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Encapsulation of poorly soluble basic drugs into enteric microparticles: A novel approach to enhance their oral bioavailability

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ABSTRACT

Poorly water soluble basic drugs are very sensitive to pH changes and following dissolution in the acidic stomach environment tend to precipitate upon gastric emptying, which leads to compromised or erratic oral bioavailability. In this work, we show that the oral bioavailability of a model poorly soluble basic drug (cinnarizine) can be improved by drug encapsulation within highly pH-responsive microparticles (Eudragit L). The latter was prepared by emulsion solvent evaporation which yielded discrete spherical microparticles (diameter of 56.4 ± 6.8 μm and a span of 1.2 ± 0.3). These Eudragit L (dissolution threshold pH 6.0) microparticles are expected to dissolve and release their drug load at intestinal conditions. Thus, the enteric microparticles inhibited the *in vitro* release of drug under gastric conditions, despite high cinnarizine solubility in the acidic medium. At intestinal conditions, the particles dissolved rapidly and released the drug which precipitated out in the dissolution vessel. In contrast, cinnarizine powder showed rapid drug dissolution at low pH, followed by precipitation upon pH change. Oral dosing in rats resulted in a greater than double bioavailability of Eudragit L microparticles compared to the drug powder suspension, although C_{max} and T_{max} were similar. The higher bioavailability with microparticles contradicts the *in vitro* results. Such an example highlights that although *in vitro* results are an indispensable tool for formulation development, an early *in vivo* assessment of formulation behaviour can provide better prediction for oral bioavailability.

1. Introduction

The oral delivery of poorly water soluble basic drugs is a common challenge for pharmaceutical scientists as a result of their pH-dependent solubility. While soluble in the acid environment of the stomach (pH 1–2), partial or complete drug precipitation in the small intestine (pH 6.8–7.4) can occur following gastric emptying, which compromises oral bioavailability (Kostewicz et al., 2004). Clinically, the bioavailability of basic drugs has been proven to be dependent on gastric pH (Ogata et al., 1986). For instance, the bioavailability of dipyridamole is lower in achlorhydric elderly patients (Russell et al., 1994) while that of cinnarizine was compromised in dogs with elevated gastric pH (Yamada et al., 1990).

Previous approaches to address this problem have included the addition of acidic excipients to reduce gastric pH and thereby increase drug solubility. Formulation scientists exploited this approach by co-compressing the basic drug with an organic acid, this can reduce the local pH around the disintegrated particles to allow a faster ionization and dissolution of basic drugs (Derendorf et al., 2005). Other formulation strategies have included super-saturated lipid formulations (Porter et al., 2008), encapsulation in liposomes (Stozek and Krowczynski, 1986) or complexation with cyclodextrins (Fregnan and Berte, 1990; Ricevuti et al., 1991; Stracciari et al., 1989; Tokumura et al., 1984, 1985).

We propose an additional approach to this problem: the use of highly pH-responsive microparticles which enhance the bioavailability of poorly soluble basic drugs through site-specific drug delivery in the gastrointestinal tract. The enteric microparticles would dissolve at the small intestinal pH conditions and release the drug at site of absorption. We have previously reported a novel pH responsive microparticle system which provides a unique platform to produce spherical and uniform microparticles with a broad ability to encapsulate drugs with a wide range of physicochemical properties (Nilkumhang et al., 2009; Alhnan et al., 2010). This system of Eudragit L microparticles showed enhanced bioavailability of a neutral drug in a rat model (Kendall et al., 2009). The microparticles presented the poorly soluble drug in the amorphous form that dissolved rapidly in the small intestine leading to a fast plasma peak following oral dosing compared to a control drug suspension. In this manuscript, we show the ability of the microparticle system to enhance bioavailability of more challenging drugs; poorly water soluble basic drugs. The microparticles were tested *in vitro* and *in vivo* in rats. The basic drug cinnarizine (an antihistamine that has been used in the treatment of vestibular disorders, such

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as vertigo, tinnitus, nausea, and vomiting in Ménière's disease and motion sickness and with pK_{a_1} 1.94 and pK_{a_2} 7.47) and which is soluble at 2110 mg/L in acid (pH 1.2) (Alhnan et al., 2010) but only at 2 mg/L at pH 6.5 phosphate buffer (Gu et al., 2005), was used as the drug model in this work to test this hypothesis in rats.

2. Materials and methods

2.1. Materials

Cinnarizine, sorbitan sesquioleate and all other reagents were purchased from Sigma Aldrich (Poole, UK). Eudragit L (Eudragit L-100) was generously received from Evonik Degussa Chemicals (Darmstadt, Germany). Methocel E4 M Premium (hydroxypropyl methylcellulose) was donated by Colorcon Limited (Kent, UK).

2.2. Preparation and characterization of microparticles

The microparticles were prepared and characterized for size, morphology, encapsulation efficiency and yield as detailed previously (Alhnan et al., 2010). Briefly, 0.3 g of cinnarizine and 3 g of Eudragit L were dissolved in 30 mL of 1:1 (v/v) ethanol:acetone mixture. The resulting solution was emulsified into 200 mL liquid paraffin containing sorbitan sesquioleate (1%, w/w) as the emulsifier by stirring at a speed of 1000 rpm (Heidolph RZR1 stirrer, Heidolph Instruments, Schwabach, Germany) for 18 h at room temperature. Solidified microparticles were collected by vacuum filtration, followed by washing 3 times using fresh batches of 50 mL *n*-hexane.

Microparticle morphology and size were characterized using scanning electron microscopy (SEM) and laser diffractometry, respectively. Span of microsphere size was calculated as $[D(v,0.9) - D(v,0.1)]/D(v,0.5)$, where $D(v,0.9)$, $D(v,0.5)$ and $D(v,0.1)$ are the particle diameters at the 90th, 50th and 10th percentile, respectively, of the microsphere size distribution curve. To characterize the crystallinity of the microparticles, a Philips PW3710 scanning X-ray diffractometer (Philips, Cambridge, UK) with a Cu Kα filter generated at 30 mA and 45 kV was used to characterize the crystallinity of the microparticles. Samples were placed in a round disc sample holder and were gently compressed and smoothed using a Perspex block. Samples were scanned at 0.02/s from 6° to 35°. The peak was calculated using XPert HighScore data analysis software (version 2.0a). Cinnarizine encapsulation efficiency was assessed by HPLC following drug extraction from the microparticles (Alhnan et al., 2010).

2.3. In vitro release study

In vitro dissolution tests were conducted using two different setups. The first method is a standard dissolution test method for enteric products while the second mimics the pH profile in rat's gastrointestinal tract (McConnell et al., 2008):

2.3.1. In vitro dissolution studies – standard conditions

The USP II paddle apparatus (Model PTWS; Pharmatest, Hainburg, Germany) was used. One hundred mg of microparticles or equivalent amount of cinnarizine (for the control) was weighed and filled into capsule No. 0. Each capsule was placed in a metal sinker (to ensure that the microparticles are submerged during the test) which was then placed in a vessel containing 750 ml of 0.1 N hydrochloric acid. After 120 min, 250 ml of 0.2 M tri-sodium phosphate (equilibrated to 37 ± 0.5 °C) was added to each vessel, and the pH of the solution was adjusted to pH 6.8. The total duration of the experiment was 5 h, the speed of the paddle was 100 rpm and the temperature of the dissolution medium was 37 ± 0.5 °C. Each dissolution test was carried out in triplicate. The released cinnarizine was assayed inline at 254 nm using a UV spectrophotometer. Data were processed using Icalis software (Icalis Data Systems Ltd, Berkshire, UK). The absorbances of (i) blank microparticles filled in gelatin capsule or of (ii) gelatin capsule only following the same dissolution procedure was measured and subtracted from those of the drug-loaded microparticles or the drug in gelatin capsule, respectively to remove any interference from the Eudragit polymer and gelatin capsules.

2.3.2. In vitro dissolution test at rats' gastrointestinal conditions

The gastric contents in the rat are at a higher pH than those in man (McConnell et al., 2008). Hence the dissolution test was conducted as described above except for the replacement of the 0.1 N hydrochloric acid with 900 ml of phosphate buffer pH 3.9 (phosphate buffer pH 4.0 B.P. adjusted to pH 3.9 with 5 N HCl) for 2 h followed by adjustment of the pH to 6.5 with sodium hydroxide 4 N.

2.4. In vivo drug absorption following oral administration in rats

Male Wistar rats (average weight 300 ± 5 g) were supplied by Harlan, UK. All procedures were approved by The School of Pharmacy's Ethical Review Committee and were conducted in accordance with Home Office standards under the Animals (Scientific Procedures) Act, 1986. Rats were kept in cages with free access to water and standard rat chow for an acclimatisation period of 1 week. Twelve hours prior to dosing, the rats were fasted but were allowed free access to water.

Rats (n = 5 for each arm) were given cinnarizine (120 mg/kg) as a drug suspension or Eudragit L microparticles suspension (2 ml) by oral gavage. The control and the test microparticles were prepared by dispensing cinnarizine (as received) or Eudragit L microparticles in an aqueous solution of 0.5% w/v methyl cellulose. Blood samples (100 μl) were taken from the rat tail at time points 0, 15, 30, 60, 120, 180, 240, 360 min post-dosing, transferred into EDTA BD Microtainer capillary blood tubes (NJ, USA) and immediately vortexed for 20 seconds, then centrifuged for 20 min at 10,000 rpm using IEC Micromax microcentrifuge (Thermo Fisher Scientific Inc., MA, USA). Plasma was collected in Eppendorf tubes and stored at −20 °C prior to HPLC assay.

2.5. Cinnarizine assay from plasma

Cinnarizine was extracted from the rat plasma samples using a method adapted from those reported by Krise et al. (1999) and Kossena et al. (2004). Briefly, 20 μl of mobile phase (1:1 mixture of acetonitrile and 20 mM ammonium dihydrogen phosphate buffer) and 20 μl of trifluroacetic acid (10%, w/v) were added to 40 μl of plasma to precipitate the plasma proteins. Methyl tert-butyl ether (800 μl) was added and vortexed for 30 s and shaken mechanically for 20 min to ensure drug extraction. The mixture was centrifuged for 10 min as above and 650 μl was taken from the organic phase and then placed into 1 ml glass vial. The organic solvent was evaporated off at room temperature in a vacuum oven and reconstituted with 120 μl of the mobile phase.

To assay cinnarizine, a 250 mm × 4.60 mm, particle size 4 μm reversed-phase C18 Synergi Max-RP80 column (Phenomenex, Torrance, USA) and a Hewlett-Packard 1050 series HPLC system (Agilent Technologies, UK) supplied with a Waters 470 Millipore scanning fluorescence detector (Milford, MA, USA) were used. The mobile phase was eluted at a flow rate of 1 ml/min, the injection volume was 100 μl, and the fluorescence detector was used at an excitation wavelength of 249 nm and emission wavelength of 311 nm. The retention time of cinnarizine was 14.8 min, an acceptable linearity (R^2 = 0.986) was established in the concentration

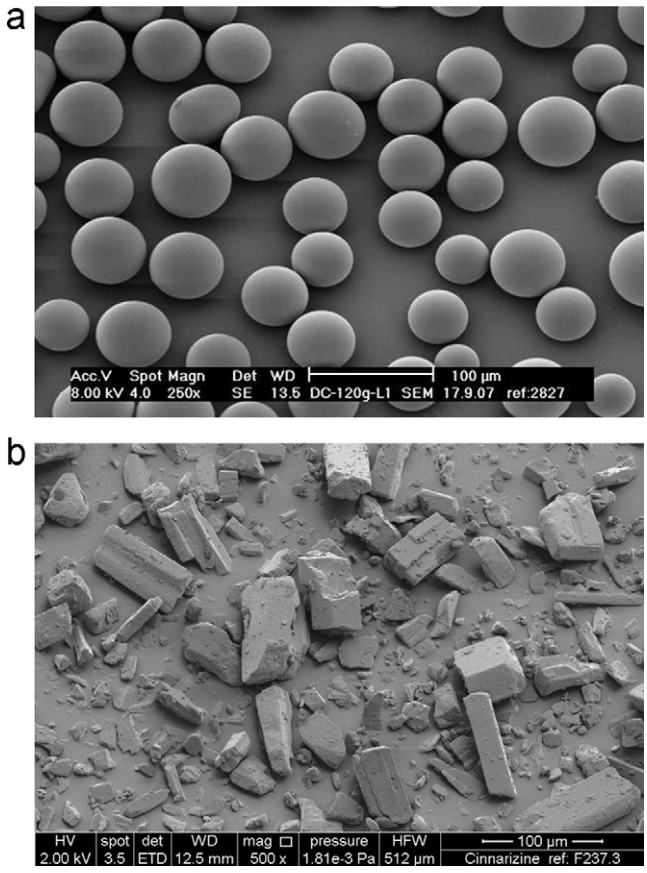

Fig. 1. SEM images of (a) cinnarizine loaded microparticles (cinnarizine:Eudragit L 1:10) and (b) cinnarizine crystals.

range of 100–1000 ng/ml. The HPLC method was tested for accuracy and precision. The limit of quantification was 100 ng/ml.

2.6. Statistical analysis

General linear model (repeated measurements) was utilized to assess the difference in cinnarizine plasma concentration over time between cinnarizine suspension and Eudragit L microparticles suspension. Maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}) and area under curve ($AUC_{0-6\,h}$) for the microparticles and drug suspensions were compared using Student *t*-test. Statistic analysis was carried out using SPSS statistics 17.0.

3. Results and discussion

The emulsion solvent evaporation process employed yielded spherical microparticles (Fig. 1a) with a median particle size (Dv 0.5) of 53.9 ± 6.8 μm and a span of 1.03 ± 0.1. Microparticle size was similar to that of cinnarizine crystals (Dv 0.5 66.3 ± 8 μm span, 2.42 ± 0.16), but had a smaller polydispersity, as can be seen from Fig. 1. The high span value of cinnarizine crystals (control) indicating high polydispersity of the crystal could be due to the oblong column shape of some of them. The encapsulation efficiency and yield of the microparticles were $68.0 \pm 3.1\%$ and $90.2 \pm 1.8\%$, respectively. The absence of peaks representative of cinnarizine crystals (Fig. 2) in the X-ray powder diffraction spectrum of cinnarizine loaded microparticles indicates that the majority of cinnarizine in the microparticles is in the amorphous form.

3.1. In vitro release

The control, cinnarizine powder, showed rapid and complete dissolution at standard gastric conditions within 40 min (Fig. 3), due to the high drug solubility in acid (2110 mg/L). Upon changing the pH to intestinal value, over 80% of the drug precipitated within 60 min, as a result of the dramatic drop in drug solubility (2 mg/L at pH 6.5 phosphate buffer) (Gu et al., 2005). A similar dissolution profile was obtained when the dissolution study was conducted at pH mimicking the rat's gastrointestinal conditions (Fig. 3). Cinnarizine dissolution was however, slower and incomplete at the higher rat's gastric pH 3.9 due to incomplete cinnarizine ionization.

The *in vitro* dissolution profile for cinnarizine loaded Eudragit L microparticles was very different to that of cinnarizine powder. The ability to prevent drug release was attributed to the relatively high molecular weight of cinnarizine (369.5 Da) as previously proposed (Alhnan et al., 2010). When the pH of the dissolution medium was changed to pH 6.8, a small amount of drug (≈10%) was detected after 25 min (as the Eudragit L microparticles dissolved at pH > 6.0) before a complete drug precipitation took place in the dissolution vessel. When the dissolution test was repeated under rat gastrointestinal pH profile, no cinnarizine release from Eudragit L microparticles was detected at gastric pH. However, changing the pH to 6.5 (average intestinal pH value in rats), no cinnarizine was detected by UV (Fig. 4). However, the formation of crystals in the dissolution vessel was observed. The crystals are expected to be of cinnarizine as Eudragit L dissolved and released the entrapped drug, which subsequently precipitated out at pH 6.5. Under the experimental conditions, it was not possible to assess the rate of microparticles dissolution or of drug precipitation.

3.2. In vivo studies

The mean plasma concentration–time profiles of cinnarizine after oral administration of the control cinnarizine suspension or cinnarizine loaded Eudragit L microparticles are shown in Fig. 5. A significant difference in cinnarizine plasma concentration over time between the two dosed formulations was observed (repeated measures ANOVA, $p < 0.05$). The mean and the individual pharmacokinetic parameters are reported in Tables 1 and 2. The $AUC_{0-6\,h}$ was significantly higher with microparticles (*t*-test, $p < 0.05$) with a relative bioavailability of 223% compared to the control cinnarizine suspension. The C_{max} of Eudragit L microparticle formulation was also higher, although not statically significant (*t*-test, $p = 0.8$) while the T_{max} was the same (*t*-test, $p = 0.82$).

Cinnarizine absorption from both Eudragit L microparticles and the control suspension occurred fairly rapidly, with T_{max} being less than or equal to 1 h in all rats. Following the oral administration of the control cinnarizine suspension, the drug is expected to mostly dissolve in the stomach. Upon gastric emptying, some of the drug would dissolve while the rest will precipitate at the higher pH in the small intestinal environment. However, this absorption is expected to be incomplete.

In contrast, following the administration of cinnarizine loaded Eudragit L microparticles, the latter is expected to remain intact in the rat stomach, and only dissolve and release the entrapped drug after gastric emptying. For the T_{max} of the five rats shown in Table 2, it appears that the gastric emptying occurs fairly rapidly (within 15 min) in some, but not all rats. Gastric emptying is expected to be rapid in the fasted rats. However, due to the fact that the rats were housed in normal cage, where they could eat their faecal pellets, strict fasted conditions are not applied. The T_{max} in both test and control rats was found to be quite variable from 0.25 to 1.0 h (Table 2) and reflects high variability observed when other drugs have been orally dosed in rats (Kendall et al., 2009). The high variability in T_{max} is likely to be

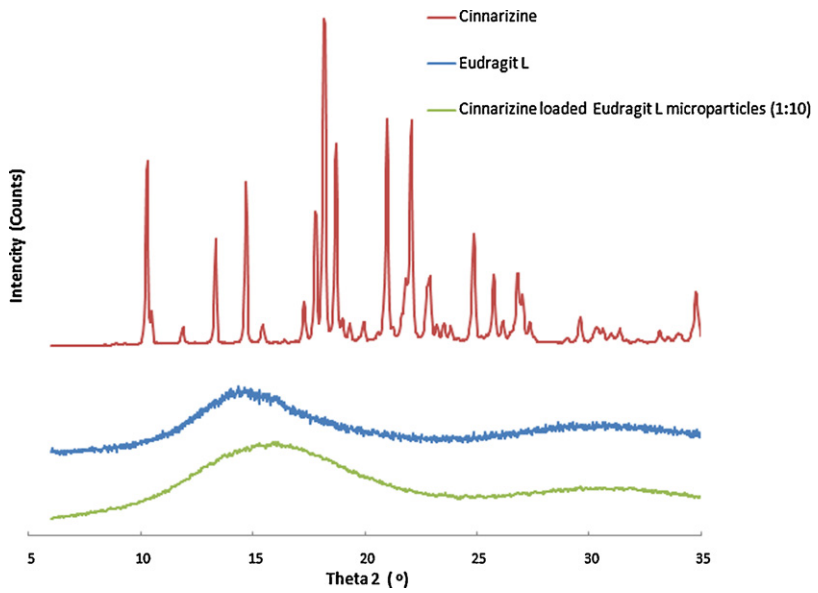

Fig. 2. X-ray powder diffraction spectra of cinnarizine powder, Eudragit L powder and cinnarizine loaded Eudragit L microparticles.

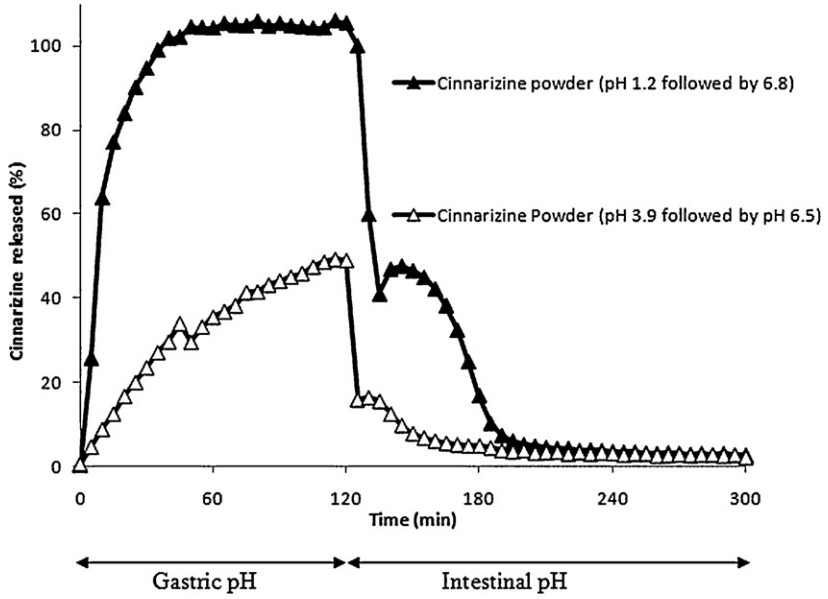

Fig. 3. *In vitro* release of cinnarizine powder using standard and rat's pH change USP II dissolution systems ($n = 3$).

Table 1
Pharmacokinetic parameters of cinnarizine powder and cinnarizine loaded Eudragit L microparticle following oral administration of equivalent dose to rats ($n = 5$).

Parameter	Cinnarizine suspension			Cinnarizine loaded Eudragit L microparticles		
	Mean	SD	Range	Mean	SD	Range
C_{max} (ng/ml)	587	206	359–866	1972	1546	437–3240
T_{max} (h)	0.55	0.27	0.25–1	0.6	0.38	0.25–1.0
AUC_{0-6} (ng h/ml)	1127	489	488–1583	2514	1021	1596–4197
F%				223	91	142–372

C_{max}, maximum plasma concentration; T_{max}, time to maximum plasma concentration; AUC_{0-6}, area under curve from time zero to last measured time point (6 h); F%, relative bioavailability of microparticles compared to the drug suspension control.

Table 2
Individual pharmacokinetic parameters of cinnarizine powder and cinnarizine loaded Eudragit L microparticle following oral administration of equivalent dose to rats.

	Cinnarizine powder suspension					Cinnarizine loaded Eudragit L microparticle suspension				
	Rat I	Rat II	Rat III	Rat IV	Rat V	Rat VI	Rat VII	Rat VIII	Rat IX	Rat X
C_{max} (ng/ml)	359	410	673	627	866	545	3240	437	384	1789
T_{max} (h)	1	0.5	0.5	0.25	0.5	0.25	1	1	0.25	0.25
AUC_{0-6h} (ng h/ml)	488	753	1256	1583	1558	1819	4197	1596	2428	2534
F%						161	372	142	215	225

F% relative bioavailability of microparticles in each individual rat compared to the mean bioavailability of the drug suspension control.

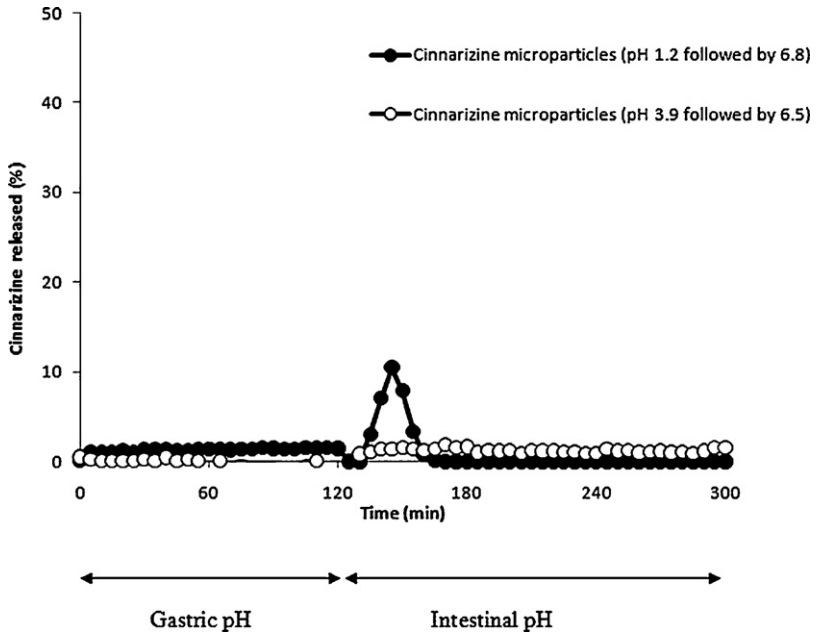

Fig. 4. *In vitro* release of cinnarizine loaded microparticles using standard and rat's pH change USP II dissolution systems ($n = 3$).

due to different gastric emptying times different rates of drug dissolution (as a result of varying amounts of gastro-intestinal liquid volumes available for drug dissolution) and different drug precipitation rate (when hostile pH environments encountered by the basic drugs) in the different rats. In addition, different rates of drug release from the Eudragit L particles due to Eudragit L dissolution (also due to varying amount of liquid volume available for polymer dissolution) is likely to contribute to the variable T_{max} in the test rats. Fluid volumes in the different sections of fed and fasted rats gastrointestinal tract have previously been shown to be small, as well as fairly variable (McConnell et al., 2008).

The higher *in vivo* oral cinnarizine bioavailability from Eudragit L microparticles compared to control cinnarizine suspension (Fig. 5) was not due to the Eudragit particle size or surface area (size being similar for both control and Eudragit particles), although the amorphous nature of the drug in Eudragit particles is expected to favour drug dissolution rate and absorption. The greater drug bioavailability from Eudragit L particles shows the promise of enteric microparticles in oral drug delivery of poorly soluble basic drugs. Incomplete drug dissolution is expected from the control suspension where drug is expected to dissolve in the acidic stomach, and precipitate at the higher intestinal pH upon gastric emptying.

In contrast, the microparticles are expected to remain intact in the acidic stomach and only dissolve and release the drug in the small intestine. The drug in turn may precipitate out in the small intestine (as observed *in vitro* dissolution experiments, Fig. 4) becoming unavailable for absorption. The *in vivo* data (Fig. 5) however, shows that a proportion of the released drug is absorbed. This is possibly due to the fact that the highly pH responsible Eudragit L microparticles can dissolve rapidly at the intestinal pH immediately following gastric emptying with drug in amorphous form, which allows drug release in the upper small intestine (site of cinnarizine absorption) and drug absorption to take place before the drug is precipitated out in the unfavourable intestinal environment. It appears that rapid absorption of the released drug can overcome the drug's insolubility in the small intestine. Interestingly, the ability of the Eudragit microparticles to enhance cinnarizine

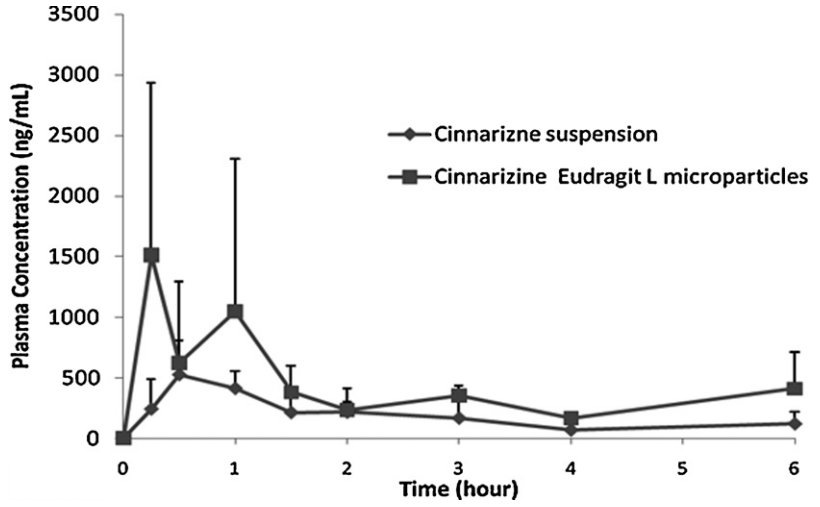

Fig. 5. Mean cinnarizine plasma concentration–time profiles of cinnarizine powder and cinnarizine loaded Eudragit L microparticles following oral administration of equivalent dose to fasted Wistar rats ($n = 5$).

bioavailability *in vivo* could not have been predicted from *in vitro* dissolution tests (Figs. 2 and 3), which lacks the absorption step.

Previous attempts to exploit enteric nanoparticles and microparticles to enhance the bioavailability of poorly soluble lipophilic drug have been reported (De Jaeghere et al., 2000; Leroux et al., 1995) and a promising increase in oral bioavailability has been established. However, the authors showed no *in vitro* drug release for these formulations at any pH values. The lack of information about the gastric resistant properties of these microparticles made it difficult to confirm the link of their high oral bioavailability to site-specific drug delivery.

The limited amount of gastric and intestinal fluids in rats was previously reported (3.2 ± 1.8 ml with <0.5 ml in stomach, 0.5 ml in small intestine, 1.5 ml in caecum and <1 ml in colon (McConnell et al., 2008)). Accordingly, only a small fraction of dosed cinnarizine (0.5%) will dissolve in the stomach. This highlights that the use of conventional dissolution protocols might be inappropriate to predict the *in vivo* performance of oral formulation in rats. In fact, an example of the lack of IVIVC was reported with microparticles in rats (Wong et al., 2006), where a decrease in particle size enhanced the *in vitro* dissolution of poorly soluble drug (griseofulvin) but failed to enhance its oral bioavailability in rats.

4. Conclusion

We show the role of enteric microparticles in the formulation of poorly soluble basic drugs. The oral bioavailability of cinnarizine in rats was more than doubled compared to the control drug powder suspension. Such promising *in vivo* results were not predicted by the *in vitro* dissolution studies. It appears that *in vivo*, drug is rapidly absorbed as the Eudragit L microparticles dissolve in the intestine following gastric emptying and hence limiting the impact of drug precipitation that is observed *in vitro*. This study shows that although a regular *in vitro* test is generally needed before formulations are tested *in vivo*, *in vitro* studies can be misleading and might result in formulations being untested when they might perform well *in vivo*.

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