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Predicting the human *in vivo* performance of different oral capsule shell types using a novel *in vitro* dynamic gastric model

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ABSTRACT

The disintegration of a capsule shell may determine the onset of drug dissolution from capsule formulations. In this study, the release of a rapidly dissolving model drug (paracetamol), from two hydroxypropyl methylcellulose capsules containing either carageenan (HPMC-C) or gellan gum (HPMC-G) and one hard gelatin (HG) capsule, were investigated using a conventional *in vitro* model, the USP dissolution apparatus I, and a novel *in vitro* model of the human gastric compartment, the dynamic gastric model (DGM). The results obtained *in vitro* were compared with *in vivo* gamma scintigraphy human data and *in vivo* gastric emptying profiles available in the literature. The drug release from HPMC-G capsules, observed with the USP dissolution apparatus I, was delayed with respect to the other two capsules, while the results obtained from the DGM in the fasted state were closer together, which was in agreement with data from the *in vivo* studies. In the fasted state, the capsule rupture times obtained from the DGM were similar to those observed by gamma scintigraphy *in vivo* studies. In the fed state, the 'apparent' rupture times observed with the DGM were delayed compared to fasted, and were even longer than those observed by scintigraphy *in vivo* for HPMC-G and HG capsules. However, these discrepancies can reasonably be explained by considering the impact of food upon dispersion of the capsule contents and the sampling from the DGM, when compared to the human scintigraphy experiments.

1. Introduction

The quality of an oral pharmaceutical product and its potential human *in vivo* performance is typically characterised using pharmacopeial disintegration and dissolution testing *in vitro* (The United States Pharmacopeial Convention, 2008). The use of these methods to predict human *in vivo* performance is difficult because such tests do not adequately represent the human physiological conditions of the *in vivo* gastrointestinal processes in order to yield good *in vitro–in vivo* correlations (IVIVC) (Donauer and Löbenberg, 2007).

In the fasted state, slow or delayed disintegration of a dosage form may result in a slow drug release potentially influencing oral absorption rate. In the fed state, where the dosage form is retained in the gastric compartment because of the presence of food, a short delay in disintegration may not be as important since delivery to the duodenum, and therefore absorption, will be dependent on the rate of gastric emptying. *In vivo* disintegration times of solid dosage forms, as determined by gamma scintigraphy, tend to be longer than those generated using *in vitro* dissolution/disintegration techniques (Gao et al., 2007). Human *in vivo* performance driven by the variable and pulsating environment of the gastric hydrodynamics is unlikely to correlate well to the continuous, high agitation/shear intensity of the standard *in vitro* disintegration test. The lack of a universally applicable gastric simulation means that a wide variety of empirical dissolution/disintegration protocols are used to test drug release from capsules.

Recent developments in modelling the chemical, biochemical and physical characteristics of the gastric compartment have resulted in the development of a computer controlled, real time physical simulation of gastric processing. The dynamic gastric model (DGM), replicates the real time changes in pH, enzyme addition, shearing, mixing and retention time (Mercuri et al., 2011; Pitino et al., 2010; Vardakou et al., 2011). The model can be fed 'meals' ranging from a glass of water to a high fat FDA type breakfast

Abbreviations: HPMC-C, hydroxypropyl methylcellulose capsules with carageenan as gelling agent; HPMC-G, hydroxypropyl methylcellulose capsules with gellan gum as gelling agent; HG, hard gelatine capsules; DGM, dynamic gastric model; IVIVC's, *in vitro–in vivo* correlations; EPI, echo planar imaging; AGJ_{wp}, artificial gastric juice without pepsin.

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Table 1
The meal used to mimic the fed state was a modification of the high fat breakfast used by Digenis et al. (2000).

Food	Mass/volume	Protein (g)	Fat (g)	Carbohydrate (g)	Energy (kcal)
Eggs (2) scrambled[a]	100	10.7	22.6	0.6	247
Sausage, pork	75	9.7	13.5	11.2	202
Toast, white	40	3.7	0.6	22.8	106
Milk, semi-skimmed	150	5.0	2.4	7.5	70
Total	365	29	39	42.1	625

[a] Prepared as per McCance and Widdowson's – The Composition of Foods. 5th ed. (McCance and Widdowson's, 1991).

and deliver samples from the 'antrum' to the duodenum in the same form and at the same rate as observed *in vivo*. The data used to program the DGM were derived from echo-planar imaging (EPI) studies (Marciani et al., 2009, 2001) and from published references detailing physiological ranges for the rate of production of gastric secretions (Geigy Scientific Tables, 1981). The discrete samples delivered from the 'antrum' over time can be collected, analysed for the concentration of drug in solution and visually inspected by means of addition of a dye to the capsule. This model offers a realistic simulation of gastric processing and retention time and raises the possibility of deriving IVIVC and a clearer mechanistic understanding of the performance of oral formulations. The purpose of this preliminary study was to compare the data derived from the DGM, with that obtained by classical dissolution tests and that obtained from published scintigraphy studies. The study used capsules made from different materials filled with paracetamol, as an example of a soluble drug, the delivery of which is capsule integrity dependent.

2. Materials and methods

2.1. Materials

Size 1 capsules, Capsugel (Bornem, Belgium) were filled by hand with 200 mg of acetaminophen (paracetamol) and 10 mg of 3,3′-dioxo-2,2′-bis-indolyden-5,5′-disulfonic acid disodium salt (indigo carmine dye). The three types of capsules used were hydroxypropyl methylcellulose capsules with carageenan as gelling agent (HPMC-C), HPMC capsules with gellan gum as gelling agent (HPMC-G) and hard gelatin capsules (HG). For the DGM reagents: egg L-R-phosphatidylcholine (lecithin, grade 1, 99% purity) was obtained from lipid products (South Nutfield, Surrey U.K.); porcine gastric mucosa pepsin (activity 3300 units/mg of protein calculated using haemoglobin as substrate) was obtained from Sigma (Poole, Dorset, U.K.); gastric lipase analogue derived from *Rhizopus orizae* (Lipase F-AP15) was purchased from Amano Enzyme Inc. (Nagoya, Japan). All other salts and chemicals used were of analytical or HPLC grade and used as received.

2.2. Methods

2.2.1. Estimation of diffusion time of paracetamol in the DGM fasted and fed states

The content of one capsule was manually emptied on top of the meal (glass of water or high fat breakfast (Digenis et al., 2000), Table 1) at different time points from the start of digestion, e.g. at 0, 20, 40 and 60 min. For the fasted state the capsule content was only added at time 0, as the first sample delivered by the DGM, already showed the presence of the dye. In the case of the fed state, samples were collected at approximately 20 min intervals.

2.2.1.1. Analytical methods. Detection of paracetamol was performed by diluting 1 mL of the aqueous phase of the digesta [obtained after centrifugation of the sample at 3700 rpm, 20 °C for 15 min (BR4i Jouan, Thermo Scientific, UK)] with 1 mL ice cold HPLC grade methanol. Samples were vortex mixed and centrifuged at 3700 rpm, 15 °C for 20 min and the supernatant was taken for analysis.

The HPLC analysis was performed on a Waters 2695 separations module with degasser connected to a Waters 2487 dual absorbance UV–vis and 996 PDA detectors. Separation was performed on a Kromasil C-18 column (4.6 mm × 250 mm, Hichrom, UK) proceeded by a analytical security guard-column equipped with a security guard C-18 cartridge (4 mm × 3 mm, Phenomenex, UK). The column temperature was kept at 40 °C by an Igloo-Cil (CIL Cluzeau Info Labo, France) column heater and the samples' temperature was kept at 5 °C.

The mobile phase was a mixture of methanol/water (1/3 v/v), which was filtered through a 0.22 μm GS membrane (Millipore, UK) prior to use. The mobile phase was pumped isocratically at a flow rate of 1 mL/min. Absorbance was monitored at 254 nm, and the PDA (photo diode array) data were acquired from 210 to 400 nm. For each sample, 10 μL was injected and each run lasted 15 min. Retention time for paracetamol was approximately 4.9 min. Chromatograms were analysed using SigmaPlot 11.0 (built 11.2.0.5 Systat Software Inc. 2008) and the presence of paracetamol was identified from the PDA spectra.

2.2.1.2. Viscosity of food digesta. The viscosity of digesta samples obtained from the DGM at different time points was measured at 37 °C on a controlled stress rheometer (TA Instruments AR2000, Crawley, UK). Samples were vortex mixed to ensure homogeneity prior to measuring their viscosity. Two geometries were used depending if the sample was used untreated (i.e. containing solid undigested particles) or after centrifugation (i.e. aqueous phase). The viscosity of untreated samples was measured in the standard concentric cylinder system (stator inner radius: 15 mm; rotor outer radius: 12 mm; cylinder immersed height: 35 mm; gap 5000 μm) under conditions of constant rotation, applying a shear rate in the range between 1 and 500 s^{-1} for 2.30 min with a conditioning step at a shear rate of 100 s^{-1} for 2.00 min. For the centrifuged samples a cone geometry was used (acrylic cone diameter 60 mm; 0°, 59°, 21°; truncation gap 22 μm) applying a shear rate in the range between 1000 and 10 s^{-1} for 2.30 min. The aqueous layer was obtained after centrifugation of the whole sample at 3700 rpm for 30 min at 25 °C. Viscosity values for the untreated samples were estimated at 100 s^{-1}, since the samples showed a pseudoplastic behaviour, while the viscosity of the aqueous phase was obtained from the linear fitting of the data, since the samples showed a Newtonian behaviour. The shear rates were chosen in order to cover a wide range of viscosities reliably and thus to be within the range of shear forces found *in vivo* in the gastrointestinal tract.

2.2.1.3. Estimation of diffusion coefficient of paracetamol in the fed state. The diffusion coefficient of paracetamol in the untreated and centrifuged digesta of the food samples delivered by the DGM was estimated using the Einstein–Stokes equation:

$$D = \frac{kT}{6\pi\alpha\rho} \quad (1)$$

where k is Boltzmann's constant, T the temperature, α is the radius of the molecule, and ρ the viscosity of the medium.

The viscosity of a medium is temperature dependent and the viscosity of water can be estimated for a certain temperature by applying the following equation (Korson et al., 1969):

$$\rho_T = \rho_{20} + 10^{[(A(20-T)-B(T-20))/T+C]} \quad (2)$$

where ρ_T and ρ_{20} are the viscosities of water at the temperature T and 20 °C, respectively, and A, B and C are constants (A = 1.1709; B = 0.001827; and C = 89.93).

Therefore, since D is inversely proportional to the viscosity of the medium, at a constant temperature we can write the following relationship, which can be used to estimate the apparent diffusion coefficient of a drug in a certain matrix $D^{app}_{(food)}$:

$$D^{app}_{(food)} = \frac{\rho_{(H_2O)} D_{(H_2O)}}{\rho_{(food)}} \quad (3)$$

2.2.2. Fasted and fed states

For both the fasted (n = 6) and fed (n = 5) state, the clean DGM 'stomach' was brought to 37 °C and primed with 20 mL of gastric secretions (0.01 M hydrochloric acid and salts without gastric enzymes) to simulate the mean residual gastric fluid volume in the stomach.

For fasting conditions ultrapure water (200 mL) (NANOpure Diamond™, Barnstead International, Iowa, USA), at room temperature, was slowly poured into the main body of the DGM at the same time as the capsule was dropped in to simulate taking the formulation with a drink of water on an empty stomach. All the capsules initially floated within the simulated fundus.

The fed state was a simulation of taking the capsule after a high calorie, high fat breakfast (Center for Drug Evaluation and Research (CDER), 2002) the composition of which is described in Table 1. The meal was prepared, chewed to the point of swallowing and then spat out. All the components of the meal were chewed together, including the semi-skimmed milk to simulate drinking during the meal, and then placed in the main body of the DGM. Physically, the chewed meal was a roughly textured paste, which did not settle to leave an obvious aqueous or lipid layer on the top. The capsule was dropped to the main body of the 'stomach' and each time settled within the top 10 mm of the gastric contents.

2.2.2.1. Gastric digestion process. For the fasted state, the model was set up to process the gastric contents at a rate of 19 mL/min (Franke et al., 2004) giving a target value of 6 gastric samples of 38 mL over a total time of 12–13 min. For the fed state, the meal composition indicated that the total gastric residence time would be between 160 and 180 min, a gastric emptying rate of approximately 3 g/min based on fat content, which translates into a mean emptying rate of 3.4 kcal/min. Samples were collected every 20 min giving up to 10 samples depending upon the rate of additions of gastric secretions. All samples collected from the 'antrum' were visually inspected, for the presence of the dye marker, weighed and centrifuged at 10,000 rpm for 10 min and the supernatant assayed for concentration of paracetamol by HPLC. The concentration of paracetamol contained in each sample, as well as the cumulative amount released from each capsule, was calculated as a percentage of the label claim (200 mg/capsule).

2.2.3. Dissolution test

Experiments were performed (n = 4) using a USP I Dissolution Tester, Diss 8000, at 37 °C, with rotating basket at 50 rpm and a temperature controller, FH 16-D, both from Copley Scientific, Nottingham, UK. It has been reported that in order to simulate the fluid amount in the stomach 250–300 mL of dissolution media should be used (Vertzoni et al., 2005); however, in the case of the rotating basket the volume of dissolution media was set at 600 mL, in order to have complete immersion of the basket. Artificial gastric juice (AGJ_{wp}) without pepsin was used as dissolution medium (British Pharmacopoeia, 2008). Dissolution samples (7 mL) were collected with 10 mL syringes every 2–5 min. The sample volume drawn was replaced with the same volume of AGJ_{wp}, maintained at a constant temperature of 37 °C in a separate vessel placed in a water bath (Grant Instrument, Cambridge, UK). Each sample solution was filtered through a Minisart® NML surfactant-free cellulose acetate, 0.45 μm filter (Sartorius Stedim UK Limited, Epsom, UK) discarding the first 1 mL. The dissolution test samples were visually inspected for the presence of marker dye and assayed for dissolved paracetamol. The cumulative appearance of the dye and paracetamol released from each capsule was calculated as a percentage of the label claim (200 mg/capsule).

2.2.4. Analytical methods for quantification of capsules rupture times

The capsule rupture times for all three tests were visually assessed by the release of the indigo carmine dye. For the fed state, because of the 20 min separation of the samples, 'rupture' time was taken as the time of the midpoint between consecutive samples, the first showing no dye or paracetamol and the next where paracetamol or dye was detected. The choice of the midpoint time was based on the assumption that 'rupture' could have taken place at any time between these two time points. For the fasted state and dissolution tests, because of the absence of food, the dissolution of the capsules was followed by tracking the changes in optical density at 615 nm (Cecil Instruments Ltd., Cambridge, UK) as a result of the continuing dye release. In all the samples, paracetamol concentration was measured with an HPLC system (Agilent 1100 series) using a Waters Sunfire C18 (4.6 mm × 150 mm) analytical column. All the HPLC parameters were as before, but the detection wavelength was 243 nm. Paracetamol was quantified by peak area against a standard curve.

2.2.5. In vivo data

In vivo data of capsules rupture times in the fasted and fed state were taken from published human data obtained from gamma scintigraphy experiments performed by: Digenis et al. (2000), Tuleu et al. (2007), Cole et al. (2004) and Casey et al. (1976).

2.2.6. Data analysis and curve fitting

Unless otherwise stated the data are given as mean ± SD.

Both the dissolution and the delivery of paracetamol from the DGM produced S-shaped curves of concentration and mass delivered respectively against time. A logistic function (Eq. (4)) was therefore used to fit the data from the dissolution of paracetamol during the dissolution test and the delivery of dissolved paracetamol from the simulated fasted and fed gastric digestions with the DGM (Costa and Sousa Lobo, 2001).

$$\%\text{diss} = \frac{A}{[1 + e^{((\beta - t)/\gamma)}]} \quad (4)$$

where %diss is the percentage of drug dissolved at time t; A is the value of the horizontal asymptote as t approaches infinity ($t \to \infty$); β is the time at which %diss = A/2; and γ is the scale parameter on the time axis which represents the distance on the time axis between β and the point where the response is $A/(1 + e^{-1}) \approx 0.73$ A.

The capsule rupture times (T_R) were calculated as average between the time of the first sample containing 1% of drug and the time of the preceding sample:

$$T_R = \frac{t_{(p \geq 1\%)} + t_{(p < 1\%)}}{2} \quad (5)$$

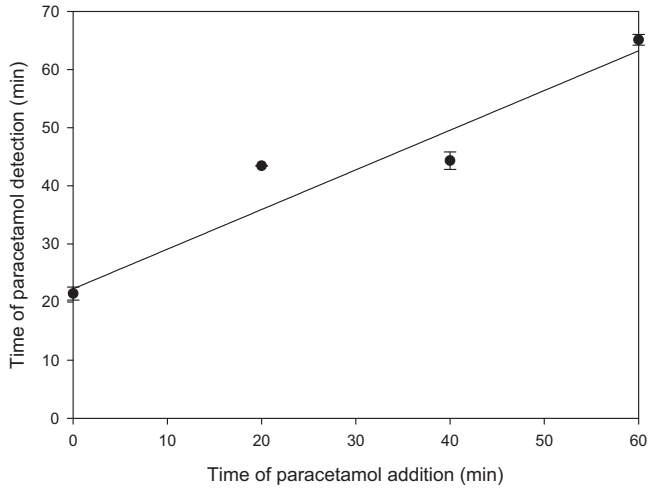

Fig. 1. Calibration graph showing the linear correlation between the time of paracetamol appearance within the DGM digesta and the time of paracetamol addition at the beginning or at specific time points during digestion.

where $t_{(p \geq 1\%)}$ and $t_{(p < 1\%)}$ are the times at which the amount of paracetamol measured is ≥1% or <1%, respectively.

Gastric emptying profiles of paracetamol in the fasted and fed states were obtained and fitted with a Lorenzian equation of the type:

$$y = \frac{H}{1 + ((x - x_c)/w)^2} \quad (6)$$

where H is the height of the centre, x_c the centre of the curve and w the width of the distribution.

3. Results

3.1. Estimation of diffusion time of paracetamol in the fasted and fed state

From the analysis of the digested samples collected from the DGM in the fasted and fed states it was found that the drug paracetamol could be detected in the first emptied sample from the DGM, in both states. Regarding the fasted state, the first sample was emptied after 3 min from beginning of digestion, indicating that the drug diffuses rapidly within the bulk aqueous phase contained within the DGM. In the fed state, dissolution and diffusion of the drug may be more difficult, as the viscosity of the bulk phase may slow the mobility of the drug molecules. Furthermore, during *in vivo* gastric digestion, the food undergoes phase separation within the stomach, as can be observed from the work of Marciani et al. (2007); this can add a further degree of complexity to the measurement of the diffusion of the drug within the bulk content of the stomach. However, the antral contraction simulated by the DGM may speed the diffusion of the drug, when compared to a static system. The addition of the capsule content at different time points from the beginning of digestion was done to estimate the length of time required for paracetamol to diffuse through the bulk meal if the capsule was ruptured at any of these different time points. The drug was detected as soon as the first sample was ejected from the DGM and by comparing the time values of paracetamol appearance against the times of paracetamol addition to the meal a linear relationship was found, as shown in Fig. 1. The linear fitting of the data (R^2 0.93) gave an intercept equal to 22.27 ± 3.130 min, which by subtracting the 20 min lag time used in this experiment, gives an estimated appearance time of 2.3 ± 3.1 min. This finding was confirmed by the analysis of a sample ejected after 5 min, which already contained traces of the drug (data not shown).

Table 2
Apparent diffusion coefficient of paracetamol in whole digested food matrixes and after centrifugation at two different times.

Food matrix	$D_{app}^{(food)}$ (cm^2 min^{-1})	
	Sample @ 20 min	Sample @ 40 min
Whole	1.000×10^{-6}	6.000×10^{-7}
Aqueous phase	2.480×10^{-4}	1.913×10^{-4}

It has been reported that the diffusion coefficient of paracetamol in buffer solution at pH 1.2 and 37 °C is 7.10×10^{-4} cm^2 min^{-1} (Grassi et al., 2003), while the water viscosity at the same temperature is 6.90×10^{-4} Pa s (calculated as from Eq. (2)). The viscosity of the samples digested by the DGM for the digesta at 20 and 40 min was very similar between time points (statistical analysis of the data did not show any significant difference) and were estimated to be in the range of 0.4825 ± 0.1557 Pa s and 0.7753 ± 0.2124 Pa s for the untreated samples and $1.98 \times 10^{-3} \pm 5.00 \times 10^{-4}$ Pa s and $2.57 \times 10^{-3} \pm 7.00 \times 10^{-4}$ Pa s for the centrifuged samples, respectively. From these data, the diffusion coefficients of paracetamol were calculated using Eq. (3) and the results for the untreated and centrifuged samples are shown in Table 2. From the apparent diffusion coefficient of paracetamol it can be estimated that the time for the drug to diffuse vertically through the DGM will require just less than one year and a half, in a static system. However, if we consider that during simulation of the fasted state in this work, paracetamol was found in the sample collected after 3 min, we can estimate that in a dynamic system like the DGM the time for paracetamol to diffuse through the bulk in the fed state can be calculated by applying:

$$t_{fed} = \frac{t_{fasted} D_{(H_2O)}}{D_{food}^{app}} \quad (7)$$

where t_{fed} and t_{fasted} are the times required for paracetamol to diffuse through the meal in the fed and fasted states, respectively. From Eq. (7) it can be estimated that the maximum diffusion time for paracetamol through the bulk food is no more than 9 min.

These experimental results, along with the theoretical estimation of the diffusion of paracetamol through the meal indicate that the appearance of paracetamol in the digesta emptied from the DGM can be used as an indicator of capsule rupture in a complex matrix.

3.2. Dissolution test

The HG and the HPMC-C capsules were seen to rupture after approximately 3.13 ± 0.48 and 6.50 ± 1.00 min, respectively. The HPMC-G capsules, however, stayed intact for much longer and the marker dye started leaking from the capsule around 18.0 ± 5.21 min after they were placed in the rotating basket. As seen in Fig. 2A the rate of paracetamol release from the HPMC-G capsules was very slow during the first hour of dissolution and only reached levels similar to the other two types of capsules after 70 min. The release of paracetamol from the capsules at 30 min for HG, HMPC-C and HMPC-G was of 27%, 19% and 1.8%, respectively. The HMPC-G capsule released paracetamol up to the end of the test (75 min), at which time only 15% had been dissolved.

3.3. Simulated fasted state digestion

The plots of cumulative paracetamol release (Fig. 2B) against time indicate that by the end of gastric emptying the HPMC-C and the HG capsules had released 70% and 73%, respectively of

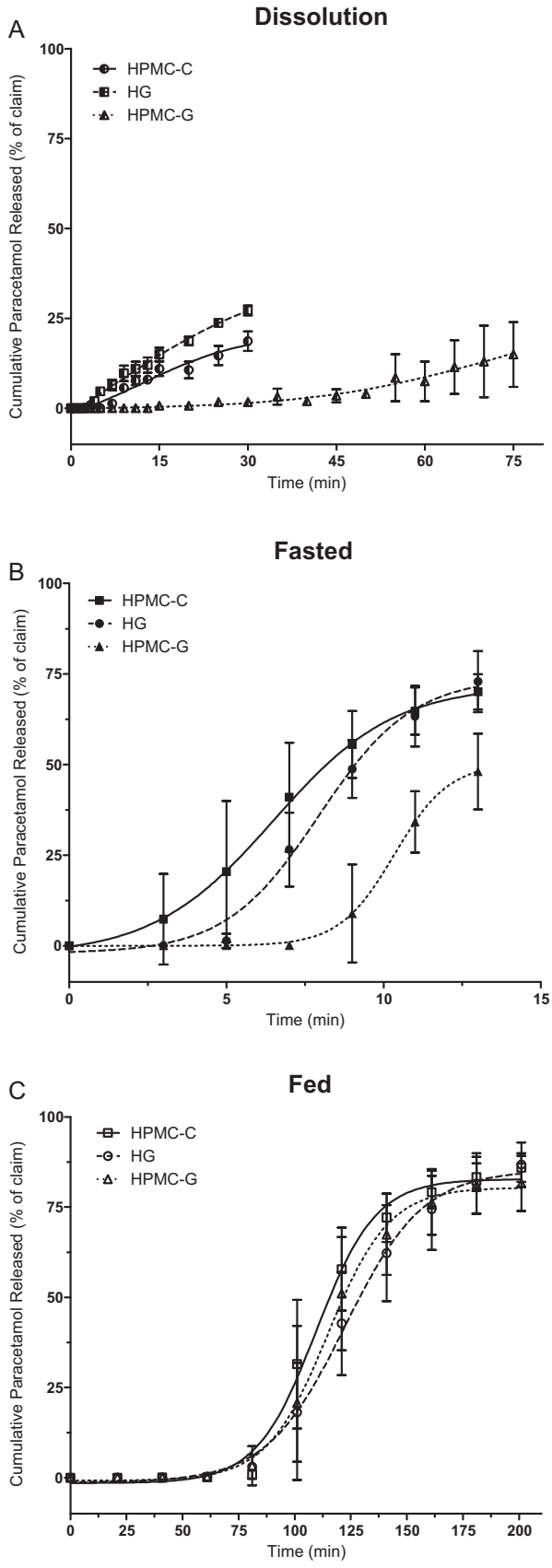

Fig. 2. Cumulative paracetamol % release (of label claim) from HPMC-C (■), HG (●) and HPMC-G (▲) capsules in: (A) USP I dissolution bath containing AJP_{wp}; (B) DGM fasted state and (C) DGM fed state. The Logistic function (Eq. (4)) was used to fit the data.

the total paracetamol, while the HPMC-G had released 48%. The rupture times of the capsules estimated from the analysis of paracetamol in the samples delivered from the antrum were 3.86 ± 1.84, 5.33 ± 1.03, and 9.33 ± 1.03 min for HPMC-C, HG, and HPMC-G capsules respectively (Table 3).

3.4. Simulated fed state digestion

The rupture times estimated from the first appearance of paracetamol were 85 ± 8.94, 75 ± 16.7 and 79 ± 11.0 min for HMPC-C, HG and HPMC-G, respectively, Table 3. HPMC-C, HG and HPMC-G capsules released on average 86%, 87% and 82% of their paracetamol content, respectively, Fig. 2C.

4. Discussion

There are a number of *in vivo* disintegration scintigraphic studies with instant release (IR) tablets (Gao et al., 2007; Kelly et al., 2003) in human volunteers reported in the literature, but it is IR capsules that have been studied in most detail, specifically in relation to: (a) the effect of cross-linking gelatin on long term storage of capsules and (b) the use of alternative shell materials such as hydroxypropyl methyl cellulose (HPMC).

In these studies it is capsule rupture time (when material first starts to emerge from the capsule) that is most frequently reported as it is considered easier to detect by gamma scintigraphy, although some reports also measure time to complete disintegration. Capsule rupture time may be difficult to assess if the capsule is embedded in the food bolus because there is virtually no bulk mixing of viscous gastric contents (Marciani et al., 2001) which is essential to the observation of capsule contents dispersal indicating the initial rupture time i.e. in the fed state, capsules may soften but may not actually rupture due to the lack of shear and even if they do, their contents may not disperse. Apparent rupture may therefore be dependant on a combination of softening rate and the level of shear to which capsules are subjected which in turn depends on the physical properties of the gastric contents. Rupture time *in vivo*, as determined by scintigraphy, will precede emptying of the drug from the stomach, which is analogous to the point of rupture that can be calculated using the DGM. The delay between capsule rupture and gastric emptying of capsule contents will be greater in the fed state than the fasted, due to the physical nature of food components.

Digenis et al. (2000) reported an *in vivo* study, which compared treated (exposed to formaldehyde to produce cross-linking that inhibits gelatin dissolution) and untreated amoxicillin filled gelatin capsules. The untreated capsules disintegrated rapidly *in vivo,* rupturing in about 7 min in the fasted state and 11 min in the fed state. If the capsules were treated so they become moderately cross-linked (but still passed a modified *in vitro* dissolution test where pepsin is added to the dissolution media) then *in vivo* rupture times became 22 and 23 min fasted and fed, and if treated to become highly cross-linked, rupture times were 31 and 71 min fasted and fed. Differences in the pharmacokinetic (PK) parameter, T_{max} could be seen even in the moderately cross-linked capsules, suggesting that ideally, *in vivo* rupture times of less than 20 min are a desirable target for IR capsules if disintegration is to be eliminated as a factor that might affect *in vivo* release, particularly if onset is important.

In a similar scintigraphy experiment by Brown et al. (1998), this time using paracetamol as the drug, the fasted state rupture times of untreated and moderately cross-linked gelatin capsules were found to be 8 and 10 min respectively. Tuleu et al. (2007) compared placebo capsules made from HPMC/carageenan and gelatin using scintigraphy. Capsule rupture times were similar for the two

Table 3
Summary of the capsule rupture times (mean ± SD) for all three (3) capsule types as measured from the dissolution apparatus (USP I), the DGM fasted and fed states, and *in vivo* scintigraphy human data.

Capsule type	*In vitro*			*In vivo*	
	USP I ± SD	DGM			
		Fasted ± SD	Fed ± SD	Fasted ± SD	Fed ± SD
HMPC-C	6.50 ± 1.00	3.86 ± 1.84	85.0 ± 8.94	9.00 ± 2.00[a]	na
HG	3.13 ± 0.48	5.33 ± 1.03	75.0 ± 16.7	7.80 ± 3.60[a] 7 ± 5[c] 30[d]	23.4 ± 21.6[b] 11 ± 7[c] 93–120[d]
HMPC-G	18.0 ± 5.2[e]	9.33 ± 1.03	79.0 ± 11.0	28.2 ± 10.2[b]	60.0 ± 22.2[b]

na – Not available.
[a] Mean disintegration time in minutes as assessed by gamma scintigraphy (Tuleu et al., 2007).
[b] Mean initial disintegration time in minutes as assessed by gamma scintigraphy (Cole et al., 2004).
[c] *In vivo* mean time of disintegration for unstressed capsules as assessed by gamma scintgraphy (Digenis et al., 2000).
[d] Initial release of non dissolving particles (Casey et al., 1976).
[e] The capsules were intact until they entered the 'antrum'.

types of capsule with mean disintegration times of 7 and 9 min for gelatin and HPMC-C capsules, respectively in the fasted state.

Cole et al. (2004), again using scintigraphy, compared HPMC/gellan gum and gelatin capsules filled with ibuprofen. The gellan gum added as a plasticizer is insoluble at low pH and would therefore be expected to delay capsule rupture in the fasted state where the gastric pH would be low. The fasted state rupture times were 8 and 28 min for gelatin and HPMC/gellan capsules, respectively confirming the role of gellan in promoting capsule integrity at low pH. However, for the fed state, where the gastric pH would be expected to be higher (and HMPC/gellan more susceptible to rupture) the rupture times were 23 and 60 min, respectively. Despite these differences, the PK parameters (AUC, C_{max} and T_{max}) for ibuprofen were statistically similar. As ibuprofen is a poorly soluble weak acid, most of the drug is unlikely to dissolve to any great extent until disintegrated particles reach the small intestine where the pH is closer to neutral, so irrespective of the gastric behaviour of the capsule, intestinal dissolution of the capsule contents is likely to be the dominant factor in controlling ibuprofen absorption.

Recently, Curatolo et al. (2011) found a direct relationship between the reduction in azithromycin bioavailability and slow capsule disintegration times in food component containing media. This finding was supported by the study of Casey et al. (1976) where, according to scintigraphy, the *in vivo* release of insoluble drug particles from gelatine capsules size 0 administered with water shifted from 30 min in the fasted state to 93–120 min in the fed state.

Peh and Yuen (1996) and Sunesen et al. (2005) used the appearance of paracetamol in the plasma as a indirect measure of gastric emptying. The lag times observed in these *in vivo* studies for the fasted state were no lag time (Peh and Yuen, 1996) or 14 ± 11 min (Sunesen et al., 2005), with the first blood sample taken at 30 and 15 min, respectively. In the fed state, the lag times measured varied between 36 ± 24 min (Peh and Yuen, 1996) and 44 ± 20 min (Sunesen et al., 2005), indicating, overall, a slower gastric emptying due to the presence of food.

In vitro prediction of capsule rupture times in the fasted state has been successfully achieved using a combination of biorelevant media and compendial dissolution apparatus with physiologically relevant hydrodynamic characteristics, thus to more closely mimic those found *in vivo* (Kalantzi et al., 2008).

Table 3 shows that in the DGM fasted state, the rupture times of all three types of capsules were in good agreement with *in vivo* scintigraphy data. When the results obtained from the USP dissolution apparatus I and the DGM fasted state are analysed, it can be observed that the DGM showed a similar rupture time for the HG and HPMC-C capsules, as observed *in vivo*, while the rupture time for HG capsules was shorter than HPMC-C capsules when the experiment was performed in the dissolution bath. However, in both *in vitro* systems, the HPMC-G capsule required longer times to rupture, and this trend is depicted in the box and whisker plots in Fig. 3A and B, for the USP dissolution bath I and the DGM, respectively.

In the fed state *in vivo* scintigraphy data there was a 3-fold increase in the rupture time of the HG capsule and a 2-fold increase in the HPMC-G capsule. Such fed state induced delays in capsule rupture would be very difficult to mimic in conventional dissolution media/apparatus. In the DGM, all three capsule types tested gave similar apparent rupture times (Table 3). A comparison of the rupture times assessed by the fed DGM is given in the box and whisker plot in Fig. 3C. In the fed state, the rupture times as detected by drug leaving the stomach compartment were significantly longer for the DGM compared to the *in vivo* scintigraphy rupture times. This difference may be the result of differences in the techniques used, as the samples were collected as they left the stomach compartment and were not sampled from the actual contents of the stomach compartment itself. The differences may also be attributed in part to the sampling procedure as samples were collected every 20 min on leaving the stomach, though the paracetamol diffusion within the food matrix was shown to be relatively fast. This would imply that rupture and subsequent dispersal/dissolution is primarily a shear and mixing related phenomenon dominated by antral processing rather than a feature of the main body of the stomach. In the DGM and *in vivo*, solid chewed food in the upper stomach is very poorly mixed because it is not subject to high shear. Therefore even if the capsules had initially ruptured there was not sufficient shear/mixing to cause significant dispersal of the contents. It may also be the case that scintigraphy in the fed state does not have the resolution to consistently observe leakage (capsule rupture) until the level of mixing produces a sufficiently diffuse image. Furthermore, from the *in vivo* studies previously described, it is evident that rupture time is highly variable in the fed state, as it can be seen for the different rupture times observed for HG capsules (Table 3).

Recent studies have shown that the *in vivo* performance of some dosage forms may be influenced by the presence of food or dietary components digestion. In relationship to the behaviour of capsules administered in the fed state, this is supported by the study performed by Curatolo et al. (2011), where the observed increased degradation of azithromycin was found to be likely due to interactions between the capsule shell and the food present within the stomach, which may alter the disintegration of the capsule. So it is possible that there could be some differences in the rate of hydration and softening of the capsule material between the fed and

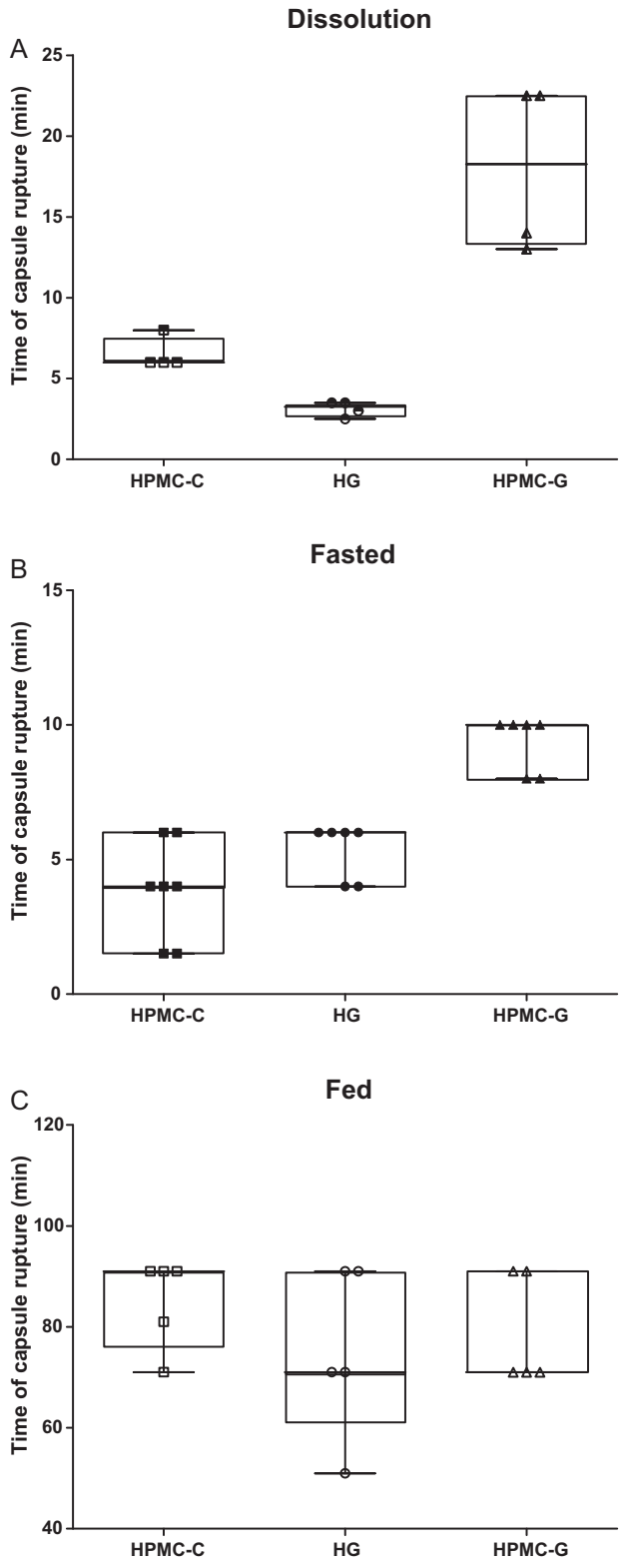

Fig. 3. Box and whisker plots of capsule rupture times determined using Eq. (5) (A) USP dissolution apparatus I containing AGJ_{wp}; (B) DGM fasted state and (C) DGM fed state.

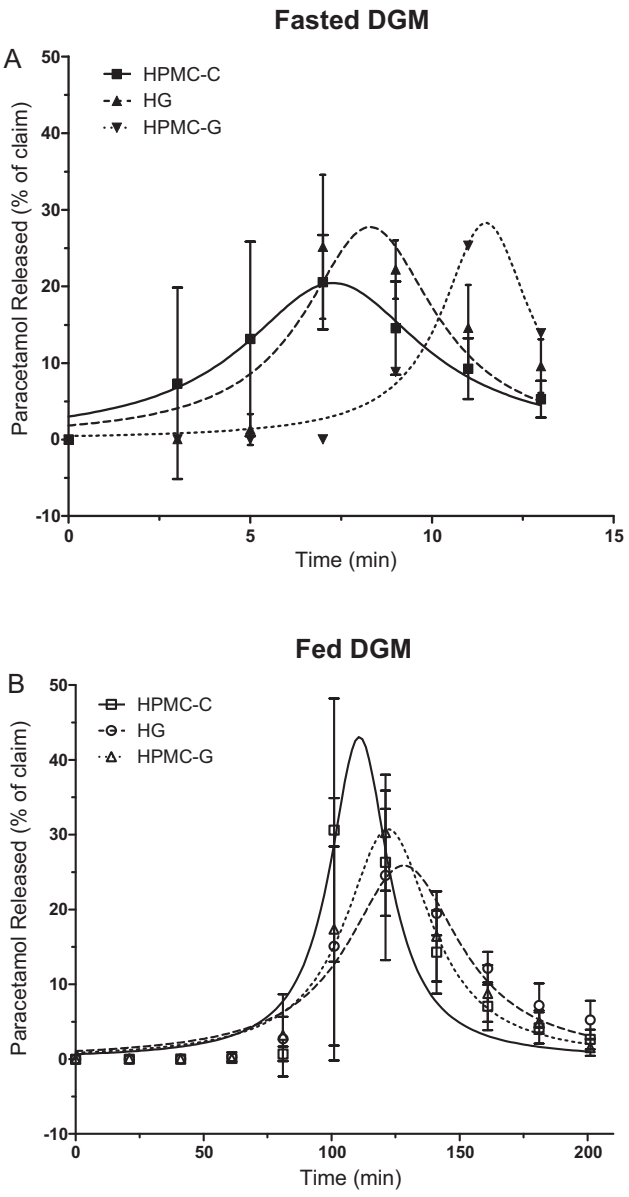

Fig. 4. Gastric emptying profiles of paracetamol from HPMC-C (■), HG (•) and HPMC-G (▲) capsules. (A) DGM fasted state; (B) DGM fed state. The Lorenzian function (Eq. (6)) was used to fit the paracetamol % release profile from the capsules.

fasted states. The differences in the results may be also influenced by the capsule size and quality between those used in this study and the *in vivo* studies.

If we consider the gastric emptying profiles of paracetamol in the fasted and fed states (Fig. 4A and B, respectively) and we compare them with the gastric emptying profiles of paracetamol observed *in vivo* using either pellets (Peh and Yuen, 1996) or tablets (Sunesen et al., 2005), we can notice a similar trend: rapid release of the drug in the fasted state and slower in the fed state. The gastric emptying times of paracetamol observed in this study were comparable with those observed *in vivo*, though the rate of release seems to be faster in this study for both fasted and fed states. This is not surprising and it should be expected as dissolution of paracetamol in this experiment will occur from a powder filled capsule after rupture of the shell, while in the case of pellets or tablets, the mechanism of dissolution will occur, after diffusion and disintegration respectively. However, whereas gastric emptying of paracetamol from the DGM was quite rapid in the fasted state, in the fed state it occurred later than the *in vivo* study.

The trend towards longer rupture times of capsules *in vivo* in the fed state compared to the fasted state, seen in the scintigraphy studies by Cole et al. (2004) and Digenis et al. (2000) are likely to be due to the low shear in the fundus. This is combined with

the inhibition of the dispersion of capsule contents due to embedding in a higher viscosity food matrix rather than differences in the hydration and softening rate. Although dissolution tests (high shear) may be a reliable method of monitoring drug release, the more complex systems seen in the DGM and *in vivo* in the fed state rely on hydration and dissolution of the capsule material and subsequent shear processes to rupture and disperse the contents. Thus, the dissolution test is far removed from the situation found in the fed state DGM and *in vivo*. This makes it very difficult to relate simple dissolution tests to *in vivo* performance in the fed state and ultimately to managing the temporal profile of disintegration and dissolution.

5. Conclusions

The fasted state rupture times of all the capsule types and gastric emptying profiles of paracetamol measured by the DGM were in agreement with those assessed by gamma scintigraphy and plasma profiling in humans.

As expected, in the fed state, rupture times were longer compared to the fasted state. The degree to which rupture times were extended, compared to the fasted state, as determined by the DGM, was compatible with the *in vivo* scintigraphy studies, but only once differences in the way samples from the DGM are taken versus the way scintigraphy is used to detect capsule rupture are accounted for. The general trend of results indicate that gastric content and thus residence time are the main controlling factor in determining the capsule behaviour and the delivery into the duodenum of the capsule contents in the fed state.

Overall, the DGM would appear to be a useful tool for the assessment of potential *in vivo* performance of different capsule formulations.

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