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Research paper

In-vitro/in-vivo correlation of pulsatile drug release from press-coated tablet formulations: A pharmacoscintigraphic study in the beagle dog

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Abstract

The aim of the current study was to investigate the in-vitro and in-vivo performance of a press-coated tablet (PCT) intended for time delayed drug release, consisting of a rapidly disintegrating theophylline core tablet, press-coated with barrier granules containing glyceryl behenate (GB) and low-substituted hydroxypropylcellulose (L-HPC). The PCTs showed pulsatile release with a lag time dependent upon the GB and L-HPC composition of the barrier layer. In-vivo γ-scintigraphic studies were carried out for PCTs containing GB:L-HPC at 65:35 w/w and 75:25 w/w in the barrier layer in four beagle dogs, in either the fed or fasted state. The in-vivo lag time in both the fed and fasted states did not differ significantly ($p > 0.05$) from the in-vitro lag time. Additionally, no significant difference ($p < 0.05$) between in-vivo fed and fasted disintegration times was observed, demonstrating that in-vivo performance of the PCT was not influenced by the presence or absence of food in the gastrointestinal tract. A distinct lag time was obtained prior to the appearance of drug in plasma and correlated ($R^2 = 0.98$) with disintegration time observed from scintigraphic images. However, following disintegration, no difference in pharmacokinetic parameters ($AUC_{0-6\ dis}$, K_{el}, C_{max}) was observed. The current study highlighted the potential use of these formulations for chronopharmaceutical drug delivery.

Keywords: Press-coated tablets; Pulsatile release; Beagle dogs; Pharmacoscintigraphy; IVIVC; Lag time

1. Introduction

Circadian phase dependent patterns have been well documented in conditions such as asthma, arthritis, epilepsy, migraine, allergic rhinitis, cardiovascular disease (myocardial infarction, angina, stroke) and peptic ulcer disease, with particular times where symptoms are more prominent and/or exacerbated [1]. Treating these diseases with immediate release dosage forms may be impractical if the symptoms of the disease are pronounced during the night or early morning. Therapy with modified release dosage forms with zero order drug release theoretically leads to controlled and constant levels of drug in plasma throughout the day. However this does not provide extra therapeutic levels at the time of increased symptoms, and unwanted plasma drug concentration at other times of day may produce adverse effects with little therapeutic benefit [2]. In order to optimize therapy in terms of safety, patient compliance and efficacy, chronopharmaceutical formulations based upon time controlled drug delivery systems (TCDDS) are considered to be potential therapeutic options [3]. TCDDS are dosage forms that are designed to mimic the circadian rhythm of the disease by releasing the drug at the appropriate time, by means of an internal pre-programmed clock that is initiated when the dosage forms come in contact with gastrointestinal (GI) fluids. TCDDS have been formulated as pellets [4], capsules [5,6] or tablets [7–13] designed to release drug only after a defined lag time. Tablet formulations generally consist

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of a rapid release core tablet encased in a barrier layer either formed by press-coating or liquid coating or a combination of both. The press-coating technique offers advantages over liquid coating as it does not involve the use of solvents, requires a relatively short manufacturing process, and allows greater weight gain to the core tablet. However, common drawbacks of the press-coating technique are the multi-step processes involved, and the requirement for reliable and reproducible central positioning of the core tablet within press-coated tablet (PCT), a major challenge for large scale industrial manufacturing. Ozeki et al. published an article demonstrating that the core tablet can be centrally positioned within the PCT, and that the formulation can be manufactured in single-step process [14]. This is a positive step toward overcoming the aforementioned limitations and providing the basic platform toward large scale manufacturing. The PCTs used in the current study are not manufactured by a single step process as mentioned by Ozeki et al. [14], because this technique requires special instrument setup which is not readily available. The lag time of drug release from PCTs depends upon the thickness and the composition of the barrier layer. Generally speaking, the thicker the barrier layer, the longer the lag time to drug release will be [9,13,14]. The composition of the barrier layer controls the mechanism of effecting a lag time. In most instances where high viscosity grades of polymers such as hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose, polyethyleneoxide are used in the barrier layer, the polymer swells upon contact with GI fluid, then dissolves and/or erodes exposing the inner core tablet [8,9]. Some researchers have produced a lag time prior to drug release from the core tablet using an outer barrier layer that consisted of a mixture of soluble excipient and wax components, with drug release from the core tablet occurring through the resultant channels created following the dissolution of water soluble excipient [7]. Other researchers have used ethylcellulose (EC) in the barrier layer with the lag time prior to drug release believed to be dependent upon water penetration through the inert EC matrix followed by destruction of the barrier layer [10]. As the particle size of EC decreased, the rate of water penetration decreased [10], while the addition of the water soluble excipient (spray dried lactose) to EC created larger channels, thus decreasing the lag time prior to release [13].

The PCT investigated in the current study retains the characteristics of a formulation originally prepared by Leaokittikul et al. [12], consisting of a rapidly disintegrating core tablet press-coated by a barrier layer consisting of varying concentrations of low-substituted hydroxypropylcellulose (L-HPC) and glyceryl behenate (GB). L-HPC is a disintegrant and had been used to cause rapid disintegration of tablets [15,16]. The other component of the barrier layer, GB, is commonly used as a lubricant, but the current study utilises its previously reported sustained release characteristics [17–19]. It was postulated that when the barrier layer was exposed to dissolution media, the L-HPC particles swell and erode, a process which was retarded to varying degrees depending upon the quantity of GB present, demonstrating that manipulation of both components controls the erosion rate [20]. In previous studies, the erosion rate of the barrier layer consisting of GB and L-HPC was found to be dependent upon the weight ratio of GB to L-HPC, but was found to be independent of the dissolution media hydrodynamics, pH, and fat content [20–22]. These findings suggested that the PCT may perform in an in-vivo environment in a manner similar to its performance in in-vitro studies, as the erosion rate of the outer barrier layer was largely unaffected by the changes to the pH of the dissolution media (pH 2–7), paddle speed of the dissolution apparatus (0–200 rpm) or the percentage fat content in milk when used as dissolution media within the range 0.1–3.6%. These parameters and others have been considered in the simulation to in-vivo conditions [23].

The dog has previously been used as the animal model to evaluate the in-vivo performance of TCDDS and colonic drug delivery systems [24,25]. However, the GI anatomy and physiology of a dog is not always directly comparable with that of human, and caution must be exercised when extrapolating findings from an animal model to expected behaviour in man [26,27]. In terms of the 'destructive force' in stomach of the dog, the overall force generated is approximately twice than that in humans [28,29]. The mechanical forces in the small intestine however are reported to be lower than those of the stomach, and are thought to be similar in dogs and humans [30].

Gamma scintigraphy is currently considered to be the non-invasive technique of choice to determine in-vivo performance of dosage forms. The pharmaceutical application of gamma scintigraphy started in 1976 as a novel method to determine in-vivo disintegration and drug release from a gelatin capsule [31]. For TCDDS, gamma scintigraphy has been well established as a useful method to track the dosage forms within the GI tract and assess in-vivo performance in terms of site and time of drug release [6,11,12]. Visually tracking the dosage forms is of great importance if the performance of the barrier layer depends upon its location within the GI tract [9], or if a time controlled drug delivery approach is utilised for site controlled release [6]. Plasma profiles of TCDDs should ideally show no drug presence for a defined period of time, followed by rapid drug absorption, in contrast to immediate or sustained release dosage forms where systemic absorption should start almost immediately following dosing. In order to accurately detect the initial rapid drug absorption phase after disruption of the barrier layer, sampling the blood at quick succession during the initial absorption phase is required. In practice, it is not feasible to perform blood sampling at quick succession throughout the entire study period, and thus the use of gamma scintigraphy is ideal as it provides the visual information required regarding tablet disintegration time, which can act as a trigger to begin pharmacokinetic blood sampling. Furthermore, scintigraphic images combined with pharmacokinetic data are a powerful tool in the interpretation of unusual or unex-

pected pharmacokinetic profiles, allowing identification of gastrointestinal location and behaviour of the formulation at the time of a particular pharmacokinetic event [32].

The objective of the current study was to evaluate the in-vitro performance of the press-coated pulsatile release tablet formulation and to correlate with in-vivo behaviour in beagle dogs using pharmacoscintigraphy. Theophylline was used in the immediate release core tablet as the model drug for in-vitro drug release and pharmacokinetic analysis. Theophylline falls under Class I (high solubility-high permeability) drug according to the Biopharmaceutical Classification System and is absorbed throughout the GI tract.

2. Materials and methods

2.1. Materials

All the materials used in the current study were of pharmacopoeial grade: Low-substituted hydroxypropylcellulose, L-HPC (LH 21 grade) (Shin-Etsu Company, Tokyo, Japan); Glyceryl behenate, GB (Compritol 888 ATO) (Gattefossé, Saint Priest Cadex, France); Acetonitrile (HiPerSolv) (VWR International Ltd., Poole, England); Water (HiPerSolv) (VWR International Ltd., Poole, England); Formic acid (VWR International Ltd., Poole, England); 3-propylxanthene (Sigma–Aldrich Chemie GmbH, Germany); Theophylline (Fluka Chemie GmbH, Buchs, Switzerland); Riboflavin (Sigma–Aldrich Chemie GmbH, Germany); Lactose (Fast-Flo) (Pfizer Ltd., Kent, UK); Magnesium stearate (Allchem International Ltd., Berks, UK); Ac-di-Sol (Pfizer Ltd., Kent, UK) and ^{99m}Tc-DTPA (Radiopharmacy, Western Infirmary, Glasgow, UK).

2.2. Manufacture of core tablet

Theophylline (85 g), Ac-di-Sol (1.8 g) and lactose (2.3 g) were mixed in a Turbula orbital mixer (Glen-Creston Instruments Ltd., Middlesex, England) for 20 min, following which magnesium stearate was introduced to the blend and mixed for a further 5 min. 90 mg of the resultant powder blend was manually compressed using an IR press (Research and Industrial Instrument Co., England) for 10 s at a pressure of 1 ton, with a 6.5 mm punch and die (Penta Punch & Die Co. Ltd., Nottingham, England) to obtain the core tablet.

2.3. Manufacture of barrier layer granules

A melt granulation process was employed to prepare the barrier granules (BG). The compositions of GB and L-HPC were varied as shown in Table 1. Firstly, the required amount of GB was melted in a beaker maintained in a water bath at 95–99 °C, followed by addition of L-HPC. The mixture was continuously stirred until homogeneous, and allowed to cool to room temperature, with continuous stirring to prevent caking during the solidification process. The granules formed were forced through a 1 mm sieve to obtain barrier granules which were stored in airtight containers prior to use.

Table 1
Formulation composition of barrier granules (BG)

BG	A	B	C	D	E
GB (%)	50	60	65	70	75
L-HPC (%)	50	40	35	30	25

2.4. Preparation of press-coated tablets

The core tablets were press-coated with 500 mg of BG (A–E) to produce corresponding PCT (A–E) formulations. Briefly, 250 mg of BG was weighed and transferred into a 13 mm IR press die (Pye Unicam, Cambridge, England). Next, the core tablet was centrally positioned on the powder bed using a locally manufactured centralising tool. The remaining 250 mg of the BG was added into the die and compressed at 5 ton for 3 min using an IR press. The thickness of the PCT was 3.82 ± 0.01 mm (mean $\pm$ SD, $n = 10$) and that of inner core tablet was 1.80 ± 0.06 mm (mean $\pm$ SD, $n = 10$), affording a barrier layer thickness of 1.01 mm on either face of the tablet.

2.5. Preparation of radiolabelled tablets for scintigraphy study

A hole of 2.4 mm depth and 1 mm diameter was drilled centrally into a previously compressed PCT. $^{99\,m}$Tc-DTPA labelled lactose giving 1 MBq of activity at the time of dosing to the dog was filled into the hole, followed by addition of approximately 0.5 mg of non-radiolabelled lactose, before adding melted GB which solidified as a plug to seal the hole. Radiolabelled, hole-drilled PCT C and PCT E were prepared (named as HPCT C and HPCT E, respectively).

2.6. Dissolution studies

In-vitro dissolution studies were performed on the theophylline formulation at 37 °C in 1 L of distilled water in USP apparatus II (Erweka GmbH, Heusenstamm, Germany) with the paddle speed 50 rpm. 5 mL of filtered aliquot was manually withdrawn at pre-determined time intervals and replaced with 5 mL of fresh distilled water maintained at the same temperature. The samples were analysed at 242 nm using a UV spectrophotometer (Helios Alpha, Thermospectronic, England). The lag time was defined as the time corresponding to 10% release of drug ($t_{10\%}$). The $t_{10\%}$, $t_{50\%}$ and $t_{80\%}$ were calculated as the intersections on the time axis corresponding to 10%, 50% and 80% of drug release, respectively.

2.7. In-vivo dog study

All procedures were performed under a UK Home Office Animals (Scientific Procedures) Project Licence with

a washout period of at least 1 week between two consecutive administrations. In-vivo pharmacoscintigraphic studies were carried out for HPCT C and HPCT E formulations in four male beagle dogs weighing 14–17.5 kg. The dogs were fasted for at least 14 h prior to the study, with free access to water. Each HPCT C or E was administered to the dogs with around 20 mL of water on each separate occasion in either the fasted state, or within 30 min of eating a FDA high fat breakfast [33], in a four-way non-randomized study. Dogs were given free access to water during the studies. During the fasted study, dogs were given access to normal dog food (Chappie and Eukanuba diet) 3 h after tablet disintegration or gastric emptying of HPCT. In the fed studies, dogs were allowed normal dog food 3–4 h after dosing.

As the animals were trained to stand supported in a sling next to the gamma camera before commencement of studies, the imaging procedure did not appear to cause stress to the animals, reducing the potential for altered GI transit. External positional reference markers of 0.1 MBq ^{99m}technetium were placed at the base of the tail and between the shoulders. A gamma scintigraphic camera (Scintron, MIE, Germany) equipped with a low energy collimator was used to acquire images at intervals of 10 min until tablet disintegration was observed. The time of disintegration was defined as in-vivo lag time. Time of disintegration was taken as the time halfway between the last image of an intact core tablet, and the first image in a consecutive series showing disintegration of the core tablet. Images were analysed for site of disintegration, gastric retention time (GRT), gastric emptying time (GET), small intestine transit time (ITT), and colon arrival time (CAT) for the HPCTs.

Blood samples of 2.6 mL were taken from the saphenous vein every 1 h until tablet disintegration was observed scintigraphically, then at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5 and 6 h post disintegration using heparinised tube. Blood samples were centrifuged (IEC Centra-7R, International Equipment Co., Damon Corp., MA, USA) at 2000 rpm for 20 min and plasma was removed and stored at −20 °C for subsequent analysis.

2.8. Plasma theophylline analysis

Plasma theophylline concentration was determined using an HPLC method. 0.2 mL of plasma, 0.4 mL internal standard (3-propylxanthene, 5 μg/mL) and 0.2 mL of water were mixed and centrifuged (Labrofuge 400, Heraeus Instruments, Germany) at 3500 rpm for 5 min. The supernatant solution was mixed with 0.5–1 mL of water, passed through a 0.22 μm Millex GP filter (Millipore, Carrigtwohill, Co. Cork, Ireland) and collected in 1.5 mL vials. Samples were then analysed using an HPLC system (ThermoSeparations P1000 system, San Jose, CA, USA) equipped with a Vydac 201SP^{M}54 C18 column (250 × 4.6 mm id, 5 μm particle size) (Hichrom Ltd, Reading, UK) and guard cartridge (Phenomenex, Cheshire, England). The sample injection volume was 10 μl using AS1000 autosampler (ThermoSeparations P1000 system, San Jose, CA, USA) and the UV detection wavelength was 272 nm. Analysis was preformed at room temperature using a mobile phase of acetonitrile and 0.1% v/v formic acid in water at a ratio of 5:95 v/v.

2.9. Data analysis and statistics

The pharmacokinetic parameters evaluated were maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), area under the curve from time of dosing to 6 h post disintegration ($AUC_{0–6\ dis}$) and K_{el} were determined using WinNonlin version 5.0.1, assuming a non-compartmental model for drug absorption and distribution. The lag time to drug appearance in plasma (T_{lag}) was defined as the time corresponding to the first appearance of theophylline in the plasma. In-vitro and in-vivo disintegration times were compared using a two-sample *t*-test with a 95% confidence interval using Minitab™ Release 14.1.

3. Results and discussion

3.1. Dissolution studies

All PCTs showed pulsatile release behaviour with a distinct lag time. Fig. 1 shows the dissolution profiles of the core tablet and PCTs (A–E). The immediate release core tablet showed 80% of theophylline release within 5 min, while incorporation of the core tablet into the PCT produced a lag time prior to drug release. When the dissolution medium reached the core after eroding the outer barrier layer, rapid drug release was observed. The mean lag time ($t_{10\%}$), $t_{50\%}$ and $t_{80\%}$ for the PCTs is represented

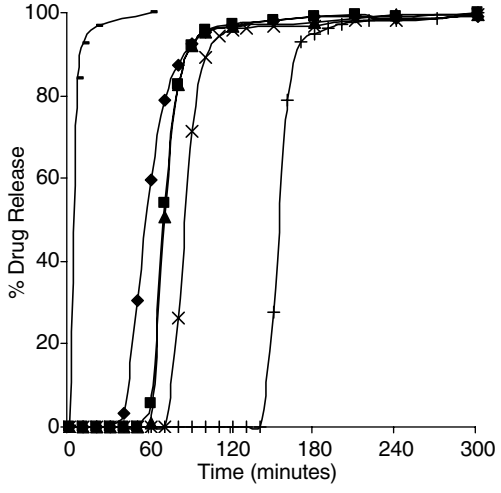

Fig. 1. Effect of barrier layer composition on the release of theophylline PCT A (◆), PCT B (■), PCT C (▲), PCT D (×) and PCT E (+) compared to core tablet without barrier layer (−). Dissolution studies were carried out in 1 L of distilled water using USP apparatus II (50 rpm, 37 °C). Each data point is the mean of $n = 6$ individual determination.

in Table 2. All of the PCT (A–E) formulations showed 80% of drug release within 30 minutes following their respective lag time. Such pulsatile release profiles differ from previous reports in the literature where sustained release of drug from the core tablet occurred following a lag period [7,10]. This is probably because the mechanism of producing a lag time for these formulations was based upon the hydration of the outer barrier layer, or water penetration through outer barrier layer, with the outer barrier layer being present when the dissolution medium reaches the core tablet, preventing rapid drug release. The PCT investigated in the current study is based upon an active erosion mechanism of the barrier layer, thus the barrier layer eroded uniformly from each axial surface providing uniform exposure of at least one surface of the core tablet, leading to rapid dissolution within a short time frame. The active erosion rate of the outer barrier layer depends upon the formulation composition, which in turn determines the lag time of the PCT. The lag time of drug release from PCT increased as the ratio of GB to L-HPC in the barrier layer increased (Fig. 2). It is postulated that L-HPC is responsible for the active erosion of the barrier layer as the characteristics of such disintegrants are absorption of water followed by swelling [34]. The presence of GB will retard the erosion process, and the extent to which it does this will depend on the relative ratio of the two excipients. The higher the ratio of disintegrant in the barrier layer, the more opportunity there will be for L-HPC to come in contact with the dissolution medium, resulting in swelling and more rapid erosion.

The radioactive material was incorporated within the formulation by drilling a hole in the tablet into which the isotope was added, before sealing up the hole as previously reported [35]. A potential disadvantage of this technique is that the performance of the formulation may be altered by the drilling technique. Therefore, the dissolution behaviour of the hole drilled PCTs (HPCTs) was determined and the profile compared to that of the corresponding PCT, with no significant difference between lag times observed for either formulation ($p > 0.05$) (Table 2). This was because less than 0.3% of available formulation surface area was being altered by drilling into the PCT. However a significant ($p < 0.05$) increase in the values of $t_{50\%}$ and $t_{80\%}$ was found for the HPCTs in comparison to PCTs. This increase is probably attributable to the use of GB as a sealant for the hole that was drilled into the core tablet within the HPCT. If a small amount of GB had solidified within the core tablet, it may have had the effect of preventing rapid explosion of the core tablet after erosion of outer barrier layer. Although the slight increase in the values of $t_{50\%}$ and $t_{80\%}$ was noticed, the lag time was not affected by this variability, and the HPCT was accepted for use in the in-vivo studies.

Table 2
Lag time, $t_{50\%}$ and $t_{80\%}$, of drug release of the PCT and HPCT formulation calculated from in-vitro dissolution profiles

Formulation	Lag time ($t_{10\%}$) (min)		$t_{50\%}$ (min)		$t_{80\%}$ (min)	
PCT A	44 ± 3		56 ± 5		72 ± 10	
PCT B	61 ± 2		70 ± 2		80 ± 5	
PCT C	64 ± 4	NS	70 ± 4	S	78 ± 4	S
HPCT C	72 ± 8		80 ± 6		93 ± 9	
PCT D	75 ± 2		85 ± 2		94 ± 5	
PCT E	148 ± 5	NS	153 ± 5	S	159 ± 5	S
HPCT E	152 ± 11		163 ± 5		175 ± 5	

Values are represented in means ± SD ($n = 6$).
PCT, press-coated tablet; HPCT, hole drilled press-coated tablets; S, significant difference; NS, no significant difference at $p = 0.05$.

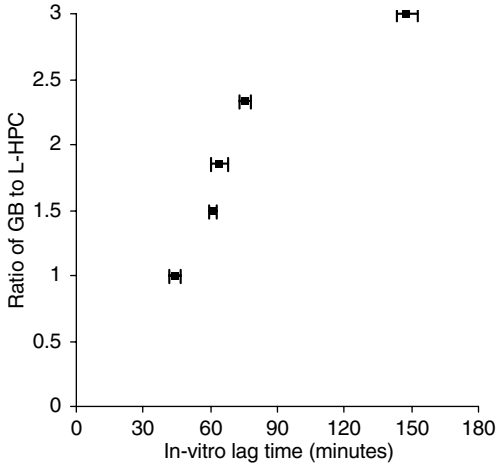

Fig. 2. Influence of GB to L-HPC ratio on in-vitro lag time of PCT formulations (time in the x-axis corresponding to 10% of drug release) calculated from in-vitro dissolution profile of the PCTs. Each point is the mean ± SD of $n = 6$ individual determination.

3.2. Gastrointestinal transit

It was possible to track the dosage form throughout the GI tract and to determine the site and time of disintegration as shown in Fig. 3. In-vivo disintegration time and transit data evaluated by scintigraphy are shown in Tables 3 and 4, respectively. Although the overall formulation composition of the barrier layer was different for HPCT C and HPCT E, both HPCTs had identical size and shape, therefore it was not expected that gastric emptying and transit data would be affected prior to disintegration. Transit data are therefore reported in this discussion for HPCTs as a whole (i.e. $n = 8$), rather than for HPCT C and HPCT E individually.

In the fasted state, gastric emptying was observed for 3 HPCTs out of 8. The mean GET for the HPCTs in the fasted state was found to be 38 ± 11 min, while the GRT for the HPCTs which did not empty intact ranged from 78 to 148 min prior to disintegration. The values for GRT and GET obtained from the current study lie in agreement with the documented variability of GET from 35 to 315 min in the fasted state for a Heidelberg capsule in the dog [36]. The GET in the fasted state depends upon the time difference between dosing and the occurrence of phase III of the migrating motor complex cycle which can vary from 91 to 110 min [37] if the physical nature of

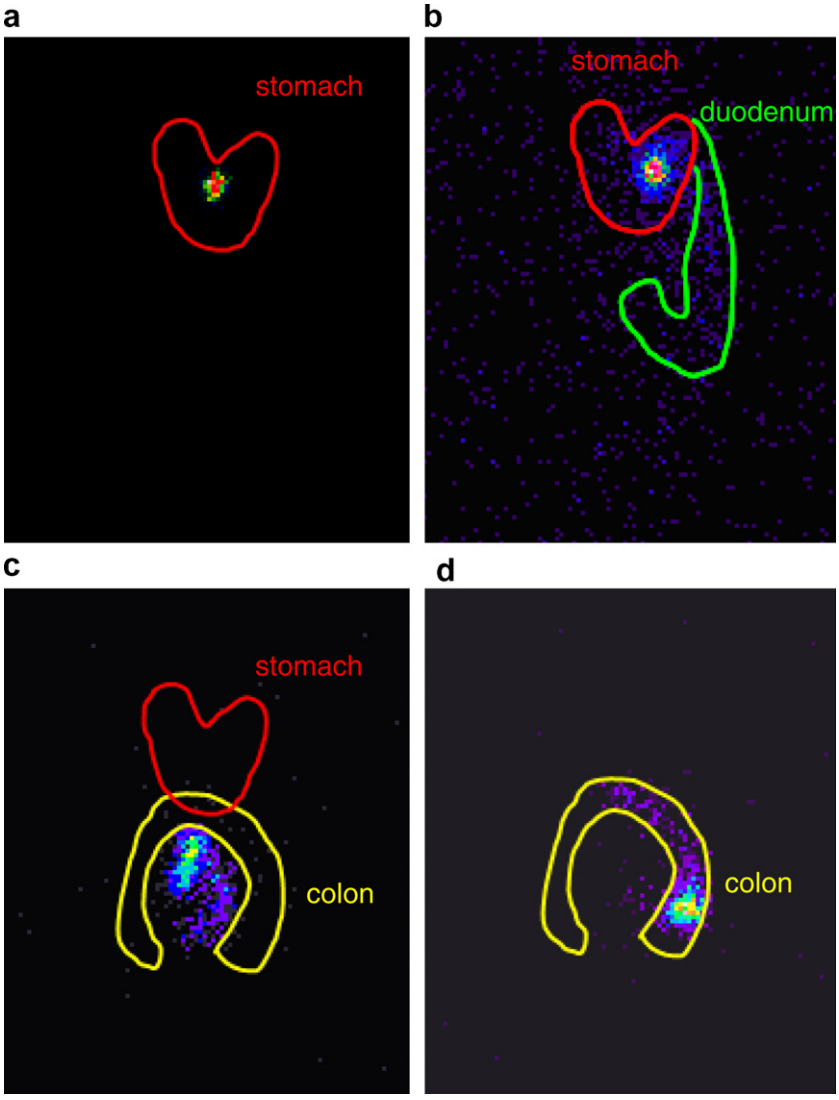

Fig. 3. Representative gamma scintigraphic images showing intact HPCT in stomach (a), and disintegrating HPCT in stomach (b), small intestine (c) and colon (d).

the dosage form or physiological variation of the animal does not play a significant role. If the size of dosage forms is greater than 5 mm, it has been reported that it may not empty in phase III of the migrating motor complex cycle in dogs [38], which may explain the low incidence of gastric emptying of an intact tablet in the current study, where the diameter of the HPCT administered was 13 mm. The mean ITT for the HPCTs which emptied intact from the stomach was found to be 68 ± 18 min ($n = 2$), with a third HPCT disintegrating in the small intestine 26 min post gastric emptying. The previous reported range for ITT in dogs is 15–206 min, and was noticed to be more variable in dogs in comparison to humans [26]. The mean colon entry time for intact tablets in the current study was found to be 112 ± 22 min ($n = 2$).

All of the HPCTs in the fed state disintegrated in the stomach, so GET could not be obtained. The GRT prior to disintegration ranged from 65 to 184 min, excluding one HPCT (dog 3) that was retained intact for 390 min before disintegration. Gamma scintigraphic images revealed that the tablet remained static in the stomach, without any movement for more than 5 h. This is similar to results reported by Ayogi et al. [39] where an enteric coated tablet attached to the surface of the stomach and was retained for more than 20 h in dogs in the fed state, and by Ishibashi et al. [24] where colon targeted capsule (size 2) was retained in the fed stomach for 14 h. In the fed state, it has been reported that solid dosage forms greater than 5 mm in size can be retained in the stomach of dog for more than 450 min [40]. The long retention time of an intact tablet in the dog stomach is due to the time taken to change from fed motility state to the MMC fasting cycle which has been observed to be between 5.4 and 13.3 h [27].

Table 3
Summary of in-vivo disintegration time of HPCTs observed by scintigraphy in beagle dogs ($n = 4$)

State/tablet	Dog	Site of disintegration	Disintegration time (min)	
			Individual	Mean ± SD
Fasted HPCT-C	1	Stomach	107	89 ± 13
	2	Stomach	88	
	3	Small intestine	81	
	4	Stomach	78	
Fed HPCT-C	1	Stomach	92	79 ± 11
	2	Stomach	82	
	3	Stomach	75	
	4	Stomach	65	
Fasted HPCT-E	1	Stomach	146	166 ± 33
	2	Stomach	148	
	3	Colon	215	
	4	Colon	156	
Fed HPCT-E	1	Stomach	184	218 ± 117
	2	Stomach	168	
	3	Stomach	390	
	4	Stomach	130	

Table 4
Summary of in-vivo transit data of intact HPCTs in beagle dogs

Parameter	n	Transit (min)
GET	3	38 ± 11
ITT	2	68 ± 18
CAT	2	112 ± 22

GET, gastric emptying time; ITT, Intestinal transit time; CAT, colon arrival time.

3.3. Correlation between in-vitro dissolution and in-vivo scintigraphic data

The in-vivo disintegration performances of the HPCTs are shown in Table 3. The in-vivo lag time for HPCT C in the fasted (89 ± 13 min) and fed states (79 ± 11 min) did not differ significantly ($p > 0.05$) from the in-vitro lag time (72 ± 8 min). Similarly for HPCT E, in-vivo disintegration time (166 ± 33 min, fasted; 218 ± 117 min, fed) did not differ significantly from in-vitro lag time (152 ± 11 min). Additionally, no significant difference ($p > 0.05$) was detected between in-vivo fasted and fed state disintegration time for both HPCTs, demonstrating that the in-vivo performance of the HPCTs was not influenced by the presence or absence of food in the GI tract.

It has been observed in previous studies that the erosion characteristics of the outer barrier layer were largely independent of the pH and hydrodynamics of the dissolution medium [21], which may explain the similar performance of the HPCTs in both the in-vivo and in-vitro environment. The similar performance of the HPCTs in the fasted and fed states can be explained partially by the results from previous studies, where the erosion performance of the outer barrier layer was found to be independent of fat content when milk was used as dissolution media (0.1%w/v to 3.6%w/v) [22]. It was also interesting to note that the site of disintegration in the upper GI tract did not have a marked effect on the in-vivo disintegration time of the HPCT C in the fasted state. The mean disintegration time of 91 ± 15 min for the three HPCT C tablets which disintegrated in the fasted stomach was similar to the disintegration time of the remaining tablet in the small intestine (81 min) (Table 3). It has been reported that the dosage forms are subjected to lower pH [36] and higher destructive force [29,30] in a dog's stomach in comparison to that of small intestine, however results obtained from the current study for HPCT C in the fasted state suggest that neither pH nor gastrointestinal mechanical forces had a marked effect on the in-vivo erosion performance of the press-coated tablets. For HPCT E in the fasted state, higher variability was observed for the HPCTs which disintegrated in the colon (186 ± 42 min, $n = 2$) than those which disintegrated in the stomach (147 ± 1 min, $n = 2$) (Table 3). As the dosage form moves distally from the small intestine the water availability decreases and fluid viscosity increases [41]. Since the erosion rate of the outer barrier layer of the HPCTs depends upon the interaction with GI fluids this could account for the more variable performance of the dosage form in the colon.

3.4. Pharmacokinetic analysis of HPCTs

The mean pharmacokinetic data for the two HPCT formulations are shown in Table 5. The pharmacokinetic blood sampling regimen for each dog started only after confirming in-vivo disintegration of the HPCT by gamma scintigraphy. Fig. 4 represents the mean plasma profiles of HPCT C and HPCT E in both fasted and fed states. Both formulations exhibit a distinct lag phase prior to drug release illustrating the robust performance of the barrier layer in controlling the lag time in the in-vivo environment. The value of $AUC_{0-6\ dis}$ and C_{max} obtained for both HPCTs (in the fasted and fed conditions) did not differ significantly ($p > 0.05$) from each other, suggesting that the lag time of the HPCT or feeding condition did not affect in-vivo pharmacokinetic performance.

3.5. Correlation between scintigraphic observation and pharmacokinetic data

Good correlation ($R^2 = 0.98$) was obtained between the first appearance of theophylline in the plasma of the dogs, and the disintegration time observed from the scintigraphic study, as shown in Fig. 5.

4. Conclusion

The lag time of drug release from the PCT formulation can be readily modulated by varying the weight ratio of GB to L-HPC in the outer barrier layer. Good correlation was observed between in-vitro and in-vivo performance of the press-coated tablet, independent of the site of disintegration

Table 5
Pharmacokinetic parameters of the theophylline time-delayed tablets in the beagle dog

Formulation	Meal	T_{lag} (min)	C_{max} (μg ml^{-1})	T_{max} (min)	$AUC_{0-6\ dis}$ (μg ml^{-1} min)	K_{el} (h^{-1})
HPCT C	Fasted	96 ± 16	4.7 ± 1.3	208 ± 58	1272 ± 394	0.0020 ± 0.0003
	Fed	82 ± 7	3.8 ± 0.7	116 ± 6	833 ± 148	0.0027 ± 0.0005
HPCT E	Fasted	166 ± 33	4.2 ± 0.9	279 ± 62	1103 ± 190	0.0025 ± 0.0014
	Fed[a]	169 ± 21	4.1 ± 0.7	230 ± 24	1046 ± 170	0.0031 ± 0.0018

T_{lag} (time of first appearance of theophylline in plasma), C_{max} (maximum plasma concentration), T_{max} (time to reach C_{max}), $AUC_{0-6\ dis}$ (area under the curve from time 0 to 6 h post disintegration), K_{el} (elimination rate constant).
Data are represented as means ± SD, $n = 4$ except [a]where $n = 3$ excluding dog 3. Pharmacokinetic results normalised to a 5 mg/kg dose.

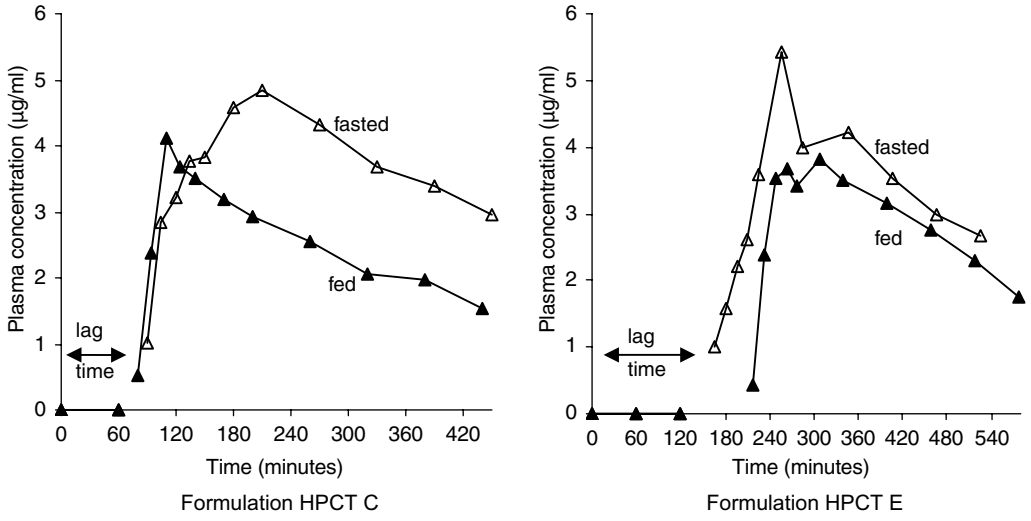

Fig. 4. Mean plasma concentration–time profiles of theophylline after oral administration of HPCT C and E in beagle dogs in fasted (△) and fed (▲) states. Each data point is the mean of $n = 4$ individual determination.

and fed state, suggesting that the robust and reliable ability to produce a lag time before drug release may make this formulation useful as a chronopharmaceutical drug delivery system. Furthermore, this study also demonstrated the value of scintigraphic images in describing the performance of time-delayed formulations.

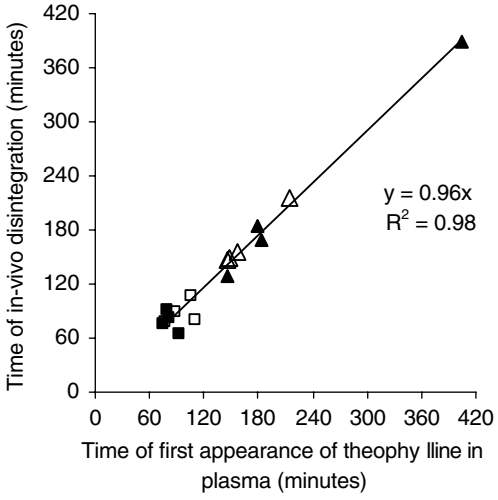

Fig. 5. Correlation between in-vivo tablet disintegration observed from gamma scintigraphic images, and time of first appearance of drug in blood plasma in four beagle dogs. HPCT C fasted (□), HPCT C fed (■), HPCT E fasted (Δ) and HPCT E fed (▲).

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