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In Vitro and In Vivo Performance of a Multiparticulate Pulsatile Drug Delivery System

A. Mohamad and A. Dashevsky
College of Pharmacy, Freie Universität Berlin, Kelchstr, Berlin, Germany

ABSTRACT The objective of this study was to investigate the in vitro and in vivo drug release performance of a rupturable multiparticulate pulsatile system, coated with aqueous polymer dispersion Aquacoat® ECD. Acetaminophen was used as a model drug, because in vivo performance can be monitored by measuring its concentration in saliva. Drug release was typical pulsatile, characterized by lag time, followed by fast drug release. Increasing the coating level of outer membrane lag time was clearly delayed. In vitro the lag time in 0.1 N HCl was longer, compared to phosphate buffer pH 7.4 because of ionisable ingredients present in the formulation (crosscarmelose sodium and sodium dodecyl sulphate). In vitro release was also longer in medium with higher ion concentration (0.9% NaCl solution compared to purified water); but independent of paddle rotation speed (50 vs.100 rpm). Macroscopically observation of the pellets during release experiment confirms that the rupturing of outer membrane was the main trigger for the onset of release. At the end of release outer membrane of all pellets was destructed and the content completely released.

However, pellets with higher coating level and correspondingly longer lag time showed decreased bioavailability of acetaminophen. This phenomenon was described previously and explained by decreased liquid flow in the lower part of intestine. This disadvantage can be considered as a limitation for drugs (like acetaminophen) with high dose and moderate solubility; however, it should not diminish performance of the investigated system in principle.

KEYWORDS Crosscarmelose sodium, Aqueous coating, Aquacoat® ECD, Pulsatile drug release

INTRODUCTION

Pulsatile drug delivery systems release the drug rapidly and completely after certain lag time and have gained increasing interest during recent years for a number of drug therapies (Lemmer, 1991; Bussemer et al., 2001). Different types of pulsatile systems have been developed, including erosion (Gazzaniga, et al., 1994a; McConville et al., 2005) and rupturable (Bussemer et al., 2003a)

Address correspondence to A. Dashevsky, College of Pharmacy, Freie Universität Berlin, Kelchstr. 31, 12169 Berlin, Germany; Tel: +49 30 838 50708; E-mail: dashevsk@zedat.fu-berlin.de

systems. The latter were developed either as single unit hard/soft gelatin capsules (Bussemer et al., 2003a; Bussemer & Bodmeier, 2003) and tablets (Sungthongjeen et al., 2004) or in the form of multiparticulates (Ueda et al., 1994).

Rupturable systems usually consist of a drug-containing core, a swelling layer, and an external water-insoluble, but permeable polymer coating (Bussemer and Bodmeier, 2003a). Aqueous release medium or gastrointestinal (GI)-fluids penetrate through the polymer coating, the swelling layer expands until the outer polymer coating ruptures and the drug is then released rapidly. Pressure developed by the swelling layer as well as the water permeability and the mechanical strength of the outer coating were the main factors controlling the lag time of pulsatile hard/soft gelatin capsules (Bussemer et al., 2003a; Bussemer & Bodmeier, 2003a) and tablets (Sungthongjeen et al., 2004). Parameters such as surfactants, pH, and ionic strength of the in vitro dissolution medium were investigated previously, in an attempt to predict in vivo performance of drug formulations (Galia et al., 1998).

Multiparticulate systems (e.g., pellets) offer various advantages over single unit. These include no risk of dose dumping, flexibility of blending units with different release patterns, and short and reproducible gastric residence time (Chebre-Sellassie, 1994, Bechgaard & Nielson, 1978). The time controlled explosion system (TES) is based on multiparticulate system and consists of a core, drug layer, swelling layer and water-insoluble polymeric membrane (Ueda, 1994). Concerning TES, the rate of absorption in vivo and drug release in vitro were consistent within initial phase (up to 3 hr). However, absorption in vivo decreased after 6 hr due to changes in the absorption behavior in small and large intestine (Hata et al., 1994). Dependence of plasma concentration on the intestinal location of the TES was also shown for the low soluble diclofenac sodium (1.2 mg/mL in JP 2nd fluid). While TES with 3 hr nominal lag time showed good performance without remarkable reduction of the AUC, in the case of TES with 6 hr lag time drug plasma level was low. This was explained by differences in liquid flow in different gastrointestinal tract parts (Murata et al., 1998). Liquid flow in human duodenum, jejunum, ileum and colon was reported to be 3–5, 1.5–2, 0.7–1.2, and 0.1 L/day, respectively (Hirtz, 1985). Drug stability and associated bioavailability was shown to be dependent on gastrointestinal transit of captopril oral pulsatile delivery system, confirmed by gamma scintigraphy. Drug was detected only when delivered to the terminal ileum; it was not detected when delivered to the colon (Wilding et al., 1992).

For in vivo experiments, blood sampling is a widely applied, but inconvenient method. A noninvasive alternative is the detection of drug concentrations in saliva, especially advantageous for frequent collections. However, there are only few drugs appropriate for saliva concentration monitoring. Suitable drugs, including theophylline, carbamazepine, phenytoin, and acetaminophen diffuse from the plasma into saliva independent of the pH (Drobitch & Stevensen, 1992; Malamud & Tabak, 1993).

The objective of this study was to investigate the in vitro drug release and in vivo parameters of the rupturable multiparticulate pulsatile system, based on acetaminophen pellets layered with 48% w/w AcDiSol® and coated with Aquacoat® ECD.

MATERIALS AND METHODS

Materials

Acetaminophen (Semi fine powder USP, Synopharm GmbH, Barsbüttel, Germany) and commercially available instant release (IR) tablets (500 mg) were used as a model drug. Other materials obtained were sugar spheres, Suglets®: NP 355–425 μm (NP Pharma S.A., Gustav Parmentier, Frankfurt, Germany), croscarmellose sodium, AcDiSol®; aqueous dispersion of ethylcellulose, Aquacoat® ECD (FMC, Newark, DE); hydroxypropylcellulose, Klucel® MF (Hercules Inc., Wilmington, DE); hydroxypropyl methylcellulose, Methocel® E5 (Colorcon, Dartford, UK); triethyl citrate, TEC (Morflex, Greensboro, NC); polyethylene glycol 6000, Lutrol®E 6000 (BASF AG, Ludwigshafen, Germany); and talc (Luzenac Deutschland GmbH). All other reagents were of analytical grade and were used as received.

Preparation of the Multiparticulate Pulsatile Systems

Acetaminophen (15% w/w) was layered on sugar spheres (NP 355–425 μm) using an ethanol/water (60:40 w/w) solution of Methocel® E5 (1.5% w/w) as a binder and Lutrol® 6000 as a plasticizer (10% w/w based on Methocel® E5) in a fluidized bed coater (Strea 1, Aeromatic-Fielder AG, Bubendorf, Switzerland).

This was done to achieve a 36% w/w drug content based on the final dosage form. The layering conditions were, batch size 600 g, inlet temperature 45°C, outlet temperature 32°C, air flow 100 m^3/h, nozzle diameter 1.2 mm, spray pressure 2 bars, spray rate 6 g/min, final drying at inlet temperature 40°C for 10 min.

Drug cores were then layered to achieve 48% w/w weight gain using a 5% w/w AcDiSol® dispersion in 2% w/w ethanolic (96% v/v) solution of Klucel® MF. The resulting pellets were then coated with Aquacoat® ECD 15% w/w dispersion also containing 25% w/w TEC and 100% w/w talc (both based on polymer) to achieve a weight gain of 15, 25, and 35% (w/w).

Drug Release

The drug release was performed in USP paddle apparatus (Vankel VK 300, Vankel Industries, Edison, NJ) at 37°C and paddle rotation speed 50 or 100 rpm. Since the orally administered drug delivery system comes into contact with gastrointestinal fluids of different pH and ionic strength, drug release was investigated in different media like phosphate buffer pH 7.4, 0.1 N HCl (both approximately 200 mosmol/kg), 0.9% NaCl solution (305 mosmol/kg) or purified water. Three mL of samples were withdrawn at predetermined time points and measured at 241 nm using UV-spectrophotometer. Drug release was characterized by two parameters, the lag time and $T_{75\%}$. Lag time was determined by extrapolation of the upward part of release profile to the time axis. $T_{75\%}$ was selected analogue to pharmacopoeia criteria for the release from instant dosage and defined as a time to release 75% of drug minus lag time. For visual observation of the pellet's rupturing of one hundred units were processed as described for drug release. At predetermined time points pellets with membrane destruction were separated and counted using macroscope with image analysis software (Inteq GmbH, Berlin, Germany). Intact pellets were put back to continue the test.

In Vivo Study

Acetaminophen was used as a model drug because it is suitable for the in vivo monitoring and measuring concentration in saliva (Drobitch & Stevensen, 1992; Malamud & Tabak, 1993). Commercially available acetaminophen tablets (dose 500 mg) and investigated pellets (amount corresponding to 500 mg acetaminophen) were administered to five healthy volunteers together with 250 mL water after a 10 hr overnight fasting. Food was allowed after 2 hr subsequent to the administration with free access to water. Samples collected at predetermined time intervals were kept frozen at −60°C until analytical assessment. Sample analysis was performed according to the method, described by Malamud & Tabak, 1993. A saturated ammonium sulphate solution (200 μm) was added to a 400 μL of thawed saliva sample, well mixed at 1000 rpm for 5 min (MS1 Minishaker IKA®, IKA-Works, Inc., Wilmington, NC). The mixture was centrifuged at 258 *g* for 10 min (Biofuge 22 R, Heraeus Instruments GmbH, Berlin, Germany) in order to precipitate saliva proteins and to remove mucus and particulate matter. The supernatant was analyzed with high performance liquid chromatography (Shimadzu Europa GmbH, Duisburg, Germany). 50 μL aliquot of the sample was injected into the column (Knauer LiChrospher 100, RP-18, 250 × 4 mm, particle size 5 μm, Knauer GmbH, Berlin, Germany) with the eluent water:methanol 75:25 at a flow rate of 1 mL/min at 40°C. Acetaminophen was detected at a wavelength of 280 nm after retention time of 3.9 min.

Bioavailability, C_{max} and T_{max} were estimated using the concentration of acetaminophen in saliva. The in vivo lag time was defined as the mean value of the sampling time when acetaminophen was first detected in the saliva.

RESULTS AND DISCUSSION

The formulation development of the multiparticulate pulsatile system used in this study was described previously (Mohamad & Dashevsky, 2006).

In Vitro Drug Release

In vitro drug release can be characterized as pulsatile with good reproducible lag time, followed by relatively fast release ($T_{75\%}$ in range 0.9–1.8 hr), as seen in Fig. 1 and Table 1.

Onset of drug release (lag time) was clearly delayed by increasing coating level of Aquacoat® ECD (Fig. 1, Table 1). Increasing the coating thickness the rate of water penetration and swelling decreased, but mechanical

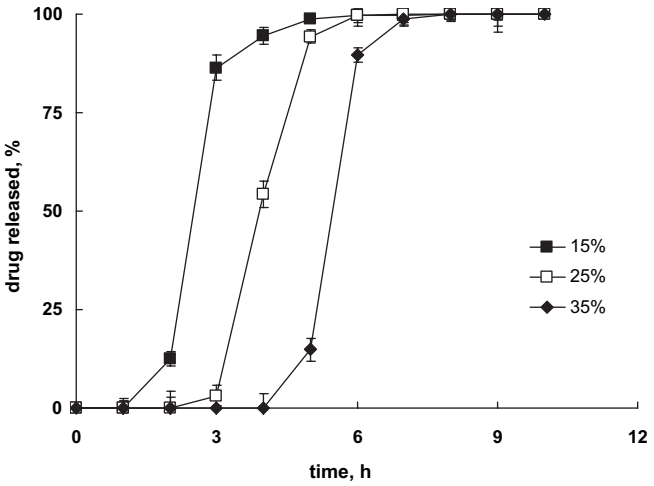

FIGURE 1 **The Effect of Coating Level of Outer Membrane on Drug Release in Phosphate Buffer pH 7.4, 100 rpm, *n* = 3.**

resistance of outer membrane increased. The lag time was dependent on pH. It was longer in 0.1*N* HCl compared to phosphate buffer pH 7.4 (Fig. 2), although the osmolality of both mediums was approximately 200 mosmol/kg. This pH effect could be explained by the presence of substances with ionisable groups in the formulation. First, water uptake and swelling energy of Ac-Di-Sol® is lower in 0.1 N HCl, compared to phosphate buffer pH 7.4, attributed to the presence of unionized carboxylic groups (Bussemer et al., 2003b). Secondly, in 0.1 N HCl/phosphate buffer pH 7.4 the sodium dodecyl sulphate present in Aquacoat® ECD is unionized/ionized, resulting in lower/higher wetting and permeability of outer membrane, respectively (Wesseling & Bodmeier, 1999). Both, swelling of Ac-Di-Sol® and permeability of outer membrane contribute to delay of water penetration, swelling and consequently rupturing of outer membrane. The ion concentration also has an influence on swelling behavior and rupturing time of the investigated pellets. As expected, lag time was prolonged (3 vs. 4 hr) by increasing salt concentration in release medium, (0.9% w/v NaCl vs. purified water) (Fig. 3). The swelling energy of AcDiSol® in this case is reduced due to competition of ions for free water (Bussemer et al., 2003b). Lag time and drug release were independent of paddle rotation speed (Fig. 4), which might mimic the influence of mechanical stress or/and hydrodynamic conditions in vivo on the performance of the pellets.

To monitor and quantify rupturing of pellets during release one hundred pellets were observed macroscopically during the dissolution in phosphate buffer pH 7.4 and the number of ruptured pellets was determined every hour. Within the first 2 hr, no ruptured pellets and correspondingly no drug release was observed. The beginning of rupturing (approximately 1.8% of pellets) after 3 hr corresponds to onset of drug release (approximately 2% released). Profiles of release and rupturing were similar in shape (Fig. 5a), but did not exactly overlay. This can be explained in two ways. First, small rupturing responsible for drug release were not recognized. Second, large pellets likely ruptured first, thus having higher than average contribution in their release. Irrespective of this discrepancy, the rupturing of the outer membrane as a trigger for release can be confirmed. Membranes of all pellets were completely destructed and content released at the end of release (Fig. 5b).

In Vivo Study

In vitro drug release was pulsatile with good reproducible lag time, followed by relatively fast release ($T_{75\%}$ 0.9–1.8 hr) (Fig. 1, Table 1). However, the lag time and T_{max} in vivo were clearly dependent on coating level of outer membrane (Fig. 6). The lag time in vivo was 1.5, 3.5, and 4.5 hr for the pellets coated with 15, 25, and 35% w/w, respectively (Fig. 6, Table 1), which is in agreement with lag time 1.9, 3.0, and 4.8

TABLE 1 **In Vitro Release and in Vivo Pharmacokinetic Parameters After Oral Administration of Acetaminophen Pellets Layered with 48% w/w AcDiSol® and Coated With Aquacoat® ECD**

	In vitro		In vivo				
Coating level, %	Lag time, h	$T_{75\%}$, h	Lag time, h	$AUC_{0.8}$ (μg/mL*h)	AUCratio (%)	C_{max}, μg/mL	T_{max}, h
IR tablet	n.d.	n.d.	No	6.7	100.0	7.8	1.0
15	1.9	0.9	1.5	5.6	83.7	6.4	3.0
25	3.0	1.8	3.5	4.2	62.1	3.6	5.5
35	4.8	1.2	4.5	2.6	38.4	1.5	6.5

n.d. not determined

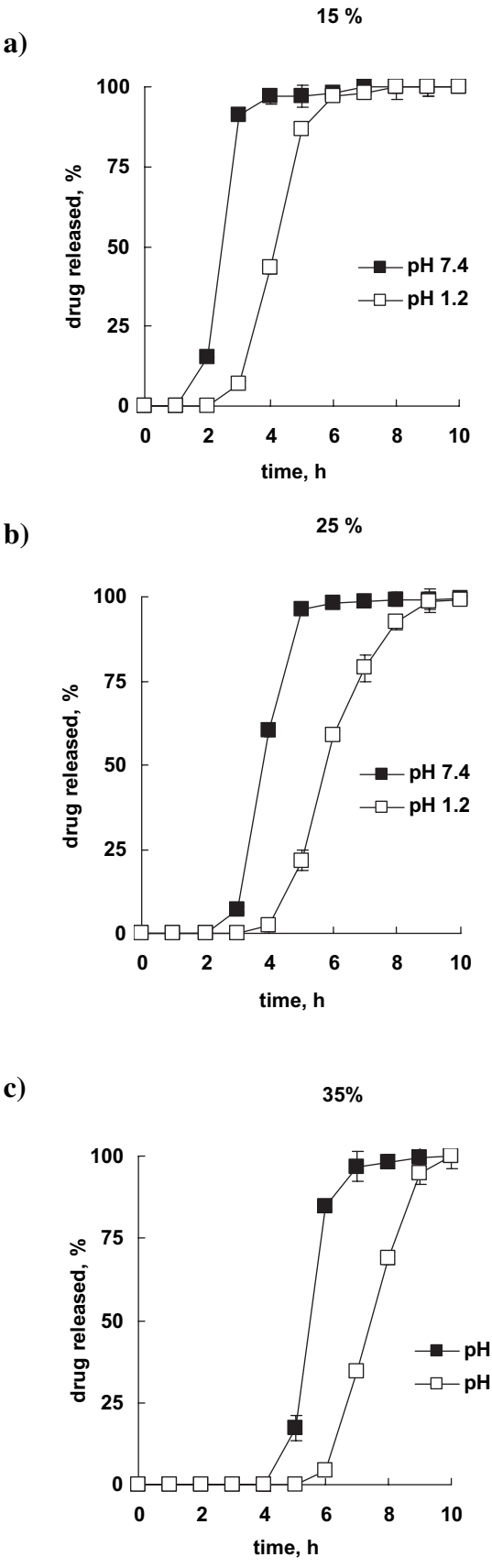

FIGURE 2 The Effect of Medium pH on Drug Release in 0.1 N HCl or Phosphate buffer pH 7.4, 100 rpm, n = 3. Aquacoat® ECD Coating Level a) 15, b) 25 and c) 35% w/w.

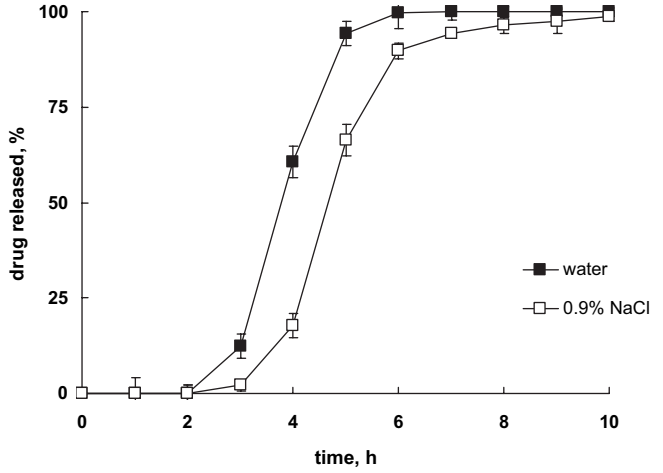

FIGURE 3 Effect of Ion Concentration on Drug Release in Purified Water and 0.9% NaCl, 100 rpm, n = 3. Aquacoat® ECD Coating Level 25% (w/w).

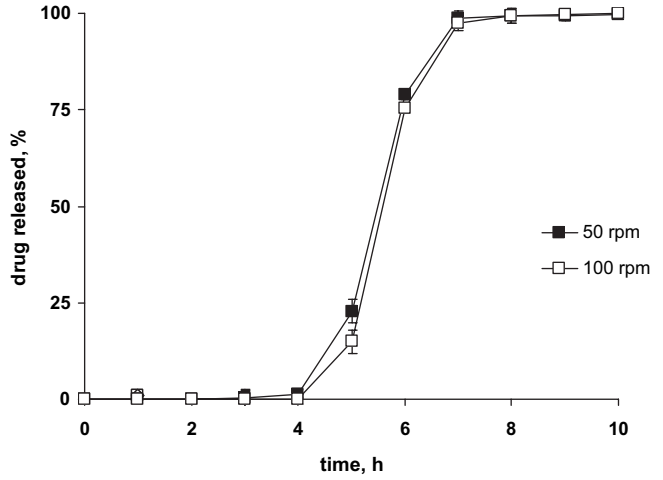

FIGURE 4 Effect of Paddle Rotation Speed (50 vs. 100 rpm) on Drug Release in Phosphate Buffer pH 7.4, n = 3. Aquacoat® ECD Coating Level 35% (w/w).

hr, respectively, recorded in in vitro experiments, performed in phosphate buffer pH 7.4 (Fig. 1, Table 1).

AUC also decreased with increasing coating level and delayed drug release (Table 1). A similar phenomenon was described earlier (Murata et al., 1998) and explained by lower liquid flow in the colon region, where pellets reached before rupturing and drug release onset. This bioavailability problem is likely a specific problem of the drugs with high dose and moderate solubility (like acetaminophen selected first because of analytical advantages) when delivered to lower regions of intestine. However, the performance of the pulsatile drug delivery system in vivo could be characterized adequately by lag time and T_{max}.

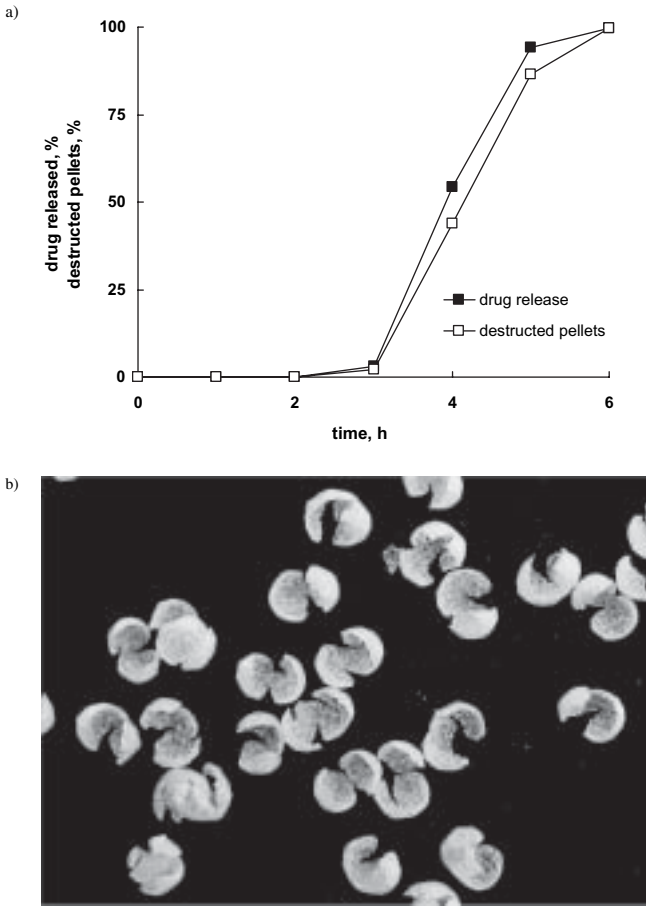

FIGURE 5 **a) Drug Release Profiles and Percentage of Destructed Pellets and b) Visual Observation in Phosphate Buffer pH 7.4, 100 rpm, *n* = 3. Aquacoat® ECD Coating Level 25% (w/w).**

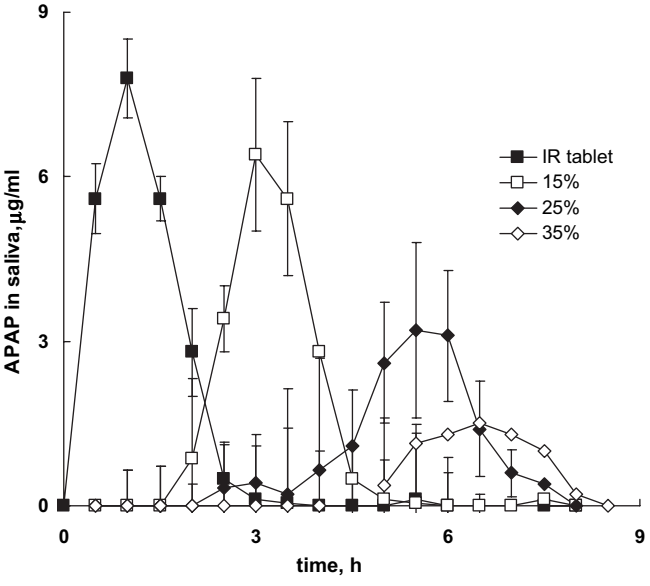

FIGURE 6 **Acetaminophen Concentration in Saliva After Administration of the Pulsatile Dosage Form. Each Data Point Represents a Mean Value and Standard Deviation for Five Volunteers.**

CONCLUSION

In vitro drug release can be characterized as pulsatile with good reproducible lag time, followed by fast release. Lag time in vitro and in vivo from the investigated rupturable pulsatile drug delivery systems was well controlled by coating level of outer membrane, followed by fast drug release. The in vitro lag time could be effected by pH and ionic concentration of the dissolution medium. The main trigger for drug release onset is the rupturing of the outer membrane. The bioavailability of the investigated model drug (acetaminophen 500 mg) decreased with longer lag time, resulting from higher coating level. However, this disadvantage can be considered as a specific limitation for drugs with high dose and moderate solubility and should not diminish performance of the system in principle.

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