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

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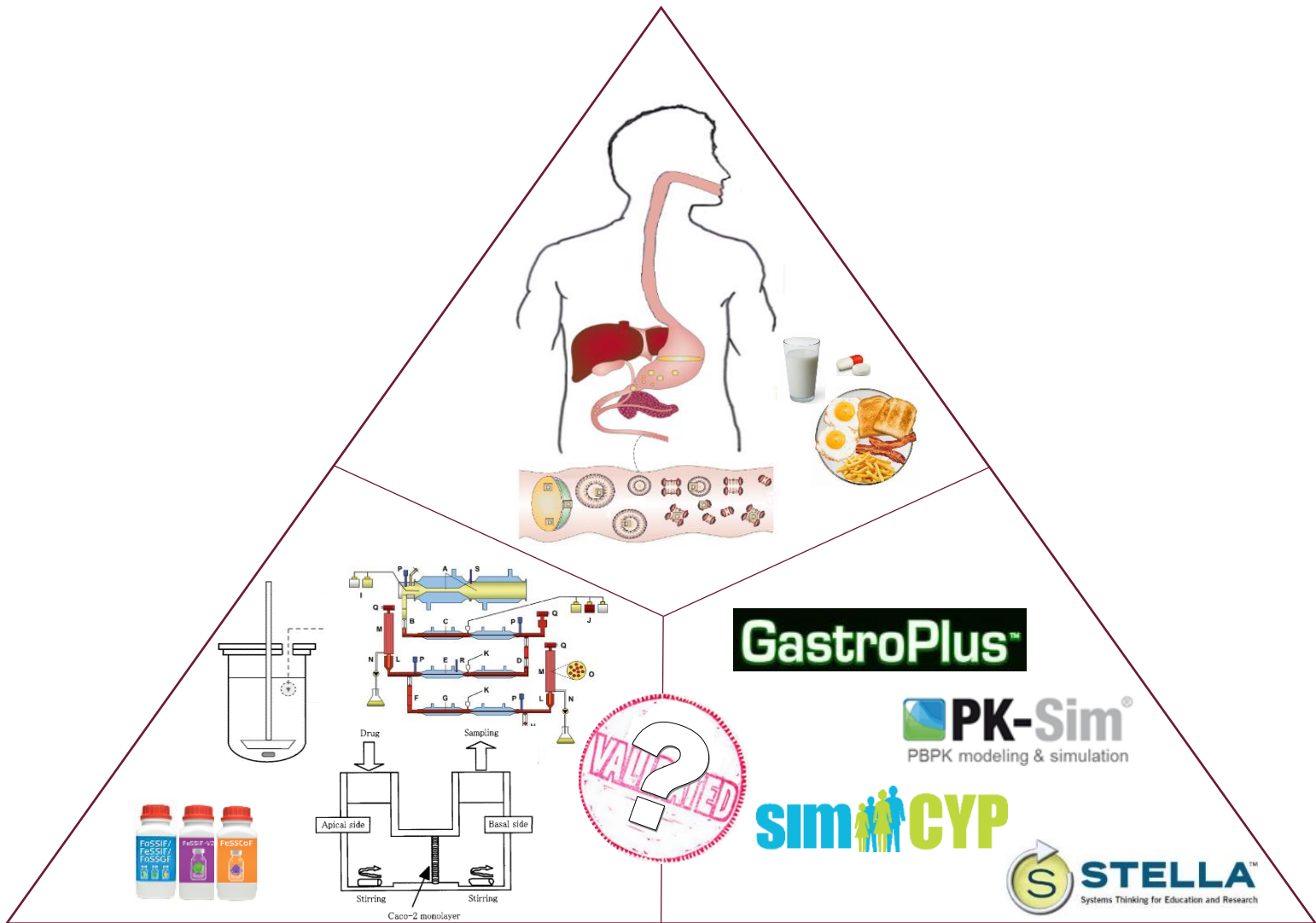
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Graphical Abstract

ABSTRACT

Objective: Using the type of meal and dosing conditions suggested by regulatory agencies as a basis, this review has two specific objectives. First, to summarize our understanding on the impact of food intake on luminal environment and drug product performance. Second, to summarize the usefulness and limitations of available *in vitro* and *in silico* methodologies for the evaluation of drug products performance after food intake.

Key findings: Characterization of the luminal environment and studies evaluating product performance in the lumen, under conditions suggested by regulatory agencies for simulating the fed state, are limited. Various *in vitro* methodologies have been proposed for evaluating drug product performance in the fed state but systematic validation is lacking. Physiologically based pharmacokinetic (PBPK) modelling approaches require the use of *in vitro* biorelevant data and, to date, have been used primarily for investigating the mechanisms via which an already observed food effect is mediated.

Conclusion: Better understanding of the impact of changes induced by the meal administration conditions suggested by regulatory agencies on the luminal fate of the drug product is needed. Relevant information will be useful for optimizing the *in vitro* test methods, and increasing the usefulness of PBPK modelling methodologies.

KEY WORDS

1. INTRODUCTION

The impact of meal intake on luminal performance of orally administered drug products is of interest at the preclinical stage of the drug development process as it may significantly affect the strategy for the development of a new active pharmaceutical ingredient (API). Knowledge of the impact of a meal on luminal product performance prior to its administration to humans is also important during the development of generic drug products.

Major issues when evaluating meal effects include the composition and timing of administration of meal in relation to the intake of the drug product. Drug regulatory agencies have made specific relevant recommendations so that the potential meal effect on dosage form performance is maximized. Both the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) propose the use of high-calorie (800 to 1000 calories), high-fat (approximately 50% of total caloric content of the meal) meals which derive approximately 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively [1, 2]. The test meal example provided by the regulatory authorities 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 (reference meal). These agencies further propose that the drug product administration should take place 30 minutes after the start of the meal, with a glass of water (240 mL) [1, 2].

The present article provides a biopharmaceutical perspective of drug and drug product performance in the gastrointestinal (GI) lumen in the fed state and has two specific objectives.

First, to summarize our understanding of the impact of food intake on the luminal environment and drug product performance. Investigation of characteristics of the luminal environment under the drug dosing conditions in the fed state recommended by regulatory agencies ("reference meal") can be challenging, especially in cases where sampling from the luminal contents is required. For example, to date, only liquid meals (having similar composition, origin of calories, calorie content and/or volume to that of the reference meal) have been used to evaluate the impact of food intake on buffer or solubilizing capacity of contents of the upper intestinal lumen. Based on the few studies

published to date on the impact of homogenization of solid-liquid meals (having a much lower calorie content than the reference meal) [3, 4], the extent to which the luminal environment after administration of liquid meals is similar to that after administration of the reference (solid-liquid) meal is not clear. In this manuscript, characteristics of the luminal environment after administration of homogenized or liquid meals with composition similar to that of the reference meal (Table 1), are presented when data after administration of solid-liquid meals are not available.

The second objective of this manuscript is to summarize the usefulness and limitations of *in vitro* and *in silico* methodologies applied today for the evaluation of drug products performance after food intake.

Table 1: Meals that have been used to explore the physicochemical properties and transit of luminal contents in the fed state. Composition, calorie content and/or origin of calories are in line with that of the meal suggested by regulatory agencies (reference meal).

Meal Texture	Meal Composition	Calorie content				Volume (mL)	Reference
		Total (kcal)	Fat (%)	Protein (%)	Carb. (%)		
Solid – liquid (Reference meal)	2 slices of toast with butter, 2 eggs fried in butter, 2 strips of bacon, 4 oz hash brown potatoes, 240 mL whole milk	800-1000	50 – 75	15 - 19	25 - 31	500	Diakidou *et al*. (2009) [5] Koziolek *et al*. (2014) [6] Koziolek *et al*. (2015) [7] Reppas *et al*. (2015) [8]
	6 oz hamburger, 2 slices of bread, 2 oz hash brown potatoes, 1 tbsp ketchup, 1 tbsp mayonnaise, 1 oz tomato, 1 oz lettuce, 8 oz milk	1000	57	17	26	Not specified	Dressman *et al*. (1990) [9]
Solid – liquid	6 oz sirloin steak, salt, 1 slice of bread, 5 g margarine, tossed salad with French dressing, 8 oz iced tea, 6.8 g sucrose	623	58	14	28	645	Fordtran *et al*. (1966) [10]
	250 mL noodle soup, chicken with rice, mixed vegetables, 200 g fruit yoghurt	803	13.7	23.1	63.2	900	Schiller *et al*. (2005) [11]
	2 slice of bread, 40 g cheese, 150 mL orange juice, 150 mL milk, 20 g cereal	511	35	17	48	Not specified	Weitchies *et al*. (2005) [12]
	1 slice of toast, 5g butter, 1 egg fried in butter, 1 slice of bacon, 2 oz hash browns, 120 mL whole milk	460	55	15	30	250	Grimm *et al*. (2017) [3]
Solid - liquid and homogenized	90 g tenderloin steak, 0.1 g salt, 25 g white bread with 8 g butter, 60 g vanilla ice cream, 35 g chocolate syrup, 240 mL water	458	40	20	40	400	Malagelada *et al*. (1976, 1979) [4, 13]
Homogenized	80 g string beans, 90 g beef, 70 g fried potatoes, 10 g butter, 15 mL oil	662	63	16	21	700	Carrière *et al*. (2005) [14]
	43 mL milk, 57 g whipping cream, 51 g instant chocolate cream, 15 g casein powder, 35 mL water	491	59	18	23	Not specified	Grimm *et al*. (2017) [3]
Liquid heterogeneous	50 g olive oil, 1 egg, 20 g sucrose, 5 mL vanilla extract, 250 mL of 0.15 M NaCl, water	604	73	14	13	400	Hernell *et al*. (1990) [15]
	70 g olive oil, 1 egg, 1 egg white, 70 g sucrose, 1.8 g NaCl	960	65.5	5.0	29.5	400	Armand *et al*. (1994, 1996) [16, 17]
	62.5 g olive oil, 1.25 eggs, 25 g sucrose, 2.7 g NaCl, vanilla flavor, water	750	73	14	13	500	Vertzoni *et al*. (2012) [18]
Liquid homogeneous	Ensure® Plus	753	32	27	41	500	Kalantzi *et al*. (2006) [19]
		602	32	27	41	400	Clarysse *et al*. (2009) [20] Riethorst *et al*. (2014) [21]
	Scandishake® Mix	598	46	8	46	300	Clarysse *et al*. (2009) [20]

2.   THE INTRALUMINAL ENVIRONMENT IN THE FED STATE

2.1. Intraluminal hydrodynamics

2.1.1. Stomach

After administration of a solid meal with half the calories of the reference meal (Table 1) [13], the volume of gastric contents remains similar to the volume of the meal (about 400mL) for the first hour and returns to baseline values about four hours post administration of the meal [13]. Despite the negligible change of volume of gastric contents during the first hour post meal administration, the flow rates of gastric contents emptying into duodenum were about 10-6 mL/min (steadily decreasing) and reached about 2 mL/min at 4 hours post meal administration [13].

Based on magnetic resonance imaging (MRI) data [6], intragastric mean(SD) volumes increase to 580(38) mL within 15 min after consumption of the reference meal i.e. about 100 mL higher than the volume of the meal (~480 mL) [22]. Gastric secretory response to the meal is enough to maintain intragastric volume constant for about 50-90 min. Then the volume starts to decrease slowly at a rate of 1.7(0.3) mL/min [6].

Gastric emptying rate of the meal itself and fluids associated with it depends on the caloric content of the meal and is about 120-240 kcal/h [23-26]. Thus, the reference meal will not be completely emptied until about 4-8 hours after ingestion [6]. However, it has been reported that water can empty from the stomach as fast in the fed as in the fasted state [3, 6, 27]. Waldeyer [28] introduced in 1908 the idea of a shortcut around the bulk contents via which any ingested water that does not mix with the bulk contents may empty within few minutes. He called this shortcut "Magenstrasse" (literal translation = stomach road) and recent research data support this claim [3, 28].

During digestion, a continuous pattern of contractions is observed in the stomach. Tonic contractions move contents downwards [29]. Peristaltic contractions are responsible for the intense grinding and mixing of contents by retropulsion of chyme back into the corpus region of the stomach

[30]. Hydrodynamics and motility are highly variable between fundus (weak forces) and antrum (high shear zone, strong forces). Antropyloric contractions cause the pylorus to partially open and let liquids and small particles (<1-2 mm) flow from the stomach into the duodenum [31, 32], whereas larger objects are retropelled back into the stomach for further grinding. Large, indigestible solids are retained in the stomach until the recurrence of the intense motility of the Phase III fasted state MMC (Migrating Motor Complexes) [33, 34]. In contrast to the maximum pressures in the fasted state which are quite variable and can be up to about 500 mbar, the maximum pressure within the stomach after ingestion of the reference meal has been reported at 293(109) mbar, typically observed shortly before gastric emptying and associated with forceful antral contraction waves [7, 35].

2.1.2. Upper Intestine (Duodenum and Proximal jejunum)

Direct sampling as well as imaging techniques have been useful in providing information on the volumes of contents in the upper small intestine. However, the quality of information varies between the two techniques.

The type of available information from direct sampling techniques, to date, does not enable conclusions to be drawn about the impact of meal intake on the volume of contents in the upper intestine. After modelling luminal data from adults, the volume of duodenal contents has been estimated to be about 30 mL in the fasted state during the first hour after administration of 240 mL of a non-caloric aqueous solution containing no osmotically active agents to fasted adults [36]. Based on data from healthy adults collected by continuously aspirating luminal contents immediately after the start of eating a solid meal of 645 mL (Table 1) [10] and for approximately two hours afterwards, the volume of contents/chyme that passed the mid-duodenum and the proximal jejunum were about 1.5 L and 0.75 L respectively [10].

Based on MRI data, food intake (Table 1) [11] reduced the mean fluid volumes in the entire small intestine from about 100 mL (in the fasted state) to about 50 mL, one hour after meal intake. In the

fasted state, i.e. 7h, after overnight fasting, during which 100 mL or 150 mL of water were administered every hour, the majority of intraluminal water is located in the distal jejunum and proximal ileum [37]. After meal ingestion, the number of fluid pockets increased but the fluid volume per pocket decreases, resulting in an overall volume decrease.

More studies are needed to understand the impact of meal on the volume of contents in the upper small intestine and at various times which are relevant to drug administration times.

Small intestinal transit times are similar for solutions and pellets both in the fasted and in the fed state, about 3 hours [38], although there appears to be a slight trend to decreased transit times in the fed state for non-disintegrating solids (198-226 min in the fed state compared with 210-352 min in the fasted state) [26, 39].

The highest pressures during small intestinal transit in the fasted state are reported at 103 (65) mbar and after the administration of the reference meal at 95 (76) mbar [35].

2.1.3. Lower Intestine (Distal ileum and Proximal colon)

Based on data from healthy adults collected by continuously aspirating luminal contents since the start of eating a solid meal (Table 1) [10], the first sample from the lower small intestine could be aspirated about 30 min after eating and collection lasted for 2-2.5 h. The total volume collected from the lower small intestine was about 250 mL [10].

Gas volume in the ascending colon is about 200 mL [40], roughly equal to its geometric capacity [41] leaving only a small percentage of the available space for liquid/solid material.

Data from direct sampling about five hours after the administration of 240 mL water or the reference meal provide the following mean(SD) total volumes of contents: for the distal ileum 3.8(2.3) mL in the fasted and 7.3(3.3) mL in the fed state; for the cecum 5.0(2.1) mL in the fasted and 8.0(2.7) mL in the fed state [8]; and for the ascending colon, up to about 30 mL with a trend for higher volumes after the reference meal [22.3(7.7) mL after water and 29.9(10.8) mL after the

reference meal] [5]. The total volume of contents in the ascending colon is 5.9 and 4.2 times bigger than the total volume of contents in distal ileum, in the fasted and fed state respectively [8]. Longer residence time and increased bacteria content in the ascending colon could contribute to this difference. Regarding the liquid/solid ratio of the contents, liquid volumes in the distal ileum were 3.4 mL in the fasted state [liquid fraction 89.9(10.0) %] and 5.1 mL in the fed state [liquid fraction 68.6(13.2) %]. In the cecum the trend did not reach significance [liquid fraction 69.7(19.2) % in the fasted state vs. 63.7(13.1) % in the fed state [8]]. In the ascending colon, liquid volumes were 15.6 mL and 18.5 mL but the respective aqueous phase percentage was 70.3(17.0) % in the fasted, decreasing to 56.0 (9.0) % in the fed state [5], in agreement with MRI data determining the free water volumes in the entire large intestine [11]. Liquid volumes increase 4.6 times in the fasted and 3.6 times in the fed state upon passage from the ileum into the ascending colon [8].

In distal regions of the small intestine slurry or fluid pockets are rarely present [11]. By contrast, based on MRI studies, in the fasted colon the fluid appears to be distributed in such pockets [42]. Postprandially in the colon, the number of pockets is significantly increased but individual pocket capacity was unchanged [11]. It should be noted, however, that endoscopic observations of the lumen in the distal ileum and especially in the proximal colon about 5 h post administration of the reference meal do not reveal distinct pockets but rather a generally wet mucosa [5, 8, 43].

Residence times in the lower intestine are considerably longer and more variable, compared with the small intestine. Longer transit times have been observed for multiparticulates compared to monolithic objects [44, 45]. For the proximal colon specifically [44, 46-48], a mean value of 11 hours with a standard deviation of 4 hours has been reported but transit times of less than 1 hour have also been reported for this region [34, 39, 49].

In the lower intestine, pressures are slightly higher than those in the small intestine, but still clearly below the gastric ones. In the fasted state pressures of 140(75) mbar have been reported, while after the reference meal they are slightly higher, at 164(29) mbar [35]. The maximum pressure

events at the ileocecal junction were measured at 60(35) mbar, significantly lower than those measured in the antropyloric region. [7]

2.2. Physicochemical characteristics and intraluminal composition

2.2.1. Stomach

After administration of a solid-liquid meal the median pH values reported from various studies 30 min after consumption were 3.6 - 4.1 and it takes more than three hours to return to baseline levels (Figure 1 and Table 2). Although median values after administration of the reference meal do not differ substantially between studies [7, 9], intersubject variability is high [7] (Figure 1). It is interesting to note that homogenization of a solid-liquid meal with half the calories of the reference meal leads to slightly higher median pH during the first hour after administration (Figure 1, [4]). Importantly, measurement after administration of a homogeneous liquid meal leads to substantially higher intragastric pH values during the first 3 hours after meal administration compared to other meal types (Figure 1, [19]).
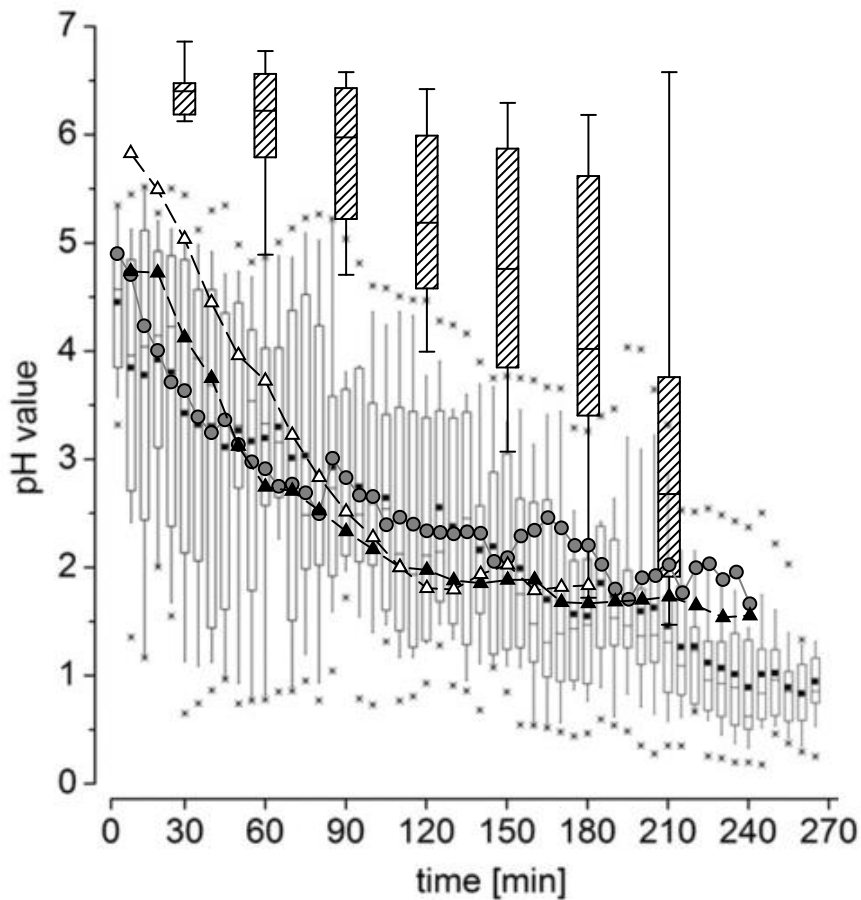
Figure 1 Gastric pH–time profiles after administration of meals with different texture based on data from various studies: reference meal [7] (white boxplot); reference meal [9] (⬤); solid-liquid meal [4] (▲); homogenized meal [4] (M); homogeneous liquid meal [19] (lined boxplot).

Data after administration of a solid meal containing 22 mM Cl^-, 25 mM Na^+, 32 mM K^+ and minimum amounts of calcium (no dairy products, Table 1, [10]), suggest that, 30 and 90 min after eating, chloride concentrations are, on average, 82 and 96 mM, respectively, lower than the values reported for the fasted stomach [50] (Table 2). Average sodium concentrations are lower than chloride concentrations and lower than values measured in the fasted state. Average potassium concentrations are lower than sodium concentrations in all cases and higher than potassium concentrations in the fasted stomach. Calcium concentrations are minimal in both prandial states (Table 2).

Information about other physicochemical characteristics and on the composition of gastric contents has been collected, after administration of liquid meals. Buffer capacity and osmolality are higher in

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the fed than in the fasted state (Table 2). Surface tension is about 30 % lower than in the fasted state. As bile salts are generally not detected in gastric contents samples, the reduced surface tension may be related to the presence of lipids and lipid digestion products (Table 2). Phospholipid and cholesterol levels decrease over time after meal administration. Concentrations of triglycerides (TG), diglycerides (DG), monoglycerides (MG) and free fatty acids (FFA) are not changed substantially during the first 3 hours after the meal. Pepsin levels are slightly higher in the fed state compared to the fasted state. Human Gastric Lipase (HGL) secretion is triggered by food intake. Due to dilution of gastric contents, HGL concentrations are lower than in the fasting state but they increase with time after meal administration, reflecting the ongoing secretion which more than compensates for losses due to gastric emptying [51].

Table 2: Physicochemical characteristics and composition of gastric contents at various times after administration of a glass of water and after administration of a meal (Table 1) to fasted adults.[a]

	Fasted state		Fed state [b]				
	10-20 min	30-40 min	0.5h	1h	2h	3h	4h
pH	1.7 - 3.3 [19, 52, 53]	1.6 - 2.7 [19, 52, 53]	**3.6 - 4.1** [4, 7, 9]	**2.7 - 3.3** [4, 7, 9]	**2.0 - 2.3** [4, 7, 9]	**1.5 - 2.2** [4, 7, 9]	**0.7 - 1.6** [4, 7, 9]
Buffer capacity (mmol/L/ΔpH)	4.7 - 21.3 [19, 52] (NaOH titration)	18 - 27.6 [19, 52] (NaOH titration)	25 [19] (HCl titration)	23 [19] (HCl titration)	23.2 [19] (HCl titration)	29.8 [19] (HCl titration)	na
Osmolality (mOsm/kg)	44.9 - 103.6 [19, 52]	117 - 178 [19, 52]	531 [19]	474 [19]	442 [19]	321 [19]	na
Surface tension (mN/m)	43.2 [19, 52]	43.0 [19]	31.2 [19]	30.3 [19]	30.6 [19]	30.7 [19]	na
Cl⁻ (mM)	41.0 - 110.1 [52]	176.3 [52]	**82** [10]	**96** [10]		na	
Na⁺ (mM)	68 [50] c		**40** [10]	**30** [10]		na	
K⁺ (mM)	13.4 [50] c		**29** [10]	**24** [10]		na	
Ca⁺⁺ (mM)	0.6 [50] c		**1.5** [10]	**1.5** [10]		na	
Bile salts (mM)	0.014 - 0.032 [19, 52, 53]	0.013 - 0.147 [52, 53]	<LOQ [19] d				
Phospholipids (mM)	na		na	2.9 [16]	1.9 [16]	0.9 [16]	0.4 [16]
Cholesterol (mM)	na		na	1.2 [16]	1.2 [16]	0.7 [16]	0.4 [16]
FFA (mM)	na		na	9.4 [16]	14.0 [16]	15.3 [16]	7.3 [16]
MG (mM)	na		na	2.5 [16]	3.2 [16]	4.6 [16]	1.1 [16]
DG (mM)	na		na	8.2 [16]	17.7 [16]	13.5 [16]	9.1 [16]
TG (mM)	na		na	157.1 [16]	150.4 [16]	154.0 [16]	42.7 [16]
Pepsin (mg/mL)	0.17 [19]	0.24 [19]	0.26 [19]	0.33 [19]	0.37 [19]	0.56 [19]	0.37 [19]
HGL (µg/mL)	108 [54]		15 [14]	32 [14]	35 [14]	77 [14]	na

FFA, free fatty acids; MG, monoglycerides; DG, diglycerides; TG, triglycerides; HGL, human gastric lipase; na, not available; [a] Range of median values for pH, range of mean values for all other parameters, based on various published relevant studies; [b] Bold data have been collected after administration of solid-liquid meals; [c] Fasted state without prior water administration; [d] LOQ: 500 µM

2.2.2. Upper Intestine (Duodenum and Proximal jejunum)

To date, no measurements of physicochemical characteristics (including pH values) and composition of contents, after administration of the reference meal have been reported in the open literature. Based on data collected, after administration of a solid meal containing 22 mM Cl^-, 25 mM Na^+, 32 mM K^+ and minimum amounts of calcium (no dairy products, Table 1, [10]), chloride concentrations were 72-137 mM in upper small intestine and 40-100 mM in mid-jejunum (Table 3). Sodium concentrations were similar to chloride concentrations and much higher than potassium concentrations whereas calcium concentrations were minimal. No clear differences from the fasted state level could be observed (Table 3).

Based on data with liquid meals, postprandial duodenal pH seems to be slightly lower than in the fasted state, with pH values decreasing through the course of digestion. Duodenal buffer capacity is higher than 20 mmol/L/ΔpH after meal intake, i.e. more than double the average value measured after the administration of a glass of water in the fasted state. It should be noted that buffer capacity values lower than those presented in Table 3 for the fasted state have been reported [55-57] but in the experiments that are not included, the relevant measurements were performed after one freeze-thaw cycle of the aspirates, which may have led to discharge of bicarbonate as carbon dioxide from the samples. Postprandial bile salts and phospholipid concentrations are highly variable (Table 3), but the bile salt /phospholipid ratio remains fairly constant at approximately 3.36 [21], significantly lower than the fasted state (median value 11.5) [58].

Dietary fat content influences gallbladder contractions and, consequently, bile secretion [20]. 2 g of lipid are enough to stimulate gallbladder contraction [59] whereas 10 g of fat is considered the threshold for a maximal gallbladder emptying stimulus [60]. Cholesterol levels and variability increase after meal intake. The main cholesterol source, apart from food, is from the bile secretions and, therefore, the cholesterol levels tend to echo those of the bile salts and phospholipids [21]. Meal lipids and digestion products in upper intestine vary with meal composition. FFA and MG levels increase whereas TG decrease with time compared to the fed stomach (Table 3 vs. Table 2). The

majority of meal related lipid species are FFA, reflecting the rapid and effective conversion of TG into FFA and MG.

Duodenal contents are hyperosmotic, in most cases (Table 3). Clarysse *et al.* [19, 20] found osmolality to range between 122-516 mOsm/kg and 174-619 mOsm/kg in fed and fat-enriched fed states, respectively. With regard to the enzyme secretions in the small intestine, a 5- to 10-fold increase in pancreatic lipase and a 5-fold increase in phospholipase-A2 are observed in the fed state, while there is no difference in esterase activity between the prandial states [21]. Duodenal surface tension is quite stable in the post-prandial state and lower than in the fasted state (Table 3). Total protein content after food intake is significantly higher than in the fasted state, which can be attributed to the increased presence of enzymes as well as the proteins in the meal, many of which are relatively resistant to enzymatic digestion [19] (Table 3).

Persson *et al.* [56, 61] characterized the physicochemical properties of the proximal human jejunum contents, after perfusing the region with the liquid meal Nutriflex® for 90 min at 2 mL/min to simulate the gastric emptying rate. Nutriflex® is usually used for parenteral nutrition of patients with mild to moderate catabolism, and contains partly metabolized TG and proteins, in similar amounts to those which would be produced via degradation in the stomach prior to emptying into the small intestine. The amount of fat administered was low, corresponding to about 1/4 of the reference meal. Jejunal fluid was collected at 10-min intervals throughout the perfusion. pH was measured at 6.1, lower than the fasted jejunal pH which was reported as 6.9. Buffer capacity was determined by titration both with acid (14.6 mmol/L/ΔpH) and base (13.2 mmol/L/ΔpH) at 37 °C after one freeze-thaw cycle, which is higher than in the fasted state where mean values were 2.8 (base) and 2.4 (acid) mmol/L/ΔpH. Mean surface tension was 27(1) mN/m, similar to the fasted state value of 28(1) were significantly

increased in the fed compared with the fasted state mN/m. The osmolality of the fed jejunal fluids was not measured, while fasted state osmolality was reported to be about 280 mOsm/kg. Bile salt concentration was measured at 8.0mM, higher than the 2.52 mM fasted state value. Phospholipid

concentration in fed jejunal fluid was between 2.0-3.0 mM and also followed the pattern of the bile

[56, 58, 61].

Table 3: Physicochemical characteristics and composition of contents of upper intestine, about 30 min after administration of a glass of water and at various times after administration of a meal (Table 1) to fasted adults.[a]

	Fasted state	Fed state **			
	30 min	30 min	1 h	2 h	3 h
pH	6.1 - 7.0 [9, 19, 20, 39, 52, 62]	6.2 - 6.6 [18, 19, 21]	6.3 - 6.5 [18, 19, 21]	5.3 - 6.1 [18, 19]	5.6 - 5.8 [18, 19]
Buffer capacity (mmol/ L/ ΔpH) (HCl titration)	6.9 - 9.0 [19, 52]	28 [18, 19]	22 - 27.4 [18, 19]	18 - 23.3 [18, 19]	12 - 25.6 [18, 19]
Osmolality (mOsm/ kg)	115 - 206 [19, 20, 52, 55, 62]	291 - 391 [18-20]	360 - 402 [18-20]	274 - 423 [18-20]	215 - 364 [18-20]
Surface tension (mN/ m)	32.7 - 35.3 [19, 20, 52]	31.3 - 35.1 [19, 20]	30.3 - 32.9 [19, 20]	30.4 - 34.0 [19, 20]	30.2 - 35 [19, 20]
Cl^{-} (mM)	106.6 [52]	72 - 137 duodenum-prox. jejunum , 40 - 100 mid-intestine [10]			
Na^{+} (mM)	142 [50] c	95 - 140 [10]			
K^{+} (mM)	5.4 [50] c	18 - 6 gradual decrease [10]			
Ca^{++} (mM)	0.5 [50] c	0.25 - 2 [10]			
Bile salts (mM)	3.66 - 7.74 [16, 20, 21, 52, 53, 58]	10.1 - 14.0 [18-21]	5 - 18.2 [15, 18-21]	3.9 - 7.7 [18-20]	3.7 - 7.3 [18-20]
Phospholipids (mM)	0.32 - 0.91 [20, 21, 52, 63]	3.9 - 6.0 [18-21]	2.87 - 7.1 [15, 16, 18-21]	1.5 - 5.6 [16, 18-20]	1.4 - 4.3 [16, 18, 20]
Cholesterol (mM)	0.08 - 0.44 [21, 52, 56]	0.75 - 1.50 [18, 19, 21]	0.68 - 3.12 [15, 16, 18, 19, 21]	0.40 - 1.2 [16, 18, 19]	0.30 - 1.4 [16, 18, 19]
FFA (mM)	0.95 - 1.55 [21, 52, 63]	30.2 - 52.0 [18, 19, 21]	21.7 - 54 [15, 16, 18, 19, 21]	42 - 46 [16, 18, 19]	34.7 - 56.9 [16, 18, 19]
MG (mM)	0.36 - 0.39 [16, 20, 21]	5.9 - 9 [18, 19, 21]	7.08 - 11 [15, 16, 18, 19, 21]	5.21 - 9.6 [16, 18, 19]	4.20 - 18.4 [16, 18, 19]
DG (mM)	na	1.1 - 6.5 [18, 19, 21]	1 - 10.7 [15, 16, 18, 19, 21]	4.20 - 12.6 [16, 18, 19]	2.6 - 33.7 [16, 18, 19]
TG (mM)	na	1.16 - 4.7 [18, 21]	0.76 - 60.7 [15, 16, 18, 21]	1.90 - 44.7 [16, 18, 19]	0.60 - 63.3 [16, 18, 19]
HPL (µg/mL)	33 [21]	845 [14]	407 [14]	250 [14]	340 [14]
Phospholipase A2 (ng/mL)	4.5 [21]	16 [21]	12.2 [21]	na	na
Esterases (U/mL)	1190.7 [21]	1148.2 [21]	759.2 [21]	na	na
Total protein content (mg/mL)	1.1 - 2.8 [19, 52, 56, 63]	6.1 - 13.7 [18, 19]	5.5 - 15.1 [18, 19]	4.3 - 11.0 [18, 19]	2.9 - 11.4 [18, 19]
Total carbohydrate content (mg/mL)	na	62.7 [19]	72.0 [19]	74.3 [19]	70.1 [19]

FFA, free fatty acids; MG, monoglycerides; DG, diglycerides; TG, triglycerides; HPL, human pancreatic lipase; na, not available. [a] Range of median values for pH, range of mean values for all other parameters, based on various published relevant studies; [b] Only data after administration of liquid or homogenized meals have been published; [c] Fasted state without prior water administration

2.2.3. Lower Intestine (Distal ileum and Proximal colon)

Most of data, to date, have been collected, five hours after the administration of the reference meal, i.e. when drugs administered as conventional products or multiparticulate modified release (MR) products are expected to reach the lower intestine after oral administration.

In the distal ileum the pH is slightly alkaline (Table 4) with values 5 hours after administration of a glass of water to fasted adults being similar with those 5 hours after administration of the reference meal [8] In the proximal colon (cecum and ascending colon) the pH 5 hours after a glass of water to fasted adults is about 7.8 whereas 5 hours after the reference meal it is lower, about 6.0 (Table 4), due to the increased bacterial fermentation activity after the meal [39].

The buffer capacities of ileal, cecal and ascending colon contents (measured by titration with HCl) were significantly increased in the fed compared to the fasted state. In the fed state, values are similar in cecum and ascending colon, while in the distal ileum the buffer capacity is generally lower (Table 4).

Lower intestinal contents are hypo-osmotic, with values lower than in the duodenum in both prandial states. [5, 8].

Due to bile acid absorption in the distal ileum, bile acid concentrations in the lower intestine are significantly lower than in the upper small intestine, regardless of the prandial state (Table 3 vs. Table 4). In the cecal contents, differences in bile salt concentrations between the prandial states are not significant. In the contents of the ascending colon bile salt concentrations are higher in the fed than in the fasted state (Table 4). Diakidou *et al*. [5] reported higher concentrations of primary bile acids (cholic acid, chenodeoxycholic acid) than secondary bile acids (deoxycholic acid, lithocholic acid) in the fed ascending colon, which is the opposite to the fasted state situation. They hypothesized that with more bile acids entering the colon after meal intake (Table 4), the capacity for conversion to secondary bile acids is saturated, resulting in more bile acids in the primary form.

Cecal and ascending colon contents have similar FFA concentrations that are two-fold higher than those observed in the ileum for both prandial states. Regarding the phospholipids, an increase from

ileum to cecum and then to the ascending colon is observed in the fasted state. In the fed state, there is no significant difference between ileal and cecal phospholipid concentrations, but the ascending colon values are increased (Table 4).

There is no significant difference between the two prandial states with regard to cholesterol levels among the three lower intestinal regions and the observed levels are similar to those in fed duodenal contents. These observations are attributed to the low net cholesterol absorption (regulated by influx and efflux transporters in the proximal small intestine) as well as the smaller fluid volume in the lower comparing to the upper intestine, and are in line with the fact that cholesterol is mainly eliminated via the fecal route [11, 64, 65]. The non-significant difference between cholesterol intracolonic levels in the fasted and the fed state (Table 4), support the claim that dietary cholesterol constitutes only 30% of total intraluminal cholesterol [64].

There is a general trend to a lower protein content in the fed state samples, which can be explained by the fact that digestion and absorption of peptides administered with the meal is almost complete by the end of the first meter of the small intestine [66]. In contrast to proteins, carbohydrates administered with the meal may or may not be digested in the small intestine and may therefore reach the colon. In the fed state, total carbohydrate concentrations were indeed higher. In the fasted state, an increase in carbohydrate content from distal ileum to cecum and then into the ascending colon is observed, but this trend is not apparent in the fed state. Total carbohydrate content in the fed colon (Table 4) was calculated to be approximately 30% of that observed in the fed upper small intestine. [5, 8, 19].

Table 4: Physicochemical characteristics and composition of lower intestinal contents 5 hours after administration of a glass of water and 5 hours after administration of the reference meal to fasted adults. [a]

	Fasted state			Fed state **		
	Distal Ileum	**Cecum**	**Asc. Colon**	**Distal Ileum**	**Cecum**	**Asc. Colon**
pH	7.7-8.1 [8, 39]	7.4 [8]	7.8 [5]	**8.1** [8]	**6.4** [8]	**6.0** [5]
Buffer capacity (mmol/L/ΔpH) [c]	8.9(3.6) [8]	19.2(10.2) [8]	21.4 [5]	**15.2(8.4)** [8]	**33.6(13.1)** [8]	**37.7** [5]
Osmolality (mOsm/kg)	60(50)[8]	144(65) [8]	81 (102) [5]	**252(245)** [8]	**267(197)** [8]	**224(125)** [5]
Surface tension (mN/m)	na	na	42.7 [5]	na	na	**39.2** [5]
Bile salts (µM)	71(151) [8]	183(221) [8]	115.2(119.3) [5]	**182(132)** [8]	**280(305)** [8]	**587.4(412.8)** [5]
Phospholipids (µM)	73(41) [8]	166(110) [8]	362(210) [5]	**40(51)** [8]	**82(77)** [8]	**539 (393)** [5]
Cholesterol (µM)	413(309) [8]	1004(1072) [8]	1703(1764) [5]	**317(426)** [8]	**640(771)** [8]	**1882(1325)** [5]
FFA (µM)	63(47) [8]	143 (118) [8]	119.8 [5]	**64 (72)** [8]	**150 (141)** [8]	**225** [5]
Total SCFA (mM)	8.6(6.6)[8]	32.2 (17.6) [8]	30.9 [5]	**5.8 (4.7)** [8]	**29.3 (15.4)** [8]	**48.1** [5]
Total protein content (mg/mL)	5.1(3.3) [8]	10.2(2.2) [8]	9.7(4.6) [5]	**3.39(0.74)** [8]	**6.2(3.2)** [8]	**6.9(2.3)** [5]
Total carbohydrate content (mg/mL)	1.55(0.99) [8]	2.3(1.0) [8]	8.1(8.6) [5]	**12.7(5.3)** [8]	**9.8(7.0)** [8]	**14.0(7.4)** [5]

FFA, free fatty acids; SCFA, short chain fatty acids; na, not available; [a] Median values for pH, mean(SD) values for all other parameters, based on various published relevant studies; [b] Bold data have been collected after administration of the reference meal. [c] HCl titration.

3. THE ROLE OF DRUG AND FORMULATION ON THE OCCURRENCE OF FOOD EFFECTS

3.1. Drug Solutions

Only a few studies in humans have dealt with the gastric emptying of drug solutions in the fed state. Hens *et al*. [67] dissolved a 250 mg paromomycin Gabbroral® IR tablet in 250 mL water and administered it 20 min after the administration of a liquid meal (Ensure® plus). Individual paromomycin concentration in stomach vs. time profiles had several distinct peaks, suggesting that distribution of the solution in the stomach with the liquid meal was heterogeneous. Another study with diclofenac potassium buffered powder for solution, which was dissolved in 30-60 mL of water prior to dosing, showed comparable onsets in drug plasma concentration, after administration in fasted state vs. after the reference meal [68].

3.2. Immediate release tablets and capsules

The impact of a meal on the luminal fate of immediate release (IR) formulations has been explored in several human studies.

Meal-induced delays in tablet disintegration [69] and in capsule disintegration [70, 71] have been documented by using scintigraphic studies in adults. Brouwers *et al.* [72, 73] observed a food-induced delay in absorption of the poorly water-soluble fosamprenavir (phosphoric ester of the week base amprenavir) after administration of the IR tablet Telzir® (700 mg) with a liquid meal (Skandishake Mix®), using a direct sampling technique. Delayed tablet disintegration played an important role. Similarly, Van den Abeele *et al.* [74] studied the GI disposition of the IR tablet Cataflam® (50 mg diclofenac potassium) administered with a liquid meal (Ensure® plus). A meal-induced delay in tablet disintegration in the stomach was observed. *In vitro* modelling methods have shed light on the potential mechanism(s) for the delayed disintegration of IR tablets in the fed stomach (as summarized later in this paper).

For BCS Class II drugs, the increased presence of solubilizing agents in the upper GI lumen after meal consumption generally enhances the dissolution of the dose. However, the delayed disintegration, the potentially decreased diffusivity of colloidal solubilizing species [18, 75-77] or other, meal related mechanisms, may adversely affect the absorption process. For example, various studies have proposed a negative food effect on both the C_{max} and AUC of IR ibuprofen tablets [78-80], despite the higher gastric concentrations (due to the increased gastric pH). Slower gastric emptying, entrapment of the drug into the chyme, and/or elevation of luminal viscosity were among the potential mechanisms hypothesized by the authors of these studies [78-80]. Also, Rubbens *et al.* [81] observed a negative food effect on indinavir (a BCS Class II weak base) bioavailability, after administration of the Crixivan® capsule (400 mg) with a liquid meal (Ensure® plus). Despite similar indinavir concentrations in solution in the stomach in both the fed and the fasted states, in subsequent experiments in which the bioaccessible fraction of indinavir was assessed across cellulose membrane strips, its permeation was 2.6-fold lower when fed state duodenal samples were applied than when fasted state samples were tested [82]. The findings were in qualitative agreement with information in the Prescribers Information for Crixivan® indicating that administration with a meal high in calories, fat, and protein results in 80 % reduction in AUC and 86 % reduction in C_{max} [83]. The authors assumed that the negative food effect may be linked to the micellar entrapment of indinavir in the abundant solubilizing compounds present in the postprandial lumen.

For BCS Class IV drugs the impact of extensive luminal solubilization in the fed state is even less straightforward as in this case transport through the mucosa may also be influenced by changes in the membrane fluidization (which may be induced by the interaction with surfactants) or the activity of membrane transport carriers [84, 85]. Geboers *et al.* [86] observed a positive food effect on IR tablets containing 250 mg abiraterone acetate (Zytiga®), a BCS Class IV drug, after administration with a liquid meal (Ensure® plus). Duodenal concentrations did not reflect the higher plasma

concentrations in the fed state, as intraluminal AUC was not significantly different between fasted and fed state. Based on *in vitro* data, it was suggested that slower intragastric degradation and hydrolysis in the fed state lead to similar duodenal concentrations as in the fasted state, whereas further down the small intestine the higher solubility in fed-state intestinal fluids leads to superior absorption in the fed state.

It is important to note that the impact of viscosity on intraluminal events may have been underestimated to date as intraluminal data have been mostly collected after administration of homogeneous liquid meals.

3.3. Modified Release Products

For MR products, especially non-disintegrating products, perhaps the most important impact of coadministration with meals is the potential for dose dumping. This can occur in the stomach due the long residence time and the mixing conditions in the fed state [12, 87, 88]. Alternatively, dose dumping can be induced by the pressures developed at the ileocecal junction, where products can remain for about 25 min (median value for monolithic dosage forms) [89]. Loss of the MR characteristics in the fed state i.e. dose dumping has been described in several studies e.g. [90], especially for hydroxypropylmethylcellulose (HPMC) based ER tablets [91-94]. In addition to dose dumping, higher drug release rates for HPMC based tablets can occur due to an increase in the erosion rate after postprandial administration, especially for formulations containing lower amounts or lower molecular weight grade of HPMC [94, 95].

3.4. Bio-enabling formulations

Typically, bio-enabling (synonym: bio-enhanced) formulations refer to lipid-based formulations, amorphous solid dispersions and inclusion compounds with cyclodextrins; formulations containing API nanocrystals are also often given this designation. Bio-enabling formulations are employed for increasing absorption primarily by improving the apparent kinetic solubility, extending the apparent supersaturation and/or facilitating the supply of the micellar phase of intestinal contents with the

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drug and reducing the impact of meal on luminal concentrations of lipophilic drugs [96, 97]. The usefulness of bio-enabling formulations in reducing food effects is reviewed in a companion article in this issue [98].

4. SIMULATING THE INTRALUMINAL PERFORMANCE OF DRUG PRODUCTS IN THE FED STATE

4.1. *In vitro* methods

The objectives of the *in vitro* evaluation of the impact of a meal on luminal performance of orally administered products include the evaluation of risks associated with the administration of a drug product in the fed state, the mapping of likely food effects, the design of better products and finally the potential waiver of *in vivo* food effect studies (provided that metabolism-related or lymphatic transport related food effects are considered separately).
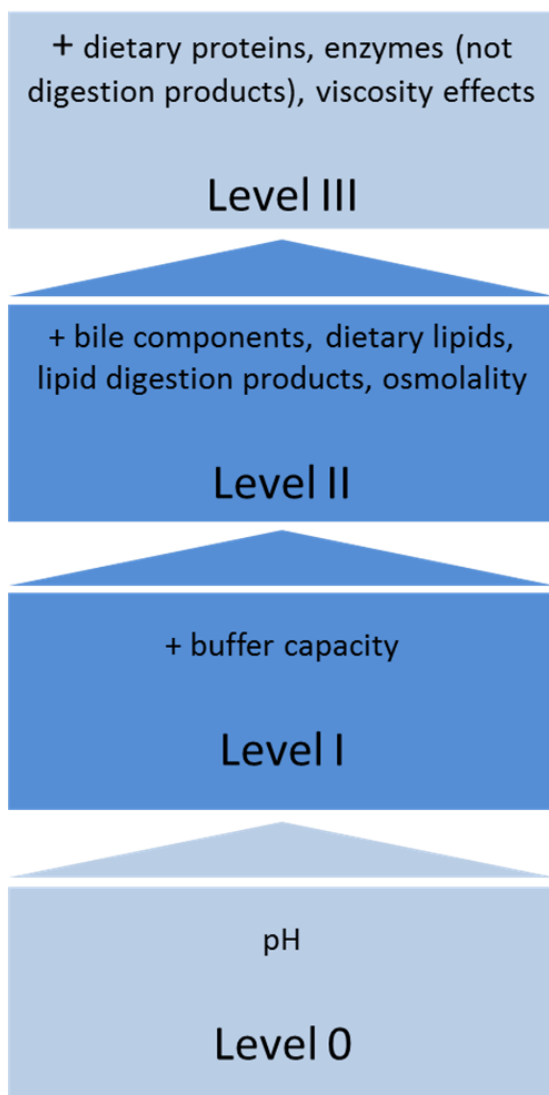
Figure 2: An overview of the four levels of biorelevant media recommended for the simulation of the luminal environment during development of oral formulations (Reproduced with permission from Markopoulos, Andreas *et al*. [99]).

4.1.1. Fed State Simulating Biorelevant media

Composition of luminal contents in the fed state can be simulated at various levels (Figure 2). Recently, the composition of media simulating the conditions in stomach in the fed state at various levels of simulation has been reviewed [51]. Composition of media used to date has been mostly based on data derived from human aspirates obtained after administration of liquid meals. Indicative Level I and Level II simulations of composition of contents in the GI lumen, after administration of liquid meals (Table 1) to fasted healthy adults, are presented in Table 5. It should be noted that, as shown in Figure 1, FeSSGF$_{middle}$ composition better reflects, at least from the pH standpoint, the period early after a solid-liquid meal administration (Figure 1); the suggestion of Markopoulos, Andreas *et al*. [99] for simulating gastric pH 75-165 min after meal intake (Table 5) was based on data collected after a homogenous liquid meal (Figure 1). Since the Markopoulos, Andreas *et al*. [99] work, updates on composition of media reflecting the environment in the lower (ascending and proximal colon) have been proposed, based on intraluminal data after administration of the reference meal obtained in more recent studies [100] (Table 5).

Table 5: Level I and Level II simulation of composition of contents in the gastrointestinal lumen, after administration of a meal (Table 1) to fasted healthy adults [99, 100] [a]

	FeSSGF$_{middle}$	FeSSIF-V2	FeSSIF$_{midgut}$ -V2	SIF$_{ileum}$-V2	FeSSCoF-V2
Lipofundin (%v/v)	9 [b]	-	-	-	-
Sodium taurocholate (mM)	-	10	5	-	-
Sodium cholate (mM)	-	-	-	-	0.6
Lecithin (mM)	-	2	1	-	0.5
Glyceryl monooleate (mM)	-	5	2.5	-	-
Sodium oleate (mM)	-	0.8	0.4	-	0.2
Sodium chloride (mM)	181.7	125.5	102.6	-	-
Glucose (mg/mL)	-	-	-	-	13
Acetic acid (mM)	18.31	-	-	-	-
Sodium acetate (mM)	32.98	-	-	-	-
Maleic acid (mM)	-	71.9	46.5	120	65
Tris (mM)	-	-	-	-	65
Sodium hydroxide (mM)	-	102.4	83	240.6	16.5
Osmolality (mOsmol/kg)	400	390	300	275	207
Buffer capacity [(mmol/L)/ΔpH]	25	25	25	7.6	15
pH	5.0	5.8	6.5	8.0	6.0

[a] Level I simulation of media composition results after eliminating data with bold characters; FeSSGF$_{middle}$, fed state simulating gastric fluid 75-165 min after ingestion of liquid meal; FeSSIF-V2, fed state simulating intestinal fluid in upper small intestine, version 2; FeSSIF$_{midgut}$-V2,Fed state simulating intestinal fluid in mid jejunum, version 2; SIF$_{ileum}$-V2, simulated intestinal fluid in distal ileum, version 2 (Level I and Level II simulation are identical for this medium); FeSSCoF-V2, fed state simulating colonic fluid in proximal colon, version 2; FeSSGF$_{middle}$, FeSSIF-V2, and FeSSIF$_{midgut}$-V2 composition was based on luminal data collected after administration of liquid meals; SIF$_{ileum}$-V2 and FeSSCoF-V2 composition was based on data collected after administration of the reference meal; [b] Equivalent to 8.75 g
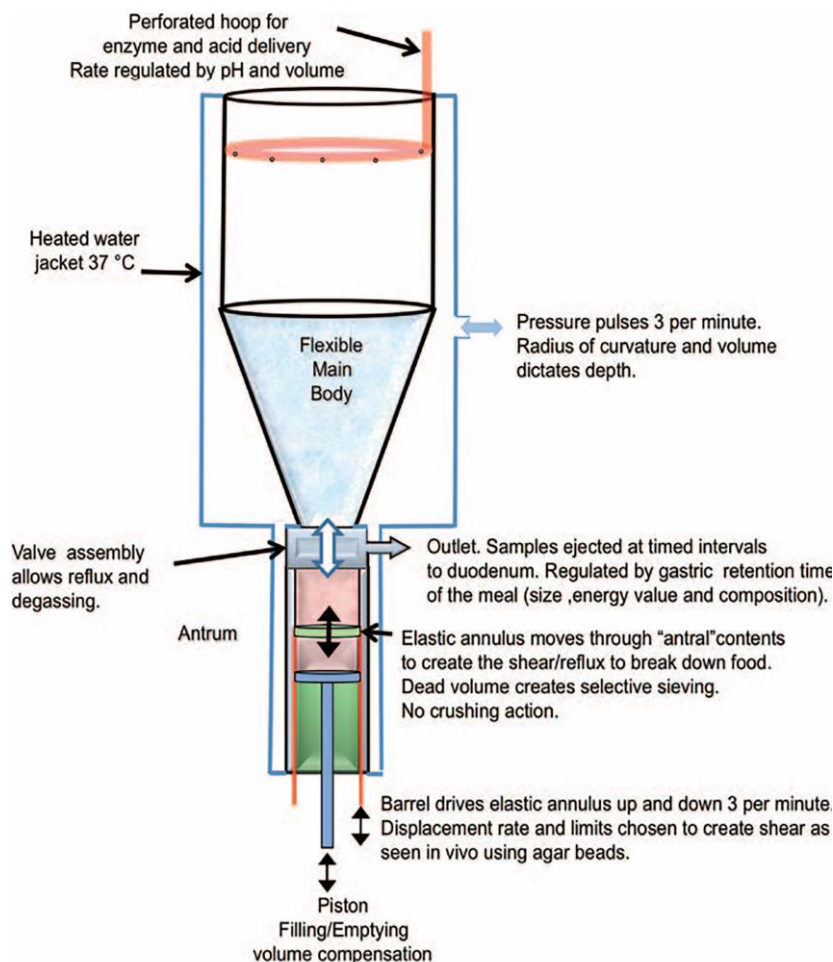
4.1.2. Simulating the intragastric disintegration of IR products

Abrahamsson *et al.* [101] simulated the disintegration of IR tablets in the fed state using the USP Apparatus II and a Level II biorelevant medium, i.e. the liquid meal Nutrison® (153 kcal/100 mL) to which protein, lipids (Intralipid®) and carbohydrates were sequentially added to study the individual effect of each nutritional component. Results revealed the formation of a film of precipitated food components, mainly proteins, around the tablets which slows water penetration and prevents effective tablet disintegration.

Cvijic *et al*. hypothesized that meal induced viscosity can impede the disintegration of IR products of BCS Class III drugs [102]. Radwan *et al.* [103] examined the negative food effect reported *in vivo* for trospium chloride tablet formulations using USP Apparatus II with compendial media and a viscosity-adjusted dissolution medium, simulating the rheological profile of the homogenized reference meal, to simulate the fed state (this corresponds to a Level III biorelevant medium, according to the nomenclature proposed by Markopoulos *et al.* [99]). The results revealed prolonged disintegration times and reduced dissolution rates in the viscous media, which were attributed to changes in the liquid penetration rates. The effect was particularly significant for film-coated tablets.

Kalantzi *et al.* [104] predicted the delayed intragastric disintegration of amoxicillin (weak acid, BCS class I) 250 mg hard gelatin capsules [71] using USP Apparatus II and Level III biorelevant medium for simulating the intragastric conditions in the fed state (cow's milk gradually digested with pepsin) to simulate the fed state [99].

Vardakou *et al*. [105] employed the Dynamic Gastric Model (DGM) [106, 107] (Figure 3) to evaluate the impact of gastric activities in the fasted and in the fed state on the relative performance of gelatin and HPMC capsules. The rupture times obtained from the DGM were similar to those observed by *in vivo* gamma scintigraphy in the fasted state but were delayed in the fed state; the observed delay was attributed to sampling issues when the fed state was simulated with DGM.

Perforated hoop for
enzyme and acid delivery
Rate regulated by pH and volume

Heated water
jacket 37 °C

Flexible
Main
Body

Pressure pulses 3 per minute.
Radius of curvature and volume
dictates depth.

Valve assembly
allows reflux and
degassing.

Antrum

Outlet. Samples ejected at timed intervals
to duodenum. Regulated by gastric retention time
of the meal (size, energy value and composition).

Elastic annulus moves through "antral" contents
to create the shear/reflux to break down food.
Dead volume creates selective sieving.
No crushing action.

Barrel drives elastic annulus up and down 3 per minute.
Displacement rate and limits chosen to create shear as
seen in vivo using agar beads.

Piston
Filling/Emptying
volume compensation

Figure 3 The Dynamic Gastric Model (DGM) (Reproduced with permission from Wickham *et al*. [106]).

4.1.3. Simulating the intraluminal dissolution of IR products

Data collected in cow's milk and in Level II FeSSIF using USP II or IV Apparatus indicate that the impact of food induced changes on intraluminal dissolution of highly soluble drugs is mediated primarily through the delayed disintegration, whereas for drugs with low solubility substantial increases in the extent of dissolution are observed, in line with *in vivo* observations [108-110].

Ratios of dissolution data in Level II FaSSIF and dissolution data in Level II FeSSIF collected over a period of 3 h with the pION µDISS Profiler™ [111] have been significantly correlated with the fasted/fed AUC ratios of corresponding clinical data (n=19; R^2=0.66)[112].

Data collected with the mini-paddle apparatus and Level II or Level III biorelevant media were useful for evaluating the dissolution kinetics of micronized aprepitant in the lower intestine. Combination

of these data with dissolution data collected under conditions simulating the upper GI luminal environment allowed for successful reproduction of the average plasma profiles in the fasted and in the fed state [100].

4.1.4. Simulating the intraluminal release from MR products

Based on data with USP Apparatus III, food components could create a hydrophobic barrier around HPMC based tablets which hampers water permeation [113]. Felodipine release from an extended release (ER) tablet was successfully simulated by using USP Apparatus II and gradually digested milk, with the addition of acidic solutions of pepsin and lipase, as the medium [114].

Based on USP Apparatus I and IV data, fat deposition on the outer surface of the rate-controlling gel layer of HPMC matrices occludes the matrix surface, thereby slowing diffusion of water-soluble drugs [115]. However, the *in vivo* relevance of these *in vitro* results is to be critically viewed, since there is not yet sufficient evidence that they are a source of *in vivo* variability [116].

Andreas *et al.* [117] studied the effect of food on drug release from different delayed and/or ER mesalamine formulations (Asacol® 400 mg, Mezavant® 1200 mg, Pentasa® 500 mg and Salofalk® 250 mg and 500 mg) and designed *in vitro* biorelevant gradient settings (sequential exposures to biorelevant dissolution media) using USP apparatus III and IV to forecast differences between the formulations and to reflect the food effect trends observed in *in vivo* studies. Both USP Apparatus III and IV coupled with Level II biorelevant media were able to capture the *in vivo* behavior of the formulations: Release changes due to meal intake were minimal for Asacol®, Mezavant®, Pentasa® and Salofalk® 500 mg, while for Salofalk® 250 mg release was predicted to occur much earlier in the fed state. The USP Apparatus III generally predicted faster dissolution rates and more pronounced food effects for Salofalk® 250 mg than the USP Apparatus IV, and there was a consistent trend toward a faster dissolution rate in Level II than Level I biorelevant media. However, the limited availability of accurate luminal mesalamine concentrations under both prandial states does not yet allow for any claim about which is the most predictive media/Apparatus combination.

Garbacz and Weitschies developed a dissolution test apparatus to simulate the impact of physiological mechanical stress during the GI passage of monolithic MR dosage forms, termed the "stress test device"[118].They evaluated the device using various ER diclofenac products (Voltaren® retard, Diclofenac-ratiopharm®) and nifedipine products (Coral tablets, Adalat OROS, and Nifedipin Sandoz retard) and found that the dissolution characteristics of some of the tested products were strongly dependent on mechanical stress events of biorelevant intensity, thus drawing attention to the sensitivity of drug release profiles from some dosage forms to GI motility. Formulation-related food effects previously reported in human studies with nifedipine Coral 60 mg (decreased AUC and C_{max} compared to other dosage forms) [92] could be predicted with the stress test device, linking the observed food effect to the lack of mechanical stability and pH-dependent dissolution profiles of Coral [119].

Koziolek *et al.* [120] proposed an *in vitro* setup specifically for modelling release and dissolution in the fed stomach, the Fed Stomach Model (Figure 4). This setup consists of a modified paddle apparatus with gastric vessels that contain two vertical blades moving with different velocities, glass beads at the bottom, a balloon that inflates to create pressures of biorelevant dimensions and a pump to control media flow. The effect of each simulated parameter (pressure, dosage form movement and pump rate) on diclofenac sodium ER tablets was investigated and different scenarios considering the variability in gastric transit of solid oral dosage forms in the fed state were tested. The results were compared and found to be equivalent to *in vivo* data from magnetic marker monitoring experiments, supporting the suitability of the setup to simulate mechanical aspects of the fed stomach [120].
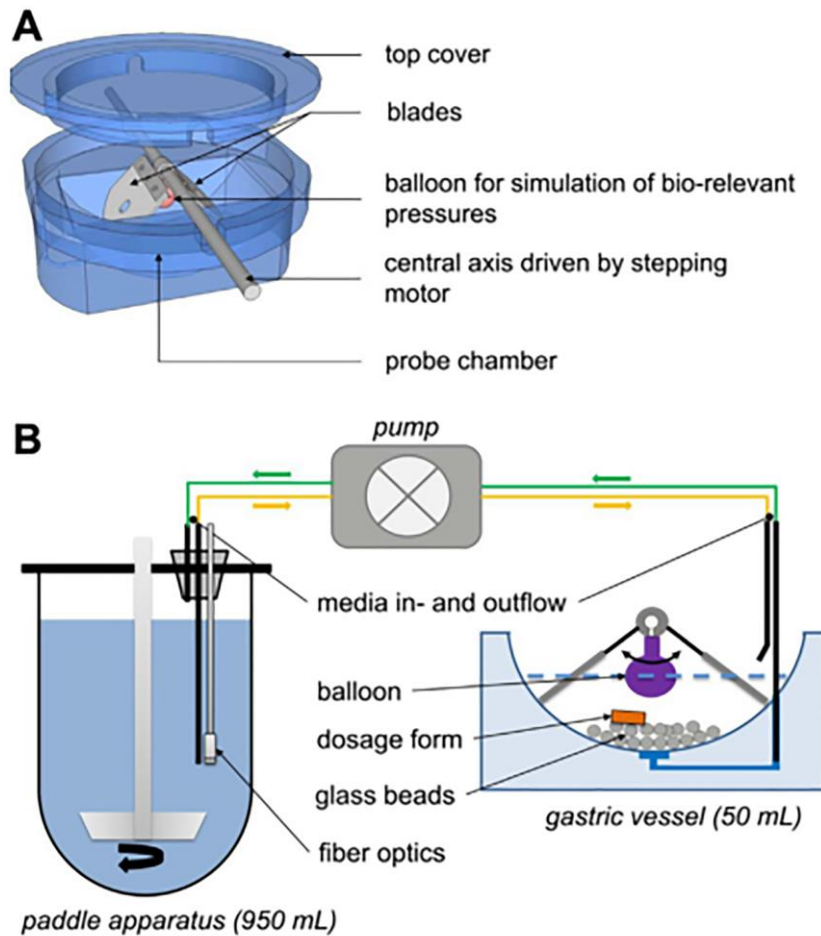
Figure 4: The Fed Stomach Model (FSM) A. The FSM gastric vessel. B. closed loop test configuration (Reproduced with permission from Koziolek *et al*. [120]).

The TIM-2 system simulates the physiological conditions in the large intestine and contains human colonic microbiota originating from healthy volunteers. Tenjarla *et al.* [121] studied the release of 5-aminosalicylate (mesalamine) from a pH-dependent, gastroresistant tablet coated with MultiMatrix System® technology, under fasted and fed conditions. The results demonstrated that 5-aminosalicylate release under simulated small intestinal conditions was minimal, while release in the TIM-2 system was extensive, in agreement with clinical observations [122, 123].

4.1.5 Simulating the dynamic nature of the absorption process

Evaluation of the luminal drug product performance, after oral administration in the fed state, with simultaneous simulation of the transport via the intestinal epithelium has been attempted with the use of compendial or non-compendial apparatus and Level II biorelevant media in conjunction with Caco-2 cell monolayers [124, 125] or a dialysis membrane [97, 126, 127]

Kataoka *et al*. [125] optimized a side-by-side dual chamber system to allow for dissolution of solid forms at the apical side of a Caco-2 cell monolayer [128] (Figure 5). 4 mg of albendazole (BCS Class II) and a self-emulsifying formulation of 1 mg Danazol (BCS Class II) were tested under fasted and fed state simulating conditions and results were in line with the *in vivo* observations [124]. As also shown by others [18], the composition of Level II FeSSIF had to be modified in order to not interfere with the integrity of the simulated epithelial barrier [124, 125].
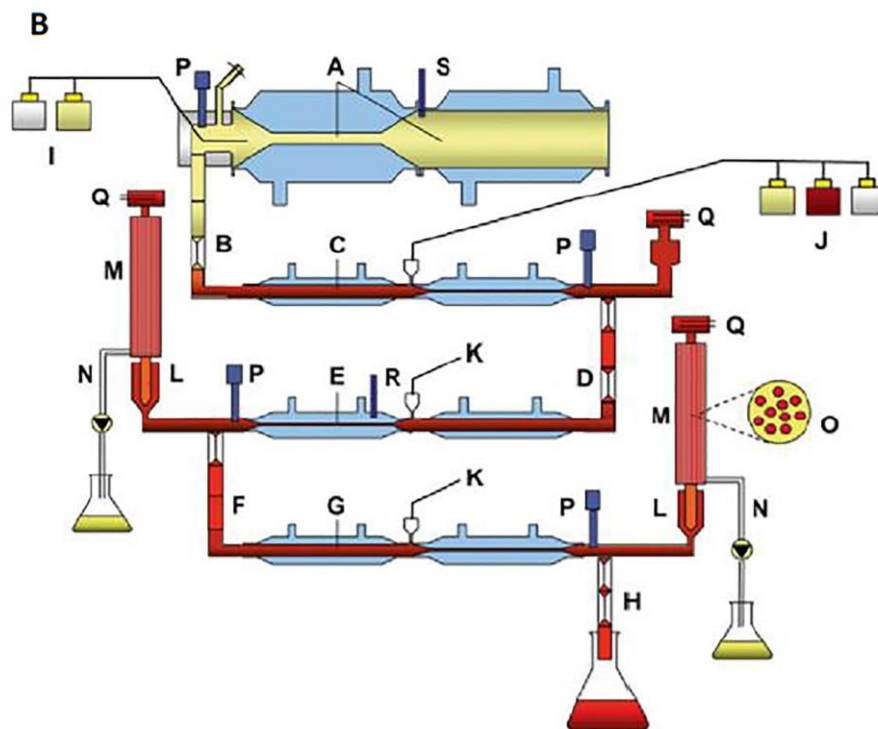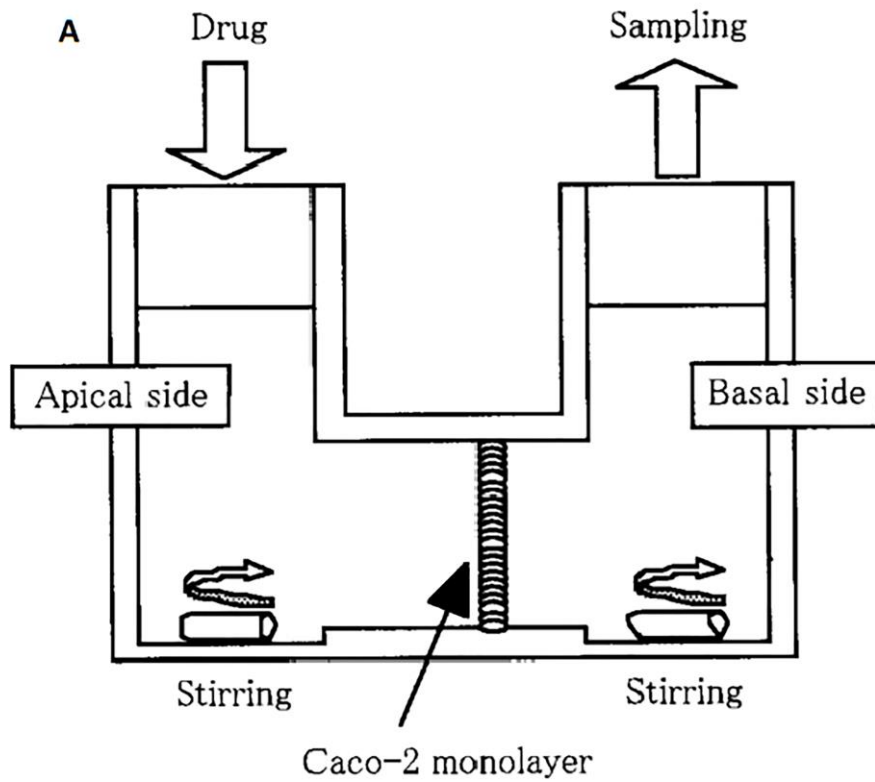
Figure 5: *In vitro* setups proposed for investigating the dynamic nature of the absorption process in the fed state. A. The dissolution/permeation system (D/P system) suggested by Kataoka *et al.*, B. The TIM-1 system. (Reproduced with permission from Kataoka *et al.* [128] and Minekus *et al.* [129]).

Hens *et al.* [97] succesfully implemented the USP Apparatus II in combination with a dialysis bag and biorelevant media (FeSSGF$_{Fortimel®}$ and FeSSIF-V2) to mimic the increased apparent solubility but lack of change in apparent permeability observed in adults after the administration of two fenofibrate (BCS Class II) products: 200 mg micro-sized capsule Lipanthyl® and 145 mg nanosized tablet Lipanthylnano®) with a liquid meal (Fortimel Extra®).

The TIM-1 system addresses the upper and middle GI tract, consisting of four serial compartments simulating the stomach, duodenum, jejunum, and ileum, with dialysis membranes employed to evaluate bio-accessibility of the API from the small intestine [127, 129] (Figure 5). TIM-1 has been shown to be useful in confirming the lack of food effect on the absorption of paracetamol (IR tablets) [126, 127], with the *in vitro* t_{max} and C_{max} values in the paracetamol study reflecting the corresponding values in adults [126, 127].

Brouwers *et al.* [72] applied TIM-1 to study release from Telzir® 700 mg fosamprenavir calcium HPMC coated tablet [73] under fasted and fed conditions. To mimic the fed state, the tablet was introduced into the gastric compartment with a liquid meal (Scandishake Mix®). The results showed delayed tablet disintegration and fosamprenavir dissolution in the fed state, in line with *in vivo* observations [72]. Hens *et al.* [67] used the TIM-1 model to simulate fed state paromomycin luminal disposition and compare it with *in vivo* human data obtained with paromomycin solutions. To obtain a reasonably good fit to the *in vivo* duodenal concentration-time profile, parameters needed to be adjusted: gastric emptying $t_{1/2}$ was decreased from 20 min to 10 min, and duodenal secretion was increased from 1 mL /min to 3 mL/min.

The tiny-TIM system, is a simplified version of TIM-1 consisting of a gastric compartment and one single small intestinal compartment instead of three [129]. Verwei *et al*. [130] used TIM-1 and tiny-TIM to test poorly water soluble drugs (PWSDs) in various formulations [ciprofloxacin: BCS class IV, 500 mg IR tablet Ciprobay® and film-coated ER tablet Ciproxin®, posaconazole: BCS class II, 40 mg/mL IR suspension Noxafil®, nifedipine; BCS class II, 10 mg IR capsule Adalat® and 60 mg ER tablet

Adalat® XL] under fasted and fed conditions. No food effect was observed on the maximum amount of bioaccessible ciprofloxacin during GI transit (BA_{max}) in line with the t_{max}, C_{max} and AUC reported for human studies [131-133]. A large positive food effect was observed for posaconazole, with a 4-5-fold increase in BA_{max}, in line with the *in vivo* 4-fold increase in C_{max} and AUC [134-136]. The bioaccessibility of nifedipine appeared to be higher under simulated fed state conditions for Adalat Eins and Adalat XL. In adults, a minimal food effect has been observed for these products [91, 93, 137, 138]. Comparing the two TIM systems, authors concluded that Tiny-TIM provides better predictions for IR formulations, whereas TIM-1 provides more detailed information on site-specific API release, relevant for MR formulations and food effects. Although TIM-1 has been used extensively within industry for predicting food effects [139], whether or not these systems provide more information than other, perhaps simpler, *in vitro* methodologies in the evaluation of food effects has not been systematically investigated.

An advanced gastric compartment (TIMagc) using an anatomically accurate gastric shape profile was recently introduced, allowing for gastric tone, antral mixing and pylorus opening to be taken into account [140] Van den Abeele *et al.* (Table 1) [74] used Cataflam® (50 mg diclofenac potassium) tablet *in vivo* data as a reference for the evaluation of *in vitro* tools with different levels of complexity, i.e. USP Apparatus II in combination with biorelevant media, a modified dynamic open USP Apparatus IV, and the TIMagc, where fed state was simulated with Ensure Plus®. The authors concluded that all three *in vitro* tools provided information on intraluminal and/or plasma concentrations in the fed state.

Based on published data to date, the usefulness of *in vitro* simulation of the dynamic nature of absorption process for understanding the luminal drug product performance in the fed state is not clear. However, it should be noted that situations where such simulations may be crucial, e.g. in the case of bioenabling products or conventional products where the API's physicochemical properties will drive variation in dissolution with changes in luminal conditions, have been studied to a very limited extent. Madsen *et al.* [141] investigated *in vitro* the reported negative food effect observed

with the amorphous formulation of zafirlukast, BCS class II, (Accolate® film-coated tablets) [142], as well as the effect of the precipitation inhibitors HPMC and PVP on supersaturation and precipitation. In the Level II biorelevant medium simulating the conditions in the fasted upper small intestine the duration of supersaturation was prolonged in the presence of HPMC and PVP. In the Level II biorelevant medium simulating the conditions in the fed upper small intestine lipolysis products caused both a negative effect on the duration of supersaturation and an increased drug concentration during supersaturation, so it was not possible to predict any positive or negative food effects in the presence of HPMC. In contrast, in the presence of PVP, a clear negative food effect was observed, with zafirlukast precipitating in the fed medium but not in the fasted.

4.2. *In silico* methods

Physiologically-based pharmacokinetic (PBPK) modelling is increasingly being used during pharmaceutical development to help to answer formulation questions, as well as to gain a greater understanding of more general biopharmaceutics questions (e.g., prediction of food effect, particle size sensitivity, impact of solubility). Early PBPK modelling approaches for the prediction of dosage form performance in the fed state were published using STELLA®, a platform that can be used to construct models for the *in vivo* performance of oral formulations [143]. The first commercial PBPK modelling software to attempt a comprehensive description of events in the GI tract in the context of a PBPK model was GastroPlus™ [144] and today, together with Simcyp®, they are the most commonly used PBPK software packages. Other software platforms, like PK-Sim®, recently released as open-source [145], gCOAS [146] and GI-SIM [147] have also been developed.

4.2.1. STELLA®

The application of user-customized models built in STELLA® (Structural Thinking Experimental Learning Laboratory with Animation: Cognitus Ltd., North Yorkshire, UK) is not uncommon. STELLA is

a simple simulation tool with a graphical interface for user-built PBPK models. Nicolaides *et al.* [148] used STELLA to simulate plasma profiles from IR products of three BCS Class II non-ionizable compounds (one atovaquone product (Wellvone®), two sanfetrinem cilexetil products (630/C078/49 and 630/C091/59), and one GV150013X product) and one BCS Class II weak acid, troglitazone, formulated as Romozin®D and two prototype formulations (157/155B and D157/155D). Fed state *in vivo* data were available for all compounds. *In vitro* dissolution data were obtained using USP Apparatus II in water, milk, USP simulated intestinal fluid without pancreatin (SIFsp), Level II FaSSIF and Level II FeSSIF. The results showed that the average plasma profiles of lipophilic drugs in the fed state can be predicted with *in vitro* dissolution data in Level II biorelevant media, provided that the absolute bioavailability of the drug is known and that drug absorption is dissolution-limited. Wagner *et al*. [149] predicted the average plasma profiles of a weakly basic BCS Class IV "Compound A", which was under development, by integrating dissolution and precipitation results obtained in biorelevant media with separately obtained permeability data into a STELLA model. Permeability restrictions were introduced into the model using an absorption rate constant calculated *a priori* from the Caco-2 permeability of "Compound A", the effective intestinal surface area and appropriate intestinal fluid volumes.

4.2.2. GastroPlus™

Gastroplus™ uses the Advanced Compartmental Absorption Transit (ACAT) mechanistic model for predictions of oral drug absorption. GastroPlus™ automatically adjusts the concentration of bile salts in each compartment for fasted and fed states. A solubilization ratio based on API logP [150] can be used for the drug if measured solubilities are not available, or it can be predicted from measured solubility in FaSSGF, FaSSIF and FeSSIF. Jones *et al.* [151] developed PBPK models in GastroPlus® to simulate the food effect on the pharmacokinetics of six lipophilic Roche compounds with alkaline properties and logP values ranging from 2.3 to 6.5. Incorporation of physiological parameters and drug solubility data in a series of biorelevant media, enabled the simulation of oral pharmacokinetics

of each drug under fasted and fed conditions. The models were able to predict the magnitude of food effects for each compound and correctly predict the observed exposure to drug. The authors concluded that PBPK modelling coupled with biorelevant solubility tests can be a reliable predictor of clinical food effects caused by solubility and/or dissolution rate limitations. Xia *et al.* [152] integrated *in vitro* and preclinical *in vivo* data in GastroPlus™ to simulate the plasma profiles of NVS123, a weak base with limited, pH-dependent solubility. The magnitude of food effects was successfully predicted and a model-guided parameter sensitivity analysis illustrated that, in this case, enhanced solubility and longer precipitation times under fed state conditions were the main reason for enhanced exposure in the fed state (Figure 6). Pandey *et al.* [153] used GastroPlus™ in order to understand the underlying cause of the dramatic positive food effect observed for a borderline BCS Class II/IV, lipophilic, weakly acidic compound. When biorelevant media solubility data, i.e. Level II FaSSIF and Level II FeSSIF, were included in the model, the model predicted the fasted state clinical data but not the fed state data. Solubility experiments showed that the drug was very soluble in digestible lipids and subsequent experiments in FeSSIF with added 10% Microlipid® (surrogate for high-fat meal media) [154, 155] indicated a greater than 10-fold increase in drug solubility. When the model was run using solubility data generated in FeSSIF with Microlipid® media, assuming a 1 h gastric emptying half-life, accurate simulations were obtained for both the fasted and fed state plasma profiles. Based on these simulations, the clinically observed food effect was attributed to the extensive solubilization of the drug into the dietary lipid content of the meal [153]. Zhang *et al.* [156] developed a PBPK model to investigate the underlying mechanisms for ways to mitigate the observed positive food effect of a weak base. The GastroPlus™ model predicted successfully the observed profiles both under fasted and fed conditions. Solubility and permeability parameters were determined *in vitro*, and human pharmacokinetic disposition parameters were estimated from preclinical data and then optimized using clinical data. A significant precipitation under fasted conditions leading to incomplete absorption explained the low bioavailability in the fasted state, whereas a prolonged precipitation time and increased *in vivo* solubility in the fed state explained the

observed food effect. Cvijić *et al.* [102] used GastroPlus® for evaluating the importance of viscosity in the fed state in delaying dosage form disintegration and reducing absorption BCS Class III APIs (atenolol, metformin hydrochloride, and furosemide). Kesisoglou *et al.* [157] predicted the pharmacokinetics in the fed state of a BCS class I compound (Compound X) based on fasted state data. Fed state exposure simulations were carried out, coupling existing oral PK data in fasted subjects with the fed state physiology in GastroPlus™. A negative food effect was precluded from the generated solubility/dissolution *in vitro* data in the fasted and fed state. Parameter Sensitivity Analysis confirmed that the fed-to-fasted AUC ratio was equal to 1, thus, no significant food effect is expected for a dose up to 5 g.
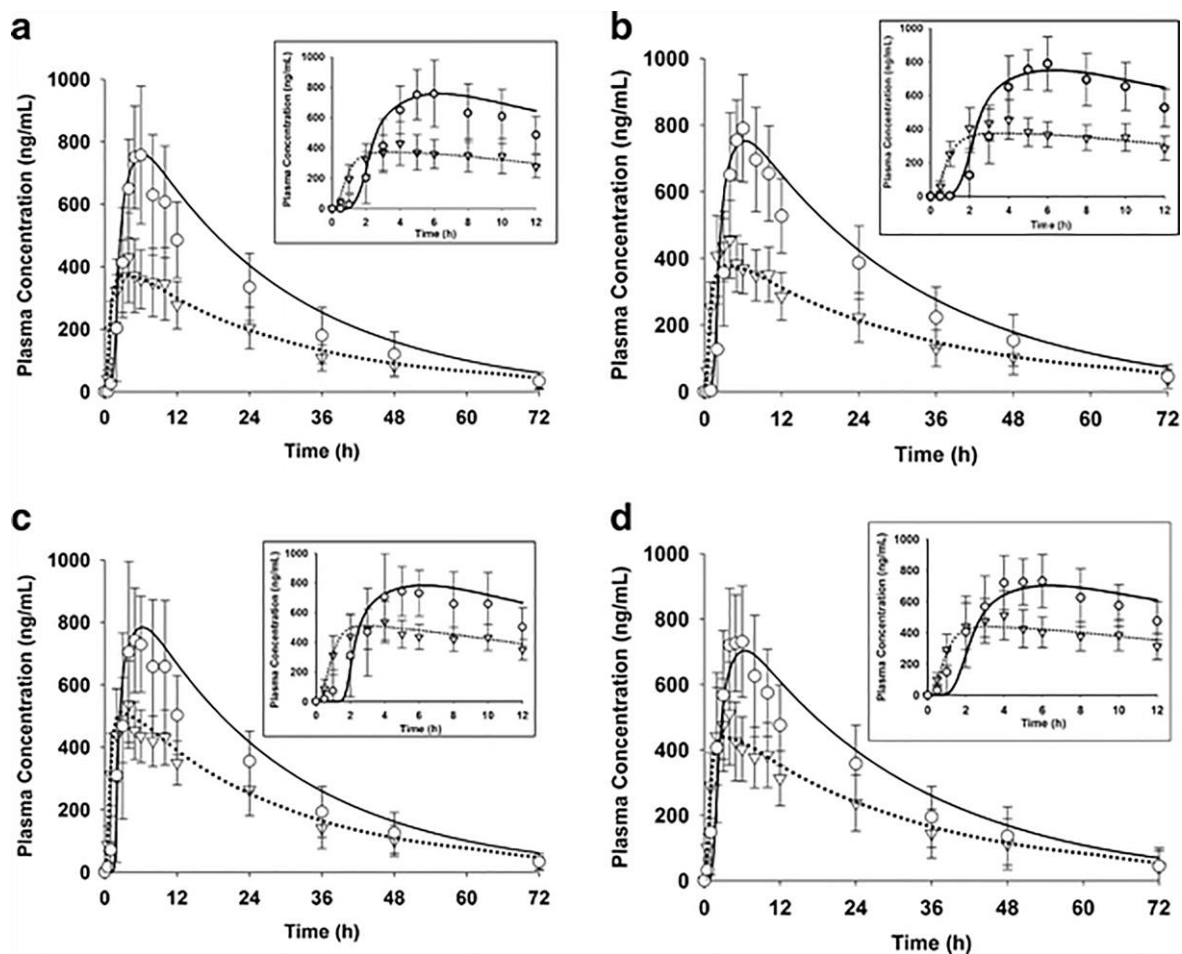
Figure 6: Positive food effect after a single administration of 200 mg of Compound NVS123, a weak base BCS Class II-IV molecule. Panels a, b, c, d represent 4 different formulations of Compound NVS123. Food effect is predicted with a PBPK model built in GastroPlus® by Xia *et al*. Symbol annotation: open triangles are the observed concentrations(SD) in the fasted state; open circles are the observed concentrations(SD) in the fed state; dotted curve is the simulated mean concentration in the fasted state; solid curve is the simulated mean concentration in the fed state. Insert panel: the observed and simulated mean plasma concentrations of each formulation from 0 to 12 h. (Reproduced with permission from Xia *et al*. [152]).

4.2.3. Simcyp®

The Advanced Dissolution Absorption and Metabolism (ADAM) model [158] is a multi-compartmental GI transit model integrated into the Simcyp® human population-based Simulator [158, 159]. The ADAM model addresses the GI tract as one gastric, seven small intestinal and a single colonic compartments. ADAM includes the population mean and inter-individual variability of regional luminal pH and bile salt concentrations in the fasted and fed states. It considers the

interplay of pH and bile salt concentration with solubility and dissolution rate [160, 161]. A fluid volume model addresses luminal fluid, biological secretions rate, fluid absorption rate, gastric emptying and intestinal transit times in the fasted or fed state [159]. Gut wall permeability can be predicted from *in vitro* permeability measurements e.g. Caco-2 cells. Bile micelle partitioning of drug affects free fraction in luminal fluids [162] and thus can lead to an additional food effect where bile salt concentrations are elevated, particularly in the fed state. Regional abundances of gut wall enzymes and transporters [163] can be modeled separately for each compartment [164]. Villous blood flows to each intestinal compartment are increased by 1.3-fold in the fed state to account for increased postprandial blood perfusion. Enterohepatic recirculation of drug is handled with different functions according to fasted or fed status. Patel *et al.* [91] used the ADAM model to describe the prediction of formulation-specific food effects for three nifedipine (BCS Class II) formulations, the IR soft gelatin capsule Procardia® and two CR formulations with different mechanisms of drug release, ADALAT OROS® and Nifedicron encapsulated mini tablets. The nifedipine ADAM model was based solely upon *in vitro* data, aside from prior knowledge of colonic absorption and negligible renal clearance. The model was successful at predicting the different food effects observed with the different formulations [161]. Cristofoletti *et al.* [165] performed simulations with Simcyp® to investigate the possible mechanisms responsible for the differences in the *in vivo* duodenal concentration-time profiles and in the magnitude of food effect between two BCS Class II weak bases, posaconazole and ketoconazole, despite their similar structure and dosing. The food effect is small for ketoconazole, whereas posaconazole exhibits a large positive food effect. Predicted and *in vivo* duodenal concentration-time profiles were compared after varying the precipitation coefficient k_{prec} and the mean gastric emptying time (GET). In a simulated fed state scenario, ketoconazole duodenal C_{max} was approximately 5.7-fold higher under fasting than fed state conditions, but the AUC was similar. In the case of posaconazole, C_{max} and AUC in jejunum were estimated to be 4.6- and 5.6- fold higher after simulating concomitant food intake. The ketoconazole fraction absorbed increased from 64% to 97%, whereas the posaconazole fraction absorbed increased from 6% to 30%,

mirroring the increase in systemic exposure for both compounds. The observed food effects were correctly reproduced *in silico* only when the gastric emptying rate was slowed down (smaller mean GET), micelle-mediated solubility enhancement was accounted for by incorporation of a bile micelle:water partition coefficient ($K_{m:w}$), and jejunal drug exposure, the main absorptive segment for both studied drugs, was considered.

4.2.4. PK-Sim®

PK-Sim® is a whole-body PBPK model distributed by Bayer Technology Services GmbH, [166-168]. Its structure is based on compartmental models [166, 167]. PK-Sim® is fully compatible with the expert modelling software tool MoBi®, allowing full access to model details and extensive model modifications [145]. Willmann *et al.* [168] presented a case-study where PK-Sim® was successfully used to predict the food effect in humans based on data obtained in beagle dogs. The model drug was a BCS class II investigational compound formulated in two IR capsules and an IR tablet, containing different types and amounts of solubility enhancers. The impact of the different formulations and feeding conditions on the plasma pharmacokinetics in Beagle dogs was investigated first, and then the model established in dogs was used to predict the plasma pharmacokinetics in humans under fasted and fed state conditions. Given the interspecies differences with respect to luminal bile salt concentrations and solubility of PWSDs, solubilities in Level II FaSSIF and Level II FeSSIF were considered to appropriately reflect the intestinal solubility. All other parameters were considered species-independent and were maintained as defined in the Beagle studies. The virtual human population was created using the population module of PK-Sim® [169]. To account for physiological changes in the GET in the fed versus fasted state, GET was changed based on literature data, assuming ingestion of the reference meal. The plasma concentration-time profiles under fasted and fed state conditions, which were based on the in-vitro solubility values in Level II FaSSIF and Level II FeSSIF respectively, were satisfactorily predicted [170].

4.2.5. PBPK modelling in the investigation of food effects: Current status

Andreas *et al.* [171] investigated the negative food effect observed *in vivo* for the BCS Class I compound zolpidem, combining *in vitro* and *in silico* approaches. They tested two formulations, an IR tablet Stilnox® 10 mg zolpidem, and two MR tablets Ambien® containing 10 mg and 12.5 mg zolpidem, using compendial methods in the USP Apparatus II and biorelevant methods in the USP Apparatus III and IV. *In vitro* tests suggested that interactions with meal components, resulting in incomplete release, may be the source of the negative food effect for both products. Plasma profiles were successfully simulated with Simcyp® and GastroPlus™, based on *in vitro* dissolution data combined with pharmacokinetics parameters estimated after intravenous administration. A Weibull function was used to describe the dissolution data in GastroPlus™, while a piecewise cubic polynomial interpolation of the observed dissolution data was applied in Simcyp®. Deconvolution of the individual profiles proved that the *in vivo* absorption rate from the MR formulation is formulation-driven in the fasted state, whereas in the fed state, it is mainly controlled by gastric emptying. This study demonstrated that combining biorelevant dissolution testing with PBPK modelling is a useful biopharmaceutical approach for the development of MR oral dosage forms, acknowledging in the same time that further efforts are needed to better characterize and model drug release in the complex and dynamic fed state environment.

In a recent review by the FDA, Li *et al* [143] aimed to build a knowledge base for establishing the predictive performance of PBPK modelling to predict food effects by using the GastroPlus™ and Simcyp® platforms. They underline that caution should be taken when assessing predictive performance of a model mainly because model parameters, most commonly the dissolution rate and precipitation time, are commonly optimized when the PBPK model does not initially capture the food effect. Also, there may be some publication bias towards "good" results. Currently, health authorities do not consider biowaivers for food effect studies for any BCS class, and PBPK modelling for fed-state pharmacokinetics is not accepted in lieu of a clinical study [143].

In another recent article, reflecting the industry view point, Tistaert *et al.* [172] proposed a workflow for PBPK food effect predictions for IR formulations of BCS I and BCS II compounds. Five case studies were used to demonstrate that food effect can be predicted well using appropriately established and validated models. Solubility and/or dissolution data were used for initial model development and a "middle-out" validation with clinical data in one prandial state was applied.

With all PBPK models, it is essential to validate the model with clinical data in both the fasted and fed state data before application to new scenarios. Once validated, the PBPK model can be used to simulate outcomes for new doses, formulations, and/or API forms, in lieu of additional clinical food effect studies.

5.   CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Within each specific GI region the inter-subject variability in the postprandial intraluminal environment is high. In an attempt to provide a picture of how a drug product would perform in the fed state phase of a bioequivalence study, the environment in the GI tract following the administration of meals with compositions (calorie content and origin of calories) similar to that of the reference meal was considered in this review. Even with this restriction, it is apparent that luminal drug concentrations, after administration of a specific drug product are highly variable and available *in vitro* and *in silico* methods do not provide yet sufficient confidence to support decisions to replace clinical studies of food effects. Improving the usefulness of *in vitro* data requires better understanding of the system, for example, how simple dosage forms empty from the stomach when administered 30 minutes after administration of the reference meal. Also, to date, the intraluminal physicochemical characteristics and composition in the fed state have been almost exclusively (apart from pH and ions) studied after administration of liquid meals. Similar analysis for solid/liquid meals would enable optimization and systematic validation of *in vitro* methodologies and increase the usefulness of PBPK modelling approaches for predicting meal effects on oral drug absorption. There is today a great interest among industry and researchers to introduce recent improvements in the GI *in vivo* prediction area into the regulatory arena [173]. Regulatory agencies also support this initiative [142, 173]. To reduce inter-laboratory variability and increase the usefulness of *in vitro* data (including potential regulatory applications), efforts should be taken to develop *in vitro* methodology for simulating the drug product performance after meal administration that is sufficiently representative of the luminal conditions but not unnecessarily complex. To date, PBPK modelling in combination with *in vitro* data is already useful for mechanistic understanding of the absorption process in the fed state. Further improvement of both the *in vitro* tools for estimating the relevant processes and simulation of GI conditions in the PBPK models would lead to more accurate *in silico* predictions, on the one hand, and facilitate the development of products with reduced food effects on the other hand.

Acknowledgements

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