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Scintigraphic evaluation of colon targeting pectin–HPMC tablets in healthy volunteers

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ABSTRACT

The *in vivo* evaluation of colon-targeting tablets was conducted in six healthy male volunteers. A pectin–hydroxypropyl methylcellulose coating was compressed onto core tablets labelled with 4 MBq ^{99m}Tc-DTPA. The tablets released in the colon in all subjects; three in the ascending colon (AC) and three in the transverse colon (TC). Tablets that released in the TC had reached the AC before or just after food (Group A). The other three tablets released immediately upon AC entry at least 1.5 h post-meal (Group B). Release onset for Group B was earlier than Group A (343 min vs 448 min). Group B tablets exhibited a clear residence period at the ileocaecal junction (ICJ) which was not observed in Group A. Prolonged residence at the ICJ is assumed to have increased hydration of the hydrogel layer surrounding the core tablet. Forces applied as the tablets progressed through the ICJ may have disrupted the hydrogel layer sufficiently to initiate radiolabel release. Conversely, Group A tablets moved rapidly through the AC to the TC, possibly minimising contact times with water pockets. Inadequate prior hydration of the hydrogel layer preventing access of pectinolytic enzymes and reduced fluid availability in the TC may have retarded tablet disintegration and radiolabel diffusion.

1. Introduction

Pectin is a ubiquitous polysaccharide found in the fruit of many edible plants, with high concentrations found in apples, quinces and oranges. Isolation from plant cell walls by stepwise processing methods produces pectins of various degrees of methoxylation (DM) (Ashford et al., 1994). The source of pectin also contributes to the type of pectin generated. Conventionally, the 50% DM value boundary divides pectin into high methoxy (HM) and low methoxy (LM) pectins (Jain et al., 2007). While the overall water solubility of pectins is relatively high, HM pectins are less water-soluble than their LM counterparts.

At acidic pH values, pectin exists as macromolecule aggregates but these aggregates dissociate and swell at neutral pH. Pectin is also resistant to proteolytic enzymes which are active in the upper gastrointestinal (GI) tract, whereas it is digested by the microflora of the colon (L. Liu et al., 2003). These properties make this polymer a suitable candidate to achieve colon-specific drug delivery. Delivery to the colon has been investigated for more than 20 years to achieve (a) sustained delivery that allows reduction in dosing frequency; (b) prevention of drug release until arrival in the colon for treatment of local diseases; (c) time-delayed delivery to coincide with periods of increased susceptibility to disease symptoms (chronotherapy); and historically, (d) delivery to a region of the gut which is more conducive to absorption of molecules which are prone to acid and enzymatic degradation (Wilson, 2000).

In order to capitalise on its characteristic selective degradation in the colon but not in the upper GI tract, the high solubility of pectin has been modified by the physical addition of other polymers such as ethylcellulose (Wakerly et al., 1996) as well as hydroxypropyl methylcellulose (HPMC) and chitosan (Macleod et al., 1999; Ofori-Kwakye et al., 2004). Addition of these polymers can also improve the compressibility of the tabletting mixture as pectin alone produces poor compacts (Kim and Fassihi, 1997a). Alternatively, chemical modification of pectin by amidation (Ahrabi et al., 2000) and calcium cross-linking (Adkin et al., 1997) also allows for manipulation of the solubility profile.

Pectin, as a physical admixture with HPMC alone, has been proposed as an excipient combination for a colon-specific delivery system (Turkoglu et al., 1999). This combination of pectin and HPMC has been used previously in a binary matrix tablet system that successfully produced zero-order release kinetics (Kim and Fassihi, 1997a,b). In the current study, this polymer mixture was

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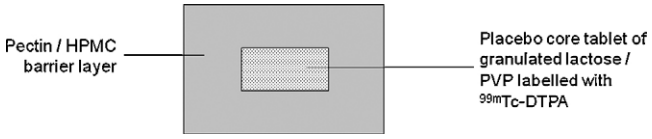

Fig. 1. Colon targeting drug delivery system.

compression coated onto a core tablet in order to delay release until colon arrival.

Fig. 1 shows a cross-section of the system under current investigation, constructed of a core tablet coated by a barrier layer of pectin and HPMC. This layer was formed by compression of a physical mixture of both polymers around the core tablet. Both are hydrophilic polymers which swell to form a hydrogel layer upon contact with aqueous media. The inclusion of high molecular weight HPMC increases the mechanical strength of the tablet, protecting the core tablet during transit in the GI tract and also partially modifies the high solubility of pectin.

In vitro tests were performed on the combination of pectin–HPMC in an 80:20% weight ratio compressed onto core tablets of 5-aminosalicylic acid (Turkoglu and Ugurlu, 2002) and nisin, a naturally occurring, ribosomally synthesised protein (Ugurlu et al., 2007). Tablets of this polymer composition ratio showed 25–35% erosion after 6 h in a pH change dissolution designed to reflect the pH environment of the GI tract. Addition of pectinase after 6 h increased the rate of tablet disintegration. Based on an average mouth–caecum transit time of 6–8 h (Washington et al., 2001), this *in vitro* observation indicated that delivery of drug to the colon was achievable with this system.

USP grade pectin was used in the tablet currently under investigation, which has a DM of approximately 70%. This grade of pectin, when tabletted as a mixture with HPMC is reported not to show any variability in gelation properties over pH 1–7.4 (Kim and Fassihi, 1997b). Lack of pH effect *in vitro* was also observed with the current tablet (Ugurlu et al., 2007). Significant increases of *in vitro* release rates were only observed when pectinase was added to the dissolution media, indicating that this system is more dependent on enzymatic activation rather than pH change for initiation of release.

Gamma scintigraphy, a non-invasive imaging technique, has been shown to be successful in determining the *in vivo* behaviour of various colon delivery systems. By incorporating small amounts of gamma-emitting radionuclides into the dosage forms, it is possible to establish the GI transit patterns of these systems within the body and determine site of disintegration and release. A common radioisotope used is technetium-99m (^{99m}Tc) which has a half-life of 6.03 h and a monoenergetic gamma emission of 140 keV. The behaviour of colon-targeting polysaccharide systems such as alginate gel beads (X. Liu et al., 2003), guar gum matrix tablets (Krishnaiah et al., 1998), enteric-coated tablets (Ibekwe et al., 2008) and xanthan/guar gum/starch matrix tablets (Sinha et al., 2005) has been successfully characterised *in vivo* using scintigraphic methods.

The radioisotope can be incorporated during the manufacturing process e.g. mixed in with the tablet granules prior to compression (Krishnaiah et al., 1998) or after complete manufacture of the film-coated tablet by 'drilling and filling' then re-sealing (Macleod et al., 1999; Ofori-Kwakye et al., 2004). However, if the manufacturing procedure prohibits the incorporation of the radiolabel at an early stage or the 'drill and fill' method is not suitable, there is the option of adding the isotopically enriched stable isotope of samarium-152 during manufacture which can then be converted to the gamma-emitting samarium-153 by neutron bombardment. This technique was used to investigate the *in vivo* behaviour of pectin-based tablets (Adkin et al., 1997) and enteric-coated HPMC capsules (Marvola et al., 2008) formulated for colon targeting purposes.

In this study, placebo systems consisting of a core tablet radiolabelled with technetium-99m-diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) and compression coated with 80:20% (w/w) pectin–HPMC were evaluated in healthy volunteers using gamma scintigraphy. Tracking of the tablets through the body enabled release parameters (sites and times of onset and complete release) to be determined.

2. Materials and methods

2.1. Materials

The materials used in the clinical study were of pharmacopoeial grade: polyvinylpyrrolidone (PVP) K30 was a gift from BASF, Germany; lactose (Flow Lac 100) was a gift from Meggle, Germany; stearic acid was a gift from H. Foster & Co. Ltd., UK; pectin (GENU pectin (citrus) type USP/100) was a gift from CP Kelco, Denmark; HPMC (Metolose 90SH–100000 cps) was a gift from Shin-Etsu Chemical Co. Ltd. (Japan). ^{99m}Tc-DTPA was supplied by the West of Scotland Radionuclide Dispensary, Glasgow, UK.

Tablets used for validation of the radiolabelling procedure were made from the same batches of excipients used for the clinical study. The following materials used for *in vitro* dissolution studies were of reagent grade: hydrochloric acid, potassium dihydrate orthophosphate and sodium hydroxide (all supplied by VWR International, UK).

2.2. Methods

2.2.1. Assembly of radiolabelled tablets

The core tablet mixture was prepared by granulation of PVP K30 and lactose with water. The wet granulate was dried in an oven for 2 h, then pressed through a 1-mm sieve. Resultant granules were dried for another hour. Stearic acid (1% weight of dried granules) was added and mixed. The radioactive marker, prepared by drying ^{99m}Tc-DTPA onto lactose, was added to the resultant mixture. This was followed by compression at 2 tons pressure into 100 mg tablets using a 6-mm punch and die set. For *in vitro* validation purposes, the tablets contained between 1 MBq and 1.5 MBq ^{99m}Tc-DTPA at the start of the testing period. In the clinical study, the tablets contained approximately 4 MBq ^{99m}Tc-DTPA at time of dosing.

The coating mixture was prepared by mixing pectin and HPMC in an 80:20% weight ratio in a Turbula mixer. The core tablet was centralised on a 200-mg bed of the coating mixture contained in a punch and die set prior to addition of a further 200 mg of the coating mixture, followed by compression at 2 tons pressure. The complete tablet was approximately 500 mg in weight and was of the following dimensions: 10 mm (diameter) × 5 mm (thickness).

2.2.2. Validation of radiolabelling process

Prior to the clinical study, an *in vitro* validation study was conducted to ensure that release of radiolabel from the tablet could be visualised and was consistent with non-radiolabelled batches.

Three radiolabelled tablets underwent dissolution testing in USP Apparatus II (50 rpm, 37 °C) placed in front of a gamma camera fitted with a low energy, high resolution (LEHR) collimator. The initial dissolution medium was 500 mL 0.1N hydrochloric acid. After 2 h, the medium was replaced with 500 mL pH 6.8 phosphate buffer.

Static acquisitions of 25 s each were taken every 15 min until the release of the radiolabel was complete and no core tablet could be visualised. The scintigraphic images were analysed to determine the times of onset and completion of radiolabel release.

2.2.3. Clinical scintigraphic study

2.2.3.1. Study design. This was a single centre, open-label, single dose study. The study followed the tenets of the Declaration of

Helsinki, was approved by the Glasgow Royal Infirmary Research Ethics Committee and the Administration of Radioactive Substance Advisory Committee and was conducted to International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines.

2.2.3.2. Study population. All screened volunteers gave written informed consent and underwent a medical examination within the 28 days prior to dosing to ensure compliance with study criteria. Six healthy male volunteers (age 24–47 years, body weight 60–90 kg, body mass index 20.0–28.1 kg/m^2) were recruited into the study, all of whom completed the study. Subjects had a follow up visit within 14 days after completion of the dosing visit.

2.2.3.3. Study day procedure. Subjects were instructed to fast from 10 pm the night prior to the study day. Fluids were allowed up to 6 am on the study day.

Upon arrival at the study centre, the subjects were questioned on compliance to the study restrictions. External radioactive markers (approximately 0.01 MBq ^{99m}Tc) were taped to the chest and back to enable accurate alignment of sequential images. The subjects were dosed with one ^{99m}Tc-DTPA-labelled tablet, swallowed with 240 mL water. The total radiation dosimetry for each subject was 0.1 mSv.

Scintigraphic imaging was performed with the subject in a standing position using a Siemens E-Cam gamma camera fitted with an LEHR collimator. At each time point, the subjects were positioned for 25 s acquisitions in the anterior followed by the posterior aspect. The imaging schedule was as follows: immediately after dosing, then every 15 min until complete release of the ^{99m}Tc radiolabel was confirmed, up to a maximum of 12 h post-dose. Adverse events were collected pre-dose, then hourly to 12 h post-dose (if applicable) and again just prior to the subject leaving the study centre.

All subjects received a standard lunch at 4 h post-dose, a snack at 7 h post-dose, and a standard dinner at 10 h post-dose if imaging was ongoing. Decaffeinated fluids were available *ad libitum* after lunch.

2.2.3.4. Data analysis. Images were analysed using the WebLink® image analysis program (Link Medical Ltd., UK). Time and site of onset and completion of radiolabel release were determined by qualitative assessment of the scintigraphic images by two independent trained personnel. The GI transit parameters of gastric emptying (GE) and arrival in the ascending and transverse colon (if applicable) were also determined in this manner. Transit times of the tablet core through the small intestine and ascending colon (if applicable) were calculated from the values of the transit parameters.

Small intestine (SI) and AC transit times are defined as the time differences between AC arrival time and gastric emptying time, and TC arrival time and AC arrival time, respectively. Similarly, length of residence at the ileocaecal junction (ICJ) is the time difference between AC arrival time and ICJ arrival time.

Table 1
Release parameters for *in vitro* dissolution studies. All values are shown in minutes.

	Tablet 1	Tablet 2	Tablet 3	Mean
Pre-onset time	375	435	450	
Post-onset time	390	450	465	
Onset time	383	442	458	428
Pre-completion time	436	480	495	
Post-completion time	450	495	510	
Completion time	443	488	503	478
Release time	60	46	45	50

3. Results and discussion

3.1. Validation of radiolabelling process

Inspection of the scintigraphic images showed that the radiolabelled tablets remained intact during the acid phase of the dissolution cycle. Onset of disintegration was clearly seen from the images with gradual release of the radiolabel observed as the tablets began to erode. Dispersion of the radiolabel throughout the dissolution media coincided with complete disintegration of the tablet. This confirmed that the release of radiolabel could be visualised scintigraphically and was representative of tablet disintegration.

From Table 1, radiolabel release was first observed 428 min into the dissolution period and complete release was noted after 478 min. From these values, the time taken for complete release after onset was calculated to be 50 min. This corresponded to the dissolution profiles obtained previously (Turkoglu and Ugurlu, 2002), confirming that the radiolabelling process did not affect the *in vitro* performance of the tablet.

3.2. Clinical scintigraphic study

The tablets arrived intact in the colon in all subjects; onset of release occurred in the ascending colon (AC) in three subjects and in the transverse colon (TC) in the other three subjects. This

Table 2
(a) Radiolabel release and (b) GI transit parameters for subjects whose tablets arrived in the colon after lunch. AC—ascending colon; ICJ—ileocaecal junction; SI—small intestine; NA—not applicable.

(a) Subject	Release of radiolabel				
	Site of onset of release	Site of complete release	Onset (min. post-dose)	Completion (min. post-dose)	Time Taken (min)
002	TC	488	TC	578	90
005	TC	458	TC	533	75
006	TC	398	TC	533	135
Mean	NA	448	NA	548	100

(b) Subject	Gastrointestinal transit				
	Gastric emptying (min. post-dose)	AC arrival (min. post-dose)	TC arrival (min. post-dose)	SI transit (min)	AC transit (min)
002	53	203	248	150	45
005	83	158	173	75	15
006	23	248	263	225	16
Mean	53	203	228	150	25

Table 3
(a) Radiolabel release and (b) GI transit parameters for subjects whose tablets arrived in the colon before or coinciding with lunch. AC—ascending colon; SI—small intestine; TC—transverse colon; NA—not applicable.

(a) Subject	Release of radiolabel				
	Site of onset of release	Onset (min. post-dose)	Site of complete release	Completion (min. post-dose)	Time Taken (min)
001	AC	338	AC/TC	503	165
003	AC	353	AC	443	90
004	AC	338	AC	443	105
Mean	NA	343	NA	463	120

(b) Subject	Gastrointestinal transit				
	Gastric emptying (min. post-dose)	ICJ arrival (min. post-dose)	AC arrival (min. post-dose)	SI transit (min)	ICJ residence (min)
001	53	248	338	285	90
003	83	278	353	270	75
004	143	278	338	195	60
Mean	93	268	343	250	75

demonstrated that the pectin–HPMC coating was sufficiently robust and successfully protected the core tablet from premature release in the upper GI tract.

Although this was a pilot study in only six subjects, it was possible to stratify the subjects based on the arrival time of the tablet in the ascending colon relative to the time lunch was consumed. In this study, subjects were fed a standard lunch at 240 min post-dose. AC arrival was recorded in Subjects 002 and 005 prior to food intake. In Subject 006, the tablet moved into the AC just after food. These three subjects were classified as one group (A) whereas Subjects 001, 003 and 004 in which colon arrival of the tablet occurred more than 1.5 h after lunch made up the second group (B).

Radiolabel release and GI transit parameters are shown in Tables 2 and 3, with subjects divided into the two subgroups described above. Precise times for transit and release of radiolabel could not be determined due to the intervals between acquisitions of images. The times presented in Tables 2 and 3 represent the mid-point between the image at which the event was observed and the

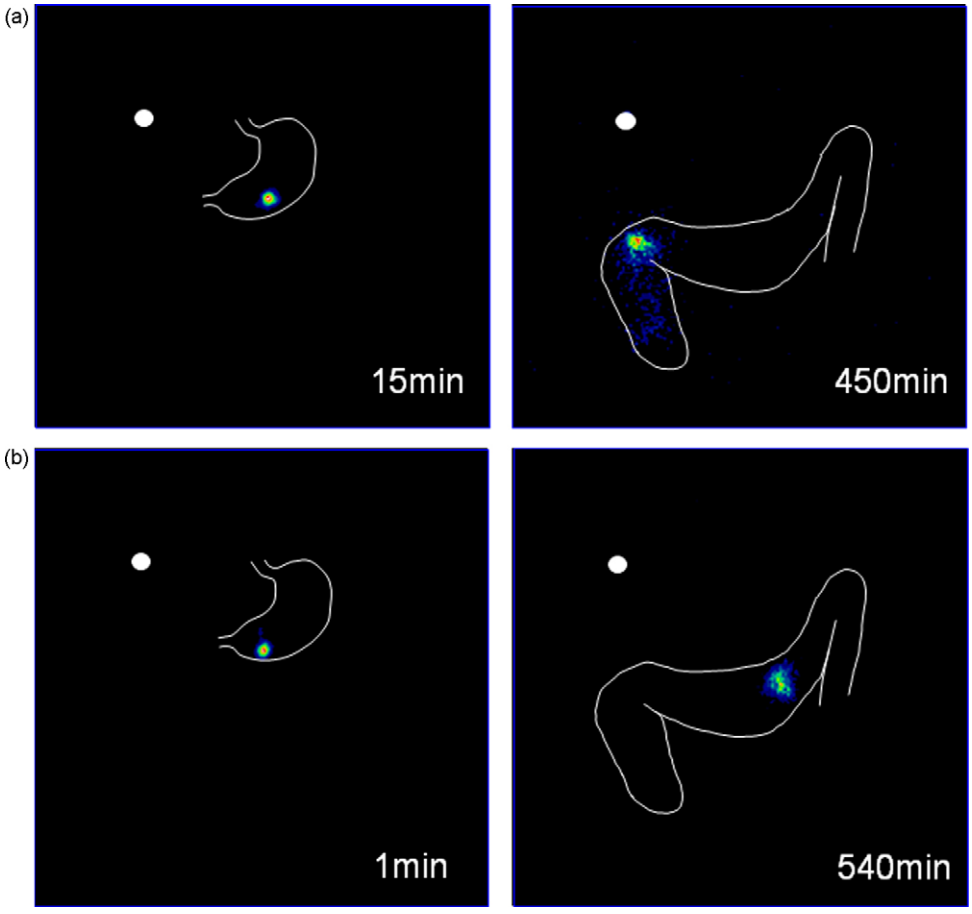

Fig. 2. Scintigraphic images of (a) Subject 003 and (b) Subject 002 showing: (i) intact tablet in the stomach and (ii) release in the ascending colon for Subject 003 and in the transverse colon for Subject 002. All times are shown in minutes post-dose.

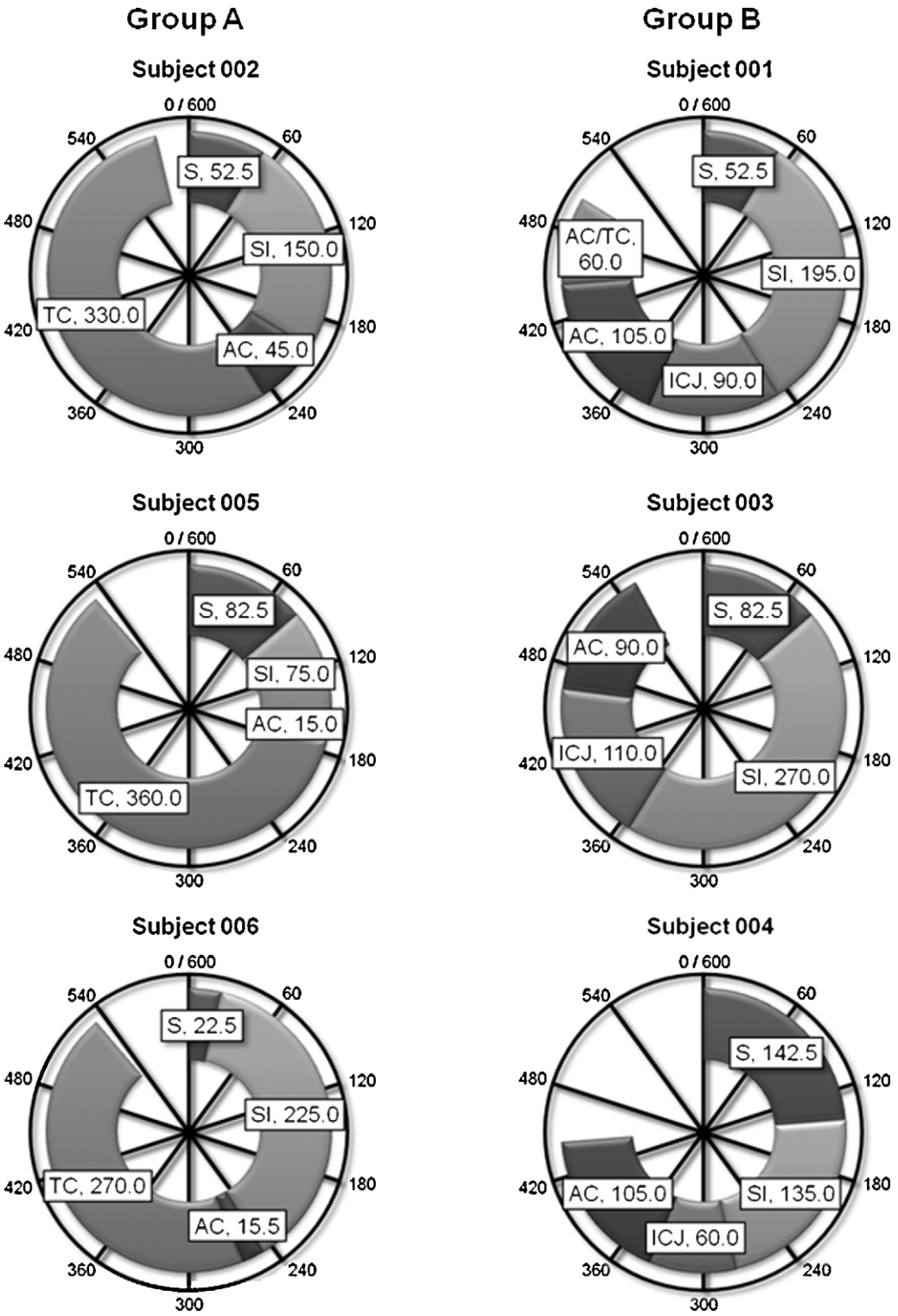

Fig. 3. Doughnut charts of residence times in GI regions for subjects whose tablets arrived in the ascending colon before or coinciding with lunch (Group A) and after lunch (Group B). The charts are overlaid on time wheels with time values shown in minutes. Values of residence time in minutes are noted. S—stomach; SI—small intestine; ICJ—ileocaecal junction; AC—ascending colon; TC—transverse colon. Tracking of the tablets were stopped once radiolabel release was complete.

previous image. Selected scintigraphic images of tablet release in the AC and TC are shown in Fig. 2. 'Doughnut' charts of residence times in the various GI tract regions for all subjects are shown in Fig. 3.

The mean gastric emptying times of the tablets were comparable between the groups, with 53 min and 93 min for Groups A and B respectively. The overall range was 23–143 min. This large variation in values may be attributed to dosing in the fasted state as the phases of the migrating myoelectric complex cycle (MMC) are not standardised. This increased the possibility of fortuitous emptying during a housekeeper wave in phase 3 of the MMC (Code and Martlett, 1975).

The tablet moved into the transverse colon within 8 min and 23 min of completion of the meal in Subjects 002 and 006

respectively. At meal time, the tablet was already in the transverse colon for Subject 005; it then moved further along the transverse colon after food. These observations may be attributed to the well-documented gastrocolonic response i.e. stimulation of colonic activity which initiates mass movement from the terminal ileum into the colon after meal consumption (Price et al., 1993; Kenyon et al., 1998).

For the remaining three subjects, food consumption moved the tablet further along the small intestine, into the region of the terminal ileum which is known to be a reservoir for undigested material (Camilleri et al., 1989; Spiller et al., 1987). Significant residence at the ileocaecal junction was noted for Subjects 001, 003 and 004 with residence times of 90 min, 75 min and 60 min respectively. This phenomenon was also observed by Adkin et al. (1993) where tablet stasis at the ICJ was more pronounced in subjects where the tablets had yet to reach the ICJ before lunch was consumed. No clear residence in the ICJ region was noted for subjects in Group A.

Residence in this region of relatively higher fluid content may have contributed to dilution of the hydrogel layer, reducing overall tablet integrity. A previous gamma scintigraphic study showed that a diluted HPMC gel layer in a controlled release matrix tablet could impair the ability of the tablet to withstand shearing forces within the GI tract (Ghimire et al., 2008). Progress through the ICJ would have subjected the tablet to high agitation forces which would result in the physical breakdown of the tablet hydrogel layer, exposing the core tablet. This initiated release of the radiolabel upon entry into the ascending colon. For these subjects in Group B, release onset was noted in the same image as ascending colon arrival; mean release onset time was 343 min. It is probable that the disruption of the protective hydrogel layer was mainly due to its aqueous dilution rather than action of pectinolytic enzymes.

For subjects in Group A, mean release onset time was 448 min post-dose. In the two subjects (002 and 005) where the tablet was resident in the colon before the meal, the tablets had resided in the TC for 240 min and 285 min prior to initiation of radiolabel release. For Subject 006 where AC arrival coincided with meal time, radiolabel release onset was noted 135 min after TC arrival.

Water availability in the transverse colon is relatively limited compared to that in the caecum, AC and even the descending colon, where pockets of water may be observed (Schiller et al., 2005). It is postulated that the rapid transit to the transverse colon in Subjects 002, 005 and 006 prevented adequate contact with water, thereby reducing the degree of hydration and gelling of the compression coat layer. It was observed that hydration of the polymer mixture *in vitro* was essential for enzyme activity (Ugurlu et al., 2007). Formation of a hydrated gel was crucial to enable inward diffusion of pectinolytic enzymes and subsequent breakdown of the protective pectin–HPMC gel structure.

The *in vitro* results predicted the *in vivo* behaviour of Group A more closely than that of Group B. Mean onset of release times were comparable; 428 min (*in vitro*) and 448 min (Group A). We hypothesise that the shorter mean onset time of 343 min for Group B could be attributed to the effect of high attrition forces acting upon the tablets during passage through the ileocaecal junction on an already weakened hydrogel layer. This reinforces the observation that a simple pH dissolution test does not mimic the hydrodynamic forces *in vivo* (Garbacz et al., 2008).

Although it is postulated that the integrity of the hydrogel layer in Group B was severely compromised by prolonged hydration in the terminal ileum and subsequent attrition forces on passage through the ICJ, the length of time taken for complete radiolabel release was comparable to that of Group A (100 min and 120 min for Groups A and B respectively). These values were more than double than that observed *in vitro* (50 min) despite the lack of pectinolytic enzymes in the *in vitro* dissolution media. While dissolution of the tablets *in vitro* was conducted in 'sink' conditions, this situation is far removed from the conditions in the body. The environment of low free water content in the colon would have prevented water ingress for dissolution and consequent diffusion of the radiolabel.

The two subgroups that were defined based on the post-meal response nicely illustrate the interplay between GI transit and tablet hydration/disintegration. The three subjects of Group A did not release until the tablet had moved into the TC. The lack of fluid within the TC may impede the release process and reduce bioavailability of the active, but if the matrix is sufficiently hydrated, dispersion will occur in this zone, aided by pectinolytic activity. The immediate release of the radiolabel upon colon entry for those subjects in Group B indicates that 20% (w/w) HPMC is sufficient to maintain barrier layer integrity until colon arrival.

4. Conclusion

The use of scintigraphic imaging provides a powerful tool in the assessment of formulation behaviour. It was fortuitous in this study that two distinct behaviours were observed and thus even in the case of small study numbers, valuable insights were obtained. There is an interesting interplay between position in the gut, the hydration of the matrix and the subsequent release pattern. The pectin–HPMC formulation absorbs sufficient water to eventually disperse. The role of the pectinolytic environment is not clearly defined in the situation although the result is satisfactory, with successful colon targeting in all six cases.

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