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Preparation and in vitro–in vivo evaluation of none gastric resident dipyridamole (DIP) sustained-release pellets with enhanced bioavailability

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ABSTRACT

The objective of this study was to develop none gastric resident sustained-release pellets loaded with dipyridamole with a high bioavailability.

Two different kinds of core pellets, one containing citric acid as a pH-modifier (CAP) and, the other without pH-modifier (NCAP) were prepared by extrusion-spheronization and then coated with mixtures of enteric soluble and insoluble polymers (referred to as CAP_1 and $NCAP_1$) or insoluble polymer alone (referred to as CAP_2 and $NCAP_2$).

The relative bioavailability of the sustained-release pellets was studied in fasted beagle dogs after oral administration using a commercially available immediate release tablet (IRT) as a reference. The in vitro release, in vivo absorption and in vitro–in vivo correlation were also evaluated.

Results revealed that the plasma drug concentrations after administration of CAP_2, $NCAP_1$ and $NCAP_2$ were undetectable, indicating that the drug release was almost zero from the preparations throughout the gastro-intestinal tract. The C_{max}, T_{max} and $AUC_{(0\rightarrow 24)}$ of CAP_1 were 0.78 ± 0.23 (μg/ml), 3.80 ± 0.30 (h), and 6.74 ± 0.47 (μg/ml h), respectively. While the corresponding values were 2.23 ± 0.32 (μg/ml), 3.00 ± 0.44 (h) and 9.42 ± 0.69 (μg/ml h) for IRT. The relative bioavailability of CAP_1 was 71.55% compared with IRT.

By combined incorporation of a pH-modifier into the core of pellets to modify the inner micro-environment and employing mixtures of enteric soluble and insoluble polymers as a retarding layer, drugs with high solubility in stomach and limited solubility in small intestine, such as DIP, could be successfully formulated as sustained release preparations with no pH-dependence in drug release and enhanced bioavailability.

1. Introduction

Dipyridamole (persantin) inhibits platelet aggregation and plays an important role in antithrombokinesis and expanding vascellum. It has been widely used to inhibit and cure angina, and prevent recurring myocardial infarction and thrombosis in clinical situations (Village, 1991).

Common preparations require frequent administration given their short half-time (Guan et al., 2008), so sustained release preparations are urgently needed. Recently, it has been proposed that DIP therapy requires the use of a sustained release formulation for prolonged action and to improve patient compliance (Zhang et al., 2005).

DIP is a weakly basic drug which exhibits a high but pH-dependent solubility with good solubility (37 °C, 36.5 g/L at pH 1.0) at low pH and poor solubility (37 °C, 0.02 g/L at pH 7.0) at a higher pH (Hasegawa et al., 1986). Because the increasing pH along the gastrointestinal tract (GI-tract) results in lower solubility of dipyridamole, the major absorption sites of DIP are the stomach and duodenum (Hu et al., 2006). Normally, preparations passed through the GI tract from the stomach to the colon constantly, thus making the sustained and durative release of DIP throughout the GI tract difficult to be realized because of the increasing pH value along the GI tract and the resulted decreasing drug solubility. What is more, the small intestine transit time is much longer than gastric emptying time in the fasted state. As a result, incomplete and irregular drug absorption in the gastrointestinal tract after oral administration of the slow release preparations of DIP is often observed and this results in a low bioavailability and clear inter-patient variability (Naonori and Masaki, 1992).

Previous research has involved the use of traditional matrix tablets, membrane-controlled release pellets and gastric floating prolonged-release beads (Zhou et al., 2005; Zhang et al., 2008). However, traditional matrix tablets and membrane-controlled release pellets were unable to overcome the difficulties associated with the pH-dependent solubility of the drug, while gastric floating preparations are not always effective due to the complexity of the gastro-intestinal tract.

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The purpose of the present study was to develop none gastric resident DIP sustained-release pellets with enhanced bioavailability by achieving a sustained drug release along the entire GI-tract to minimize the influence of the physiological factors. More specially, the objectives were to evaluate the suitability of combined use of citric acid as a pH-modifier and Eudragit® NE 30D and Eudragit® L30 D-55 polymer blend as a coating material in slow release and enhanced drug dissolution applications.

(Jijun et al., 2011) has demonstrated that the bioavailability of diclofenac potassium sustained release pellets was greatly enhanced by achieving durative as well as sufficient drug release in the small intestine. Although the pH-dependent nature of diclofenac potassium and dipyridamole were opposite, similar conclusions that drugs should be released from the preparations gradually and as much as possible in less than 4 h were drawn in both investigations. Thus, the reliability of the hypothesis was confirmed to an extent.

2. Materials and methods

2.1. Materials

The materials used in the test were as follows: dipyridamole (The Sixth Pharmaceutical Factory in Shanghai, China), citric acid (Suichang Huikang, Zhejiang), stearic acid (Bodi, Tianjin), hydroxypropyl methyl cellulose (HPMC; Huzhou Zhanwang, Zhejiang), Compritol® 888 ATO (GATTEFOSSE, Shanghai), microcrystalline cellulose (MCC; Huzhou Zhanwang, Zhejiang), talc (Yulin Talc Factory, Shanxi), Eudragit® NE30D and Eudragit® L30D-55 (Degussa, Germany), diethyl-o-phthalate (DEP; Huzhou Zhanwang, Zhejiang), sodium dodecyl sulfate (SDS; Bodi, Tianjin), and diazepam (Biocause Pharmaceutical Co., Ltd., Hubei).

Other excipients used to prepare the pellets were of standard pharmaceutical grade. Acetonitrile was HPLC grade and other reagents were of analytical grade. Dipyridamole standard substance (99.9%) was provided by the Chinese National Institute for the Control of Pharmaceutical and Biological Products. Commercially available immediate release DIP tablets (25 mg, Tianjin Jinshi, China) were chosen as the reference in the bioavailability study. The hard gelatine capsules used in this study were supplied by Suzhou Capsule Company (Suzhou, China).

Male beagle dogs from the Laboratory Animal Center of Shenyang Pharmaceutical University were used. Principles of good laboratory animal care were followed and animal experimentation was carried out in compliance with the Guidelines for the Care and Use of Laboratory Animals in Shenyang Pharmaceutical University.

2.2. Preparation of drug-loaded pellets

Pellets were prepared by the extrusion/spheronization technique. There are different pelletizations and granulation techniques available to prepare drug loaded spherical particles or granules. Extrusion spheronization is one of them and utilized in formulation of beads and pellets. It is more labor intensive than other granulation method, but it is useful when uniform spherical shape, reproducibility in packing and smooth surface of granules is desired. Today this technology has gained attention because of its simple and fast processing. Extrusion spheronization is widely utilized in formulation of sustained release, controlled release delivery system (Rajesh Gandhi, 1999).

Five formulations are listed in Table 1. After mixing dipyridamole, EDTA, Compritol® 888 ATO, proper citric acid with sufficient MCC by passage through an 80 mesh screen three times, a homogeneous physical mixture was obtained. Then, a 5% (w/v) HPMC aqueous solution was used as an adhesive agent and added to obtain a wet mass. The plastic mass of this material then underwent extrusion using a Granulator (WL350, Wenzhou Pharmacy Equipment Factory, China) with a 1–2 mm screen carried out on a laboratory scale (0.5 kg) and then spheronized in a spheronizer (WL350, Wenzhou Pharmacy Equipment Factory, China). Finally, the beads were dried at 40 °C for 12 h and sized by passage through 18–24 mesh sieves. Core pellets containing CA and those without CA in formulation were named CAP and NCAP, respectively.

2.3. Pellets coating

2.3.1. Stearic acid hot-melt subcoating

Hot-melt subcoating is another method for coating water-soluble drugs. Instead of using a fluidized bed coater, a coating pan (B-300 Coating Pan, BaoJi JianHua Co. Ltd., Baoji, China) with a diameter of 30 cm was used in the holt-melt subcoating method and stearic acid (melting point 60 °C) was selected as the subcoating material to prevent drug migration during polymer coating (Yang et al., 2008). In this study, the hot-melt subcoating was carried out using a traditional coating pan although it has always used a fluidized bed (Philippe Barthelemya et al., 1999; Mitrevej, 2004). Compared with the fluidized bed coating method, not only production costs could be reduced but also time as well as labor could be saved by this technique.

An amount of pellets weighing 550 g was transferred into the coating pan with a rotation speed of 30 rpm. Heating was maintained until the temperature of the particles reached to 60 °C, then stearic acid was added to the pan in batches over a period of 10 min and the weight gain of stearic acid was 7%. Rolling stearic acid melt rapidly resulted in the formation of a uniform layer on the surface of the pellets. After rolling for another 15 min, the heating containing the air chest was turned off. Then, a suitable quantity of talc was added to avoid particles aggregation. Finally, the pellets continued to be rolled until the temperature became equal to the room temperature.

2.3.2. Polymer coating

In this study, Eudragit® NE 30D or a blend of Eudragit® NE 30D and Eudragit® L30 D-55 were used for polymer coating separately by the fluidized bed technique. The method reduced capital investment in equipment and ongoing maintenance when compared to electrostatic spray, which was basically a low-tech coating method, and once the coating parameters were established, there were not that many things that could go wrong. The coating formulations were designed as follows:

Table 1
The main formulations of core pellets.

Formulation no.	Composition of core pellets (%, w/w)					
	Dip	MCC	EDTA	Citric acid	Compritol® 888 ATO	5% HPMC solution (w/v)
1	12	56	1	–	20	11
2	12	55	1	3	20	9
3	12	48	1	7	20	12
4	12	50	1	10	20	7
5	12	45	1	12	20	10

Formulation a (Fa): coated with Eudragit® NE 30D: Eudragit® L30 D-55 (E-E; 1:1, w/w), resulting in a 7% coating level;

Formulation b (Fb): coated with Eudragit® NE 30D, resulting in a 3% coating level.

For Fa, DEP was used as a plasticizer and added to the desired amount of Eudragit® L30 D-55 aqueous dispersion and stirred for at least 3 h before diluting with distilled water to 15% (w/w) based on the dry polymer weight. Then, Eudragit® NE 30D was first diluted with distilled water to 15% (w/w) based on the dry polymer weight and added and stirred until a homogenous solution containing the two polymer was obtained. For Fb, Eudragit® NE 30D aqueous dispersion was diluted with distilled water to 15% (w/w) based on the dry polymer weight and stirred for at least 30 min.

A batch of 300 g subcoated pellets were polymer-coated in the fluidized bed coater with a bottom spray (FD-MP-01, Powrex, Japan). The process parameters were as follows: inlet temperature 25 °C, outlet temperature 23 °C, spray rate 1.5–2 ml/min, atomization pressure 1.2 bar, and rate of air flow 80 m^3/h. After application of the polymer coating, the pellets needed to be cured for 24 h in a 40 °C oven to form a homogeneous film. Fig. 1 shows a schematic diagram of the extended release pellets.

2.3.3. Dissolution testing

In vitro drug release was determined using the USP31-NF26 apparatus I (basket) at 100 rpm in 900 ml dissolution medium at 37 ± 0.5 °C. Coated pellets containing 250 mg dipyridamole were transferred to the dissolution medium, and 6 ml samples were withdrawn and replaced with an equal volume of fresh medium at pre-determined intervals. The samples were analyzed in an ultraviolet spectrophotometer (752 Violet Visible Spectro Photometer, Shanghai Spectro Equipment Company.) at a wavelength of 283 nm. A solution of 0.1 mol/L HCl solution, purified water, pH 4.5 acetate buffer and pH 6.8 phosphate buffer were used as different dissolution media and 0.05% (w/v) SDS was added to the last three media.

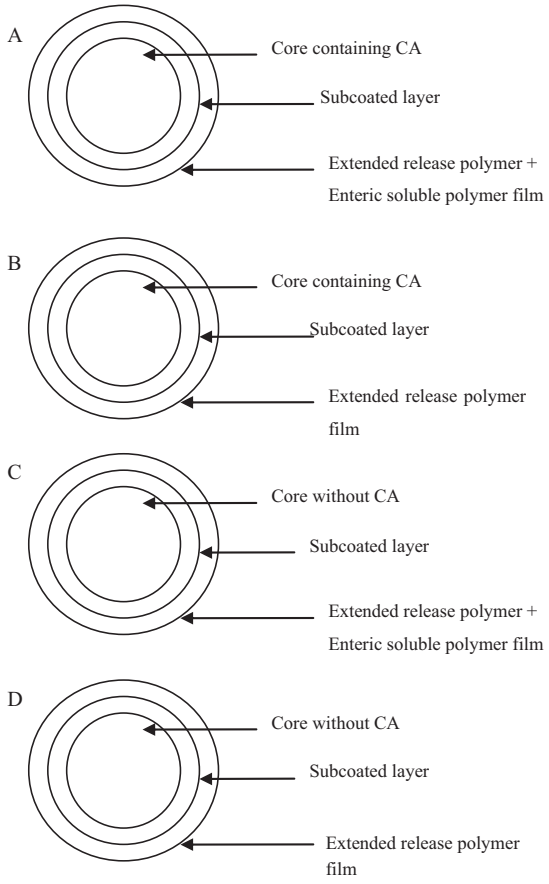

Fig. 1. Schematic presentation of different coated pellets: (A) CAP_1; (B) CAP_2; (C) $NCAP_1$; (D) $NCAP_2$.

2.4. Scanning electron microscopy

A scanning electron microscope (SSX-550, Shimadzu, Japan) was used to record the surface and cross-section of the pellets, which had been subjected to gold-coating beforehand.

3. Bioavailability study

3.1. Administration and sampling protocol

The study protocol was approved by the Ethics Committee of Shenyang Pharmaceutical University responsible for bioavailability studies. The study was carried out in accordance with a single-dose of five treatments and five periods. Each dog was given five preparations and there was a 1 week washout period between each treatment. Six beagle dogs were used for each treatment group. The dogs were randomly selected to receive a single dose of the preparations containing 50 mg DIP with 50 ml of water after an overnight fast (10 h). Blood samples (3 ml) were collected at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h after dosing. Plasma was separated from samples by centrifugation at 4000 rpm for 10 min and stored at −20 °C until analysis. Liquid–liquid extraction was employed to extract DIP from the above samples. For this, 100 μl plasma was mixed with 20 μl internal standard in a 7 ml screw-capped glass centrifuge tube. After vortexing for 5 min in a Liquid Fast Mixer (YKH-3, Liaoxi Medical Apparatus and Instrument Factory, China), the mixture were extracted with 3 ml ethyl ether by vortexing for 10 min and then centrifuged at 4000 rpm for 10 min. Then 2 ml of the organic supernatant was transferred to a clean 5 ml tube and evaporated to dryness at 45 °C in a centrifugal concentrator (Labconco Corp., MO, USA). The residue was then reconstituted in 200 μl mobile phase (water containing 0.1% formic acid (v/v)/acetonitrile = 4:1, v/v) and an aliquot (5 μl) was subjected to UPLC/MS/MS.

3.2. Analysis conditions and methods validation

Liquid chromatography was performed on an ACQUITYTM UPLC system (Waters Corp., Milford, MA, USA) fitted with an autosampler and column oven. The separation was carried out on an ACQUITY UPLC® BEH C18 column (50 mm × 2.1 mm i.d., 1.7 μm; Waters Corp., Milford, MA, USA) with the column temperature maintained at 40 °C. The analysis was achieved with gradient elution using a mobile phase composed of acetonitrile and water containing 0.1% (v/v) formic acid at a flow rate of 0.20 ml/min. The gradient elution started at 20% acetonitrile, increased linearly to 80% acetonitrile over 1.4 min, was maintained at 80% for 0.2 min and then returned to the initial percentage over 0.4 min.

A Waters ACQUITYTM TQD triple quadrupole tandem mass spectrometer (Waters Corp., Manchester, UK) with an electrospray ionization (ESI) interface was used for mass analysis. The ESI source was operated in positive ionization mode with the capillary voltage, extractor and RF voltage set at 2.5 kV, 3.0 V and 0.1 V, respectively. The source and desolvation temperature was set at 100 °C and 400 °C, respectively. Nitrogen was used as the desolvation gas (450 L/h) and cone gas (50 L/h). For collision-induced dissociation (CID), argon was used as the collision gas at a flow rate of 0.15 ml/min. The multiple reaction monitoring (MRM) mode was selected for transition quantification and the optimized fragmentation transitions for MRM were m/z 505.27 → m/z 429.22 amu for dipyridamole and m/z 285.12 → m/z 193.07 amu for diazepam,

with a scan time of 0.02 s per transition. All data collected in centroid mode were acquired using Masslynx™ NT4.1 software (Waters Corp., Milford, MA, USA) and the quantification was performed using peak areas.

The linear response of this method was 5–2500 ng/ml with an r (correlation coefficient) value of not less than 0.99. The lower limit of quantification (LLOQ) was set at 5 ng/ml and the R.S.D.s reflecting the intra-day and the inter-day precision of the QC samples were both not more than 13.3%. The method had an acceptable recovery of 63.8 ± 8.1% for dipyridamole and 61.6 ± 4.4% for diazepam (internal standard).

3.3. Data calculation

The pharmacokinetic parameters were calculated using a non-compartmental model. The area under the plasma concentration–time curve from time zero to time t h (AUC_{0-t}) was calculated using the trapezoidal method. The peak concentration (C_{max}) and time to reach the peak concentration (T_{max}) were obtained directly from the individual plasma concentration–time profiles. The relative bioavailability (F) was calculated as: $F = AUC_T/AUC_R$.

4. Results

4.1. The effect of citric acid (CA) on drug release

Solubilization of poorly water-soluble drugs has gained much interest in the pharmaceutical industry (Usui et al., 1998; Riis et al., 2007). Poorly water-soluble dipyridamole is weakly basic drug in nature, and shows pH-dependent solubility. For this reason, incorporating pH-modifier into the core pellets and modulating the pH in the dosage form will improve the release rate of dipyridamole, namely, the lower micro-environmental pH (pH_M) of the dosage forms created by the pH-modifier could enhance drug dissolution under basic conditions. Drug-loaded pellets with different amounts of CA (percentage based on pellet weight) were prepared and the dissolution testing was conducted in pH 6.8 medium (containing 0.05% SDS, w/v). The effect of the addition of different amounts of CA on drug release is shown in Fig. 2. It can be seen that with the increase in CA in the formulation from 0% to 12%, the release rate of dipyridamole became faster, with more drug being released from the core pellets within the same time period. The incorporation of CA significantly promoted the drug release by creating a more acidic micro-environment and keeping the pH within the drug formulation low, particularly in the basic medium. Drug-loaded core pellets containing 10% (w/w) CA and those without CA were selected for further study.

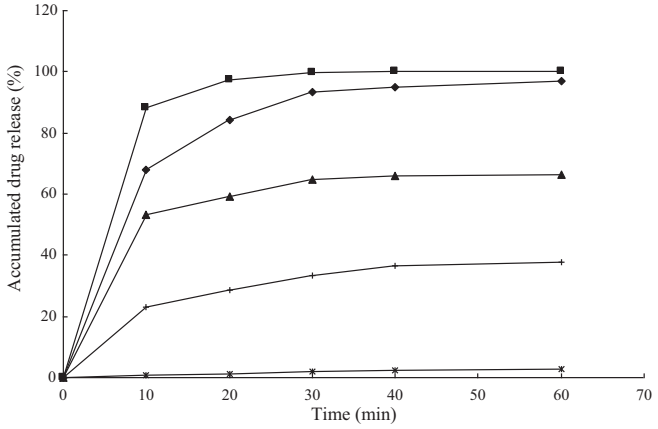

Fig. 2. The effect of CA on drug release from DIP pellets ($n = 3$) (*) 0% CA; (+) 3% CA; (▲) 7% CA; (♦) 10% CA; (■) 12% CA.

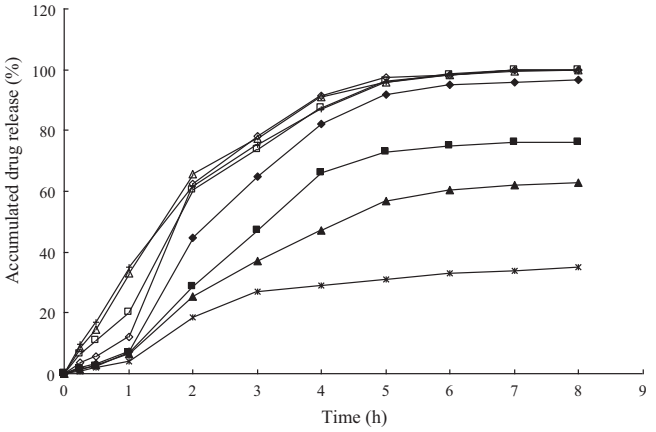

Fig. 3. Drug release profiles of CAP_1 and $NCAP_2$ in media with different pH values ($n = 3$) (◊) CAP_1, pH 1.2; (□) CAP_1, pH 4.5 (containing 0.05% SDS, w/v); (△) CAP_1, pH 6.8 (containing 0.05% SDS, w/v); (+) CAP_1, water (containing 0.05% SDS, w/v); (♦) $NCAP_2$, pH1.2; (■) $NCAP_2$, pH 4.5 (containing 0.05% SDS, w/v); (▲) $NCAP_2$, pH 6.8 (containing 0.05% SDS, w/v); (*) $NCAP_2$, water (containing 0.05% SDS, w/v).

4.2. In vitro release profiles of coated pellets

The profiles of DIP released from CAP_1 and $NCAP_2$ in media with different pH values are shown in Fig. 3.

It is clearly that the drug release rate of CAP_1 was not significantly affected by different pH -environments (pH_M) while that of $NCAP_2$ decreased steadily on increasing the pH-value of the dissolution medium due to the pH-dependence of DIP. For CAP_1, the pH-independent release was a result of the interaction with the pH-modifier and the retarding layer exhibiting different solubility properties. In acidic medium, the dissolution rate was promoted by the high solubility independent of the low pH_M or intrinsic good solubility while it was retarded by the insoluble E–E coating layer; when the pellets were treated at pH 6.8, Eudragit® L30 D-55 would dissolve (Lehmann, 1997) and the film appeared to be porous and permeable, leading to reduced prevention of the drug dissolution. The lower solubility of dipyridamole was overcompensated by the low pH_M created by CA and the increased permeability of the coating layer due to the enteric soluble Eudragit® L30 D-55. In current study, the release rate and amount of dipyridamole took these two aspects into account. Because Eudragit® NE 30D is insoluble

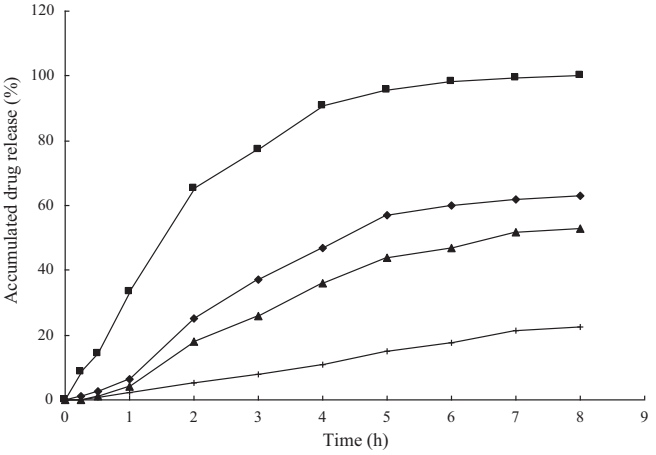

Fig. 4. Dissolution profiles of different formulations at pH 6.8 (containing 0.05% SDS, w/v; $n = 3$) (■) CAP_1; (♦) CAP_2; (▲) $NCAP_1$; (+) $NCAP_2$.

(Bodmeier and Paeratakul, 1989; Watano et al., 1997) and contributed to diffusion resistance at any pH-value, drug release from $NCAP_2$ is mainly controlled by its pH-dependent solubility. Considering the good solubility of dipyridamole, the pH-independent release profile of CAP_1 is expected to enhance the in vivo absorption of DIP throughout the GI tract.

As shown in the figure, a lag-time appeared in the dissolution curves of $NCAP_2$ and, resulted in not more than 10% drug release in

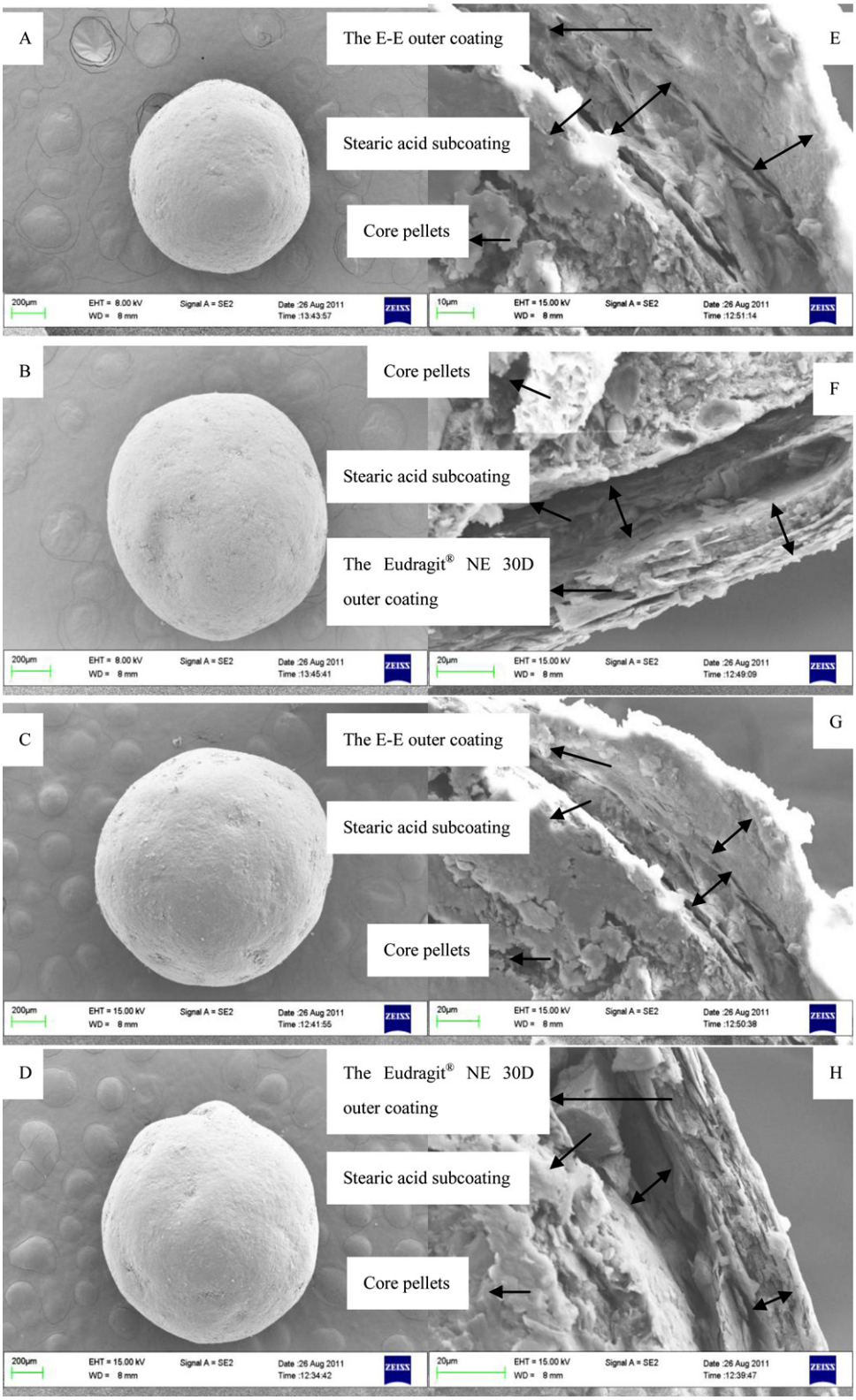

Fig. 5. (A) SEM photograph of CAP_1; (B) SEM photograph of CAP_2; (C) SEM photograph of $NCAP_1$; (D) SEM photograph of $NCAP_2$; (E) SEM photograph of a cross-sectional view of CAP_1; (F) SEM photograph of a cross-sectional view of CAP_2; (G) SEM photograph of a cross-sectional view of $NCAP_1$; (H) SEM photograph of a cross-sectional view of $NCAP_2$.

Table 2
Pharmacokinetic parameters ($n = 6$, mean ± S.D.).

Parameters	C_{max} (μg/ml)	T_{max} (h)	MRT (h)	$AUC_{0\to24}$ (μg/ml h)
CAP_1	0.78 ± 0.23	3.80 ± 0.30	8.74 ± 0.84	6.74 ± 0.47
IRT	2.23 ± 0.32	3.00 ± 0.44	5.71 ± 0.36	9.42 ± 0.69

1 h. The reason for this might be as follows: the insoluble Eudragit® NE 30D required some time to expand and then permitted the medium to penetrate into the core pellets and dissolve the drug, which is regarded as the rotational interaction time (τR) and at last resulted in a lag-time. These results corresponded well with the study undertaken by Nisar-Ur-Rahman (Rahman and Yuen, 2005), which indicated that a lag time was generally present in dissolution test when Eudragit® NE 30D was used for coating. Regarding the different formulation composition of CAP_1, a favorable sustained-release profile for CAP_1 with an ideal drug release amount (33.2%) over an hour was obtained at pH 6.8, while only 9.6% at pH 1.2 due to the presence of a lag-time. In the medium with high pH-value, the enteric soluble Eudragit® L30 D-55 decreased the τR by increasing the permeability of the polymer coating. However, a significant retarding effect was supposed to be produced by the insoluble polymer film at low pH, so the τR of CAP_1 seemed to be much longer and then, a lag-time was observed. These findings have implications that the therapeutic effect of CAP_1 in basic environment would be produced faster than that in acidic condition.

The profiles of DIP released from CAP_1, CAP_2, $NCAP_1$ and $NCAP_2$ at pH 6.8 were investigated as shown in Fig. 4. Apparently, the accumulative drug release from CAP was higher than that of the other three kinds of pellets, indicating that the percentage release of CAP_1 in vivo might be markedly higher than that of CAP_2, $NCAP_1$ and $NCAP_2$ when the release time was the same. A lag-time was also observed in the release curve of CAP_2 and $NCAP_1$ at pH 6.8. The diffusion resistance from the insoluble polymer coating films of CAP_2 could well explain for the present lag-time. While the delay in drug release from $NCAP_1$ was considered to be due to the fact that the enteric polymer was unable to fully compensate for the lower solubility of the drug in basic medium.

4.3. Scanning electron photomicrographs

Fig. 5 showed the scanning electron photomicrographs of CAP_1, CAP_2, $NCAP_1$ and $NCAP_2$. The surfaces of the pellets were smooth (Fig. 5A–D), and the subcoating layer and polymeric layer were clearly observed (Fig. 5E–H), which presented the cross-section of the pellets and matched the schematic diagram in Fig. 1. This showed that the preparation process of this study were satisfactory to obtain pellets with content uniformity of double-layer films.

4.4. Pharmacokinetic studies

The in vivo pharmacokinetics of four types of sustained-release DIP pellets and IRT were investigated. However, the plasma concentration of DIP released from CAP_2, $NCAP_1$, $NCAP_2$ was below the detection limit (data not shown), suggesting that the drug release might be limited throughout the GI tract. The mean plasma concentration versus time curves are shown in Fig. 6 and all the pharmacokinetic parameters (C_{max}, T_{max}, MRT and AUC_{0-24}) are listed in Table 2. It can be seen that the plasma level of IRT rose quickly and the maximum concentration was reached 3 h after administration. There was then a marked fall in plasma concentration between 3 and 12 h. For CAP_1, the maximum concentration (0.78 μg/ml) was reached 3.8 h after administration and the drug concentration of CAP_1 fell slowly even at 24 h after administration, when the drug concentration was 0.12 μg/ml, in contrast with

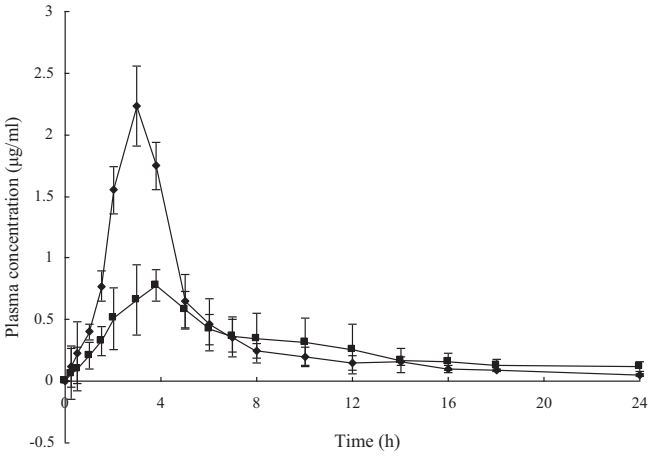

Fig. 6. Mean plasma concentration–time curves of dipyridamole in beagle dogs after a single oral administration of sustained-release capsules and commercial tablets ($n = 6$) (■) CAP_1; (♦) IRT.

the almost undetectable drug concentration in plasma for the IRT 16 h after administration. CAP_1 prolonged the MRT from 5.71 to 8.74 h, and reduced the C_{max} to 0.78 from 2.23 μg/ml compared with the IRT, demonstrating the sustained drug release. The relative bioavailability of CAP_1 was found to be 71.55% compared with the IRT. Apparently, the behavior in vivo of CAP_1 was superior to the other three kinds of pellets, which indicated that the preparation designed as CAP_1 (combined use of CA and E–E coating) was a good method of achieving sustained release of DIP with enhanced bioavailability.

5. Discussion

The gastric emptying time (GET) of the fasted beagle dogs has been reported to be only 0.27 h (Murata et al., 1998). The point was evidenced by the fact that plasma drug concentration of CAP_1 was detected immediately at 15 min in this study. The gastric pH was found to be 1.5, with a range of 0.9–2.5 (Dressman, 1986; Willmann et al., 2010). As was shown in Fig. 3, a lag time in the dissolution process of CAP_1 was present in acidic environment while it was absent under basic condition. As a result, the rapid absorption of CAP_1 could only be explained by the short GET, which corresponded well to the literature. The low gastric pH will greatly increase the dissolution rate of dipyridamole in the fasted stomach due to the weakly basic nature of dipyridamole (pKa 6.4) (Gu et al., 2005). However, the absorption of dipyridamole from the fasted stomach might be expected to be poor because of a lag-time in drug dissolution and the short GET. In addition, it could be predicted that the release rate will fall dramatically after the conventional prolonged release preparations have been delivered to the duodenum. The pH of the fluid in the small intestine in fasted beagle dogs is between 6.2 and 7.5 (Willmann et al., 2010). Owing to the high pH and the poor solubility in the small intestine, the absorption will be low in the small intestine for the conventional sustained release dosage forms. Hence, it is essential to employ a solubilization method to increase the release rate of DIP in the small intestine.

The experimental log P (n-octanol/water) of dipyridamole is 2.74 (Kalantzi et al., 2006), which is greater than that of the highly permeable marker drug, metoprolol (log $P = 1.72$) (Kasim et al., 2004). According to the biopharmaceutics classification system (BCS), metoprolol belongs to class I (high permeability/high solubility) (Lobenberg and Amidon, 2000) while DIP was confirmed to be class II (high permeability/low solubility) with respect to the BCS in later research (Zhang et al., 2009). In addition, it has been shown that the absorption of dipyridamole is incomplete and

exhibits site-specificity along the GI tract (Wilding, 2003; Zhang et al., 2009). In contrast, previous dog studies (Ueda et al., 1994) have demonstrated that the TES system containing metoprolol exhibited controlled drug release in all regions from the small intestine to the colon. Obviously, it was the difference between the solubility or the dissolution rate of the two drugs that caused the differences seen in absorption in the GI tract. For the class II drugs, the release rate may be controlled by the water content in the GI tract, especially in the colon. This means that, the fluid movement around preparations would be restricted and then drug dissolution was retarded due to the small volume of GI fluid and viscosity of the colon contents. Narisawa et al. (1995) also demonstrated that, in the case of controlled release preparations, the drug solubility strongly affected the dissolution rate in the lower regions of the canine GI tract. In summary, due to the low solubility of DIP in water, the limited water content in the colon, the short GET in the fasted state and the present lag time in the dissolution process, we propose that the drug is not released in the lower intestinal tract and the stomach from sustained-release pellets in the present investigation and the small intestine is the only site of absorption for dipyridamole under these conditions.

When the drug is absorbed in the small intestine but not the large intestine, it is said to have an absorption window (Jijun et al., 2011). If the dosage form passes beyond this absorption window before it starts to disintegrate and the drug dissolves, the drug absorption will be incomplete. The small intestinal transit time (SITT) of the beagle dogs has been estimated to be about 2 h (Dressman, 1986; Kabanda et al., 1994). Moreover, no difference has been found in the transit performance for solutions, pellets and single units concerning SITT (Davis et al., 1986). Undoubtedly, the short SITT in dogs would lead to only a small a low fraction being absorbed for extended-release pellets of slightly soluble drugs, namely, restricted absorption. The lag-time for $NCAP_2$ was about 1 h during the vitro dissolution process (under any pH conditions), which was similar to that of CAP_2 and $NCAP_1$. That is to say, because of the short GET in the fasted state, only after about 1 h since the preparation was delivered to the small intestine does the drug start to release. In addition, the drug release rate was rather slow in the small intestine due to the high pH, as indicated in Fig. 4. What is more, it has been reported that the drug release in vivo of the coated sustained release preparations was slower than that observed in vitro (Levy et al., 1963; Brockmeier et al., 1985; Dietrich et al., 1988; Yuen et al., 1993). So it was estimated that the drug release from CAP_2, $NCAP_1$ and $NCAP_2$ in the small intestine was limited. Taking the fact that the drug release was terminated in the colon because of the limited water content and the high pH environment into account together, the reason for the undetectable plasma drug concentration of $NCAP_1$ and the E–E coated pellets in present study became clear.

As demonstrated in the in vitro dissolution test for CAP_1, a lag-time appeared in the dissolution curve at a low pH while it was not present at a high pH. Drug release in the stomach was expected to be poor owing to the short GET of the fasted dogs and the presence of a lag-time. However, it could be predicted that the release would increase quickly once the CAP_1 reached the small intestine. Due to the suitable low pH_M provided by the included CA, the solubility of dipyridamole in the small intestine was significantly increased and then the release rate was further accelerated owing to the increased permeability of the retarding layer produced by the enteric soluble Eudragit® L30D-55. Subsequently, the absorption of dipyridamole in the small intestine reached a maximum and, hence, the bioavailability was greatly improved. Obviously, the small intestine is the main absorption site for dipyridamole because of the high effective surface area and the favorable formulation factors.

Considering the relative bioavailability of CAP_1, only 71.55% was obtained based on the $AUC_{(0\rightarrow 24)}$ of the IRT, which is much lower than the previous report that dipyridamole is 100% absorbed after oral administration, compared with IRT (Naonori and Masaki, 1992; Sugawara et al., 2005). The poor absorption of CAP_1 compared with IRT in dogs was believed to be caused by the incomplete release of the drug in the upper GI tract when using prolonged-release preparations owing to the short SITT and the termination of drug release in the colon owing to its low solubility and the limited water content. This finding was very similar to the previous studies in dogs showing that dipyridamole was not released from preparations after reaching the colon and no absorption took place in the colon (Sugawara et al., 2005), reflecting the fact that the colonic release from prolonged release formulations should be taken into account in addition to the colonic permeability of drugs. The drug could be released rapidly and totally in the stomach from the IRT, so the corresponding C_{max} and $AUC_{(0\rightarrow 24)}$ were high enough. A short mean small intestinal residence time has been reported, namely about 4 h in humans (Dressman, 1986; Kabanda et al., 1994). So this suggests that the bioavailability of CAP_1 would be higher in patients.

By combining incorporating a pH-modifier with polymer blend coatings to maximize the absorption of DIP in the small intestine, the problem of low solubility to realize sustained release of DIP was solved. At the same time, due to the independent property of the slow release of the preparation on its residence in the stomach, the influence of physiological factors on its behavior was minimized and resulted in relatively small variation.

In summary, drug release from sustained release dosage forms is affected by various GI factors and properties of these compounds, such as the pH value of the GI fluid, GET, SITT, the environment in the colon, and the permeability and solubility of the drug, as detailed above. These results suggest sustained release of the poorly soluble and pH-dependent dipyridamole could be achieved by the combined use of a pH-modifier and polymer blend coatings to maximize the absorption of DIP in the small intestine due to counteract its restrict absorption. Consequently, the formulation factors affecting its in vitro release and the factors related to its in vivo absorption need to be considered when designing a sustained-release dosage form for a drug with pH-dependent solubility.

6. Conclusions

None gastric resident sustained release pellets of dipyridamole with enhanced bioavailability (CAP_1) were prepared successfully by incorporating a pH-modifier in the core pellets and coating with a polymeric blend composed of Eudragit® NE 30D/L30 D-55 (1:1, w/w) after subcoating. The incorporated CA reduced the pH_M in the core pellets and the enteric Eudragit® L30 D-55 modified the permeability of the E–E coating and, in addition, the interaction of the two in the GI tract significantly increased the rate and extent of drug release in the small intestine, thereby increasing the bioavailability.

The formulation CAP_1 offers a promising strategy for the development of extended-release preparations of a drug with pH-dependent solubility.

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