A Pharmacoscintigraphic Study of Three Time-Delayed Capsule Formulations in Healthy Male Volunteers

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ABSTRACT: Three time-delayed capsule (TDC) formulations were investigated in a pharmacoscintigraphic study, using a three-way crossover design in eight healthy male volunteers. Additionally, the pulsed release of a TDC was investigated with time-lapse photography, using a nondisintegrating riboflavin tablet. The photographic study indicated how the release characteristics of the TDC relied on the erosion of a tablet containing hypromellose (HPMC). Each TDC was duel radio labelled with indium-111 and technetium-99 m DTPA complexes, to observe drug release scintigraphically (theophylline was a marker compound). Three formulations, having *in vitro* dissolution release times of 1.8, 2.9 or 4.0 h were shown to compare favourably with mean *in vivo* scintigraphic release times of 2.7, 3.0 and 4.0 h for each formulation containing 20, 24 or 35% (w/w) HPMC concentrations respectively. An increase in HPMC concentration was associated with a delayed technetium release time, and followed the same rank order as the *in vitro* dissolution study. Observed radiolabel dispersion always occurred in the small intestine. In conclusion, the study established that the TDC performs and demonstrates an *in vitro–in vivo* correlation. Additionally, time and site of release were accurately visualized by gamma scintigraphy, and confirmed with determination of theophylline absorption.
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Keywords: controlled delivery; gastrointestinal transit; scintigraphy; site-specific delivery; targeted drug delivery

INTRODUCTION

Pulsed release drug delivery is of especial interest in the area of controlled release pharmaceuticals. For example, drug release at a specific time of day (i.e., from chronopharmaceutical formulations)

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could potentially be tailored to coincide with the circadian rhythms exhibited by certain disease states.[1–4] In addition, time-delayed preparations may be helpful for delivery to specific regions in the gastrointestinal (GI) tract,[5–7] assuming that small intestinal transit is constant. However, variable gastric emptying times will limit the success of such time-delayed delivery as a means of targeting specific regions in the GI tract unless such formulations are also rendered gastroresistant.[8]

Drug delivery systems targeted to specific areas of the GI tract are generally self-modulated by one or more elements that may interact with components endogenous to that region. For example, delivery to the large intestine may include the use of protective coatings and biodegradable polymers such as, azo bond-containing hydrogels,[8–10] enteric coatings,[11–13] glycosidic bond-containing hydrogels,[8,14] guar gum, pectin and xylan[15–20] and enzyme-containing substrates.[21] The action of some of these polymers is dependent on susceptibility to enzymatic degradation by bacteria resident in the colon.

Investigations into the GI transit of oral preparations have frequently utilised the technique of gamma scintigraphy.[8] The simultaneous use of two different isotopes has been shown to aid the detection of pulsed release from an oral dosage form.[22] Indium-111 (^{111}In) and technetium-99m (^{99m}Tc) have different photopeak energies and hence can be used to visualise two separate components of complex pulsed release dosage forms.[23] Both radiopharmaceuticals have suitable half-lives, high water solubility and form nonabsorbable chelates when complexed with diethylenetriaminepentaacetic acid (DTPA). Pharmacoscintigraphy, a combination of pharmacokinetic analysis of drug levels in biological fluids and concurrent scintigraphic imaging, offers a convenient means of correlating pharmacokinetic parameters to the sites of absorption in the GI tract.[24]

This present study investigates the performance in man of three time-delayed capsule (TDC) formulations with the potential for delivery to various sites throughout the GI tract, such as the duodenum/jejunum, the ileum and the colon. It is hypothesised that delivery to these sites might be achieved by varying the time of drug release.

The performance was evaluated using time-lapse photography study *in vitro* with a highly visible marker tablet, and as a pharmacoscintigraphic study in humans. Theophylline was chosen as marker drug as it is absorbed effectively throughout the GI tract. This delivery device differs from other systems reported in the literature as it makes use of a relatively simple design, and relies on a simple well published erosion process that is inherent for HPMC containing tablets.

Previously studied capsule based pulsed delivery systems were found to be subject to operational difficulties. The Chronset®,[25] PORT System®[26] and Pulsincap™[27,28] devices rely on swelling mechanisms that result in the contribution of frictional forces. In the case of the Pulsincap device the internal structure of the capsule is not of uniform surface roughness.[29] This means that frictional forces vary as the hydrogel plug swells, leading to some degree of lag-time variability. The Chronset device is subject to frictional forces on the external surface of the capsule as the cap is removed by an increase in osmotic pressure. In addition the rigid barrier layer, which acts as a swelling block, also has the potential to restrict the complete dissolution of drug from the core. The PORT System is subject to the influence of frictional forces as the osmotic core swells to expel the waxy plug. However, additional studies have highlighted the importance of a tight fitting seal with the PORT System achieved by the use of hot-melt wax plugs that were shown to prevent premature drug release.[30] The Egalet™ device[31,32] may be subject to a large amount of variability due to the reliance on uniform erosion at its terminal ends. A previous study involving a similarly constructed device highlighted the problem of asymmetrical erosion.[33] The TDC presented in this study attempts to correct the problem that were observed with the Pulsincap™ device, that relied on a swelling process associated with a hydrogel plug insert in a capsule body, which ultimately led to poor reproducibility *in vivo*.

Each TDC functions by means of the erosion of a tablet containing HPMC and lactose which seals a theophylline tablet within an ethylcellulose-coated, impermeable hard gelatin capsule. In the presence of fluid, this erodible tablet gradually disintegrates permitting entry of fluid into the capsule. The expulsion of the theophylline tablet from the capsule is assured by the hydration and subsequent swelling of a layer of low-substituted hydroxypropyl cellulose (L-HPC) positioned below the theophylline tablet. Since the erosion of the erodible tablet is independent of pH,[2–4] manipulation of the HPMC to lactose weight ratio controls the rate of erosion. This control affords

the potential for preprogramming of a specific lag time.

The objectives of this paper are threefold. Firstly, to fully describe the mechanism of drug release from a Time-Delayed Capsule *in vitro*, by using time-lapse photography (and riboflavin as a highly visible marker tablet). Secondly, to investigate whether the TDC could be used to target specific areas of the GI tract by an erosion controlled process, as indicated in a human study. And, finally, to evaluate and describe whether or not an *in vitro–in vivo* correlation is present, by examination of the data.

MATERIALS AND METHODS

Assembly of the TDC

The assembly of the TDC (Fig. 1) was completed in three steps: (a) L-HPC (200 mg) was filled into the coated capsule body and tapped lightly followed by a further 50 mg of L-HPC before lightly compressing to a uniform density using a 2 N force (200 g weight); (b) a theophylline or riboflavin containing tablet was placed on top of the compacted L-HPC; (c) the selected erodible tablet was inserted flush into the end of the capsule body, to seal the contents inside the capsule.

Preparation of Water Impermeable Capsule Body

Methods were employed as described previously.[2–4] Briefly, size 0 hard gelatin capsule bodies (Capsugel, Basel, Austria) were coated with a 5% (w/v) organic ethylcellulose (Ethocel®; 100 Premium grade) (a gift from The Dow Chemical Company, Midland, MI) solution [50:50 volume ratio of HiPerSolv™ grades of acetone and isopropanol (Sigma–Aldrich, Poole, UK)] using a Strea-1 coater (Aeromatic-Fielder, Bubendorf, Switzerland). The ethylcellulose solution was plasticised with 5% (w/w) USP grade triacetin (Merck-Chemicals Ltd, Poole, UK). To achieve internal and external coating and to ensure impermeability, coating was continued until a target weight gain of 120% was achieved; as predetermined by a capsule integrity study (data not shown).

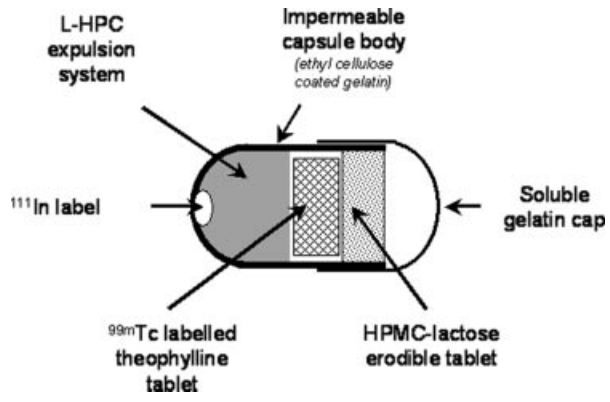

Figure 1. Assembly of the TDC used in the clinical study.

Manufacture of Riboflavin Tablet (Photographic Study)

Dicalcium phosphate (47.5 g) (a gift from Pfizer Ltd, Sandwich, Kent, UK) and riboflavin (2 g) (Sigma–Aldrich, Poole, UK) were mixed using a Turbula™ orbital mixer (Glen-Creston Instruments Ltd, Stanmore, UK) for 20 min at 42 rpm. Magnesium stearate (0.5 g) (Merck-Chemicals Ltd) was then added to the mixture before mixing in the Turbula for a further 10 min. The resultant direct compression mixture was then tableted with a 5 mm punch and die set (I Holland Ltd, Nottingham, UK) using a Manesty E2 tablet machine (BWI-Manesty Ltd, Liverpool, UK). A target tablet weight of 100 ± 2 mg and a crushing strength of 100 N were required (at this weight and strength the tablets were predetermined to have a disintegration time >300 min; data not shown).

Manufacture of Radiolabelled Theophylline Tablet

A theophylline mixture was prepared using USP grade theophylline (10 g) (BASF, Minden, Germany), lactose (0.49 g) (Fast-Flo®) and croscarmellose sodium (0.225 g) (Ac-Di-Sol®) (gifts from Pfizer Ltd, Sandwich, Kent, UK), and Aerosil®; R728 grade colloidal silica (0.0225 g) (Evonik Degussa Ltd, Milton Keynes, UK). The components were mixed using the Turbula for 20 min before adding magnesium stearate (0.1125 g) and mixing for a further 10 min. [^{99m}Tc]-DTPA-labelled lactose [prepared by drying [^{99m}Tc]-DTPA (Radiopharmacy, Western Infirmary, Glasgow, UK) onto lactose] was mixed *ad hoc* with 85 mg of the theophylline mixture before compression, to produce tablets labelled with 2MBq [^{99m}Tc]-DTPA at the time of dose administration (up to 24 h later). As a radioactive component was used an

isolated tablet compression procedure needed to be followed in compliance with the regulations of Bio-Images Research Ltd at the Glasgow Royal Infirmary. In this case, tableting was performed using a 5 mm punch and die set (I Holland Ltd), with a 1 ton compression force applied using a 3628 25-Ton IR Bench Press (SPEX Certiprep, Stanmore, UK). A target tablet weight of 90 ± 2 mg and a crushing strength of 75 N were required (predetermined to afford the tablets a disintegration time of less than 3 min; data not shown). The final tablet composition was: theophylline (87% w/w); [^{99m}Tc]-DTPA-labelled lactose (5.5% w/w); lactose (4.3% w/w); croscarmellose sodium (2% w/w); magnesium stearate (1% w/w); colloidal silica (0.2% w/w). Disintegration time of the theophylline tablet was determined to be identical to that obtained for the *in vitro* theophylline tablet formulation (data not shown). It should be noted that for the correct functioning of the TDC, the size theophylline tablet, corresponding to the tablet weight, had to remain constant despite the changes afforded by controlling the lag time (indicated later by changing the concentration of the HPMC containing tablet). It should also be noted that a considerably larger drug containing tablet could be used for any number of therapeutic end-points; it would mean that extensive *in vitro* dissolution characterization was undertaken for any change in the drug loading, but it is entirely feasible that a larger or smaller dose could be used.

Preparation of Erodible Tablet Formulations

In a healthy human adult it is generally accepted that gastric emptying times vary considerably, however it was assumed from previous literature that the stomach 'housekeeper wave' might be approximately 2 h following commencement of phase 1 of the migrating myoelectric complex of the stomach,[34] in a fasted state. As such, a formulation with an erosion time of 2 h was deemed necessary for the earliest release capsule. By some trial and error, a HPMC concentration of 20% (w/w) was found to correspond to a release time of 1.8 h *in vitro*. Due to the inherent variability associated with human studies, this erodible table composition was considered suitable for the earliest release time. At the opposite end of the spectrum a small intestine transit time was estimated to approximate to anywhere from 2 to 4 h, from information obtained in the literature.[35] As such an upper pulsed release time of 4 h was deemed suitable for the study; this corresponded to a HPMC concentration of 35% (w/w) for the erodible tablet formulation used. A mid-point formulation at 3 h was found to correspond to an HPMC concentration of 24% (w/w) in the erodible tablet component of the time-delayed capsule.

Formulations containing HPMC at 20, 24 or 35% (w/w) (Methocel®; K100LV grade) (a gift from The Dow Chemical Company, Midland, MI) with lactose at 79%, 75% or 64% (w/w) respectively were mixed in the Turbula for 20 min. Magnesium stearate at 1% (w/w) was then added to each blend before mixing for a further 10 min. The mixtures were then compressed (60 ± 2 mg) using a 6.75 mm punch and die set (I Holland Ltd) and the Manesty E2 tablet machine. For the erodible tablet used in the photographic study, a 15% (w/w) HPMC containing tablet was prepared (80 ± 2 mg), at this tablet weight previous studies had shown a performance *in vitro* having a lag-time of 1 h[2] (a 60 mg HPMC tablet was not used in the photographic study due to the smaller thickness of the riboflavin tablet, and this effect this might have on movement of the TDC internal components prior to use).

All tablets, for each stage of the study were compressed to give a 40 N crushing strength.

Radiolabelling of the TDC

For the radiolabelling steps during the TDC assembly, the [^{111}In]-DTPA label was incorporated into the inner surface of the base of the capsule to allow the position of the capsule to be followed through the gut. 0.25 MBq [^{111}In]-DTPA lactose [prepared by drying [^{111}In]-DTPA (Radiopharmacy, Western Infirmary, Glasgow, UK) onto lactose] was added to the base of the capsule prior to addition of the first aliquot of L-HPC. [^{99m}Tc]-DTPA was incorporated in the theophylline tablet to allow the time and site of expulsion and disintegration to be determined scintigraphically.

In Vitro Dissolution Studies

Dissolution tests were carried out on coated assembled TDC formulations weighted with stainless steel wire coils, using a USP II paddle type dissolution apparatus (model ST7) (Caleva GB Ltd, Sturminster Newton, UK) in 1 L deionised water set at 37°C with a 50 rpm paddle

speed. Samples were analysed at 5 min intervals using a Cecil 500 UV spectrophotometer at a wavelength of 273 nm (Cecil Instruments Ltd, Cambridge, UK), using a flow return setup. Excipients used in the various tablet formulations showed no interference at the specified wavelength (data not shown).

Tablet hardness was determined using a tablet testing station, model TBH30 (Erweka GmbH, Hausenstamm, Germany). Briefly, 10 tablets were placed into 10 compartments of the carousel unit in the test station. The carousel rotated to present each tablet sequentially to the crushing load cell, which subsequently crushed each tablet in turn. The individual measurements were recorded and an average hardness was determined.

Photographic Study

A TDC with a riboflavin tablet was immersed in deionised water and maintained at 37°C with stirring. Images were obtained using an RDC50 digital camera (Ricoh UK Ltd, Cambridge, UK) at 5 min intervals until the first signs of expulsion of the yellow tablet were observed. Images were then acquired manually in rapid succession until complete tablet expulsion had occurred from the impermeable capsule body.

Clinical Study Design

This was a single centre, randomised, three-way cross-over study. The study followed the tenets of the Declaration of Helsinki, was approved by the North Glasgow Universities NHS Trust Ethics Committee and the Administration of Radioactive Substance Advisory Committee and was conducted to Good Clinical Practice. Following a randomised table prepared for each study day, each subject received one TDC containing 20%, 24% or 35% HPMC in the erodible tablet component (from here on referred to as 20% TDC, 24% TDC and 35% TDC respectively). Each TDC contained 80 mg theophylline and 0.25MBq [^{111}In]-DTPA and 2.0MBq [^{99m}Tc]-DTPA. A washout period of seven days was adhered to between each dose, and the dose of administered theophylline was set below a normal therapeutic dose to minimise any risk of adverse effects associated with a narrow therapeutic window for this drug.

Study Population

Eight healthy male volunteers aged 25–35 years (mean $\pm$ SD 29.7 $\pm$ 4.5) participated in the study. All volunteers gave written informed consent and underwent a medical examination to ensure compliance with study criteria. Volunteers were required to be nonsmokers, with no history of GI tract disorders and taking no regular medication. The exclusion of smokers, and those with known GI tract disorders was designed to minimise the potential for theophylline absorption issues.

Study Day Procedure

Subjects fasted for 10 h before each study day. In order to facilitate alignment of gastric emptying, 30 min prior to dosing each volunteer received 200 mL apple juice and a crispbread with jam. It should be noted that alignment of gastric emptying by providing such a specific calorific intake was useful for the purposes outlined in this study: that is to investigate delivery from the TDC that are only reproducible under the highly controlled conditions outlined in these procedures. This study is not intended to translate directly to a patient setting, only merely to provide a controlled *in vivo* analysis model. On study days the TDC was given with 240 mL of water according to a randomisation schedule. All subjects were given a 310 calorie lunch (consisting of a cured ham roll) at 4 h postdose, and a 143 calorie snack (an individual yoghurt) at 7 h postdose. A 573 calorie evening meal (comprising lasagne and salad) at 10 h postdose was given to subjects who received the 35% TDC.

External radioactive markers were taped to the anterior and posterior abdomen to allow accurate alignment of sequential images. Following dosing, scintigraphic imaging was performed with the subject in a standing position using a Siemens E-Cam gamma camera (Siemens AG, Munich, Germany) fitted with a low-energy high-resolution collimator. Anterior and posterior static acquisitions of 30 s duration were collected immediately after dosing then every 15 min until dispersion of the ^{99m}Tc radiolabel was observed then hourly until the end of the study period. The study period was dependent on the formulation dosed which were 8 h for the 20% TDC, 10 h for the 24% TDC and 12 h for the 35% TDC.

Predose blood samples were taken 15 min before dosing. Following dosing, blood samples were taken according to the following schedules: (i) 20%

TDC—every 15 min to 3 h, then every 30 min for 1 h, then hourly until 8 h; (ii) 24% TDC—hourly until release was observed by scintigraphy, then every 15 min for 2 h, then every 30 min for 1 h, then hourly until 10 h; (iii) 35% TDC—hourly until release was observed by scintigraphy, then every 15 min for 2 h, then every 30 min for 1 h, then hourly until 12 h. A further blood sample was taken at 24 h postdose for each of the three formulations. Blood samples were placed in lithium-heparin tubes then centrifuged at 2000*g* for 10 min before the plasma fraction was removed for assay. The plasma fraction was stored in deep freeze conditions at −20°C, until the time of analysis.

Scintigraphic Data Analysis

Images were analysed using the WebLink® image analysis program. Each TDC gastric emptying time was determined by qualitative assessment of the scintigraphic images. Precise gastric emptying times could not be determined due to the gap between image acquisitions. The times presented represent the midpoint between the image at which gastric emptying was observed and the previous image.

The time of theophylline tablet disintegration was determined by spreading of the ^{99m}Tc radiolabel in the GI tract contents. Due to rapid disintegration of the theophylline tablet (2.8 min) and the imaging interval used, it was not possible to assess separation of the capsule and tablet.

Plasma Theophylline Analysis

For each plasma sample obtained, plasma (100 μL), internal standard solution (100 μL) of β-hydroxyethyl-theophylline (1 μg/mL) and 6% (v/v) perchloric acid (100 μL) were transferred into a 1.5 mL micro-centrifuge tube and vortex mixed for a few seconds. The tubes were then centrifuged at 4500*g* for 10 min. An aliquot (120 μL) of the supernatant was transferred to a 150 μL small volume insert of an HPLC vial. Samples were analysed using a HP1050 integrated HPLC system (Agilent Technologies Ltd, Stockport, UK) equipped with a Hypurity® C18 column, 150 mm × 3 mm (ID), 5 μm particle size (Thermo Hypersil, Runcorn, UK) and Securityguard® cartridge 4 mm × 3 mm (ID), 5 μm particle size (Phenomenex Ltd, Macclesfield, UK). A sample injection volume of 20 μL was used and the UV detection wavelength was set to 273 nm. The limit of quantification (LOQ) for theophylline was 0.1 μg/mL. Samples were analysed at ambient room temperature using a 10 mM sodium acetate buffer mobile phase (pH 4.1) at a 0.4 mL/min flow rate. Plasma data were fitted to a noncompartmental pharmacokinetic model using PC Modfit (G.D. Allen, Felsted, UK).

RESULTS

Photographic Study

The result of the photographic study is shown in Figure 2. At 5 min the surface appears roughened with expulsion of air from surface pores caused by hydration and swelling of the erodible tablet. At 10 min the erodible tablet has formed a smooth gel layer. By 15 min erosion of the surface of the erodible tablet is visible. By 40 min the internal riboflavin tablet is visible through the thinned erodible. At 46 min the riboflavin tablet is partially exposed in the upper left quadrant of the erodible tablet. Erosion continues (52 min 30 s) allowing fluid to enter the capsule and contact the L-HPC. At 54 min and 20 s the riboflavin tablet is partially expelled by expansion of the L-HPC. And the riboflavin tablet is completely expelled from the TDC at 54 min and 40 s together with a portion of the now swollen L-HPC.

The importance of an inner coating of ethylcellulose becomes clear at the 40 min time point. Flaws or weakness in the coat may lead to premature expulsion due to water migration through to the contents via the wall of the gelatin capsule. Remnants of the erodible tablet are still associated with the capsule body (lower right quadrant) after expulsion of the riboflavin tablet. This was consistent with earlier data which indicated that only 80% of the ET needed to erode before expulsion.[2] An encroaching 'tidemark' was also seen through the translucent coated capsule wall, initially present after 54 min and 20 s. This 'tidemark' became visible at the moment of ejection of the riboflavin tablet and so coincided with the wetting and swelling of the L-HPC. Even after final ejection the 'tidemark' appeared no further than half way down the length of the capsule body, suggesting an excess of L-HPC was present. Indeed following repeated dissolution experiments the L-HPC at the base of the capsules was observed to remain dry (this was important

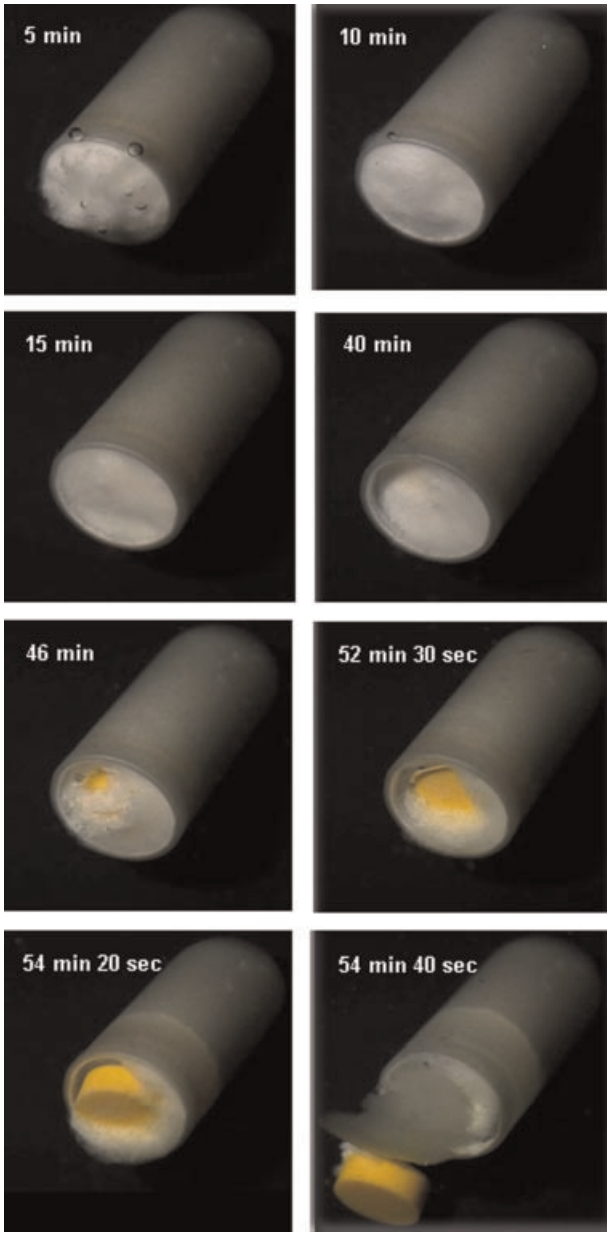

Figure 2. The process of pulsed release drug delivery from a TDC fitted with an 80 mg 15% hypromellose ET showing release of a riboflavin tablet.

when considering the placement of the ^{111}In-DTPA marker for the clinical study). During the entire process of expulsion the riboflavin tablet, which contained no disintegrant, showed no sign of disintegration.

Dissolution

The *in vitro* theophylline release times of the 20%, 24% and 35% TDCs were found to be 1.8 ± 0.3 h, 2.9 ± 0.3 h and 4.0 ± 0.3 h respectively. The value was determined at the $T_{50\%}$ release time. This value was used in the study as it had previously been shown that there was no significant difference *in vitro* for determining the pulsed release time at any point from the onset of drug release to the complete release (due to the rapid disintegration of the drug containing tablet).[2,4]

Scintigraphic Analysis

All subjects completed all three study arms. Representative scintigraphic images are shown in Figure 3 and individual gastric emptying times are shown in Tables 1–3. Gastric emptying times for 20, 24 and 35% TDCs were 1.4 ± 0.7 h, 1.4 ± 0.6 h and 1.4 ± 0.5 h respectively ($n = 8$ in all cases). The data were analysed using one-way ANOVA and no significant differences were found in gastric emptying times ($p > 0.05$).

No dispersion of the ^{99m}Tc-DTPA marker was observed in a total of six TDC doses. This was confirmed by failure to see the separation of the ^{99m}Tc-DTPA marker from the ^{111}In-DTPA marker, by observing the different photopeak energy dispersion images in each case. Two failures were seen with the 35% TDC (Subjects 005 and 008). Both subjects had relatively rapid colonic arrival times of 4.1 and 3.6 h respectively. Three doses in the 24% TDC group failed and of these, two again had relatively rapid colonic arrival times of 3.6 h (Subject 003) and 3.5 h (Subject 008). In Subject 004, the TDC remained stationary postgastric emptying for a considerable period with a prolonged SI transit time of greater than 9 h. One capsule failed from the 20% TDC group (Subject 006); in this case there was also a prolonged residence at the ileo-caecal junction (ICJ).

In Vitro–In Vivo Correlation

A correlation was shown between expected release time (from dissolution testing) and actual release time observed *in vivo* (Fig. 4), on those subjects where a release time could be assigned. An increase in HPMC concentration (as a component in each erodible tablet) was associated with a delayed ^{99m}Tc release time, and followed the same rank order as the *in vitro* dissolution study (indicated above). Mean scintigraphic release times were 2.7 ± 0.5 h ($n = 7$), 3.0 ± 0.4 h ($n = 5$) and 4.0 ± 0.7 h ($n = 6$) respectively. In those cases

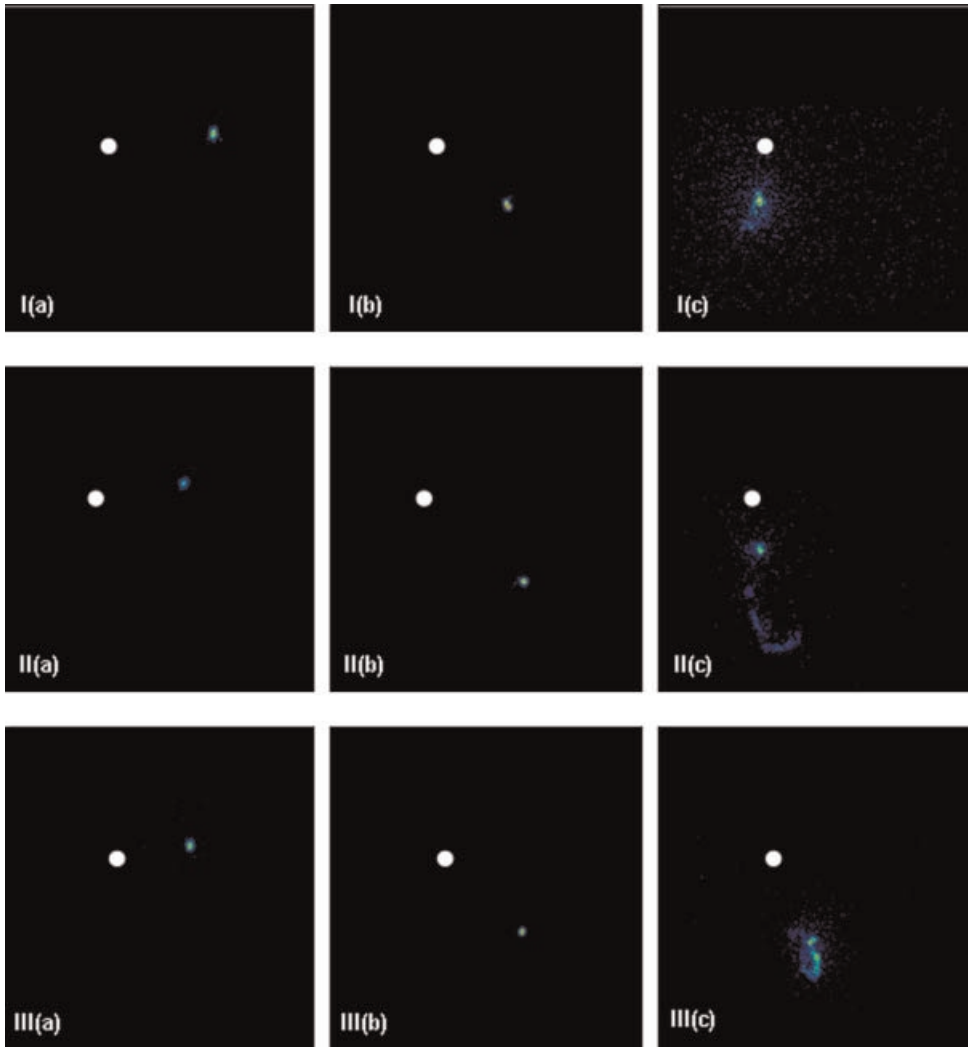

Figure 3. Representative scintiscans obtained for Subject 007 for (I) 20% TDC, (II) 24% TDC and (III) 35% TDC; [a] Pregastric emptying, [b] Postgastric emptying and [c] Release. A white circle represents the radioactive marker used for positioning sequential images.

where dispersion of the ^{99m}Tc-labelled theophylline tablet was observed, this occurred in the small intestine. One-way ANOVA revealed no significant differences between observed scintigraphic release-times of the 20% and 24% TDC ($p > 0.05$). However, a significant difference was observed between both these and the 35% TDC ($p < 0.05$ in both cases).

Pharmacokinetic Data

Pharmacokinetic parameters were calculated for study subjects (Tabs. 1–3), that had a quantifiable amount of theophylline in their plasma, and mean plasma profiles for subjects releasing in the small intestine are shown in Figure 5 (error bars were not included in the interests of clarity). Additionally as many time points were obtained for the 35% TDC formulation (due to the study design), points are omitted where fewer than 50% or less of the subjects had a quantifiable amount at a given time point.

The absorption of theophylline was rapid when released in the small intestine with T_{max} occurring within 1 h of first appearance of theophylline in the plasma. Mean observed T_{max} values were 4, 4.7 and 8.2 h for 20%, 24% and 35% TDCs respectively and the order correlated well with TDC HPMC content. Interestingly it can be seen in all cases that similar average pharmacokinetic profiles are observed (i.e., C_{max} and elimination

Table 1. Gastric Emptying Time, Scintigraphic Release and Pharmacokinetic Parameters for 20% TDC

Subject	Gastric Emptying Time (h)	Scintigraphic Release (h)	Site of Release	First Appearance in Plasma (h)	T_{max} (h)	C_{max} (μg/mL)	$AUC_{(0-24)}$ (μg · h/mL)
001	1.1	2.6	SI	2.8	4.0	1.37	14.3
002	2.9	3.4	SI	3.5	5.0	0.96	10.9
003	1.1	2.6	SI	2.5	2.8	1.38	18.1
004	0.9	2.6	SI	2.8	4.0	1.37	17.9
005	0.9	1.9	SI	2.0	3.0	1.02	9.9
006	1.6	NO	—	5.0	6.0	1.58	22.4
007	1.6	2.4	SI	2.8	3.0	1.46	14.2
008	0.9	3.4	SI	3.5	4.0	1.58	21.5
Mean	1.4	2.7		2.8[a]	4.0	1.3	16.2
SD	0.6	0.5		0.5[a]	1.1	0.2	4.6

NO, not observed; SI, small intestine.
[a]Calculated with observed scintigraphic release.

slope). This suggests that rapid absorption of the drug occurs once the TDC has been activated following the predetermined lag time *in vivo*. Additionally, a one-way ANOVA revealed no significant differences between AUC_{0-24} ($p > 0.05$) for the three formulations.

The pharmacokinetic data analysis failed to detect a quantifiable amount of theophylline for one subject in the 35% TDC group (Subject 005) and two subjects from the 24% TDC group (Subjects 003 and 004). The limit of quantification (LOQ) for theophylline was 0.1 μg/mL.

DISCUSSION

The *in vitro* analysis, and in particular the photographic study demonstrated the mechanism of drug release from the assembled TDC. Importantly, it was clearly observed that the erosion rate of the HPMC tablet was solely responsible for the commencement of the pulsatile drug release. Additionally, it could be seen that the depth of media penetration into the capsule following the drug release, and swelling of the expulsion excipient, was well above the site of the ^{111}In-DTPA marker. This was deemed a critical requirement for the successful incorporation of the two radio-labelled markers within the capsule so that adequate separation of the markers (at their respective photopeak energies) could be used to confirm drug release.

The TDCs were well tolerated and would be treated as undigested matter without any pharmacological effects on transit. In the fasted state, medium-sized intact tablets or capsules are typically retained in the stomach until they are

Table 2. Gastric Emptying Time, Scintigraphic Release and Pharmacokinetic Parameters for 24% TDC

Subject	Gastric Emptying Time (h)	Scintigraphic Release (h)	Site of Release	First Appearance in Plasma (h)	T_{max} (h)	C_{max} (μg/mL)	$AUC_{(0-24)}$ (μg · h/mL)
001	1.4	2.9	SI	3.0	3.0	2.09	15.0
002	1.9	2.6	SI	3.0	4.0	1.07	11.1
003	0.9	NO	—	<LOQ[a]	—	—	—
004	0.9	NO	—	<LOQ	—	—	—
005	1.1	2.9	SI	3.0	3.3	2.42	21.2
006	2.6	3.6	SI	4.0	4.5	1.60	18.5
007	1.1	3.1	SI	3.3	3.5	1.17	12.5
008	1.4	NO	—	6.0	10.0	0.59	7.4
Mean	1.4	3.0		3.3[b]	4.7	1.5	14.3
SD	0.6	0.4		0.4[b]	2.6	0.7	5.0

NO, not observed; SI, small intestine.
[a]Below limit of quantification.
[b]Calculated with observed scintigraphic release.

Table 3. Gastric Emptying time, Scintigraphic Release and Pharmacokinetic Parameters for 35% TDC

Subject	Gastric Emptying Time (h)	Scintigraphic Release (h)	Site of Release	First Appearance in Plasma (h)	T_{max} (h)	C_{max} (μg/mL)	$AUC_{(0–24)}$ (μg · h/mL)
001	1.1	4.1	SI	4.6	4.6	1.5	12.9
002	2.4	4.6	SI	5.0	7.0	1.3	13.6
003	0.9	3.1	SI	3.5	5.3	1.2	14.9
004	1.1	3.6	SI	4.0	4.3	1.5	15.6
005	0.9	NO	—	<LOQ[a]	—	—	—
006	1.6	5.1	SI	5.0	8.0	1.1	17.0
007	1.9	3.6	SI	4.0	4.3	1.5	12.7
008	1.4	NO	—	9.0	24.0	0.5	7.2
Mean	1.4	4.0		4.3[b]	8.2	1.2	13.4
SD	0.5	0.7		0.6[b]	7.1	0.4	3.1

NO, not observed; SI, small intestine.
[a]Below limit of quantification.
[b]Calculated with observed scintigraphic release.

removed by the 'housekeeper wave' of the stomach's migrating myoelectric complex (MMC) which occurs approximately every 2–3 h in fasted individuals.[36] The close grouping of the gastric emptying times in this study is notable and is attributable to the administration of a minimal calorific meal 15 min before dosing with the tablet. This had the desired effect of aligning the phases of the MMC for each subject.

It should be noted that this dosing schedule is useful for the purposes outlined in this study, that is, to compare formulations, but in clinical practice it may be difficult to accurately reproduce these exact dosing conditions. Due to the difficulty of reproducing the exact calorific requirements, it is not anticipated that the TDC in this present form, could be used outside of very specifically controlled clinical GI tract targeting studies. The TDC is designed to represent a departure from externally modulated GI tract delivery systems, and alignment of the gastric emptying cycle goes some way to achieving this, in a controlled clinical setting.

Postgastric emptying, the erosion of each the HPMC tablets should have been able to afford a specific lag-time to the TDC, as had been established *in vitro*. As it has been previously described, the transit time of the small intestine has been demonstrated to be more uniform than the emptying time of the stomach between individuals,[28] which has implications for regional targeted delivery of the TDC if gastric emptying is

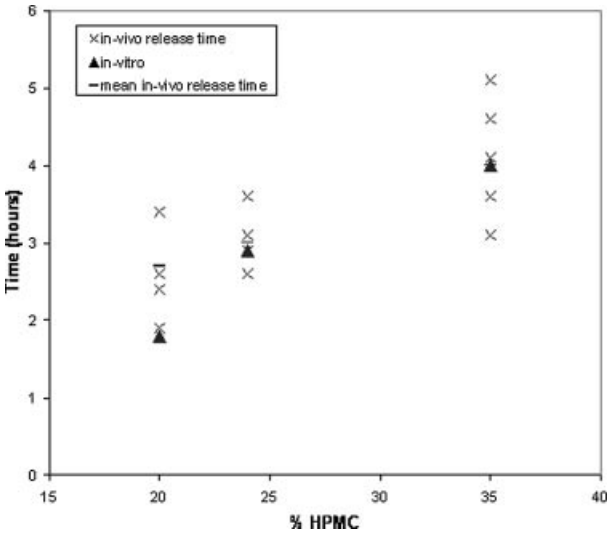

Figure 4. Correlation between *in vitro* and *in vivo* release times obtained by scintigraphic observation.

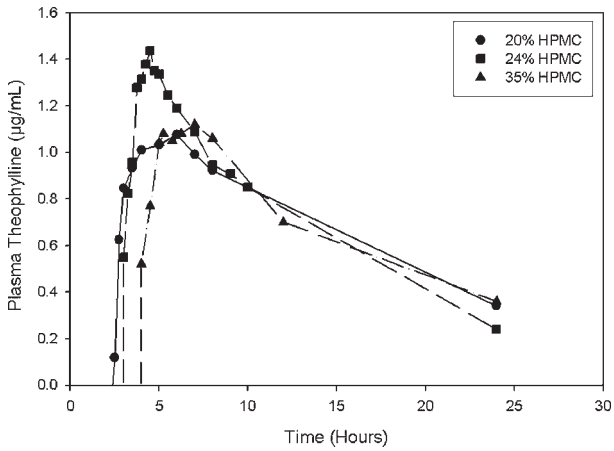

Figure 5. Mean plasma concentration time profiles.

uniform. Regional targeting would provide an improved therapeutic effect. Conditions such as irritable bowel disease (IBD): Crohn's disease or ulcerative colitis; and bowel cancer also have the potential to be treated in this way. Conventional therapies for the treatment of IBD rely on daily administration of high doses of drug. Crohn's disease is often treated with high dose anti-inflammatory agents,[37] and in the case of this corticosteroid treatment there is a high risk of dependency, relapse and the development of serious irreversible adverse effects.[38] Thus, localised treatment would also reduce the dosage level and subsequent side effects. Preferential absorption of drugs has also been demonstrated for different sites of the GI tract. For example leuprolide shows absorption variability throughout the GI tract in rats, with maximum absorption available in the colon.[39]

For Subjects 001, 002, 003, 004, 005 and 007 a good correlation in trend is observed between the scintigraphic separation and the time of theophylline first detection in the patient plasma. Table 1 shows that with the 20% TDC observed scintigraphic release typically can be seen at between 0.1 and 0.2 h prior to detection of plasma levels of drug. In Table 2, this observed release is seen at 0.1–0.4 h for the 24% TDC, and in Table 3 it seen to be at 0.4–0.5 h. Additionally, the mean times for the first observation of theophylline in the plasma (Tabs. 1–3), reflect differences in the onset of the pulsed release times *in vivo*, as indicated in Figure 5.

In vitro dissolution testing resulted in no observed failures for each of the TDC formulations; this was not the case *in vivo* as the scintigraphic analysis showed failure on six occasions. In three of these cases this was confirmed by the failure to detect theophylline in the plasma samples. However, in the remaining three cases, theophylline was detected in the plasma samples after dosing. The 20% TDC taken by Subject 006 showed a first appearance in blood plasma of 5 h with scintigraphic separation after 2 h. But, re-analysis of the scintigraphic images suggested that this observed scintigraphic release was in fact due to small movements of the capsule during the imaging period. Practically it is likely that separation of the two radiolabels occurred between long sampling times and was missed. Prolonged residence was observed at the site of the ICJ, and it is highly probable that this statis retarded dispersion of the radiolabel from the capsule.

In Subject 008, no distribution of ^{99m}Tc-DTPA was seen by scintigraphy for either the 24% or 35% TDCs. However, theophylline was detected in the plasma at 6 and 9 h in both cases respectively, when the capsules were observed to be in the ascending colon. Plasma profiles indicated prolonged absorption of theophylline in this subject. In fact comparable C_{max} levels of theophylline were observed in all subjects, where detection was preceded by observed scintigraphic dispersion of ^{99m}Tc-DTPA, except for Subject 008. It has been documented that the available fluid is generally less than 30 mL;[29] therefore it is probable that reduced motility and low water content in the colon were insufficient to disperse the drug content of the tablet, as well as contributing to poor swelling of the expulsion excipient in the TDC.

In earlier studies with Pulsincap™ (another type chronopharmaceutical capsule), protracted drug absorption has been attributed to low fluid levels which are inadequate to enter the capsule and cause swelling of the expulsion system and ejection of the drug.[24] Rapid colonic arrival would also certainly have resulted in compromised erosion of the ET (as it is so dependant on water). On reaching the transverse colon, the space is largely occupied by gas, and further into the distal colon the GI contents are too viscous to allow effective ejection of a drug containing tablet from the TDC. This prevents subsequent dissolution and transport to the gut wall of the drug, eliminating absorption. Increased peristalsis of the SI is largely propulsive and ineffective as the TDC possesses sufficient strength to resist over shorter periods of exposure to gut fluids. To improve the *in vitro–in vivo* correlation it has been suggested that dosage forms should be tested *in vitro* using a novel technique in low liquid surroundings,[40] when the dosage form is targeted to a site of administration where fluid volumes and agitation are reduced.

Overall, as 25% of the formulations were not observed to show scintigraphic release it is probable that some degree of this may be attributed to the study design setup. It may be possible to include more scintigraphic snapshots per patient over the course of the study so that separation of the two radio isotopes is not easily missed. In order to achieve this it could be appropriate to reduce the number of subjects included for each study day and extend the allotted time for the entire study. Another possibility is to increase the level of ^{99m}Tc-DTPA in the theophylline

containing tablet. This might have the effect of increasing the sensitivity of the gamma camera to any dispersion that might occur. Any such modification would first need to meet the approval of the ethics committee.

Taking into account the release times observed *in vivo* in this study, it is apparent that a degree of control is attained by varying the concentration of HPMC in the erodible tablet. At the highest concentration of 30% HPMC it is apparent that erosion of this tablet may run into difficulties later in the GI tract due to an overall reduction in moisture available. It is clear that the best control of delivery is imparted on the TDC in the upper GI tract which is moisture rich. In order to make this delivery system therapeutically significant for chronopharmaceutical therapy, further study is needed to improve and increase the lag window following dose administration.

CONCLUSIONS

The mechanism of drug release from the Time-Delayed Capsule was shown using a time-lapse photographic study, which corroborated the importance of an erodible HPMC tablet in controlling the onset of pulsed release *in vitro*. The site of drug release in the gastrointestinal tract was confirmed with the pharmacoscintigraphic study, and the potential to administer drugs to specific sites in the GI tract was observed under strictly controlled dosing conditions which would only be suitable for study purposes, and not easily reproducible for out-patient dosing situations. With adherence to a rigid study design, a good correlation was observed by comparing the average *in vitro* and *in vivo* data, with the same rank order of results being maintained throughout the HPMC concentrations studied.

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