Characteristics of contents in the upper gastrointestinal lumen after a standard high-calorie high-fat meal and implications for the in vitro drug product performance testing conditions

Christina Pentafragka[1], Maria Vertzoni[1], Jennifer Dressman[2,3], Mira Symillides[1], Konstantinos Goumas[4], Christos Reppas[1]*

[1]Department of Pharmacy, National and Kapodistrian University of Athens, Zografou, Greece

[2]Goethe University, Frankfurt/Main, Germany

[3]Present address: Fraunhofer IME, Department of Translational Pharmacology and Medicine, Frankfurt/Main, Germany

[4]Department of Gastroenterology, Red Cross Hospital of Athens, Athens, Greece

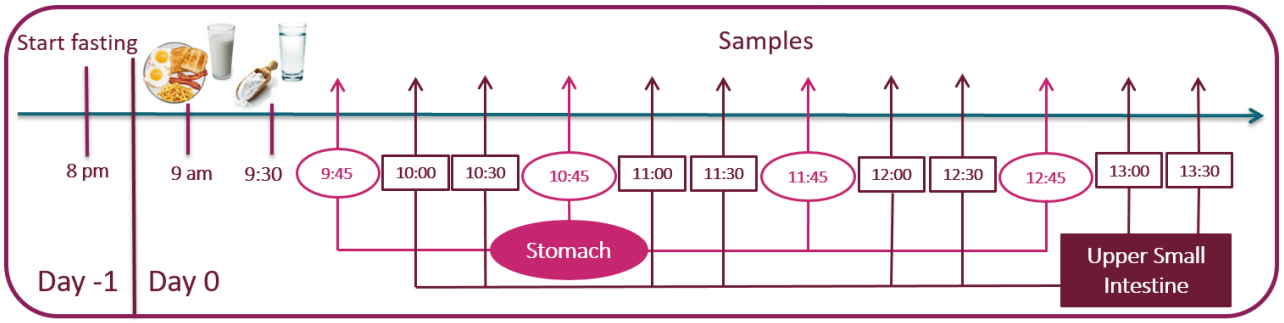
*To whom correspondence should be addressed:

Professor Christos Reppas, Department of Pharmacy, School of Health Sciences,

National and Kapodistrian University of Athens, Panepistimiopolis, 15784 Zografou, Greece

Tel. (+30) 210 727 4678 / Fax: (+30) 210 727 4027 / reppas@pharm.uoa.gr

Graphical Abstract



ABSTRACT

Objectives: To measure the pH, buffer capacity, lipid content, bile acid content, and viscosity in the upper gastrointestinal (GI) lumen after a standard high-calorie, high-fat meal as well as the osmolality, lipid content and bile acid content in the aqueous phase of the gastric contents and the micellar phase of the upper small intestine contents. To evaluate the implications of these findings for the composition of biorelevant media employed in *in vitro* oral drug product performance testing representing the upper GI conditions after ingestion of the standard meal.

Methods: Eight healthy male adult volunteers participated in a two-phase, crossover study in which a homogenized standard meal was administered to the antrum via the gastric port of a naso-gastro-intestinal tube. A glass of tap water and single paracetamol and danazol doses were administered to the antrum of the stomach 30 min after the initiation of meal administration (Pentafragka et al., 2020a). Samples were aspirated from the antrum and the upper small intestine over the next four hours. The pH and the buffer capacity of the samples were measured immediately upon aspiration, while viscosity, osmolality, and presence of solubilizing agents were measured after storage at -70°C.

Results: The composition of gastric contents over time fluctuated less after the homogenized standard meal than after liquid meals with similar composition. Intra-subject variability of pH and buffer capacity in the stomach and in the upper small intestine was low. Mean viscosity values in the stomach at $100 s^{-1}$ were 80-800 times higher than in the fasted state for more than 3 hours after the standard meal. In the upper small intestine, mean viscosity values at $100\ s^{-1}$ were at least 100 times higher than in the fasted state for 4h after the standard meal.

Conclusions: Based on data collected in this study, Level I and Level II biorelevant media simulating the intragastric conditions after ingestion of a standard meal could be simplified whereas FeSSIF-V2 composition was confirmed to be representative of the composition of the micellar phase of contents in the upper small intestine. Representative values of viscosity in the stomach and the upper small intestine and Level II composition of the aqueous phase of gastric contents, after the standard meal, are proposed for first time.

KEYWORDS

physicochemical characteristics, viscosity, micellar phase, upper gastrointestinal lumen, fed state, biorelevant media

1. INTRODUCTION

Oral drug product performance is typically evaluated in healthy adults both under fasted state and fed state conditions. Nevertheless, in addition to ethical issues, relevant studies are associated with high costs and lengthy procedures.

During the last two decades, substantial progress has been made in developing *in vitro* methodologies which are useful for decreasing the likelihood of failing a relevant *in vivo* study in the fasted e.g. (Butler et al., 2019) and, in certain cases, for waiving an *in vivo* study in the fasted state e.g. (EMA, 2020).

In vitro methodologies for the evaluation of oral drug products in the upper gastrointestinal (GI) lumen in the fed state have also been proposed and, in some cases, in combination with physiologically based pharmacokinetic modelling techniques, they have succeeded in reproducing the average plasma profile after drug product administration and/or understanding the mechanism(s) of previously observed food effects on oral drug absorption (Pentafragka et al., 2019). However, the simulation of luminal characteristics has been based predominantly on data collected after administration of liquid meals with similar caloric content and, in some cases, similar origin of calories to that of the standard high-calorie, high-fat test meal employed in *in vivo* studies in adults (standard meal), but not after the standard meal itself (Pentafragka et al., 2019). Both the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) request the standard meal to contain 800 to 1000 calories in total with approximately 150, 250, and 500-600 calories to derive from protein, carbohydrate, and fat, respectively (EMA 2010; U.S. FDA 2019). The standard meal example suggested by the regulatory authorities today consists of two eggs fried in butter, two strips of bacon, two slices of toast with butter, four ounces of hash brown potatoes and a glass of whole milk (EMA 2010; U.S. FDA 2019). Furthermore, in *in vitro* methodologies proposed to date, certain luminal characteristics which could potentially affect product performance and/or drug absorption, e.g. luminal viscosity and composition of the micellar phase in the upper small intestine have not been adequately addressed.

Recently, the concentrations of two model highly permeable drugs were quantified in the upper GI lumen of healthy adults after administration of the standard meal (Pentafragka et al., 2020a). In the present paper, we report on

- the characteristics of aspirates collected in that study, i.e. the pH, buffer capacity, lipid content, and bile acid content in the upper GI lumen; apart from the gastric pH data, relevant data in adults have been published after administration of liquid meals with similar caloric content and origin of calories similar to that of the standard meal, but not after the standard meal itself.
- the viscosity of contents in the stomach and in the upper small intestine; data in adults on the viscosity of contents in the GI lumen in the fed state have not been published
- the osmolality, the lipid content and the bile acid content of the aqueous phase of gastric contents and of the micellar phase of contents of the upper small intestine; relevant data in adults, after the standard meal, have not been published.

The model drugs employed in our recent study (Pentafragka et al., 2020a) , paracetamol and danazol, are non-ionisable at physiological pH values and are therefore expected to have minimal, if any, effects on the physicochemical characteristics of luminal contents (Litou et al., 2020). Thus, the implications of luminal characteristics reported in the present paper for the composition of biorelevant media to be employed in *in vitro* oral drug product performance testing are also discussed.

2. MATERIALS AND METHODS

2.1 Collection and treatment of aspirates

Aspirates were collected from eight healthy male adult volunteers (Pentafragka et al., 2020a). On two different occasions, each volunteer reported to the clinic in the morning after fasting for at least 12 h. The volunteer was intubated using a sterile naso-gastro-intestinal double lumen tube. The standard meal (containing, in addition, 100 mg phenol red as non-absorbable water flux indicator) was homogenized and then administered over a period of 15-20 min through the gastric port of the tube to the antrum using 60 mL-capacity syringes. A glass of water [with paracetamol and danazol (Pentafragka et al., 2020a)] was administered 30 min after initiation of meal administration. Samples (up to 18 mL) were aspirated from the antrum and from the upper small intestine at various times after water administration. Details on the inclusion/exclusion criteria, the regulatory approvals, the clinical study protocol, the composition of the standard meal, the impact of meal homogenization on meal's viscosity and size of particles, and the impact of the naso-gastro-intestinal tube on the GI transfer process have been reported (Pentafragka et al., 2020a).

The sample volume, pH, and buffer capacity were measured immediately upon each aspiration. After adding a cocktail of lipase/protease inhibitors (Hernell et al., 1990; Pentafragka et al., 2020a) the sample was divided in two sub-samples. The first was immediately centrifuged (11000 g, 37 °C, 10 min) to eliminate solid particles and subsequently ultracentrifuged (410174 g, 37 °C, 2 h) to obtain the aqueous phase (antral aspirates) or micellar phase (aspirates from the upper small intestine). It is known that samples aspirated from the upper small intestine in the fed state contain several co-existing colloidal species e.g. (Elvang et al., 2019). Ultracentrifugation of aspirates from the upper small intestine under the specific conditions applied in the present investigation leads to the formation of four phases, i.e. triglyceride phase, interphase, primarily micellar phase, and pellet (Hernell et al., 1990; Vertzoni et al., 2012). Even under the conditions applied in the present study collection of micellar-phase-only is not possible

(Müllertz et al., 2012). However, for simplicity and in accordance with previous articles (Vertzoni et al. 2012; Pentafragka et al. 2020) the term micellar phase is used in this article. The aqueous phases of gastric contents and the micellar phases of contents of the upper small intestine were stored at -70 °C for quantifying osmolality, lipid content and bile acid content. The second sub-sample was stored at -70 °C for quantifying lipid species, bile acids and viscosity. In all cases, to avoid multiple freeze-thaw cycles, the material to be stored was distributed to smaller vials prior to initial freezing.

2.2. Chemicals

All chemicals were of analytical grade and purchased from Sigma Aldrich Chemie GmbH or E. Merck (Germany), except for egg lecithin and mono-oleyl-rac-glycerol, which were kindly donated by Lipoid GmbH (Germany) and Danisco A/S (Denmark), respectively.

2.3. Analytical Methods

Buffer capacity of each sample aspirated from the antrum and from the upper small intestine was assessed by titrating with 0.1N NaOH and 0.1N HCl, respectively (Kalantzi et al., 2006a).

Triglycerides (TGs), diglycerides (DGs), monoglycerides (MGs), fatty acids (FAs), phosphatidylcholine (PC), lyso-phosphatidylcholine (lyso-PC), and cholesterol (CHO) in each aspirated sample, the aqueous phase of each sample aspirated from the stomach, and the micellar phase of each sample aspirated from the upper small intestine were quantified as described previously (Diakidou et al., 2009). Glyceryl trioleate and glyceryl trilinoleate were used to quantify TGs, 1,2 dioleoyl-rac-glycerol and dipalmitin were used to quantify DGs, and 1-monooleyl-rac-glycerol was used to quantify MGs. Oleic acid, linoleic acid, and stearic acid were used to quantify FAs.

Taurocholic acid (TC), glycocholic acid (GC), taurochenodeoxycholic acid (TCDC), ursodeoxycholic acid (UDC), glycochenodeoxycholic acid (GCDC), and glycodeoxycholic acid (GDC) in each sample aspirated from the upper small intestine and its micellar phase were quantified as described previously (Vertzoni et al., 2008).

The viscosity of each aspirated sample was measured in triplicate (37^{o}C) with a cone and plate rotational viscometer (RM 100 CP 2000 PLUS, LAMY Rheology, France) at 50 s^{-1}, 100 s^{-1} and 200 s^{-1}.

Osmolality of the aqueous phase of gastric aspirates and of the micellar phase of samples from the upper small intestine was measured using the freezing point depression technique (semi-micro osmometer Typ Dig L; Knauer, Berlin, Germany), after subtracting the osmolality of the lipase/protease inhibitors cocktail (427 mOsm/kg).

2.4. Data treatment

Since the meal was administered to each volunteer twice, at two different occasions, individual data were summarized as mean (SD) values or as Box-Whisker plots by employing up to sixteen individual data points. Each Box-Whisker plot shows the 10th, 25th, 75th, and 90th percentiles, the individual outlying data points, the mean value (dotted line in the box) and the median value (solid line in the box).

Individual differences between the first and the second meal administration enabled evaluation of the intra-subject variation of pH and buffer capacity of contents in the stomach and in the upper small intestine, after the standard meal. Other characteristics of luminal contents investigated in this study were highly variable.

3. RESULTS AND DISCUSSION

3.1. Antral contents

3.1.1. pH and buffer capacity

The median pH values at 15, 75, 135 and 195 min, were 2.9, 3.1, 2.9, and 1.9 (Figure 1), similar to the median pH values observed previously at 45, 105, 165 and 225 min after initiation of consumption of the standard meal by Dressman et al. and Koziolek et al., i.e. pH 3.3, 2.3, 2.3, 1.8 and pH 3.1, 2.5, 1.3, and 1.0, respectively (Dressman et al., 1990; Koziolek et al., 2015). The data are also in line with previous data showing that the pH of gastric contents after a solid meal is not affected significantly by prior homogenization of the meal (Dressman et al., 1990; Malagelada et al., 1979).

During the first 4 hours after initiation of meal administration, the average buffer capacity of antral contents remained practically unchanged at approximately 20 mmol/mL/ΔpH. Mean buffer capacity increased over time from 11.9 to 27.5 mmol/mL/ΔpH (Figure 1). There are no published data on buffer capacity after administration of the standard meal. Previously collected data, after administration of a homogenized liquid meal with different caloric breakdown but similar caloric content of the standard meal are similar to the data in this study (Kalantzi et al., 2006a).

At each aspiration time point, the median difference between the pH after the second administration and the pH after the first administration in a given individual ranged from -0.3 to 0.9 pH units. At each aspiration time point, the mean difference between the buffer capacity after the second administration and the buffer capacity after the first administration in a given individual ranged from -3.4% to 15%. These data suggest low intra-subject variability of pH and buffer capacity of gastric contents after administration of the homogenized standard meal.

3.1.2. Lipid species

Concentrations of lipid species in gastric aspirates over time after water administration revealed high inter-subject variability (Table 1) and high intra-subject variability (data not shown). Based on mean values, TGs, DGs, FAs and PC were comparatively the most abundant species for more than 3 hours after water administration (Table 1), in line with previous data suggesting that intragastric lipolysis of TGs mainly generates DGs and FAs (Amara et al., 2019).

Previously, only data after administration of a heterogeneous liquid meal with composition and origin of calories similar to those of the standard meal have been published (Armand et al., 1996). However, in addition to differences in composition, differences in meal texture can also affect concentrations of lipid species in the stomach. For example, gastric lipase output is higher after administration of liquid meals (Amara et al., 2019; Koziolek et al., 2018). It has also been shown that TGs in an emulsified meal are more accessible to lipase than in a minced solid meal, such that intragastric TG lipolysis is approximately 25% and 10%, respectively (Carrière et al., 2001).

3.1.3. Viscosity

Based on data collected at 50, 100 and 200 s^{-1}, intragastric viscosity is highly variable and aspirated samples are pseudoplastic, i.e. viscosity is higher at lower shear rates (Figure 2). Mean values in the stomach at 100 s^{-1} are lower than the viscosity values of the homogenized meal [1582(166) mPa·s at 100 s^{-1} and 37 ^{o}C (Pentafragka et al., 2020a)] but they are 80-800 times higher than the viscosity values of gastric contents in the fasted state [1.4-6.4 mPa·s at 100 s^{-1} (Pedersen et al., 2013)].

3.1.4. Characteristics of the aqueous phase

At all aspiration time points, osmolality data and concentrations of lipid species revealed high inter-subject variability (Table 2) and high intra-subject variability (data not shown).

Osmolality of a limited number of aspirated samples was found to be similar to the osmolality of the respective aqueous phases (data not shown). The reduced intragastric osmolality, compared to the osmolality of the aqueous phase of the meal (423 mOsm/kg), suggests significant amounts of gastric secretions in response to the administration of the (hyperosmotic) standard meal (Pentafragka et al., 2020a). Previously, only data after a homogenous liquid meal with different caloric breakdown but similar caloric content with the standard meal have been published (Kalantzi et al., 2006a), in which higher intragastric osmolality was reported than in the data collected in the present study. Since prior homogenization of a solid meal does not affect intragastric osmolality values (Malagelada et al., 1979), the difference is attributed to differences in texture and composition between the standard and the liquid meal.

Compared to the data in total gastric contents, mean concentrations of lipid species in the aqueous phase of antral contents were at least ten times lower (Table 2 vs. Table 1). As in total antral contents, FAs and PC were the most abundant lipid species for more than 3 hours after water administration (Table 2).

3.2. Contents of upper small intestine

3.2.1. pH and buffer capacity

Median pH values at 30, 60, 90, 120, 150, 180, 210 and 240 min were pH 6.3, 5.8, 5.4, 5.2, 5.1, 5.0, 5.6 and 4.9, respectively (Figure 3). The extended meal effect on the pH of contents in the upper small intestine is in line with previous data suggesting that gastric emptying of the standard meal lasts more than 3h; median pH value in the stomach becomes consistently lower than 2 about 3 hours after meal ingestion (Dressman et al. 1990; this study) and intragastric volumes return to baseline levels more than 4h after the standard meal (Koziolek et al., 2014). Overall median value estimated in this study (pH 5.3) is lower than the overall median pH value reported previously for the period between 60 and 240min, after initiation of consumption of a similar but not identical to the standard meal example suggested by the regulatory authorities today [pH 6.3,

(Dressman et al., 1990)]. Previous data show that the pH of duodenal contents is not affected significantly by homogenization of a solid meal (Malagelada et al., 1979).

Mean buffer capacity decreased from 27.6 mmol/L/ΔpH at 30 min to 15.5 mmol/L/ΔpH at 240 min (Figure 3). There are no published data after administration of the standard meal but our observations are generally in line with data collected after administration of liquid meals with similar caloric breakdown and/or caloric content (Kalantzi et al., 2006a; Vertzoni et al., 2012).

At each aspiration time point, the median difference between the pH after the second administration and the pH after the first administration in a given individual ranged from -0.3 to 0.9 pH units, identical to the intra-individual differences observed in the antrum. At each aspiration time point, the mean individual difference between the buffer capacity after the second administration and the buffer capacity after the first administration ranged from -50% to 30%. These data suggest that intra-subject variability of buffer capacity in upper small intestine, under conditions simulating the oral drug absorption studies performed in the fed state, is somewhat higher than in the antrum.

It should be mentioned that pH values in the upper small intestine (and in the stomach) observed in the present study, are in line with previously published data collected after ingestion of the standard meal and diclofenac (Rubbens et al., 2019), suggesting that the higher buffer capacity of contents in the upper small intestine (and in the stomach), after the homogenized standard meal, can eliminate potential effects of co-administered acidic drugs on luminal pH, in contrast to the fasted state (Litou et al. 2020).

3.2.2. Lipid species and bile acids

Concentrations of lipid species and bile acids revealed high inter-subject variability (Table 3) and high intra-subject variability (data not shown).

In line with data for the antrum, FAs and PC were comparatively the most abundant lipid species for at least 4 hours after water administration. However, mean FAs concentrations were much higher in the upper small intestine (Table 3 vs. Table 1). Previously, only data after liquid meals with similar caloric breakdown and/or caloric content to that of the standard meal have been published (Armand et al., 1996; Hernell et al., 1990; Kalantzi et al., 2006b; Vertzoni et al., 2012). Differences from the data obtained in this study cannot be evaluated, due to the differences in composition and texture of the meals. For example, pancreatic lipase secretion is higher after a liquid meal than after a solid-liquid meal with the same fat amount, and thus, duodenal lipolysis rates are lower when solid-liquid meals are ingested (Amara et al., 2019; Carrière et al., 2001).

The predominant bile acids were glycoconjugates and the relative mean luminal concentrations were GCDC > GC > GDC > TC ~ TCDC > UDC (Table 3). Mean total bile acid content peaked at 30 min to 13.1 mM and decreased gradually to reach 4.4 mM at 240 min. There are no published data after the standard meal, but, unlike lipids, it has been shown that meal texture does not affect bile acid output (Malagelada et al., 1979). Previous data, obtained after liquid meals with similar caloric breakdown and/or caloric content (Armand et al., 1996; Hernell et al., 1990; Kalantzi et al., 2006a; Vertzoni et al., 2012), are generally in line with data of this study.

3.2.3. Viscosity

Aspirated samples showed pseudoplastic behavior and data were highly variable (Figure 4). Based on mean values, viscosity in the upper small intestine varies with time after water administration (Figure 4). To date, only the kinematic viscosity in the fasted upper small intestine has been reported (Litou et al., 2016). Assuming that the density of contents in the upper small intestine in the fasted state is slightly higher than that of water, viscosity in the fasted state is estimated to be slightly higher than 1 mPa·s, i.e. much lower than mean values estimated in this study (e.g. 103-516 mPa·s at $100s^{-1}$, at aspiration time points employed in this study). Also, based on data collected in this study, the viscosity in the upper small intestine after the

standard meal is much higher than the viscosity in the canine midgut [mean(SD)=30(50) mPa·s at $100s^{-1}$] after a similar solid meal (525 kcal, 43% fat, 1310 mPas at $100s^{-1}$) (Greenwood, 1994). Although the difference may be partly attributed to the different aspiration sites (upper small intestine vs. midgut) or non-identical composition of the two meals, it may also indicate differences in processing of solid meals between humans and dogs.

3.2.4. Characteristics of the micellar phase

Mean osmolality values peaked at 90 min, close to the corresponding time to peak in the antrum (Table 4 vs. Table 2). At similar periods after water administration, however, they fluctuated less than in the antrum and by 4h they returned to fasted state levels (Clarysse et al., 2009; Kalantzi et al., 2006a; Litou et al., 2016; Moreno et al., 2006). Mean data collected in the total contents of the upper small intestine, after liquid meals with similar caloric content and origin of calories with the standard meal (Clarysse et al., 2009; Kalantzi et al., 2006a; Vertzoni et al., 2012) were in line with data of this study.

Lipid species concentrations and bile acids concentrations indicated high inter-subject variability (Table 4) and high intra-subject variability (data not shown).

Major lipid species in the micellar phase are FAs and, unlike in the aqueous contents of the stomach or in total aspirates from the upper small intestine, lyso-PC (Table 4) rather than PC (Table 3). The difference between lyso-PC and PC was more pronounced than previously observed after a liquid meal with similar caloric content and origin of calories to that of the standard meal (Vertzoni et al., 2012), apparently due to differences in composition between the two meals.

At 30 min, 95.7% of total bile acids are present in the micellar phase of contents (Table 4 vs. Table 3). However, at later times the bile acid content in the micellar phase is reduced to 44.2-76.7% of the bile acid content in the total aspirate (Table 4 vs. Table 3). Potential reasons for this phenomenon include the

association of bile acids in intermediate colloidal structures during the course of lipid digestion (Sadeghpour et al., 2018) and the adsorption of bile acids onto residues of standard meal contents (Rubbens et al., 2019).

4. IMPLICATIONS FOR IN VITRO DRUG PRODUCT PERFORMANCE TESTING UNDER CONDITIONS SIMULATING THE FED STATE IN THE UPPER GI LUMEN

In the fed state, characteristics of contents in the upper GI lumen are important for dosage form disintegration, drug dissolution/precipitation and drug degradation. Composition of media that are currently used in relevant *in vitro* methodologies have mostly been based on luminal data collected after administration of liquid meals with similar caloric breakdown and/or caloric content with the standard meal, but not after the standard meal (Markopoulos, Andreas et al., 2015).

Fluctuations of median pH values and mean buffer capacity values in the antrum and upper small intestine up to 3 hours after water administration, are low (Figures 1 and 2), especially if one considers that data in stomach at 15 minutes after water administration may have been affected by the direct administration of water to the antrum; under normal administration conditions, the water will empty rapidly via the lesser curvature of the stomach (Koziolek et al., 2014). Therefore, Level I simulation of gastric contents and Level I simulation of contents in the upper small intestine (Markopoulos, Andreas et al. 2015) during the first 3 hours after water administration could be achieved by employing one medium per region. Level I $FeSSGF_{late}$ and Level I FeSSIF-V2 proposed by Markopoulos, Andreas et al., 2015 adequately reflect the pH and buffer capacity of gastric contents and contents of the upper small intestine after the standard meal, respectively; $FeSSGF_{late}$ should better be termed Level I FeSSGF-V2 (Table 5).

Despite the high inter-subject variability of osmolality and lipid species concentrations in the antrum, mean values during the first 3 hours after water administration, point to approximately iso-osmotic gastric contents containing TGs, DGs, FAs, each at about 3 mM, PC at about 5mM and cholesterol at about 2mM (Table 1). As a result, a single Level II biorelevant medium simulating the fed state in stomach is likely appropriate for representing average conditions. Level II $FeSSGF_{late}$ proposed by Markopoulos, Andreas et al., 2015 reflects the osmolality and total molar concentration of lipid species in gastric contents observed in the present study, as it is isoosmotic and contains a total of 14.5 mM lipid species (13.7 mM TGs, 0.044 mM FA and 0.674 mM

PC), based on the amount of Lipofundin® employed for its preparation. Therefore, Level II FeSSGF$_{late}$ could be used to reflect Level II simulation of gastric contents in the drug absorption studies performed in the fed state and should be termed Level II FeSSGF-V2 (Table 5). Based on the data collected in this study, FEDGAS®, a commercially available product for simulating the conditions in the stomach after administration of the standard meal (https://biorelevant.com/Fed_Gastric_Dissolution_Media/buy/, accessed, July 1st, 2020) may also be considered, after some modifications, i.e. pH and fat content should be maintained at 3 and at ~1%, respectively, at all times. In addition, buffer capacity, osmolality, lipid composition, and viscosity need to be reconsidered in view of the data reported in this study (currently no data in relation to these parameters are published).

The composition of Level II FeSSIF-V2 as proposed by Markopoulos, Andreas et al., 2015 slightly underestimates the total lipid and bile acid content in the upper small intestine after the standard meal (Table 3) whereas it slightly overestimates relevant species in the micellar phase of aspirated samples (Table 4). However, in order to avoid the formation of emulsions (Jantratid et al., 2008) the use of lipid and bile acid content of Level II FeSSIF-V2, after replacing PC with lyso-PC, is recommended for *in vitro* evaluation of drug dissolution/precipitation in the upper small intestine after ingestion of the standard meal. The need to include cholesterol and, at least partly, replace taurocholate with glycocholate may require further investigation. Level II simulation of contents in the upper small intestine in the fasted state containing equal amounts of glycocholate and taurocholate plus 0.2 mM cholesterol (Level II FaSSIF-V3) was shown to have some advantages over previous FaSSIF versions for estimating drug solubility in aspirates collected from the fasted upper small intestine (Fuchs et al., 2015), although in a more recent paper FaSSIF (V1) was shown to be more favourable for studying dissolution of poorly soluble drugs (Klumpp et al., 2020). Recently, compositions of Level II biorelevant media simulating the conditions in the upper small intestine in the fed state have been proposed to take into account the variability in luminal composition (McPherson et al., 2020). Since relevant compositions have been based on data collected after administration of liquid meals with not identical caloric breakdown and/or similar caloric contents of the standard meal, data on pH and buffer

capacity in upper small intestine collected in the present study may be useful in narrowing the range of relevant variations.

Level II simulation of the aqueous phase of gastric contents or Level II simulation of the micellar phase of contents in the upper small intestine may need to be considered when drug transport towards the intestinal mucosa is to be investigated. Although drug absorption from stomach is believed to be negligible in most cases, it has recently been shown that this may be possible for certain drugs (Buckley et al., 2018). Based on data collected in this study, Level II simulation of the aqueous phase of gastric contents in the fed state could be achieved by including 0.2mM FAs and 0.5mM PC in Level I FeSSGF-V2; the resulting medium should be termed Level II biorelevant medium simulating the conditions under which transport to the gastric mucosa could occur (Level II $FeSSGF_T$) (Table 5). Based on data collected in this study, Level II simulation of the micellar phase of contents in the upper small intestine should be prepared by including 6 mM bile acids, 0.2 mM MGs, 2 mM FAs and 0.9 mM lyso-PC in Level I FeSSIF-V2 and the resulting medium should be termed, Level II $FeSSIF_T$. However, until Level II $FeSSIF_T$ is proved to be adequately stable and compatible with the cell cultures, the use of previously proposed $FeSSIF\text{-}TM_{Caco}$ (Markopoulos et al., 2014) which contains 6.8 mM taurocholate, 3.4mM MG, 0.8 mm FAs and 6.8 mM PC, is recommended.

Data of this study enable better design of Level III biorelevant media by taking into account the increased luminal viscosity after the standard meal. A viscous, non-caloric HPMC meal (order of magnitude similar with intraluminal levels in this study) has been shown to significantly decrease indinavir plasma concentrations after administration of Crixivan® capsules as compared to fasted state after administration of an equal volume of water (Carver et al., 1999). Although it is not known whether viscosity effects in the fed state are similar to those in the fasted state, data from this study provide, for the first time, a basis for investigating the potential impact of increased luminal viscosity on oral drug absorption in the fed state. Level III biorelevant media simulating the environment in the fed stomach should have a viscosity of about 800 mPa.s, at $100s^{-1}$ (Figure 1) whereas Level III biorelevant media simulating the environment in the upper small intestine should be about 400 mPa.s at 100 s^{-1} (Figure 2).

5. CONCLUSIONS

Based on data collected in this study,

- In the antrum, composition of contents over time after standard meal administration fluctuates less than after liquid meals with similar caloric content. Also, viscosity of contents after the standard meal is 80-800 times higher than in the fasted state.
- In the upper small intestine viscosity of contents after the standard meal is at least 100 times higher than in the fasted state.
- Differences in composition between the aqueous phase and total gastric contents and between the micellar phase and total contents in the upper small intestine after administration of the standard meal were documented, for the first time.

As a result, Level I and Level II biorelevant media simulating the intragastric conditions, after ingestion of the standard meal can be simplified, whereas FeSSIF-V2 composition was confirmed to be representative of the composition of the micellar phase of contents in the upper small intestine. Representative values of viscosity levels in the stomach and in the upper small intestine and Level II simulation of the aqueous phase of gastric contents after the standard meal, were proposed for first time.

ACKNOWLEDGMENTS

Part of this work was presented at AAPS 2019 PHARMSCI 360, San Antonio, Texas. This work was supported by the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 674909 (PEARRL).

Table 1: Mean ± SD (n = 12-16) concentrations of lipid species in samples aspirated from the antrum after ingesting the homogenized standard meal and a glass of water 30 minutes after initiating meal ingestion*

Time (min)	15	75	135	195
TGs (mM)	3.0 ± 3.7	2.7 ± 2.2	2.8 ± 1.8	2.2 ± 1.3
DGs (mM)	2.7 ± 2.8	4.1 ± 3.0	3.5 ± 2.3	2.6 ± 1.9
MGs (mM)	0.27 ± 0.31	0.44 ± 0.36	0.42 ± 0.38	0.26 ± 0.18
FAs (mM)	2.84 ± 3.08	4.0 ± 2.4	3.8 ± 2.5	3.1 ± 2.2
Phosphatidylcholine (mM)	3.45 ± 3.05	5.78 ± 3.05	5.5 ± 2.3	3.9 ± 1.6
Lyso-phosphatidylcholine (mM)	0.053 ± 0.065	0.11 ± 0.11	0.15 ± 0.17	0.13 ± 0.16
Cholesterol (mM)	1.4 ± 1.4	2.0 ± 1.4	2.1 ± 1.4	1.3 ± 1.0

* Time zero is the time at which the glass of water was administered, i.e. 30 minutes after initiation of administration of the standard meal.

Table 2: Mean ± SD (n = 10-15) values for osmolality and concentrations of lipid species in the aqueous phase of samples aspirated from the antrum after the standard meal*

Time (min)	**15**	**75**	**135**	**195**
Osmolality (mOsm/kg)	153 ± 126	259 ± 112	304 ± 109	260 ± 61
TGs (mM)	< 0.001	< 0.001	< 0.001	< 0.001
DGs (mM)	0.027 ± 0.041	0.038 ± 0.045	0.037 ± 0.037	0.012 ± 0.013
MGs (mM)	0.029 ± 0.064	0.027 ± 0.027	0.036 ± 0.024	0.016 ± 0.014
FAs (mM)	0.215 ± 0.339	0.246 ± 0.234	0.309 ± 0.190	0.112 ± 0.072
Phosphatidylcholine (mM)	0.51 ± 0.93	0.65 ± 0.69	0.77 ± 0.58	0.23 ± 0.20
Lyso-phosphatidylcholine (mM)	0.009 ± 0.024	0.0057 ± 0.0077	0.010 ± 0.011	0.009 ± 0.010
Cholesterol (mM)	0.067 ± 0.065	0.13 ± 0.13	0.125 ± 0.094	0.051 ± 0.044

* Time zero is the time at which a glass of water was administered, i.e. 30 minutes after initiation of administration of the standard meal.

Table 3: Mean ± SD (n = 10-15) concentrations of lipid species and bile acids in samples aspirated from the upper small intestine after the standard meal*

Time (min)	30	60	90	120	150	180	210	240
TGs (mM)	1.5 ± 2.0	1.4 ± 1.2	1.9 ± 2.7	3.1 ± 5.5	1.01 ± 0.95	1.5 ± 2.8	0.24 ± 0.32	0.18 ± 0.21
DGs (mM)	3.4 ± 4.6	2.4 ± 1.9	2.3 ± 1.5	2.2 ± 1.0	1.7 ± 1.0	1.5 ± 1.3	0.56 ± 0.76	0.48 ± 0.51
MGs (mM)	1.13 ± 0.59	1.02 ± 0.62	0.92 ± 0.52	0.80 ± 0.46	0.65 ± 0.23	0.5 ± 0.3	0.27 ± 0.19	0.27 ± 0.16
FAs (mM)	21.3 ± 9.6	18.2 ± 8.5	16.4 ± 5.4	15.9 ± 4.7	13.8 ± 6.1	11.8 ± 6.4	6.3 ± 2.6	5.2 ± 3.6
Phosphatidylcholine (mM)	4.2 ± 5.0	4.7 ± 3.1	4.6 ± 3.0	4.1 ± 2.2	3.6 ± 2.3	3.5 ± 4.7	1.0 ± 1.1	0.98 ± 0.99
Lyso-phosphatidylcholine (mM)	2.6 ± 1.4	2.6 ± 1.3	1.8 ± 0.9	2.0 ± 1.1	1.53 ± 0.89	1.5 ± 1.0	1.20 ± 0.63	0.78 ± 0.63
Cholesterol (mM)	3.3 ± 3.0	2.7 ± 1.4	2.1 ± 1.2	2.0 ± 1.1	1.72 ± 0.86	1.30 ± 0.76	0.74 ± 0.54	0.66 ± 0.43
TC (mM)	1.52 ± 0.99	1.19 ± 0.76	0.93 ± 0.50	0.65 ± 0.59	0.83 ± 0.47	0.71 ± 0.54	0.64 ± 0.49	0.46 ± 0.40
GC (mM)	3.7 ± 2.0	3.4 ± 2.1	2.80 ± 0.89	2.3 ± 1.5	2.6 ± 1.1	2.1 ± 1.1	1.85 ± 0.99	1.37 ± 0.84
TCDC (mM)	1.48 ± 0.91	1.29 ± 0.76	1.00 ± 0.45	0.77 ± 0.66	0.94 ± 0.61	0.80 ± 0.56	0.67 ± 0.49	0.49 ± 0.44
UDC (mM)	1.2 ± 1.5	0.53 ± 0.50	0.36 ± 0.23	0.34 ± 0.29	028 ± 0.15	0.27 ± 0.20	0.17 ± 0.12	0.140 ± 0.095
GCDC (mM)	4.2 ± 2.4	3.9 ± 2.1	3.2 ± 1.0	2.7 ± 1.8	3.0 ± 1.4	2.4 ± 1.4	2.0 ± 1.2	1.5 ± 1.2
GDC (mM)	1.5 ± 0.6	1.70 ± 0.92	1.38 ± 0.74	1.27 ± 0.94	1.21 ± 0.74	0.97 ± 0.58	0.77 ± 0.41	0.46 ± 0.29
Total Bile Acids (mM)	13.1 ± 6.5	11.8 ± 5.9	10.1 ± 2.7	8.0 ± 5.1	8.9 ± 4.0	7.3 ± 3.9	6.1 ± 3.3	4.4 ± 3.1

* Time zero is the time at which a glass of water was administered, i.e. 30 minutes after initiation of administration of the standard meal.

Table 4: Mean ± SD (n = 8-13) values for osmolality, concentrations of lipid species, and concentrations of bile acids in the micellar phase of samples aspirated from the upper small intestine after the standard meal*

Time (min)	30	60	90	120	150	180	210	240
Osmolality (mOsm/kg)	303 ± 71	335 ± 122	392 ± 101	339 ± 56	322 ± 41	293 ± 52	214 ± 56	200 ± 61
TGs (mM)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
DGs (mM)	0.22 ± 0.47	0.10 ± 0.10	0.091 ± 0.094	0.042 ± 0.048	0.028 ± 0.058	0.039 ± 0.078	0.039 ± 0.090	< 0.001
MGs (mM)	0.79 ± 0.49	0.7 ± 1.3	0.35 ± 0.54	0.29 ± 0.34	0.18 ± 0.19	0.20 ± 0.23	0.25 ± 0.23	0.19 ± 0.30
FAs (mM)	8.0 ± 7.4	3.1 ± 2.4	2.0 ± 1.7	2.2 ± 1.9	1.4 ± 1.3	1.5 ± 1.3	2.1 ± 1.8	1.03 ± 0.92
Phosphatidylcholine (mM)	0.24 ± 0.25	0.15 ± 0.12	0.14 ± 0.11	0.15 ± 0.12	0.12 ± 0.11	0.078 ± 0.056	0.074 ± 0.075	0.052 ± 0.056
Lyso-phosphatidylcholine (mM)	2.0 ± 1.4	0.90 ± 0.91	0.64 ± 0.81	0.61 ± 0.54	0.50 ± 0.62	0.35 ± 0.52	0.60 ± 0.54	0.30 ± 0.32
Cholesterol (mM)	0.94 ± 0.78	0.41 ± 0.46	0.24 ± 0.30	0.17 ± 0.14	0.134± 0.14	0.12 ± 0.14	0.18 ± 0.17	0.10 ± 0.12
TC (mM)	1.05 ± 0.88	0.56 ± 0.38	0.45 ± 0.25	0.60 ± 0.45	0.45 ± 0.32	0.50 ± 0.36	0.57 ± 0.51	0.37 ± 0.29
GC (mM)	3.6 ± 2.4	2.1 ± 2.2	1.8 ± 1.6	1.8 ± 1.1	1.44 ± 0.88	1.47 ± 0.91	1.5 ± 1.1	0.99 ± 0.70
TCDC (mM)	1.3 ± 1.2	0.52 ± 0.45	0.31 ± 0.29	0.42 ± 0.38	0.32 ± 0.21	0.31 ± 0.31	0.53 ± 0.50	0.26 ± 0.22
UDC (mM)	0.41 ± 0.40	0.18 ± 0.17	0.13 ± 0.13	0.15 ± 0.16	0.12 ± 0.11	0.094 ± 0.092	0.19 ± 0.12	0.083 ± 0.061
GCDC (mM)	4.2 ± 3.5	2.0 ± 2.2	1.3 ± 1.8	1.3 ± 1.1	1.06 ± 0.83	0.9 ± 1.0	1.4 ± 1.2	0.68 ± 0.67
GDC (mM)	1.9 ± 1.5	1.0 ± 1.3	0.8 ± 1.2	0.60 ± 0.68	0.54 ± 0.60	0.34 ± 0.46	0.43 ± 0.32	0.32 ± 0.33
Total Bile Acids (mM)	12.5 ± 9.4	6.3 ± 6.5	4.7 ± 5.0	4.9 ± 3.6	3.9 ± 2.6	3.7 ± 2.8	4.7 ± 3.5	2.6 ± 2.1

* Time zero is the time at which a glass of water was administered, i.e. 30 minutes after initiation of administration of the standard meal.

Table 5: Biorelevant media simulating the gastric contents (FeSSGF-V2) and the aqueous phase of gastric contents ($FeSSGF_T$) during the first 3.5 hours, after initiation of administration of the standard meal. Level I simulation results after eliminating the grey highlighted cells in the table.

	FeSSGF-V2*	**$FeSSGF_T$**
TGs (mM)	13.7**	-
Phosphatidylcholine (mM)	0.674**	0.5
Sodium oleate (mM)	0.044**	0.2
Ortho-phosphoric acid (mM)	5.5	5.5
Sodium dihydrogen phosphate (mM)	32	32
HCl/NaOH	qs pH3	qs pH3
Sodium Chloride (mM)	127.5	127.5
pH	3	3
Buffer capacity [(mmol/L)/ΔpH)]	25	25
Osmolality (mOsmol/kg)	300	300
Viscosity at 100 s^{-1} (Level III)	800 mPa.s	-

*Identical to $FeSSGF_{late}$ proposed by Markopoulos, Andreas et al. 2015
**Included by using Lipofundin, 4.5% v/v

FIGURE CAPTIONS

Figure 1: pH and buffer capacity in the antrum, after the standard meal. Time zero designates the time at which a glass of water was administered, i.e. 30 min after initiation of administration of the standard meal into the antrum. The number above each box indicates the number of individual aspirates used for constructing the box and its whiskers. Dotted lines indicate the mean values.

Figure 2: Viscosity (37 °C) of antral contents, after the standard meal, at three shear rates. Time zero designates the time at which a glass of water was administered, i.e. 30 minutes after initiation of administration of the standard meal into the antrum. The number above each box indicates the number of individual aspirates used for constructing the box and its whiskers. Dotted lines indicate the mean values.

Figure 3: pH and buffer capacity in the upper small intestine, after the standard meal. Time zero designates the time at which a glass of water was administered, i.e. 30 minutes after initiation of administration of the standard meal into the antrum. The number above each box indicates the number of individual aspirates used for constructing the box and its whiskers. Dotted lines indicate the mean values.

Figure 4: Viscosity (37 °C) of in the upper small intestine, after the standard meal, at three shear rates. Time zero designates the time at which a glass of water was administered, i.e. 30 minutes after initiation of administration of the standard meal into the antrum. The number above each box indicates the number of individual aspirates used for constructing the box and its whiskers. Dotted lines indicate the mean values.

Figure 1

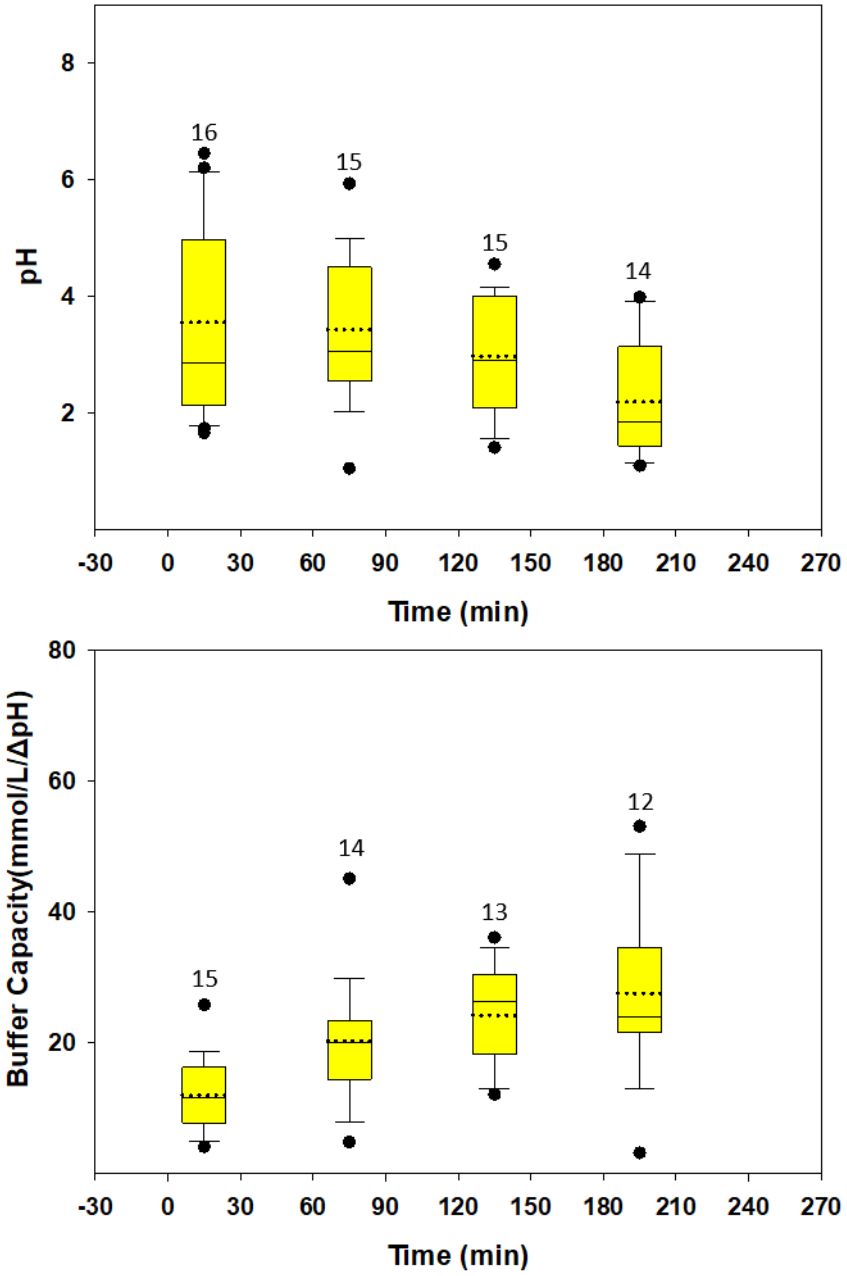

pH
8
6
4
2
16
15
15
14
-30
0
30
60
90
120
150
180
210
240
270
Time (min)
Buffer Capacity(mmol/L/ΔpH)
80
60
40
20
15
14
13
12
-30
0
30
60
90
120
150
180
210
240
270
Time (min)

Figure 2

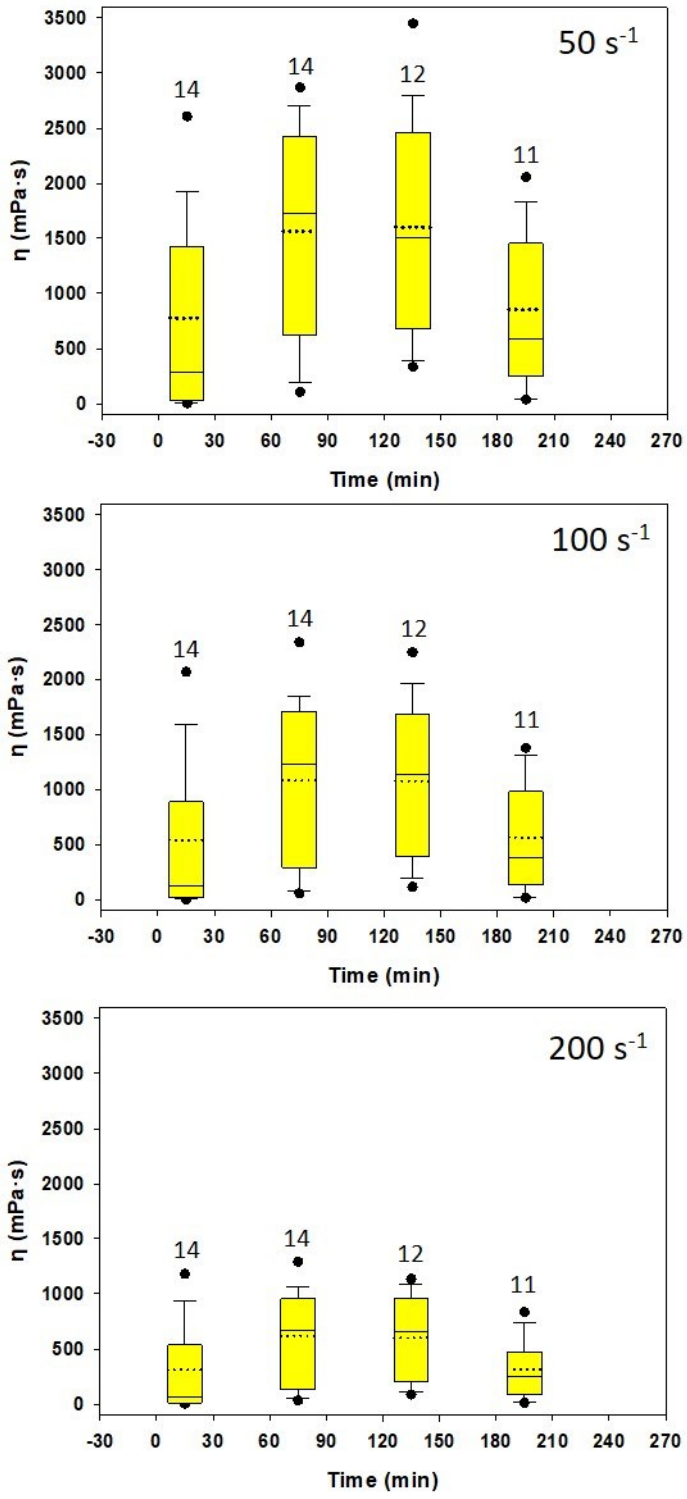

50 s-1
100 s-1
200 s-1
η (mPa·s)
Time (min)
14
14
12
11
0
500
1000
1500
2000
2500
3000
3500
-30
0
30
60
90
120
150
180
210
240
270

Figure 3

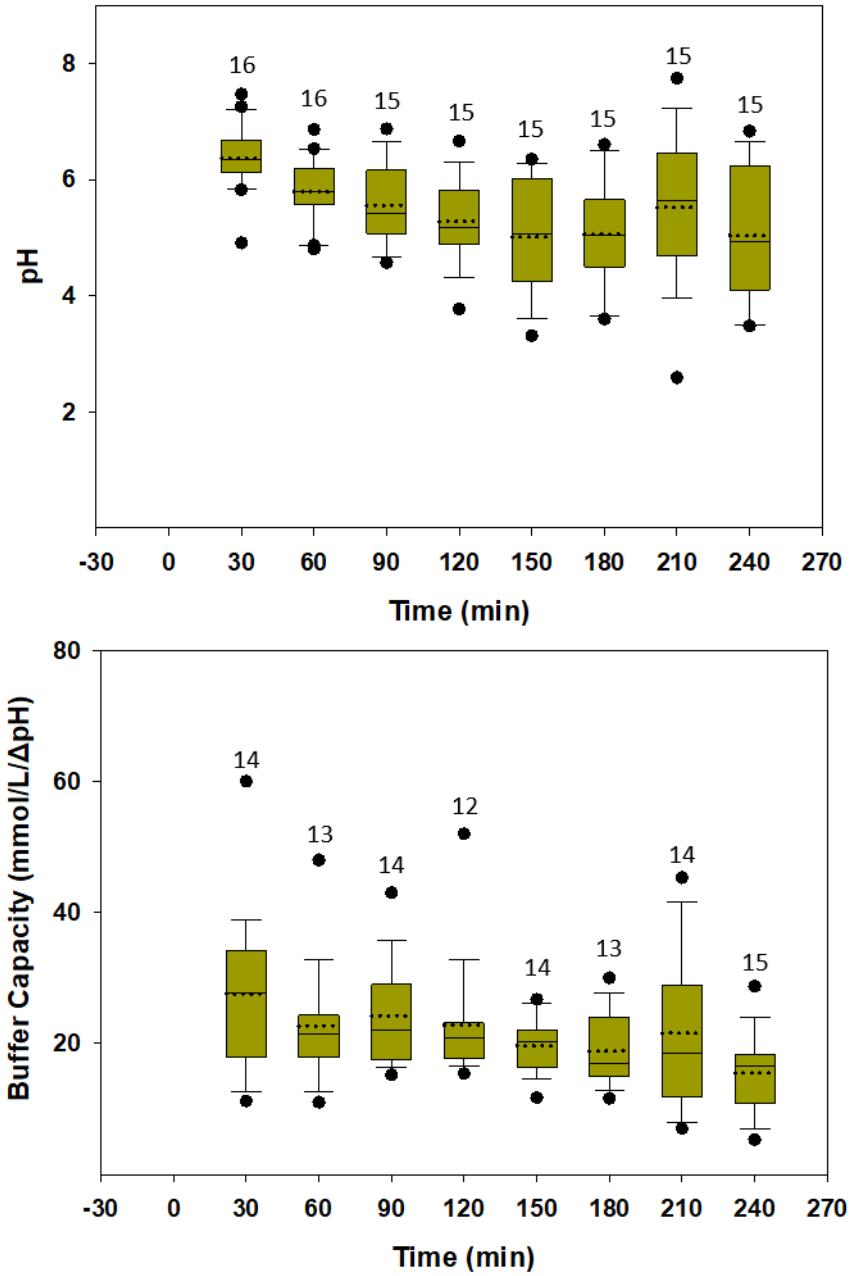
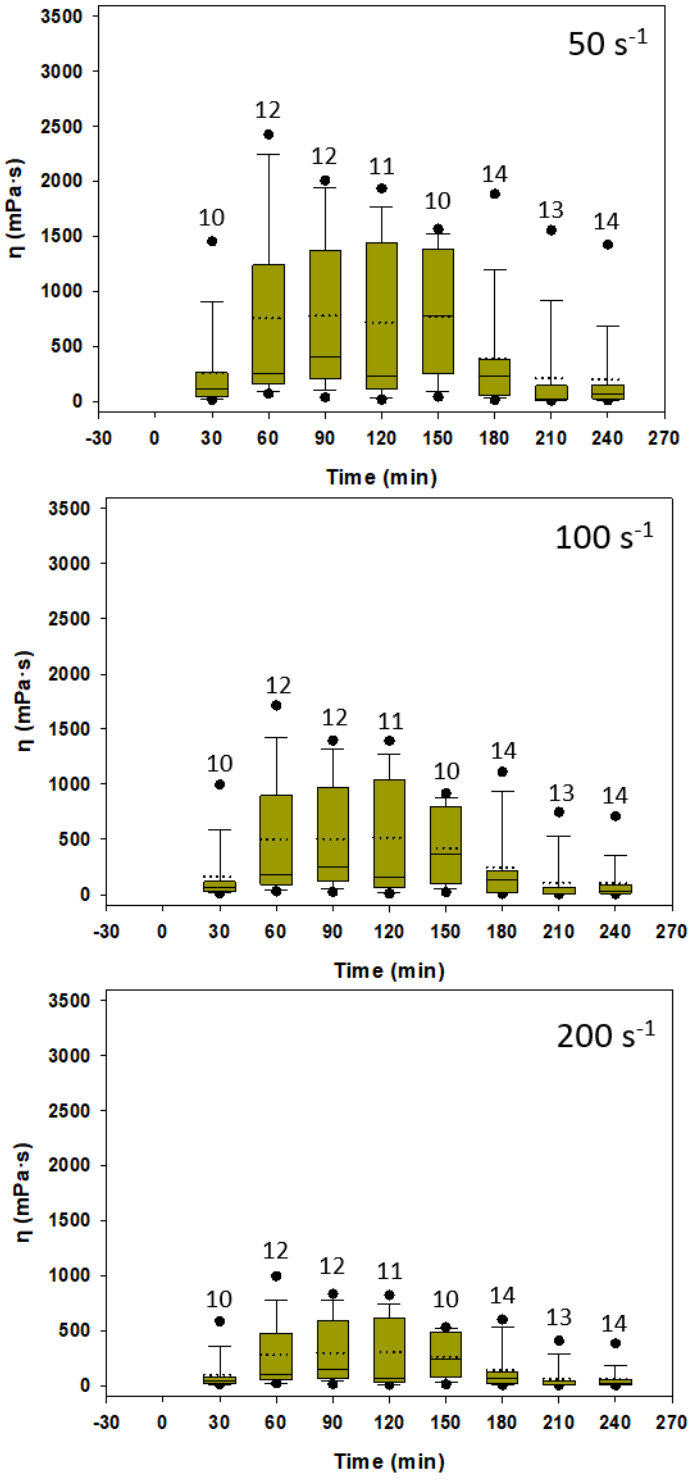

pH
8
6
4
2
16
16
15
15
15
15
15
-30
0
30
60
90
120
150
180
210
240
270
Time (min)
Buffer Capacity (mmol/L/ΔpH)
80
60
40
20
14
13
14
12
14
13
14
15
-30
0
30
60
90
120
150
180
210
240
270
Time (min)

Figure 4


50 s-1
100 s-1
200 s-1
η (mPa·s)
Time (min)
3500
3000
2500
2000
1500
1000
500
0
-30
0
30
60
90
120
150
180
210
240
270
10
12
12
11
10
14
13
14

References

Amara, S., Bourlieu, C., Humbert, L., Rainteau, D., Carrière, F., 2019. Variations in gastrointestinal lipases, pH and bile acid levels with food intake, age and diseases: Possible impact on oral lipid-based drug delivery systems. Adv Drug Deliv Rev. 142, 3-15.

Armand, M., Borel, P., Pasquier, B., Dubois, C., Senft, M., Andre, M., Peyrot, J., Salducci, J., Lairon, D., 1996. Physicochemical characteristics of emulsions during fat digestion in human stomach and duodenum. Am J Physiol 271, G172-183.

Buckley, S.T., Bækdal, T.A., Vegge, A., Maarbjerg, S.J., Pyke, C., Ahnfelt-Rønne, J., Madsen, K.G., Schéele, S.G., Alanentalo, T., Kirk, R.K., Pedersen, B.L., Skyggebjerg, R.B., Benie, A.J., Strauss, H.M., Wahlund, P.O., Bjerregaard, S., Farkas, E., Fekete, C., Søndergaard, F.L., Borregaard, J., Hartoft-Nielsen, M.L., Knudsen, L.B., 2018. Transcellular stomach absorption of a derivatized glucagon-like peptide-1 receptor agonist. Sci Transl Med. 10, 476.

Butler, J., Hens, B., Vertzoni, M., Brouwers, J., Berben, P., Dressman, J., Andreas, C.J., Schaefer, K.J., Mann, J., McAllister, M., Jamei, M., Kostewicz, E., Kesisoglou, F., Langguth, P., Minekus, M., Müllertz, A., Schilderink, R., Koziolek, M., Jedamzik, P., Weitschies, W., Reppas, C., Augustijns, P., 2019. In vitro models for the prediction of in vivo performance of oral dosage forms: Recent progress from partnership through the IMI OrBiTo collaboration. Eur J Pharm Biopharm. 136, 70-83.

Carrière, F., Renou, C., Ransac, S., Lopez, V., De Caro, J., Ferrato, F., De Caro, A., Fleury, A., Sanwald-Ducray, P., Lengsfeld, H., Beglinger, C., Hadvary, P., Verger, R., Laugier, R., 2001. Inhibition of gastrointestinal lipolysis by Orlistat during digestion of test meals in healthy volunteers. Am J Physiol Gastrointest Liver Physiol 281, 16-28.

Carver, P.L., Fleisher, D., Zhou, S.Y., Kaul, D., Kazanjian, P., Li, C., 1999. Meal composition effects on the oral bioavailability of indinavir in HIV-infected patients. Pharm. Res. 16, 718-724.

Clarysse, S., Tack, J., Lammert, F., Duchateau, G., Reppas, C., Augustijns, P., 2009. Postprandial Evolution in Composition and Characteristics of Human Duodenal Fluids in Different Nutritional States. J Pharm Sci. 98, 1177-1192.

Diakidou, A., Vertzoni, M., Goumas, K., Söderlind, E., Abrahamsson, B., Dressman, J.B., Reppas, C., 2009. Characterization of the Contents of Ascending Colon to Which Drugs are Exposed After Oral Administration to Healthy Adults. Pharm Res. 26, 2141-2151.

Dressman, J.B., Berardi, R.R., Dermentzoglou, L.C., Russell, T.L., Schmaltz, S.P., Barnett, J.L., Jarvenpaa, K.M., 1990. Upper Gastrointestinal (GI) pH in Young, Healthy Men and Women. Pharm Res. 7, 756-761.
EMA, 2010. Guideline on the investigation of bioequivalence. CPMP/QWP/EWP/1401/98.

Elvang, P.A., Bohsen, M.S., Stein, P.C., Bauer-Brandla, A., Riethorst, D., Brouwers, J., Augustijns, P., Brandl, M., 2019. Co-existing colloidal phases of human duodenal aspirates: Intraindividual fluctuations and interindividual variability in relation to molecular composition. J Pharm Biomed Anal. 170, 22-29.

EMA, 2020. ICH M9 guideline on biopharmaceutics classification system-based biowaivers, Step 5, Science Medicines health, EMA/CHMP/ICH/493213/2018. Committee for Medicinal Products for Human Use.

Fuchs, A., Leigh, M., Kloefer, B., Dressman, J.B., 2015. Advances in the design of fasted state simulating intestinal fluids: FaSSIF-V3. Eur J Pharm Biopharm. 94, 229-240.

Greenwood, D.E., 1994. Small intestinal pH and buffer capacity: implications for dissolution of ionizable compounds. PhD Thesis. The University of Michigan, USA.

Hernell, O., Staggers, J.E., Carey, M.C., 1990. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. Biochemistry. 27;29(8):2041-56.

Jantratid, E., Janssen, N., C., R., Dressman, J.B., 2008. Dissolution Media Simulating Conditions in the Proximal Human Gastrointestinal Tract: An Update. Pharm Res. 25, 1663-1676.

Kalantzi, L., Goumas, K., Kalioras, V., Abrahamsson, B., Dressman, J.B., Reppas, C., 2006a. Characterization of the Human Upper Gastrointestinal Contents Under Conditions Simulating Bioavailability/Bioequivalence Studies. Pharm Res. 23, 165-176.

Kalantzi, L., Persson, E., Polentarutti, B., Abrahamsson, B., Goumas, K., Dressman, J.B., Reppas, C., 2006b. Canine intestinal contents vs. simulated media for the assessment of solubility of two weak bases in the human small intestinal contents. Pharm Res. 23, 1373-1381.

Klumpp, L., Leigh, M., Dressman, J., 2020. Dissolution behavior of various drugs in different FaSSIF versions. Eur J Pharm Sci 142.

Koziolek, M., Carrière, F., Porter, C.J.H., 2018. Lipids in the Stomach - Implications for the Evaluation of Food Effects on Oral Drug Bioavailability. Pharm Res. 35, 55.

Koziolek, M., Grimm, M., Garbacz, G., Kühn, J.P., W., W., 2014. Intragastric Volume Changes after Intake of a High-Caloric, High-Fat Standard Breakfast in Healthy Human Subjects Investigated by MRI. Mol. Pharm. 11, 1632-1639.

Koziolek, M., Schneider, F., Grimm, M., Modeβ, C., Seekamp, A., Roustom, T., Siegmund, W., Weitschies, W., 2015. Intragastric pH and pressure profiles after intake of the high-caloric, high-fat meal as used for food effect studies. Journal of Controlled Release 220, 71-78.

Litou, C., Psachoulias, D., Vertzoni, M., Dressman, J., Reppas, C., 2020. Measuring pH and Buffer Capacity in Fluids Aspirated from the Fasted Upper Gastrointestinal Tract of Healthy Adults. Pharm Res. 37, 42.

Litou, C., Vertzoni, M., Goumas, C., Vasdekis, V., Xu, W., Kesisoglou, F., Reppas, C., 2016. Characteristics of the Human Upper Gastrointestinal Contents in the Fasted State Under Hypo- and A-chlorhydric Gastric Conditions Under Conditions of Typical Drug - Drug Interaction Studies. Pharm Res. 33, 1399-1412.

Malagelada, J.R., Go, V.L., Summerskill, W.H., 1979. Different gastric, pancreatic, and biliary responses to solid–liquid or homogenized meals. Dig. Dis. Sci. 24, 101-110.

Markopoulos, C., Andreas, C.J., Vertzoni, M., Dressman, J., Reppas, C., 2015. In-vitro simulation of luminal conditions for evaluation of performance of oral drug products: Choosing the appropriate test media. Eur J Pharm Biopharm. 93, 173–182.

Markopoulos, C., Thoenen, F., Preisig, D., Symillides, M., Vertzoni, M., Parrott, N., Reppas, C., Imanidis, G., 2014. Biorelevant media for transport experiments in the Caco-2 model to evaluate drug absorption in the fasted and the fed state and their usefulness. Eur J Pharm Biopharm. 86, 438-448.

McPherson, S., Perrier, J., Dunn, C., Khadra, I., Davidson, S., Ainousah, B., Wilson, C.G., Halbert, G., 2020. Small scale design of experiment investigation of equilibrium solubility in simulated fasted and fed intestinal fluid. Eur J Pharm Biopharm. 150, 14-23.

Moreno, M.P.d.I.C., Oth, M., Deferme, S., Lammert, F., Tack, J., Dressman, J., Augustijns, P., 2006. Characterization of fasted-state human intestinal fluids collected from duodenum and jejunum. Journal of Pharmacy and Pharmacology 58, 1079-1089.

Müllertz, A., Fatouros, D.G., Smith, J.R., Vertzoni, M., Reppas, C., 2012. Insights into intermediate phases of human intestinal fluids visualized by atomic force microscopy and cryo-transmission electron microscopy ex vivo. Mol Pharm. 9, 237-247.

Pedersen, P.B., Vilmann, P., Bar-Shalom, D., Müllertz, A., Baldursdottir, S., 2013. Characterization of fasted human gastric fluid for relevant rheological parameters and gastric lipase activities. Eur J Pharm Biopharm. 85, 958-965.

Pentafragka, C., Symillides, M., McAllister, M., Dressman, J., Vertzoni, M., Reppas, C., 2019. The impact of food intake on the luminal environment and performance of oral drug products with a view to in vitro and in silico simulations: a PEARRL review. J Pharm Pharmacol 71, 557-580.

Pentafragka, C., Vertzoni, M., Symillides, M., Goumas, K., Reppas, C., 2020. Disposition of two highly permeable drugs in the upper gastrointestinal lumen of healthy adults after a standard high-calorie, high-fat meal. Eur J Pharm Sci.

Rubbens, J., Brouwers, J., Tack, J., Augustijns, P., 2019. Gastric and Duodenal Diclofenac Concentrations in Healthy Volunteers after Intake of the FDA Standard Meal: In Vivo Observations and in Vitro Explorations. Mol Pharm 16, 573-582.

Sadeghpour, A., Rappolt, M., Misra, S., Kulkarni, C.V., 2018. Bile Salts Caught in the Act: From Emulsification to Nanostructural Reorganization of Lipid Self-Assemblies. Langmuir. 34, 13626-13637.
Vertzoni, M., Archontaki, H., Reppas, C., 2008. Determination of intralumenal individual bile acids by HPLC with charged aerosol detection. J Lipid Res 49, 2690-2695.

U.S. FDA, 2019. Assessing the Effects of Food on Drugs in INDs and NDAs — Clinical Pharmacology Considerations. Guidance for Industry. Center for Drug Evaluation and Research. Food and Drug Administration.

Vertzoni, M., Markopoulos, C., Symillides, M., Goumas, C., Imanidis, G., Reppas, C., 2012. Luminal Lipid Phases after Administration of a Triglyceride Solution of Danazol in the Fed State and Their Contribution to the Flux of Danazol Across Caco-2 Cell Monolayers. Mol. Pharm 9, 1189−1198.