Ethics Committee for the use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals. The study had an open, randomized, cross-over and multi-dose design. Six male beagle dogs were divided into 2 groups. One were given 0.4 g of test preparation (ofloxacin sustained release pellets, each capsule containing ofloxacin 0.2 g) once a day at 7:00 orally, while the other were given reference drug 0.2 g (ofloxacin capsule, each capsule containing ofloxacin 0.1 g) every 12 h, twice a day via the same route (7:00 for the first dose, 19:00 for the second dose). All the dogs received the drug repeatedly for 6 days. Then the 2 groups received the other treatment schedule after a 2-week washout period. For the test group, blood samples were collected before administration of the fourth and the fifth doses on day 4 and day 5, and at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 24 h immediately after the sixth dose on day 6. For the reference dogs, blood samples were collected before administration of the seventh and the ninth doses on day 4 and day 5, and at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 h after the eleventh dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 h after the twelfth dose on day 6. At each time point, 3 ml blood

was withdrawn from the superficial vein of the forelimbs. Plasma samples were obtained after centrifugation (3500 rpm) for 15 min and stored in a refrigerator at −20 °C until analysis.

The plasma was thoroughly mixed with 50 μl internal standard solution (ciprofloxacin methanol solution). Then, 100 μl aqueous trichloroacetic acid solution was added (100 mg/ml) to precipitate plasma proteins. The mixtures were vortexed for 3 min to allow complete mixing, followed by centrifugation (8000 rpm) for 10 min. Then a 10 μl aliquot of supernatant was directly injected into the high performance liquid chromatography system.

Chromatographic conditions: Diamonsil C_{18} column, 150 mm × 4.6 mm I.D., 5 μm (DIKMA, USA); mobile phase acetonitrile: 0.05 mol/l citric acid (pH of the aqueous phase was adjusted by triethylamine to 3.5) (18:82, v/v); flow rate 0.9 ml/min; UV detector wavelength 293 nm.

The plasma concentration of ofloxacin was found to be linear over the range 0.2–20 μg/ml. The correlation coefficient ranged from 0.9957 to 0.9983. The lowest value of the standard curve, i.e. 0.2 μg/ml was taken as the lower limit of quantitation (LLOQ). The relative standard deviations (R.S.D.) of accuracy and precision for three different plasma concentrations of ofloxacin (0.5, 5.0 and 17.5 μg/ml) were 4.60%, 0.57% and 1.00% for intra-day analysis, and 8.50%, 6.99% and 4.83% for inter-day analysis. The recovery for the three plasma concentrations of ofloxacin was found to be 79.1%, 66.0% and 58.4%, with standard deviations (S.D.) less than 4%.

2.3.2. Data analysis

The concentrations of ofloxacin in plasma were calculated, and all the data were processed by DAS statistical software. The area under plasma concentration–time curve at steady-state on day 6 (AUC_{ss}) was calculated by the trapezoidal method, and the time to maximum plasma concentration (day 6) (T_{max}), maximum and minimum plasma concentration at steady-state (day 6) (Css_{max} and Css_{min}) of the test preparation were actual observations, and the values of T_{max}, Css_{max} and Css_{min} of the reference preparation were the mean values of the two doses on the last day (day 6) of administration. The mean drug concentration at steady-state on day 6 (C_{av}) was calculated as follows: AUC_{ss}/τ, where τ = dose interval. And degree of fluctuation (DF) was acquired by $(Css_{max} - Css_{min})/C_{av}$. The relative bioavailability was calculated by dividing the AUC_{ss} of the test preparation by that of the reference preparation.

3. Results and discussion

3.1. Preparation of ofloxacin sustained release pellets

The solubility of ofloxacin in different media at room temperature is shown in Fig. 1. It can be seen that the solubility of ofloxacin was very dependent on the pH. In agreement with earlier publications, it was much higher in acidic solutions than in alkaline ones (Chavanpatil et al., 2005). Therefore, as reported by Zhang et al. (2005) previously, it was essential to control the amount of ofloxacin released in acid media. Eudragit® L30D55 was suitable for this. Eudragit® L30D55 is often used to obtain enteric dissolution. When the pH is higher than 5.5, it loses the ability to inhibit the release of drug gradually (Song et al., 2002) and, according to Huyghebaert et al. (2005) the dissolution of Eudragit® L30D55 at a pH higher than 6.5 is almost independent of its amount So, the formulation could be dominated by Eudragit® L30D55. Eudragit® NE30D, when used as a film material having medium permeability, can effectively block the release of drug over a wide pH range and it was used to promote the effect of sustained release under both acidic and alkaline conditions. Due to the small amount of Eudragit® NE30D in the formulation, DEP or polyethylene glycol 6000 (PEG 6000) needs to be used as a plasticizer to assist the film formation of Eudragit® L30D55, which renders the coating membrane more robust and stable. Since it is soluble in aqueous solutions, PEG 6000 can make the pellets more porous when dissolved in water. Consequently, it may adversely affect the sustained release effect of the coating membrane on ofloxacin pellets. Therefore, PEG 6000 is not an option when DEP is used.

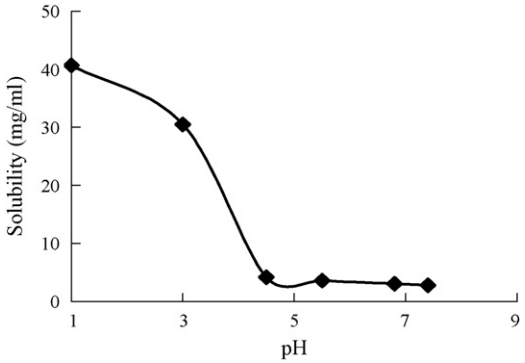

Fig. 1. Solubility of ofloxacin in different pH solvents at room temperature.

3.2. In vitro release tests

3.2.1. Release of uncoated ofloxacin pellets in different media

Fig. 2 shows that the ofloxacin in the pellets without a coating membrane released completely in less than an hour in 0.1N hydrochloride acid and phosphate buffer solutions with pH values of 4.5 and 7.4. In particular, all the ofloxacin in the uncoated pellets was released in 20 min in 0.1N hydrochloride acid. Thus, it was essential for ofloxacin pellets to be coated to control its release.

3.2.2. Release of ofloxacin pellets with different coating formulations in different media

It can be seen from Fig. 3 that the coating suspensions dominated by Eudragit® L30D55 successfully prolonged the release of ofloxacin in 0.1N hydrochloride acid, basically obtaining the desired goal. However, the release of the pellets with three different ratios of coating materials showed similar profiles due to the insolubility of both Eudragit® NE30D and Eudragit® L30D55 under acidic conditions. Consequently, it was difficult to select the proper ratio relying on the release profile in a single medium. Therefore, the pellets with three different coating films with ratios of Eudragit®

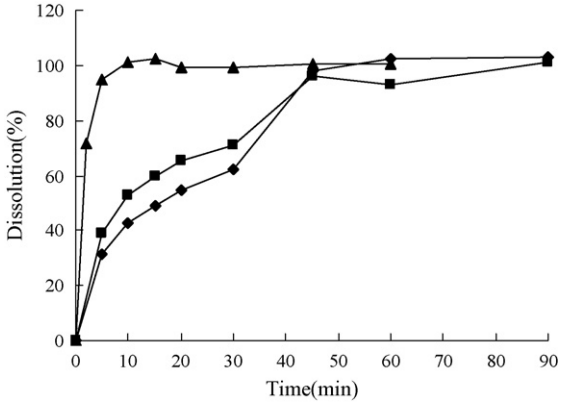

Fig. 2. Release profiles of ofloxacin pellets without coating membrane in different media. (♦) pH 7.4 phosphate buffer; (■) pH 4.5 phosphate buffer; (▲) 0.1N hydrochloride acid.

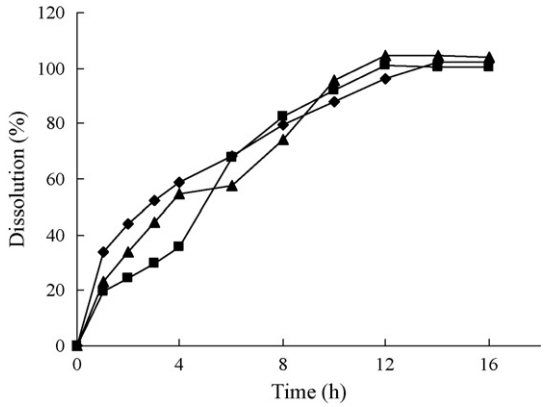

Fig. 3. Release profiles of ofloxacin coated with three different ratios of methacrylic acid copolymers with the same coating level of 8% in 0.1N HCl. (♦) NE30D:L30D55 = 1:8 (F2); (■) NE30D:L30D55 = 1:6 (F1); (▲) NE30D:L30D55 = 1:10 (F3).

NE30D to Eudragit® L30D55 of 1:6, 1:8 and 1:10, were further tested in other media.

From Fig. 4, it can be seen that the order of the release rate in phosphate buffer with a pH of 4.5 (from the fastest to the slowest) was the pellets with a copolymer ratio of 1:10, 1:8 and 1:6. The higher the content of Eudragit® L30D55 in the coating material, the faster the drug release from the pellets. Taking into account the solubility of ofloxacin in pH 4.5 medium and the release characteristics in both pH 1.0 and 4.5 media, the pellets with a 1:8 ratio of Eudragit® NE30D to Eudragit® L30D55 was chosen for further investigation.

3.2.3. *Influence of coating levels on the release of ofloxacin*

Fig. 5 shows that almost all the ofloxacin released in the medium in the first hour when the coat loading was 3% (F4). Also, the 6% coating (F5) did not properly control the release of ofloxacin and all the drug was released after 4 h. With a 10% coat loading (F6), nearly 20% of the ofloxacin was released from the pellets at 12 h and no more than 60% was released at the end of dissolution at 24 h. Among all the coat loadings, F2 showed the best ofloxacin release in 0.1N HCl medium, so a percentage coat loading of 8% was chosen.

3.2.4. *Release profiles comparison*

According to literatures (Chavanpatil et al., 2005, 2006), an Indian company has developed a gastric retention sustained release

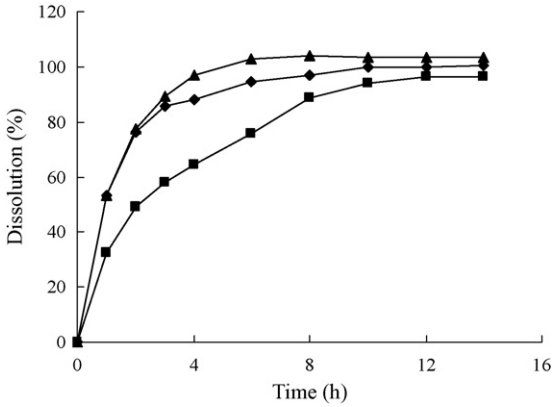

Fig. 4. Release profiles of ofloxacin coated with three different ratios of methacrylic acid copolymers with the same coating level of 8% in the pH 4.5 phosphate buffer. (♦) NE30D:L30D55 = 1:8 (F2); (■) NE30D:L30D55 = 1:6 (F1); (▲) NE30D:L30D55 = 1:10 (F3).

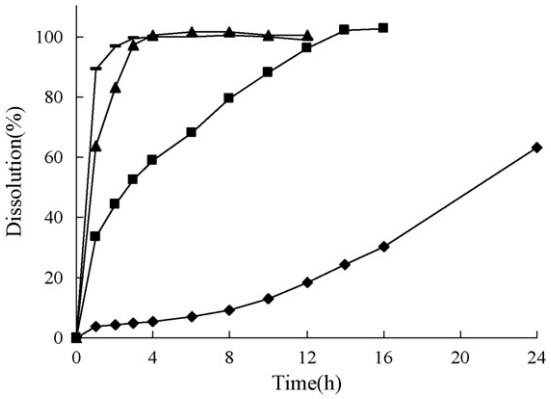

Fig. 5. Release profiles of ofloxacin pellets with different coating levels in 0.1N HCl. The ratio of Eudragit® NE30D to Eudragit® L30D55 was 1:8. (♦) 10% (F6); (■) 8% (F2); (▲) 6% (F5); (-) 3% (F4).

ofloxacin tablet which had bioadhesive and swellable characteristics. The release of ofloxacin was controlled by polymers and the preparation had bioadhesive effects which prolonged the retention time in the gastrointestinal tract. These advantages ensured steady and effective absorption of ofloxacin. The illustration in the paper showed that the release of ofloxacin from the tablets in 0.1N HCl at 1, 2, 3, 4, 8, 12 h was approximately 22%, 30%, 40%, 49%, 68% and 76%, respectively (Chavanpatil et al., 2005, 2006). The profile of sustained release pellets showed the same trend and shared a similar slope compared with gastric retention sustained release tablets under the conditions described in the paper during the first 8 h. However, after 8 h, more drug released from the pellets at each time point than from the gastroretentive preparation.

Chavanpatil et al. also applied a pH changing method to evaluate their gastroretentive preparation in which the tablets were initially kept for 2 h in a pH 1.2 medium. Then, they were transferred to a second medium with a pH of 4.5 for a further 2 h and, finally, the medium was changed to pH 7.4 for the remainder of the test until 24 h (Chavanpatil et al., 2005). Because of the high dissolution *in vitro* it was assumed that there would be a high bioavailability *in vivo* (Okonogi et al., 1997). Ofloxacin in the gastroretentive tablets was not released completely (approximately 65% was released) at the end of the test, and this might cause a reduced bioavailability. Fig. 6 shows the release profile of ofloxacin sustained release pellets under the conditions mentioned above. In the pH 1.2 medium for 2 h and subsequently in the pH 4.5 medium for another 2 h, no more than 35% of the drug was released from the ofloxacin pellets, which exhibited good sustained release characteristics. A similar

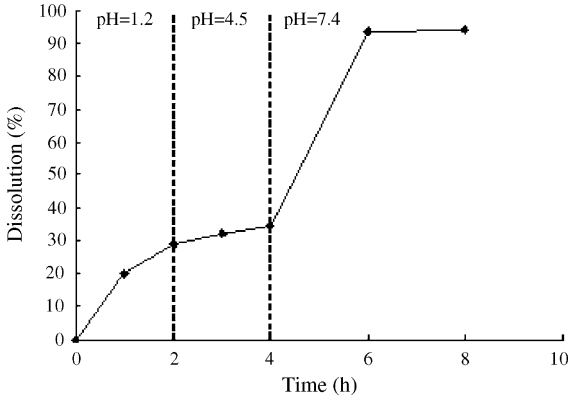

Fig. 6. Release profile of ofloxacin sustained release pellets F2 by pH changing method.

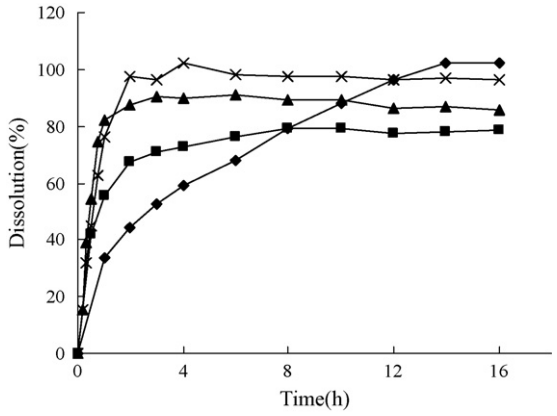

Fig. 7. Release profiles of ofloxacin sustained release pellets F2 in 0.1N HCl with 0%, 0.9%, 2.0% and 5.0% NaCl. (♦) 0.1N HCl without NaCl; (■) 0.1N HCl with 0.9% NaCl; (▲) 0.1N HCl with 2.0% NaCl; (×) 0.1N HCl with 5.0% NaCl.

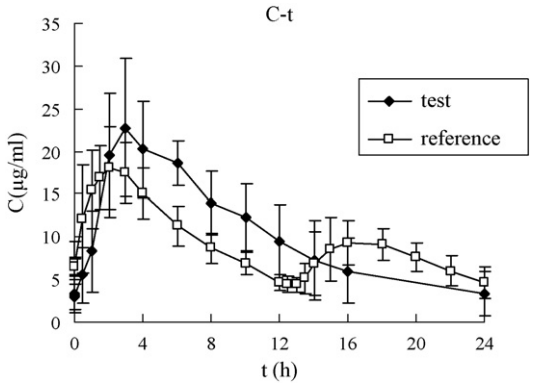

Fig. 8. Mean ofloxacin plasma concentration–time curves of six beagle dogs at steady-state on day 6. Each point represents the mean concentration ± S.D. (♦) test preparation; (□) reference preparation.

release profile was seen for the gastroretentive tablets. However, when pellets were placed in pH 7.4 phosphate buffer, it took less than 2 h for the remaining drug to be released from the pellets. During the process, it was observed that the pellets lost their physical integrity in the pH 7.4 dissolution medium. This might be caused by the dissolution of Eudragit® L30D55 in the medium and the thin layer of Eudragit® NE30D not having the ability to maintain the structure of pellets. In addition, all excipients were wetted and dissolved, which might also contribute to the breakdown of the pellets. Basis on these findings, it appears that ofloxacin could be slowly and steadily released from the pellets in the stomach and, after the pellets reached the jejunum, the rest of drug in the pellets could be released over a short period of time. This probably provides a good chance for the complete dose of drug being taken up into the blood, ensuring a high bioavailability.

3.2.5. *Effect of osmotic pressure on the release of ofloxacin from the pellets*

Release profiles of pellets in 0.1N hydrochloride acid media with 0%, 0.9%, 2.0% and 5.0% sodium chloride are shown in Fig. 7. In the media with sodium chloride, there was an obvious burst release in the first hour. More than 70% of the drug dissolved from the pellets in media containing 2.0% and 5.0% sodium chloride, which was almost 2-fold the amount of drug released in the medium without salt. Also, for pellets in dissolution medium containing 0.9% sodium chloride, the cumulative release of drug exceeded 55% in the first hour. This showed a trend that with the increase in salt concentration, the phenomenon of burst release became more and more marked. This implies that a higher osmotic pressure enhanced the release of drug from the pellets. A previous article has confirmed that the presence of Na^+ did not lead to an interaction with ofloxacin (Fresta et al., 2002). Knop (1996) previously reported that the addition of NaCl slowed the release of pellets coated with Eudragit® RS which probably meant that there was an interaction between Cl^- and the coating membrane. These results suggest that the release of ofloxacin from sustained release pellets is not driven by osmotic pressure. So, the mechanism for release from pellets was diffusion, which agrees with the conclusion reached above.

3.3. Bioavailability

The mean concentration–time curves for the test and reference preparations in six beagle dogs at steady-state are illustrated in Fig. 8.

Six beagle dogs were given test (capsules filled with ofloxacin sustained release pellets) and reference preparations (ofloxacin capsules) orally; all the pharmacokinetic parameters are listed in Table 2. The mean relative bioavailability of ofloxacin sustained release pellets to ofloxacin capsules, which was calculated from the AUC_{ss} of ofloxacin, was 116.35 ± 33.31%. There was a significant difference between the test and reference preparations. The main pharmacokinetic parameters were transformed logarithmically and standard deviations were calculated. The results indicated that the AUC_{ss}, Css_{max}, Css_{min}, C_{av} and DF of ofloxacin for test and reference preparations exhibited no intra-cycle and intra-individual differences and the AUC_{ss}, Css_{max}, Css_{min} and C_{av} exhibited no intra-preparation difference, either. The only marked differences were in the intra-preparation Css_{max} and DF. Furthermore, all the data were analyzed by a two-sided *t*-test and the $(1–2\alpha)$ confidence interval method, and the 90% confidence interval of the AUC_{ss} of the test preparation was 87.5–144.9% of reference preparation. It was also found that the bioavailability of the test preparation was higher than that of the reference preparation.

After the beagle dogs received multiple doses of ofloxacin, it was found that the Css_{max} of the reference preparation was 18.80 ± 4.01 μg/ml at the eleventh administration on day 6. After the twelfth dose on the same day, the Css_{max} was 10.14 ± 2.79 μg/ml, approximately half that of the eleventh administration. There was a clear difference between the two administrations. The reduced mean Css_{max} of the reference preparation resulted in a significant difference in the DF of the two preparations. As was shown in Fig. 8, when the reference preparation reached steady-state, the AUC_{ss} of the eleventh administration

Table 2
Pharmacokinetic parameters of test and reference preparations at steady-state on day 6

	T_{max} (h)	Css_{max} (μg/ml)	Css_{min} (μg/ml)	C_{av} (μg/ml)	DF
Test	3.3 ± 1.4	25.55 ± 2.87	3.05 ± 1.98	10.38 ± 2.14	2.26 ± 0.78
Reference	3.3 ± 0.5	14.47 ± 2.92	4.68 ± 1.23	9.26 ± 1.95	1.06 ± 0.12

All the data are presented in the form of mean ± S.D.

at 7:00 differed markedly from the twelfth administration at 19:00. The latter was much lower than the former. This phenomenon led to the improved bioavailability of the test compared with the reference. Chrono-pharmacokinetics may provide a plausible explanation for the reduction in the AUC_{ss} and Css_{max} for the reference preparation, which has not been reported so far in multi-dose pharmacokinetic studies in either beagle dogs or human volunteers (Marier et al., 2006). This deserves further investigation.

If the chrono-pharmacokinetic interference in the absorption of ofloxacin in beagle dogs is ignored, i.e. the twelfth administration of the reference preparation at the steady-state was disregarded, the Css_{max} was 25.55 ± 2.87 μg/ml for the 400 mg test dose, while, for the reference it was 18.80 ± 4.01 μg/ml for 200 mg after the first administration. As reported previously, the activity of ofloxacin relies on the amount taken up by the body (Marier et al., 2006). It was clear that the peak concentration of the test preparation was much higher than that of the reference. So this suggests the superior activity of ofloxacin sustained pellets compared with standard preparations. However, the peak value of the plasma concentration in the test group which had received a double dose was not twice as high as that of the reference. This demonstrated that the test preparation exhibited a sustained release effect, compared with the reference drug. The test preparation prolonged the T_{max} from 2.0 ± 0.5 h to 3.3 ± 1.4 h, which could also be an evidence for the sustained release effect of the test preparation.

4. Conclusion

In the dissolution test, it was found that ofloxacin pellets exhibited a sustained release effect after coating with Eudragit® NE30D and Eudragit® L30D55 with DEP as a plasticizer. The 1:8 ratio of these two copolymers and the amount of DEP equal to 10% of solid in the copolymer suspension made the release of ofloxacin from pellets sustained and dependent on the environment pH in the gastrointestinal tract. Ofloxacin sustained release pellets had similar release profiles *in vitro* under acidic conditions to those of the gastroretentive ofloxacin tablets which were developed by an Indian company. Due to sustained release effect, the ofloxacin sustained release pellets achieved the desired goal. Meanwhile, ofloxacin sustained release pellets improved the release of ofloxacin *in vitro* in pH 7.4 medium compared with the gastroretentive tablets. From the bioavailability test data, it was obvious that ofloxacin sustained release pellets not only improved the bioavailability *in vivo* but also exhibited a prolonged release effect compared with the reference drug which was an ordinary ofloxacin capsule commercially available in China. It can be concluded that the ofloxacin sustained release pellets are a suitable preparation of ofloxacin exhibiting sustained release characteristics.

References

Chavanpatil, M.D., Jain, P., Chaudhari, S., Shear, R., Vavia, P.R., 2006. Novel sustained release, swellable and bioadhesive gastroretentive drug delivery system for ofloxacin. Int. J. Pharm. 316, 86–92.

Chavanpatil, M.D., Jain, P., Chaudhari, S., Shear, R., Vavia, P.R., 2005. Development of sustained release gastroretentive drug delivery system for ofloxac: in vitro and in vivo evaluation. Int. J. Pharm. 304, 178–184.

Dévay, A., Mayer, K., Pál, S., Antal, I., 2006. Investigation on drug dissolution and particle characteristics of pellets related to manufacturing process variables of high-shear granulation. J. Biochem. Biophys. Methods 69, 197–205.

Fresta, M., Guccione, S., Beccari, A.R., Furneri, P.M., Puglisi, G., 2002. Combining molecular modeling with experimental methodologies: mechanism of membrane permeation and accumulation of ofloxacin. Bioorg. Med. Chem. 10, 3871–3889.

Fünfstück, R., Wolfram, M., Gerth, J., Schubert, K., Straube, E., Stein, G., 1999. The influence of ofloxacin (Tarivid®) on the parasite–host inter-relationship in patients with chronic urinary tract infection. Int. J. Antimicrob. Agents 11, 297–303.

Hemati, M., Cherif, R., Saleh, K., Pont, V., 2003. Fluidized bed coating and granulation: influence of process-related variables and physicochemical properties on the growth kinetics. Powder Technol. 130, 18–34.

Hu, L.D., Liu, Y., Tang, X., Zhang, Q., 2006. Preparation and in vitro/in vivo evaluation of sustained-release metformin hydrochloride pellets. Eur. J. Pharm. Biopharm. 64, 185–192.

Huyghebaert, N., Vermeire, A., Remon, J.P., 2005. In vitro evaluation of coating polymers for enteric coating and human ileal targeting. Int. J. Pharm. 298, 26–37.

Knop, K., 1996. Influence of buffer solution composition on drug release from pellets coated with neutral and quaternary acrylic polymers and on swelling of free polymer films. Eur. J. Pharm. Sci. 4, 293–300.

Lyer, R.M., Sandhu, H.K., Shah, N.H., Phuapradit, W., Ahmed, H.M., 2006. Scale-up of extrusion and spheronization. In: Levin, M. (Ed.), Pharmaceutical Process Scale-Up, vol. 157. Taylor & Francis, New York, London, pp. 325–369.

Marier, J.F., Ducharme, M.P., DiMarco, M., Di Spirito, M., Morelli, G., Tippabhotla, S.K., Badri, N., Rampal, A., Monif, T., 2006. Two open-label, randomized, crossover studies assessing the bioequivalence of ofloxacin administered as immediateand extended-release formulations in healthy subjects. Clin. Ther. 28, 2070–2080.

Mouton, Y., Leroy, O., 1991. Ofloxacin. Int. J. Antimicrob. Agents 1, 57–74.

Okhamafe, A.O., Akerele, J.O., 1989. Some aspects of the bioavailability of orally administered ofloxacin in healthy human volunteers. Int. J. Pharm. 50, 83–86.

Okonogi, S., Oguchi, T., Yonemochi, E., Puttipipatkhachorn, S., Yamamoto, K., 1997. Improved dissolution of ofloxacin via solid dispersion. Int. J. Pharm. 156, 175–180.

Rashid, H.A., Heinämäki, J., Antikainen, O., Yliruusi, J., 2001. Influence of the centrifugal granulating process on the properties of layered pellets. Eur. J. Pharm. Biopharm. 51, 227–234.

Song, H.T., Guo, T., Zhang, R.H., Zheng, C.L., Ma, Y., Li, X., Bi, K.S., Tang, X., 2002. Preparation of the traditional Chinese medicine compound recipe heart-protecting musk pH-dependent gradient-release pellets. Drug Dev. Ind. Pharm. 28, 1261–1273.

Tomer, G., Podczeck, F., Newton, J.M., 2002. The influence of model drugs on the preparation of pellets by extrusion/spheronization: II spheronization parameters. Int. J. Pharm. 231, 107–119.

Zhang, X.R., Chen, X.Y., Hu, L.D., Tang, X., Li, S.M., Zhong, D.F., 2005. Evaluation of in-vitro dissolution and in-vivo absorption for two different film-coated pellets of clarithromycin. Arch. Pharmacal. Res. 28, 977–982.

Zivanovic, Lj., Zigic, G., Zecevic, M., 2006. Investigation of chromatographic conditions for the separation of ofloxacin and its degradation products. J. Chromatogr. A 1119, 224–230.