Fed-state Gastric Media and Drug Analysis Techniques: Current status and Points to Consider

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1. Introduction

In vitro dissolution studies are an integral part of quality control and drug development processes. During drug development, they are used as a tool for the selection of the appropriate excipients and the most suitable formulation type [1] and also as an *in vitro* surrogate for *in vivo* performance [2]. In quality control, they are used to ensure the batch-to-batch consistency. [3-5]. Dissolution tests, as dictated by the Pharmacopoeias though, cannot always provide information about the *in vivo* behaviour of the drugs, even though there are cases in which these tests can provide good *in vitro–in vivo* correlations (IVIVC). The dissolution media described in Pharmacopoeia monographs are mainly used for quality control purposes, and are not often able to predict *the in vivo* behaviour of poorly soluble drugs for which the fat content and the bile salts concentration in the gastrointestinal environment will affect their solubility and dissolution rate [6, 7]. Due to the limited ability of the simple aqueous media suggested by the Pharmacopoeias to simulate the characteristics of the gastrointestinal (GI) tract, the need for media simulating the GI physiological environment in the fasted and fed states (usually called biorelevant media) arose; in these media the physicochemical properties of the GI contents (pH, osmolality, surface tension, buffer capacity) are taken into account and physiological components such as bile salts and lecithin are incorporated [2]. Use of biorelevant media during the drug development process enables the assessment of drug's biopharmaceutic characteristics and the prediction of *in vivo* performance [2, 8].

While the fasted state gastric environment has been well studied, the more complex conditions of the fed state stomach have made the prediction of food effect a challenging task. Several *in vitro* biorelevant gastric media have been used for the simulation of the gastric fed state environment and as far as the sample treatment is concerned, there is no specific protocol available and sample treatment and drug analysis are developed on a case by case basis. A good understanding of the *in vivo* conditions of the fed state stomach could lead towards the

development of a suitable medium being able to simulate the gastric content and ideally overcoming the extensive treatment before the analysis that is needed with the current gastric fed state media [9, 10]. The dependence of the drug food effect on the meal content, the role of the fat content in the solubilisation of drugs, the gastric emptying rate and the interaction with certain formulations [11] as well as the binding of drugs with metal ions and meal components are some of the parameters which have rendered the *in vitro* prediction of food effect extremely complicated.

In the current review, initially we describe the available information for the characterisation of the *in vivo* gastric fed state conditions after the administration of standard meals with an aim to provide an understanding of the effect of drug's physicochemical parameters on its *in vivo* behavior. Then, the standard meals and the gastric biorelevant media currently being used and their interaction with drugs of different physicochemical properties are presented. In the last part the analytical techniques used *in vitro* for sample treatment and quantification of the drug along with their challenges are discussed.

2. *In vivo* gastric conditions in the fed state

Gastric conditions in fasted state have been characterised in terms of pH, osmolality, surface tension, buffer capacity and protein content [12-14]. In the fed state though, the determination of absolute values is more complicated than in the fasted state. The food type is an additional factor on top of other parameters responsible for the interindividual variation of the above properties such as the individual's age [15] and administered medication [16]. The role of several physicochemical parameters of the contents of the fed state stomach on drug's dissolution and absorption is reviewed.

2.1. Gastric secretions in the fed state

The main components of the gastric juice are hydrochloric acid (HCl), pepsinogens, mucus and water; pepsinogen is the inactive form of pepsin, activated by the presence of HCl [17]. Pepsin content is higher in the fed state stomach than in the fasted state (fasted state values = 0.11–0.22 mg/mL). Samples of gastric antrum content of twenty healthy volunteers after administration of Ensure Plus®, demonstrated pepsin values within a range from 0.26 to 0.58 mg/mL in a time period from 30 to 210 minutes after administration of the liquid meal [13]. Gastric lipase is also present in the stomach. It is the enzyme responsible for the digestion of fat in the upper gastrointestinal tract. Its role involves the hydrolysis of exogenously administered triglycerides to di-glycerides and fatty acids [18]. Gastric lipase is has been reported to account from 10-30% of the total hydrolysis of triglycerides contained in a meal [19, 20] with the activity of the enzyme measured at 11.4–43.9 U/mL [21]. Its total output after administration of a liquid meal was 22.6 ± 8.1 mg (concentration 16.7 ± 0.7 μg/mL) after administration of a liquid meal in human subjects [19].

2.2. Bile salts in gastric contents in the fed state

Bile salts can increase the dissolution of poorly soluble drugs by decreasing the energy barrier between the drug and the medium, by increasing the active surface area, or via micellar solubilisation [22]. Bile salt concentration in the stomach is much smaller than in the small intestine, where the bile salts are released by the gall bladder, with their concentration in the intestinal environment in the fasted state demonstrating an approximate 4-fold decreased value in comparison to the fed state (1-4 mM and 10-20 mM, respectively) [23, 24]. In the gastric fed state (after administration of 500 mL Ensure Plus®) only traces of bile salts have been reported (60 μM) [13]. Similar bile salts concentration (51 μM) were measured in the fed state

stomach of healthy subjects after a standard lunch (13.5 g protein, 18 g corn oil, and 53 g carbohydrate in 300 mL water) [25].

2.3. Proteins, lipids and carbohydrates in gastric contents in the fed state

The protein, lipid and carbohydrate content in the fed state stomach is dependent on the type of meal consumed before the administration of the drug, therefore their concentration is highly variable and cannot be expressed solely by the results of a single study. Indicatively, the concentrations of proteins and carbohydrates after administration of 500 mL Ensure Plus® to healthy subjects were found to be 23.3 mg/mL and 152.1 mg/mL at 30 min respectively, decreasing to 11.2 mg/mL and 49.1 mg/mL at 210 min after the liquid meal's administration [13].

2.4. pH of gastric contents in the fed state

The pH affects dissolution and absorption of both actively and passively absorbed drugs. The non-ionised fraction of the drug is more efficiently absorbed during passive absorption, while the affinity of the drug carrier for the ionised or non-ionised fraction defines the rate of active absorption [26, 27]. The pH of the stomach in the fed state is significantly higher than in fasted state (pH ≈ 1.7) [13, 28] with a wide range of values between 3-7 [1]. The pH increases up to approximately a value of 6.5 after a meal and decreases exponentially reaching a value of 2-2.7, similar to the pH value measured in the fasted state after 3-4 hours. In case of patients suffering from hypochorhydria/achlorhydria due to pathological conditions (i.e. AIDS; Acquired Immune Deficiency Syndrome) or administered medication (H_2 receptor antagonists or Proton-Pump Inhibitors), initial fasted pH values are elevated compared to the values mentioned above, reducing the dissolution rate of basic drugs [29, 30]. Thirty minutes

after administration of 500 mL of a nutrient drink (Ensure Plus®), Kalantzi et al. reported a pH value of 6.4 in the gastric aspirates of twenty human subjects (Figure 1) and a decrease of the gastric pH to a value close to the fasted state three and a half hours after the liquid meal's administration [13]. The gradual decrease in gastric pH values is attributed to the induction of secretion of gastric acid after the administration of a meal and to the meal's buffering properties [22]. Another *in vivo* study [21] showed that after administration of a liquid standard meal containing 65% fat, 29.5% carbohydrate and 5% protein, the pH reaches a maximum of 4. A study by Yamaguchi et al. [31], which monitored the gastric pH of human subjects using a Bravo® pH monitoring system, with the aid of a capsule placed on the gastric wall, confirmed also the immediate burst and gradual decrease to the fasted state level pH, with it returning to its initial value 2 hours after the administration of a meal. The subjects of the above study were monitored for 48 hours and did not follow any restriction in their dietary routine. The absolute values of these two studies cannot be compared though, as the subjects of the latter did not follow a specific diet. The time needed for the gastric pH levels to return to the initial values and the pH "peak" value of the fed state are dependent upon the type of standard meal administered during each *in vivo* study, the age of the subject and the experimental protocol followed [32]. For instance, the pH decreases to the fasted state value is faster after the administration of a liquid meal than after a solid meal. Gastric pH mostly affects the dissolution of drugs with a pKa value close to the physiological pH values, as when ionised behave as week electrolytes with their solubility being increased in comparison to their un-ionised form [27]. Therefore, changes in gastric pH mostly affect weak acids and weak bases with the increased values in the fed state enhancing the dissolution of acids and reducing the dissolution of bases. Gastric pH can also affect drug release. Coatings with pH-dependent disintegration

properties, like enteric coatings which dissolve rapidly in pH values of 4.5-8 [21] may experience different disintegration profiles due to elevated fed pH value.

2.5. Osmolality of gastric contents in the fed state

Osmolality can affect drug's dissolution rate by inducing changes in the swelling behavior of the formulation. Osmolality is linked with water penetration in the formulation; when the difference in osmotic pressure between the inner and outer (GI environment) part of the formulation decreases, water penetration decreases as well, affecting negatively drug release [33]. The gastric fluids in the fed state are slightly hyperosmotic 30 minutes after administration of Ensure Plus® (559 mOsm kg^{-1}), and their osmolality is decreased to 217 mOsm kg^{-1} 3.5 h after the administration [13], revealing that the osmolality of the gastric contents returns to the fasted state value during this time period (191± 36 mOsm kg^{-1} based on measurements of 24 healthy subjects) [12].

2.6. Surface tension of gastric contents in the fed state

The surface tension of the fed gastric fluids is lower than the one of an aqueous solution due to the presence of surface tension lowering compounds, such as bile salts entering the stomach through duodenal reflux and acting as surfactants, pepsin and food components [34]. Pepsin is an enzyme produced in the mucosal lining of the stomach and acts as a digestive protein in the gastric environment. Since the lowest surface tension values acquired after the addition of biorelevant concentrations (0.003-0.195 mg/mL) of the enzyme (fasted state) in an acidic solution (pH 1.6, HCl solution with 2 g/L NaCl) were 57 mN/m, it can be assumed that other surfactants are present as well in both fasted and fed state gastric fluids, as their surface

tension value was significantly lower with a value of about 30-31 mN/m in the fed state and 33-43 mN/m in the fasted state [4, 35, 36].

2.7. Buffer capacity of gastric contents in the fed state

The buffer capacity of the medium can have a great effect on drug's dissolution in combination with its pH, as change in pH can affect the ionisation percentage and consequently the solubility of ionisable drugs and excipients. In the fasted state, bicarbonate is the buffer mainly present in the stomach [37]. In the fed state though, the buffer capacity is dependent mainly on the meal contents than on the stomach's mucosa, making this chemical property highly meal-dependent [37]. Buffer capacity at gastric fed state conditions after administration of Ensure Plus® is around 14-28 mmol/L·DpH based on *in vivo* measurements [13].

Table 1 summarises the physicochemical properties of the contents of the gastric fed state environment, as measured *in vivo* in human subjects.

3. Drug properties that relate to potential food effect

Food effects can be induced via the direct interaction of drugs, due to their unique physicochemical properties, with food components [38]. Such interactions include formation of insoluble complexes (i.e. tetracyclines and calcium ions) [39], binding to proteins (i.e. phenytoin) [40], or interaction/exchange of drugs with anionic or cationic sites of dietary fibers (i.e. metformin) [41]. In this section the drug properties that can affect drug dissolution and absorption leading to a potential food effect are described.

3.1. Ionisation (pKa)

The pKa determines the percentage of a drug's charged/uncharged form under certain pH conditions and affects the solubility of drugs at differing media pH. Solubility and dissolution of weekly acidic drugs is low at the pH of the fasted stomach as they are mostly in their unionised form. At fed state conditions, where the pH is higher, their gastric solubility and dissolution increase with a subsequent effect on their pharmacokinetics [6]. The uncharged state of the drug has a positive effect on membrane permeability, as the fraction of the unionised form of a drug is proportional to its lipophilicity [42]. As far as weekly basic drugs are concerned, their gastric solubility and dissolution is lower due to the higher pH of the stomach in the fed state in comparison to the fasted state. For compounds which are non-ionisable in the gastric environment a gastric fed state dissolution test is essential [6], as the type of meal consumed affects the surface tension of the gastric contents and thus, the active surface area which is available for drug solubility and dissolution [43].

3.2. Lipophilicity (log P, log D)

Partition coefficient, log P, is indicative of the lipophilicity of a compound and determines the partition of a compound in a system of n-octanol/water. For an ionisable molecule the apparent partition coefficient (log D) is the value which expresses the partition in the aqueous and organic phase in a more accurate way as it takes into consideration its ionised/unionised percentage and therefore log D values vary according to the pH of the environment. Log P values are related to drug's affinity for biological membranes and target sites affecting its biological activity [42]. Ideally, the drug should have such a hydrophilic-lipophilic balance so that it can be dissolved in the biological fluids, where the site of absorption is, and also be able to permeate the membranes of the site of action. Drug's lipophilicity is believed to have an important role in its dissolution in the gastric fed state, as solubility and

dissolution of lipophilic drugs in the fed stomach is performed through their partitioning in the lipid fraction of the meal during its breaking into particles throughout digestion before reaching the small intestine [6].

3.3. Solubility

Solubility is a key drug property for its potential oral absorption. Due to the prolonged residence of the drug in the gastric environment during the fed state, the solubility in the gastric contents will affect drug's dissolution and subsequent absorption. The wetting and solubilisation of drugs co-administered with food can be increased by the digestion products of lipolysis products in the gastrointestinal tract (Figure 2) [22]. The gastric compartment is the part of the gastrointestinal tract where the dietary lipids are emulsified at first place. Facilitated by gastric lipase, gastric agitation and emptying, protein and polysaccharide contents along with lypolytic triglyceride products stabilize the interface between lipid and aqueous phase [18]. Therefore, the presence of gastric lipase in the fed stomach is important when biorelevance need to be achieved for *in vitro* assays. Aqueous media usually give an underestimation of the drugs' solubility in the gastric environment. An underestimation of solubility was also observed for undigested milk, suggesting that apart from having the same pH, buffer capacity, protein content and osmolality, the presence of enzymes should be considered for a good prediction of drug's solubility in the gastric environment [44]. If not adequately soluble in the gastrointestinal fluids, drugs orally administered can have a solubility limited dissolution and non-linear dose responses due to inadequate drug in solution in the site of absorption [42].

3.4. Biopharmaceutics Classification system (BCS) and Food effect

Amidon and co-workers [45] defined drug aqueous solubility and permeability as determining parameters which control a drug's systemic *in vivo* absorption and introduced the Biopharmaceutics Classification System (BCS) which categorizes the drugs in four classes according to their aqueous solubility and permeability:

BCS I compounds: high solubility-high permeability

BCS II compounds: low solubility-high permeability

BCS III compounds: high solubility-low permeability

BCS IV compounds: low solubility-low permeability

Fleischer et al. [46] proposed a food effect predictive model for the drugs' absorption according to their drugs' BCS class (delayed/no effect for BCS class I, increased with fat content for BCS class II, decreased for BCS class III, low and non-predictable for fed/fasted states for BCS class IV); this model is only a general guideline as many drugs do not follow this pattern. The reason is that except for BCS class I drugs, the drugs belonging to the other BCS classes have a wide range of properties and consequently different rate limiting steps for drug absorption. For example, a low solubility compound with absorption just under 90% is classified as a class IV compound, but it is unlikely that its permeability would be the rate limiting step for its absorption [47]. Based on the BCS classification, Wu and Bennet [48], correlated the interactions of the different BCS class drugs with intestinal efflux and reflux transporters. It was suggested that for BCS class II compounds, the relative magnitude of the inhibition between efflux and influx transporters with additional solubilisation in the intestinal environment and gastric emptying are the two parameters affecting the drugs' absorption, with the latter process being the detrimental one. Inhibition of influx transporters was suggested for

the negative food effect for most BCS class III compounds, while BCS class IV compounds combine all the above mechanisms for BCS class II and III drugs (Table 2) making the prediction of food effect difficult [49].

4. Standard meals used in BA/BE studies

Homogenised standard meals have been used as an attempt to simulate gastric fed state conditions. In order to determine the effect of food on drug absorption, both the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) recommend the use of a high-fat meal for the determination of drug's pharmacokinetic parameters in the fed state as the worst case scenario [50]. Meals which are of a high caloric and fat content are recommended in BA/BE studies as these are more likely to affect gastric physiology and have a more pronounced effect on drugs [51]. As a high-fat and high-calorie meal FDA suggests a meal of 800-1000 caloric content with ~50% of the calories deriving from its fat content with 150, 250 and 500-600 kilocalories (kcal) being obtained by protein, carbohydrate and fat, respectively [51]. An example of a typical high fat standard breakfast as proposed by the FDA used in a bioequivalence study for Cicloral® and Neoral®, (100 mg cyclosporine A formulations) [52] is: "2 eggs fried in butter, 2 strips of fat bacon, 120 g hash brown potatoes, 250 mL whole milk, and 1 croissant". For food effect studies, EMA suggests a similar standardised high fat meal (800-1000 kcal caloric content, 500-600 and 250 of which derive from fat and carbohydrates, respectively) and a moderate meal of ~400-500 kcal with ~150 kcal deriving from fat [50]. As far as the dosage strength to be tested in fed state studies is concerned, FDA recommends the testing of the highest dose to be marketed and lower doses if the testing of the former is not possible for safety reasons [51]. According to EMA, the highest

and lowest doses in the drug therapeutic range have to be tested when the drug follows nonlinear pharmacokinetics [50].

Apart from the meals described previously, other types of meals can also be used in the investigation of the effect of food in drug's pharmacokinetics in cases of a specific food effect mechanism. For example, a high-protein meal (80 g protein, 52 g carbohydrate, 9 g fat) was used in the investigation of the pharmacokinetics of gabapentin, an anticonvulsant, whose transport through the biological membranes is controlled by System-L, the L-amino acid transport system [53]. Klein et al. [43] characterised two standard meals (GSK high fat-(62%) standard meal and FDA intermediate fat (37%) standard meal), constituted by homogenised eggs, bacon, butter, milk and other ingredients indicative of a median diet (Table 3). The purpose of the study was the comparison of their physicochemical properties with those of the meals, such as milk and nutrient drinks currently being used for the simulation of fed-state conditions in dissolution studies.

5. *In vitro* simulation of gastric conditions in the fed state (Biorelevant dissolution media)

Even though actual homogenised meals are able to simulate gastric state conditions the best, problems in the analysis of the drugs led to the development of alternative approaches [37, 54]. A range of dissolution media have been developed in order to simulate the *in vivo* conditions of the fed state stomach (Table 4). These media were developed with the aim of having the same physicochemical properties with the standard meal recommended by FDA for BA/BE studies [51].

5.1. Milk-based media

❖ **Milk**

Milk started being used as a dissolution medium for gastric fed state simulation about twenty years ago. Machairas et al. successfully used low fat milk (0.75% fat) with a flow injection serial dynamic dialysis technique (FISDD) as a food simulation medium for drug dissolution, for four drugs of different physicochemical properties; salicylamide, acetaminophen, propantheline and nitrofurantoin. Milk was selected as a dissolution medium in this study due to its potential as substrate of the gastric fed conditions and also due to its use as a vehicle in drug delivery systems [55]. Furthermore, its energy content is similar to that of a standard meal administered to the subjects participating in bioavailability/bioequivalence studies [56]. Despite its similarities with the gastric environment in the fed state, the use of milk does not always simulate the gastric fed state ideally. The issues of the use of milk as a dissolution medium relate to its lower values in osmolality (285 ± 2.7 mOsmol kg^{-1}) and buffer capacity (13.9 ± 0.2 mEq pH^{-1} L^{-1}) at 37 °C compared to the standard high-fat breakfast proposed by the FDA (771 ± 10 mOsmol kg^{-1} and 30.1 ± 1.8 pH^{-1} L^{-1}, respectively) [43]. Other issues relate to its higher pH value (pH ≈ 6.5) than the equivalent pH of gastric media after a meal (5.8 ± 0.2 after 50% of gastric emptying after liquid meal administration) [56], and the possible need of supplementary enzyme addition due to the digestion of milk's lipids and proteins taking place *in vivo* [6].

In 1998, Galia et al. [58], assessed the suitability of full fat milk as a biorelevant gastric fed state medium for the evaluation of the dissolution behavior of one BCS class I drug (acetaminophen) and two BCS class II drugs (danazol, mefenamic acid). The results of this study demonstrated that for BCS class I drugs there is a strong dependence between the absorption and the type of formulation, with the interaction between the fed matrix and excipients controlling the absorption rate. Milk's high content in lipids enhances the solubility and dissolution of lipophilic drugs; for instance, release of danazol, a BCS class II drug, in

milk, was substantially higher than in water. Furthermore the pH of milk (pH ≈ 6.5) favours the dissolution of weak acids, such as mefenamic acid [58]. Diakidou and co-workers showed that despite milk's similarity in pH and protein contents with human aspirates after administration of a liquid meal, the solubility values of two BCS class II weak bases [dipyridamole (pKa 5.7–6.4, log P 2.7) and ketoconazole (pKas 2.9, 6.5; log P 4.4)], were 4.7 and 3.6 times lower in milk (after the addition of pepsin and lipase from *Rhizopus niveus*) than in the gastric fed-state aspirates, respectively [44].

❖ **Digested milk**

After administration of a meal *in vivo*, digestion takes place. *In vitro* digestion milk models have been used for simulation of the *in vivo* digestion of gastric contents. These *in vitro* milk based models, take into consideration the role, amount and activity of the physiologically existing gastric enzymes in the fed state.

In a milk based medium, HCl, lipase and pepsin have been added [44, 59, 60]. Two models using bovine milk were considered for the simulation of gastric environment. In the first model the dissolution of L-sulpiride, a hydrophilic weak base was studied in milk digested with pepsin and HCl [60], with 4.4 mg of pepsin from hog stomach dissolved in HCl being added every 15 min for a 90 min time period. The dissolution assay was performed in USP Apparatus 2 (100 rpm, 500 mL volume). The second model used milk digested with pepsin/HCl/lipase from *Rhizopus niveus* aiming to simulate the fed gastric environment after food intake [44, 59]. In solubility studies of two lipophilic bases, dipyridamole and ketoconazole, it was shown that milk digested with the HCl solution of pepsin gave a good prediction of the ketoconazole's solubility in human gastric aspirates after administration of 500 mL of Ensure® Plus while a solubility overestimation was observed for dipyridamole.

When milk digested with pepsin/HCl/lipase was used the prediction of the drug's solubility in the gastric aspirates was dependent on the time that the *in vivo* sample was collected [44]. The biorelevance of the addition of lipase in the milk in terms of pH and protein content was shown in a release study of felodipine from an extended release matrix. Gastric pH decreased slower and protein content faster than an identical medium in the absence of lipase, giving pH and protein content values closer to the ones observed *in vivo* [13].

- **Fed State Simulated Gastric Fluid (FeSSGF)**

As an effort to improve the biorelevance of milk as dissolution medium and simulate the postprandial conditions of the gastric tube, a medium called Fed State Simulated Gastric Fluid (FeSSGF) was developed. This medium was developed by Jantratid and his co-workers [37] and is comprised of 3.5% fat milk diluted with acetate buffer. In order to mimic the three phases of gastric digestion with the pH value being 6.4, 5.0 and 3.0 for the early, middle and late phases, respectively, a FeSSGF for each phase was prepared (Table 4) [37]. The pH was adjusted with the use of 0.1 N HCl and NaOH solutions and it was suggested that the "middle" medium reflects in a satisfactory manner the sum of the physiological gastric conditions during meal ingestion. Accepting this compromise, FeSSGF could potentially be used as a universal medium potential for fed-state gastric dissolution [37]. It should be noted though that this medium does not contain any enzymes, so the presence of the gastric pepsin and lipase are not taken into account. As milk in the absence of enzymes can only simulate the gastric fed state condition in its early phase [8], the use of the three "snapshot" media can simulate the intraluminal changes in pH, osmolality and protein contents accurately.

5.2. Nutrient drinks/Emulsions

The use of various nutrient drinks and emulsion for parenteral administration have been employed to studies as an attempt to mimic the gastric stomach conditions in the fed-state, as they have similar composition (Table 5) to the standardised meals used in drug food effect studies [6].

Scandishake Mix® is a nutrient drink used for the simulation of gastric environment in the fed state. It was used in the form of powder mixed with whole milk with simulated gastric secretions containing lipase and pepsin in a dynamic *in vitro* system (TNO TIM-1), simulating the stomach and small and large intestines' environment. Scandishake mix® was used for the simulation of a high-fat meal in the development of a dissolution model for fosamprenavir (prodrug of the antiretroviral aprenavir) [61]. Food-induced disintegration of fosamprenavir's tablets was assessed using Magnetic Resonance Imaging (MRI). The stomach compartment in the dynamic system was comprised of two units separated by a silicon wall with the surface between the outer and inner tubes being thermostated (37 °C). The simulated gastric contents were mixed by application of water pressure to the walls, causing three "contractions" and "relaxations" per minute. Simulated gastric lipase and pepsin were pumped to the compartment at a flow of 0.5 mL/min. The nutrient drink, compared with simulated gastric fluid (SGF), which was used for the gastric fasted state simulation, predicted the formulations' proprandial delay in disintegration observed *in vivo*. This effect on the tablet's disintegration can be attributed to the competition of the nutrient drink with the water molecules for the interaction with the matrix and by the formation of a water layer of increased viscosity around the tablet [61].

The nutrient drinks Ensure® [62] and Ensure Plus® [13, 62] have been used in several studies as biorelevant fed–state gastric media. According to the manufacturer [63], both

emulsions contain water, corn maltodextrin, sugar, milk protein concentrate, canola oil and corn oil (Ensure® Plus) or soy oil and sucromalt (Ensure®). Intralipid®, an emulsion for of similar fat content with Ensure® and Ensure Plus®, has also been used for the simulation of gastric fed state conditions after the administration of a high-fat breakfast [10]. Ensure® contains fat (3.7%), protein (3.7%) and carbohydrate (14.5%), while Intralipid® is available in 10, 20 and 30% fat concentrations (soya oil), with the emulsion also containing egg lecithin protein (12 g/1000 mL) and glycerol (22, 22 and 16.7 g/1000 mL for 10, 20 and 30% fat, respectively. Ensure® and Intralipid® 10% were used at a 1:6 dilution with universal buffer (pH 2.5 and 5.6) in dissolution (USP Apparatus 2, 100 rpm) and microcalorimetry studies of aminophylline controlled release tablets comprised of an aliphatic alcohol and hydroxyalkylcellulose [68]. Each dissolution profile was the expression of two distinct processes; dissolution of the alcohol and diffusion of the drug though the cellulose derivative. The profile was comprised of two first order rates separated by a mixed rate. The composition of the biorelevant medium mainly affected the rate of the initial first-order process and also the onset time of the second apparent first order, indicating that differences in the medium can affect the drug release mechanism.

The similarities of the physicochemical properties (pH, osmolality, buffer, capacity, viscosity and surface tension) between standard meals used in *in vivo* studies and gastric fed state media used *in vitro* have been assessed [43]. Ensure Plus® demonstrated better resemblance to the gastric conditions after the administration of a homogenized standard breakfast than milk and Ensure® but had significantly different viscosity, an issue resolved after addition of 0.45% w/v pectin, a water soluble polysaccharide. *In vitro* dissolution studies performed with Ensure® (USP Apparatus 2) [62] and Ensure Plus® [64] (USP Apparatus 3) demonstrated that dissolution behavior is greatly affected by interaction between medium component and formulation. In the above studies, food effect observed *in vitro* was attributed

to a formation of a hydrophobic layer around the formulation (HPMC matrix) decreasing water ingress in the tablet [64], or by granting a more effective hydration of a matrix component [62].

Another nutrient drink, Nutrison®, was used for the simulation of gastric fed state conditions in dissolution studies (USP Apparatus 2, 100 rpm) for the study of possible food-drug interactions between food components and metoprolol tartrate IR tablets [5]. The nutrient drink contains 6 g/100 mL protein, 6 g/100 mL vegetable oil fat, 18 g/100 mL carbohydrate, vitamins and minerals. For its use as a biorelevant medium it was diluted to 60% with a solution containing HCl, NaCl, KCl and sucrose, giving values of pH (pH= 5.4) and osmolality (420 mOsm/kg) similar to the *in vivo* fed state conditions. A potential excipient dependent mechanism of delay in tablet disintegration was indicated through the formation of a protein film from the medium's components around the tablets (visual observation) attributed to electrostatic or hydrophobic interactions between the proteins and the excipient a confirmed by scanning electron microscopy (SEM). The dependence of the formation of this layer on certain tablet excipients was confirmed by preparation of single excipient-medium mixtures and observation of a precipitation layer in the vessel for each mixture. The effect of meal type was further evaluated by comparing tablet disintegration times in media containing a single (protein, fat or carbohydrate) or mixtures of the mentioned components present in the fed state medium. The presence of proteins increased the tablet disintegration time the most, with a more profound effect when proteins were combined with fat, carbohydrates or both.

Even though the composition of nutrient drinks like Ensure Plus® is more similar to the high-fat meals administered in BA/BE studies, milk based media can simulate the fed gastric content taking the presence of secretions into consideration [65]. Moreover, with the two approaches mentioned before (gradual digestion and snapshot media), the changes in the fed gastric environment during time can be more closely simulated.

Another version of the FeSSGF "snapshot" media has been recently proposed replacing milk with Lipofundin® MCT 20, [66] an emulsion administered parenterally to patients, providing essential fatty acids. The emulsion: buffer ratios were different to those of the milk based FeSSGF "snapshot media" (17.5, 8.75 and 4.375% v/v content for early, middle and late media, respectively compared to 100, 50 and 25% for the milk-based media). Lipofundin® does not contain any proteins, which facilitates the drug's analysis in the medium and has similar fat content to the fed stomach aspirates [67].

6. Drug and formulation-related food effect

Under fed state conditions, tablet disintegration is generally delayed. For example, as mentioned previously, a nutritional drink used both in *in vivo* (canine) and in *in vitro* studies delayed disintegration and dissolution of metoprolol tartrate tablets by creating the formation of a food- induced thin layer around the tablet which not only did prevent the water penetration in the tablet but also the drug particles from leaving the matrix. [5].

Food can have a significant effect on the absorption of drugs. This can be affected by differences in the interaction of the active pharmaceutical ingredient or/and the formulation with the fasted and fed state environments. Examples of two drugs (itraconazole and nifedipine) demonstrating drug-related and formulation-related gastric food effect, respectively, are discussed below.

Itraconazole, an antifungal agent, is a well-studied drug in terms of its food effect. The positive effect of food on itraconazole's absorption has been verified by both *in vivo* and *in vitro* studies. An *in vivo* study on itraconazole capsules (2 × 100 mg capsules administered) containing sugar coated pellets in healthy subjects was performed with the use of the FDA

standard breakfast for the determination of the drug's food effect [68]. The study showed a significant increase for both C_{max} and $AUC_{0\rightarrow\infty}$ values ($C_{max(fasted)} = 0.59\ C_{max(fed)}$, $AUC_{0\rightarrow\infty\ (fasted)} = 0.61\ AUC_{0\rightarrow\infty\ (fed)}$) (Figure 3) with the increase in the drug's absorption being attributed to the drug's increased solubility in the food components. A similar study by Zimmermann et al. [69], (one 100 mg capsule administered with a standard breakfast) demonstrated similar results, with the relative bioavailability in the fasted state being 0.54 times the one observed after meal administration. The differences were attributed to the high-fat content of the meal and also to the longer gastric retention time in the fed state.

In vitro studies were in agreement with the fact that the drug's bioavailability could be significantly affected by food [70]. An *in vitro* study used milk of different fat concentrations mixed with simulated gastric fluid (SGF) (pH adjusted to 3) as fed state biorelevant dissolution media and demonstrated that except for fat, other food components may be responsible for the food effect observed with itraconazole *in vitro*. Despite a pronounced difference between dissolution rates in the fasted (SGF pH 3) and the fed state simulated media, the fat content did not lead to statistically significant dissolution rates among the milk-based media. The presence of different carbohydrates (1% w/v glucose, lactose and starch in SGF) increased the dissolution rate at a small extend, possibly by formation of hydrogen bonds between the drug and the carbohydrates' hydroxyl functional groups. On the contrary, increased protein content, appeared to have a positive effect of drug dissolution. Drug dissolution rates in media containing albumin (0.5-4% w/v) concentration in SGF, increased by increasing the protein content. One possible mechanism could be through protein-drug binding possibly by the development of electrostatic and lipophilic interactions between the drug and albumin.

Nifedipine is a calcium channel blocker with a complicated formulation-related food effect [5]. Immediate release tablets, prolonged (modified) release tablets, capsules and soft capsules of nifedipine (5–60 mg) are commercially available [71]. Significant differences in

the bioavailability of nifedipine modified – release formulations after oral administration have been observed *in vivo* between fasted and fed state conditions [72, 73]. The bioavailability of the brand formulation, Adalat OROS® (Osmotic-Release Oral System), has not been significantly affected by the presence of food [72, 74, 75]. Dose dumping and unusually long lag times, possibly due to lack of robustness of the mechanism of drug release in the fed state or due to prolonged stay of the formulation in the stomach, have been observed after administration of generic nifedipine formulations. During the gastric residence of nifedipine, the rate of absorption is limited, which led to rapid and high absorption when it reached the small intestine, altering the formulation's controlled release mechanism characteristics of some generic formulations [76, 77]. Nifedipine Sandoz® retard 30 mg, (eroding matrix system) [74], Nifedicron® 60 mg, (capsule with mini-tablets, Pharmatec International, Milano, Italy)] [75], Slofedipine® XL 60 mg (eroding matrix system) [72] and Nifedipine ER 90 mg test tablet (hydrophilic matrix, pilot formulation, Astra AB, Sweden,) [78] demonstrated a formulation induced food effect after co-administration with a high-fat standard breakfast. Nifedipine Sandoz® retard demonstrated significant differences in its pharmacokinetic behavior between fasted and fed states, possibly due to the inability of the matrix to release the drug in a controlled way, with the exact mechanism not having been experimentally proven [76]. Dose dumping and a three-fold increase of the C_{max} was observed for Nifedicron® under the fed state conditions compared to the fasted state [75]. Slofedipine® XL's profiles between the fasted and the fed state (high-fat breakfast) were also significantly different. Nevertheless, even though the geometric $AUC_{0\rightarrow 24}$ mean in the fed state was approximately half the one in the fasted state for Slofedipine® XL, the $AUC_{0\rightarrow tn}$ values were identical [72]. Similar behavior was observed with Nifedipine ER tablets (90 mg), which demonstrated a higher absorption rate than Procardia® XL (90 mg) (osmotic pull-push system tablet), which was used as a reference

product. The effect was attributed to the increased erosion rate as a result of the gastric motility and alterations in the gastric content after meal administration [78].

It is worth mentioning that in several of the above studies, differences in the fed state-pharmacokinetic behavior between the brand and generic formulations have also been observed. In the case of nifedipine Sandoz® retard, great inter-patient variability often accompanied with fast, uncontrolled drug release was observed in plasma concentration vs time profiles, demonstrating failure of the controlled release behavior of the formulation under fed conditions [74]. In case of Slofedipine® XL [72], the authors concluded that the differences between the test (Slofedipine® XL) and the reference (Adalat® OROS) formulations in the fed state could be attributed to the prolonged transit time of the former. Slofedipine® XL had a significant delay on the onset of its therapeutic action in 15 out of 24 patients of the study (15h lag time), which resulted in 29% decreased $AUC_{0\rightarrow tn}$ compared to Adalat®. Slofedipine® XL's lag time was attributed to the fact that undissolved particles of the formulation of a diameter above 10 mm were not able to pass through the pylorus, until the onset of phase III of the migrating motor complex (MMC; the cylindrical series of gastric electrical activity, taking place between meals) [79]. In the same study, *in vitro* dissolution studies in acetate buffer pH 4.5 demonstrated that at pH values similar to the fed state, the generic formulation remained undissolved for 24 hours, in contrast to Adalat® OROS which was almost 100% dissolved.

7. Meal– related food effect

Drug food effect relates to the nature of the meal. Meal characteristics such as fat content, viscosity, caloric content size, and volume are parameters which can affect its absorption.

Increased fat content in the fed state relates to delayed drug absorption due to slower gastric emptying rate [23] and to decreased drug dissolution due to retarded wetting of the formulation [80]. Increased viscosity of the gastric contents due to the administered meal delays the rate of gastric emptying [81]. Increased meal viscosity can decrease the diffusion coefficient of a compound according to Stokes-Einstein equation [82], which could in turn decrease drug absorption if it is only absorbed in a specific part of the gastrointestinal tract, as the drug goes past this site. The effect of meal viscosity is impaired in the small intestine due to secretions and digestion products. The most common effect of high meal viscosity is an increase in the T_{max} values of drugs. In an *in vivo* study using canine subjects, when a calorie free viscosity enhancer, HPMC (hydroxypropylmethylcellulose), was added, a delay in the T_{max} of the antiarrhythmic bidisomide was observed [83]. The meal's residence time depends also on its caloric content [26]. 2-4 kcal of caloric content per minute are transferred to the duodenum [17], meaning that meals with high caloric content will reside in the stomach for longer periods, affecting drug's transit time in the gastrointestinal tract. Nutrient drinks of 1 kcal/mL content are emptied at a rate of 2-2.5 mL/min, while nutrient drinks of 0.2 kcal/mL content have an emptying rate of 10 mL/min [17]. A calorie-dependent decrease of the gastric emptying rate was observed for the solid portion of the meal (45± 3.4% of the meal mass retained in the stomach for the 68 kcal solid meal and 65± 4% for the 633 kcal solid meal at 100 min), while its liquid portion is emptied to the duodenum at a rate independent of its energy content [11]. Meal size and volume relate to its gastric residence time [11]. Meals of fourfold mass and similar caloric content resulted in a 388% higher emptying rate, attributed to activation of stretch or volume receptors in stomach, increasing peristalsis. Using different volumes of isocaloric meals, a statistically significant increase in gastric emptying rate with the larger volume was noted for volumes of liquid meals between 200 mL and 800 mL [84].

8. Analytical techniques and challenges for sample treatment and drug quantification

Most of the gastric fed state media used until now are milk based or o/w emulsions; therefore, several sample preparation processes have been developed for the extraction of the drugs from these matrices. Milk is a complex biological fluid containing proteins, lipids, carbohydrates, divalent and trivalent cations which can be bound to the compounds of interest making drug analysis challenging. The analytical techniques, issues and challenges described in this section refer mainly to fed state gastric biorelevant media which are at least partly comprised by milk or contain other types of lipid and protein sources. The quantification of drugs in these media is usually performed using high performance liquid chromatography (HPLC) and therefore, the analytical challenges and problems presented below refer mainly to this technique.

8.1. Filtration

The first challenge with drug analysis when biorelevant media are used is filtration itself. Filtration is an essential step in the analytical procedure, as biorelevant media contain a range of particles deriving from lipids, carbohydrates, fat and salts, which have to be removed before the sample's injection in the HPLC. Moreover, when fed biorelevant media like milk or FeSSGF are used, the use of small pore size filters for the sample analysis during solubility or dissolution studies cannot be used due to clogging from the presence of large proteins [9]. Several types of filters like 0.45 or 0.22 μm polytetrafluoroethylene (PTFE) [64] or regenerated cellulose (RC) [44] have been used for sample analysis prior to HPLC injection for drug quantification in both milk and lipid-based media but a sample cleanup step is required before for these type of media. Glass microfiber (GF) 2.7 μm filters have also been used in drug

solubility and dissolution studies in milk based media [85], in order to remove undissolved drug or formulation particles prior to sample treatment and filtration through the filters of smaller pore size. Filters of bigger pore size, attached to the sampling cannulas of the USP Apparatus 1/2 (polyethylene sticks, 10 μm and nylon membrane filters, 5 μm) have also been used in dissolution studies with FeSSGF and milk for the same reason [56, 85].

Adsorption of the analyte on the filters should be studied in order to evaluate and choose the appropriate filters. Salicylic acid and sodium saccharine are example of drugs which demonstrate significant adsorption on Nylon filters as shown in a study by Carlson et al. [86], where 85.8% and 60.4% of salicylic acid at 0.005 mg/mL and 0.05 mg/mL concentrations respectively was adsorbed on 25 nm nylon filters. Sodium saccharine demonstrated even higher adsorption with the entire drug (100%) being adsorbed on the same filters at a 0.1 mg/mL concentration [86, 87]. It should be noted that if the first few drops of the filtrate are not discarded, the percentage of adsorption can reach extremely high values.

8.2. Medium

The analysis of the drug content in milk based media can be challenging as the content of the medium itself is comprised of a lipid and an aqueous phase that requires separation of the phases before an HPLC analysis can be performed. Analysis could be affected by the differential distribution of the drug in the multiple phases of the milk based media, as it could distribute either in the aqueous or lipid phase or even bind to the proteins or fat contained in the medium. Several drugs have shown binding in milk at amounts higher than 50%; diazepam, indomethacin, grizeofulvin and dicumarol demonstrated binding percentages from approximately 55% to 95% in low and full fat milk (37 °C) after equilibrium dialysis against a phosphate buffer solution (pH 6.5). For some drugs, their percentage bound was more significantly affected by milk's temperature (dicumarol, prednisolone) and fat content (e.g.

binding of diazepam increased more than 13% in full fat milk in comparison to low fat milk at temperatures 15–37 °C) [88]. A factor affecting the analysis of milk is its variable fat, carbohydrate and lipid content. Its composition differs among different mammalian species, and is also affected by parameters such as their diet or the onset of their lactation period [89]. Therefore, milk of the same commercial brand and batch should be used when different drugs and dissolution conditions are compared, as changes in the medium composition may affect parameters like recovery, precision, and analytical method compatibility.

8.3. Sample treatment and analysis

i. Protein precipitation

One simple method of sample cleanup prior to drug quantification in gastric milk- based fed state media is protein precipitation. Protein precipitation is the technique mainly used until now with the addition of a volume of an organic reagent to a volume of medium, followed by a centrifugation and a filtration step before its analysis in HPLC. The precipitation of the milk's proteins can be performed using an organic reagent followed by filtration and centrifugation steps [9].

A range of solvents like acidified MeOH [90], acetone [91] and HCl [92] have been used as protein precipitation reagents for the extraction of drugs from milk-based media. Parameters such as their compatibility with the analytical technique chosen, their volatility in case organic phase evaporation is needed, their selectivity and their cost have to be considered during the selection of an appropriate protein precipitation solvent [88].

Fotaki et al. [60] suggested a precipitation and centrifugation method for the quantification of L-sulpiride, a BSC class III drug, in a milk based dissolution medium,

proposing an assay involving centrifugation at 4000 rpm for 10 min (8 ºC) for the separation of the aqueous phase from the lipid phase with acetonitrile being added in the aqueous filtrate (1:2) and a last centrifugation and filtration step (Titan® filters 0.45 μm) following. Sample treatment in nutrient drinks and emulsions requires a more time consuming cleanup procedure. Ensure® Plus requires phase separation via a centrifugation step (e.g. 11500 rpm, 1.5 h) [64] and possibly an extra filtration step with a larger pore size filter (5 μm) prior to filtering with a 0.45 μm filter and injecting the supernatant in the HPLC.

A study by Williams et al. [10] used 1:2 with ice-cold 12% (w/v) trichloroacetic acid for milk protein precipitation and then centrifugation at 10000 rpm for 15 min extracting 0.2 mL of the supernatant for the HPLC analysis [10] for the quantification of caffeine in both milk-based (0.1%, 1.7%, 3.6% fat milk) and fat emulsion type (30% fat emulsion (Intralipid®)) media. Despite the fact that a protein precipitation step, followed by centrifugation and filtration, was adequate for both types of media, the recovery of the drug in the fat emulsion was significantly lower. A challenge associated with the selection of protein precipitation reagents is their compatibility with the HPLC method for the analysis of the selected drug. Peak fronting is a common issue when organic reagents of higher strength than the mobile phase are used; an issue which can usually be resolved with evaporation of the reagent under nitrogen and reconstitution in the mobile phase. Another disadvantage of this technique is its inability of complete removal of the lipid part of the medium [89], as for the selective removal of lipids, a supplementary step is required. This step may be a wash with hexane, given that the compound of interest is ionised or not extracted in hexane, so as not to be lost during the washing step [93].

ii. Solid Phase Extraction (SPE)

673 Another method used for the extraction of the analyte of interest from milk is Solid
674 Phase Extraction (SPE). Solid phase extraction (SPE) is widely used for the extraction of drugs
675 from biological fluids such as whole blood [94], plasma [95], urine [96] and milk [97], and is
676 often preceded by a protein precipitation step. SPE cartridges are comprised by a polypropylene
677 tube with their sorbent between two porous frits. A typical experimental protocol involves 4
678 main steps: i. cartridge conditioning, ii. sample loading, iii. sample washing and iv. sample
679 elution [98].

680 Most cartridges are either comprised of bonded silica phases, similar to the material of
681 the reversed phase HPLC columns but with bigger diameter particles (10–60 μm), or of
682 polymeric resins (e.g. polystyrene-divinylbenzene). [99] SPE C_{18} cartridges have been used for
683 the quantification of several drugs, like β-lactam antibiotics [100] or nonsteroidal anti-
684 inflammatory drugs (NSAIDs) [101], in milk. In the above studies, a protein precipitation step
685 similar to the one previously described [60] took place before the extraction, due to the milk's
686 high content in proteins and lipids, which render the sample too "dirty" to be loaded straight
687 on the cartridge. An elution solvent of high water percentage is preferable for extraction from
688 milk-based media, and has to be able to elute the drug and retain most of the lipids on the
689 cartridge [10]. The extraction yield of lipid drugs using SPE may be low due to the drug's
690 interaction with milk's fat globules. A way to disrupt this drug-fat globule interactions would
691 be sonication and dilution of the medium before its loading on the cartridge [89]. A challenge
692 for the analysis with SPE when it comes to biorelevant media, is the interference from the
693 matrix in HPLC analysis. Therefore, cleanup with a solid phase extraction cartridge can help
694 towards the development of more sensitive and robust methods in drug analysis in fed state
695 biorelevant media. Disadvantages of SPE as a drug's extraction method from gastric fed state
696 media include the quick drying of the cartridges and the difficulty to adjust the vacuum during

the multiple steps without the presence of an automated manifold, affecting the reproducibility of the method [102, 103].

iii. Liquid-liquid extraction (LLE)

Liquid-liquid extraction is based on the analyte partitioning between an aqueous phase and a water-immiscible solvent [99]. Several extraction protocols have been successfully applied for a wide range of drugs such as mycotoxins [104], mycrocyclic lactones [105], vitamins [106] and analgesics [107]. The main advantages of LLE is the short time required for method development and its low cost. A serious drawback is the fact that it is a time-consuming and labour intensive method. Moreover, the possible presence of the milk's lipid content in the extraction solvent after the LLE process, leads to phase separation of the sample and lipid partitioning in the stationary phase of the HPLC column during drug analysis. Therefore a washing step with hexane is usually required, so as to remove the lipids [89], making the whole procedure even more time consuming. The following protocol used in milk for the quantification of Ochratoxin A is a typical case of liquid-liquid extraction [104]; 0.2 mL saturated NaCl solution and 2.4 mL chloroform were added to 1 mL milk, mixed gently for 3 minutes, centrifuged (4500 rpm, 20 minutes) and after the removal of the chloroform layer, evaporation to dryness under nitrogen steam and reconstitution in acetonitrile followed. Lipid removal was performed by double extraction (2 × 0.4 mL petroleum ether for 1 min). After discarding the etheric layer, acetonitrile was blown to dryness, reconstituted in 1 mL of mobile phase, filtered and analysed in HPLC.

iv. Ion Selective Electrode (ISE) sensor

An online monitoring system, in an attempt to avoid the sample treatment traditionally required for the extraction of the analytes of interest from FeSSGF has been published [9]. An Ion Selective Electrode (ISE) sensor system was used, with two electrodes placed constantly

721 in the dissolution vessel which were able to monitor the changes in drug concentration through
722 the changes in potential. With this proposed methodology, diphenhydramine hydrochloride
723 was successfully quantified in a dissolution study using the USP Apparatus 2 in several fasted
724 [Fasted State Simulated Gastric Fluid (FaSSGF), Fasted State Simulated Intestinal Fluid
725 (FaSSIF) and Fasted State Simulated Intestinal Fluid Version Two (FaSSIF-V2) and fed
726 (FeSSGF) state biorelevant media. Sample preparation steps needed with extraction techniques
727 were avoided, and a continuous dissolution profile and a much faster and less laborious
728 alternative were offered. Disadvantages of this method are: a. its limitation to the analysis of
729 ionised drugs b. the complicated correction of the baseline needed for the heterogeneous
730 biorelevant media and c. its inability for the analysis of compounds of extremely low aqueous
731 solubility [9].

732

733 **v. Other techniques**

734 Several other techniques have been used for drug quantification in milk and may have
735 the potential to be used in fed state gastric media, some of which are briefly listed below:

736 **Matrix solid-phase dispersion (MSPD):** The matrix solid-phase dispersion (MSPD)
737 technique was firstly developed by Barker et al. [108]. MSPD involves the grinding of
738 biological samples with sorbent particles producing a column material acting as a solid support
739 from which the drugs in the matrix can be selectively extracted [109, 110]. MSPD has been
740 successfully used in drug quantification in milk with sorbents, like C_{18} [111], C_8, silica gel
741 [112], mixed-mode/cationic-exchange (MCX), mixed-mode/anion-exchange (MAX) and weak
742 anion–exchange (WAX) [113], and more recently molecularly imprinted polymers (MIPs)
743 [110]. An advantage of this method is the combination of homogenization, fractionation and

744 purification in one single step and could be also used to milk-based gastric dissolution media
745 [109]. It is also cheap and environmentally safe but relatively labour demanding [114].

746 **Solid-phase microextraction (SPME):** SPME is a sample preparation technique
747 which is based on a concentration equilibrium between an extracting phase associated with a
748 solid support and a biological matrix [115]. Two different SPME designs are the most
749 commonly used: in-tube mode and fibre design. In this technique, small quantities of the
750 sorbent are exposed to the headspace or solution of the biological sample using a suitable
751 format. After a predefined amount of time and when equilibrium between the coating and the
752 matrix has been reached, the sorbent does not absorb any additional quantity of the analyte of
753 interest, meaning that the amount of drug extracted for a specific concentration is constant
754 [116]. The main advantages of SPME are its low cost and the fact that it is time saving and
755 environmentally friendly [117]. Some of its disadvantages are the slow time for equilibrium
756 between the analyte and the extraction phase to be reached [118], the poor selectivity and the
757 limited type of fibres commercially available [119].

758 **Ultrafiltration:** Ultrafiltration is a technique extensively used in food industry and
759 water treatment. It is based on the selective passage of drugs of low molecular weight through
760 the pores of a membrane of a specific MW cut-off, which inhibits the passage of molecules of
761 higher MW. This technique does not involve time-consuming steps but its use is limited by
762 reduced sensitivity due to interferences from the matrix in drug analysis and is usually used in
763 combination with other cleanup techniques [89]. Ultrafiltration was successfully employed for
764 the quantification of tetracyclines in milk, by adding solid EDTA at 10 mM concentration,
765 before sample centrifugation at 5000 rpm for 1 hour. Ultrafree MC/PL devices (nominal
766 molecular weight limit 5000) were used in this study [120].

9. Conclusion

Prediction of gastric food-effect of drugs has been a challenge of the pharmaceutical industry. Even though the *in vivo* properties of the fed state gastric environment have been determined in some cases, the complicated fed environment due to differences in the meals administered makes the determination of the precise gastric conditions difficult. Despite the fact that some progress has been made with the development of gastric biorelevant media, a universal robust predictive analytical method has not been yet developed. The development of suitable biorelevant media in combination with a simple and robust analytical method could potentially provide a means of understanding of a potential food effect in regards of a drug's solubility and dissolution. Several biorelevant dissolution fed state media like milk, nutrient drinks or Fed State Simulated Gastric Fluid (FeSSGF) have been developed in an attempt to simulate the human postprandial conditions. Nevertheless, none have managed to achieve precise representation and fully overcome issues such as the need of a time consuming preparation for the quantification of the drug, possible matrix interferences and compatibility with the analytical methods used.

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List of Tables

Table 1. Physicochemical Properties of the contents of the fed state stomach based on *in vivo* measurements.

	Value	Technique employed	Meal	Sample	Reference
pH	6.23–6.84 (during meals and 1-2 hours after meal administration)	Bravo wireless pH monitoring capsule	No restriction in meal composition	11 volunteers (8 healthy)	[31]
	median 6.4–2.7 (from 5 min to 3h 30min)	Aspiration through nasogastric tube	500 mL Ensure Plus®	20 healthy volunteers	[13]
	median 5.0 (peak 6.7)	Heidelberg capsule	Standard meal 1000 kcal	34 healthy volunteers	[28]
Osmolality	559 mOsm kg^{-1} - 217 mOsm kg^{-1}, (from 30 to 210 min)	Aspiration through nasogastric tube /freezing	500 mL Ensure Plus®	20 healthy volunteers	[13]

		point depression			
Buffer capacity	14-28 mmol L^{-1} DpH (from 30 to 210 min)	Aspiration through nasogastric tube /titration with HCl	500 mL Ensure Plus®	20 healthy volunteers	[13]
Surface tension	30-31 mN m^{-1}	Aspiration through nasogastric tube /titration with HCl	500 mL Ensure Plus®	20 healthy volunteers	[13]

1262

1263

1264 **Table 2.** Biopharmaceutics classification system, predictability of food effect and transporter
1265 effect [46, 48].

BCS class	Solubility/ permeability	Food effect	Drug examples	Transporter effect on drug disposition	Transporter effect by high fat meal
I	+/+	no effect	Disopyramide Ketoprofen Verapamil	Minimal	No effect
II	-/+	+	Cyclosporine Danazol Dapsone	Efflux transporter effects predominate	Efflux transporters inhibition, intestinal drug solubilisation (drug passively absorbed) Inhibition of both absorptive and efflux transporters. Food effect according to relative inhibition (drugs actively absorbed)
III	+/-	-	Fesofenadine Nadolol Valsartan	Absorptive transporter effect predominate	Inhibition of absorptive transporters in the intestine
IV	-/-	+, -,	Chlorothiazide Furosemide	Possible substrates for both	All effects mentioned above for classes II and III

		no effect	Neomycin	absorptive and efflux transporters	

1266

1267 **Table 3.** Examples of meals used in for the determination of drug- food interactions *in vivo*.

Meal	Composition	Fat content	Protein content	Carbohydrate content	Reference
GSK high-fat standard meal	2 slices of toasted white bread with butter, 2 eggs fried in butter, 2 slices of bacon, 2 ounces of hash browned (fried shredded) potatoes, 8 ounces of whole milk	67 g (603 kcal, 62% of total calories)	33 g (132 kcal, 14% of total calories)	58 g (232 kcal, 24% of total calories)	[43]
FDA high-fat standard meal	2 eggs fried in butter, 2 strips of bacon, 2 slices of toast with butter, 4 ounces of hash brown potatoes, 8 ounces of whole milk (800-1000 kcal)	500-600 kcal	150 kcal	250 kcal	[51]
FDA intermediate-fat standard meal	1 English muffin with butter, 1 fried egg, 1 slice of cheese, 1 slice Canadian bacon, 1 serving of hash browned (fried shredded potatoes), 6 ounces of orange juice, 8 ounces of whole milk	27 g, (240 kcal, 37 % of total calories)	29 g (116 kcal, 18% of total calories)	73 g (292 kcal, 45% of total calories)	[43]
Light standardised breakfast	Not specified (+100 mL of black coffee)	26 g	28 g	51 g	[121]

Standardised high-carbohydrate meal	Not specified (1000 kcal)	Not specified	Not specified	600 kcal	[122]
Low-fat meal	1 slice of white spread bread with jelly, 6 ounces of orange juice, 8 ounces of skim milk (250 kcal)	1 g	12 g	51 g	[83]
Long chain truglyceride meal	Fat: 80% w/w medium chain triglycerides (C_6-C_{12}) Proteins: whey, casein and soy hydrolysates Carbohydrates: monosaccharides, oligosaccharides, polysaccharides	36% of total calories	16% of total calories	48% of total calories	[123]
Medium chain triglyceride meal	Fat: 31% w/w medium chain triglycerides (C_6-C_{12}) Proteins: whey, casein and soy hydrolysates Carbohydrates: monosaccharides, oligosaccharides, polysaccharides	36% of total calories	16% of total calories	48% of total calories	[123]

High-protein meal	2% low fat milk, Carnation Instant Breakfast® and Pro Pac® Plus (protein supplement)	9 g	80 g	52 g	[53]
High-protein meal	Not specified (439.5 kcal)	17.1 g	30.5 g	43.5 g	[124]
Low-protein meal	Not specified (417.8 kcal)	14.9 g	10.5 g	64.3 g	[124]

[a] 25 °C
[b] 37 °C
[*]Calories derived from fat

1274 **Table 4.** Physicochemical properties of gastric fed state biorelevant media used *in vitro*.

Medium	Mass (g)	Volume (mL)	Density (g/ mL)	pH	Buffer capacity (mEq pH^{-1} L^{-1})	Osmolality (mOsmol kg^{-1})	Surface tension (mN m^{-1})	Viscosity (mPas)	Reference
Standard breakfast (62% fat*)	516± 6[a]	474±7.7[a]	1.09± 0.03[a]	6.51± 0.01[a], 6.61± 0.03[b]	29.3± 0.9[a], 30.1± 1.8[b]	771± 10	52± 1[a], 44± 1[b]		[43]
Standard breakfast (37% fat*)	540± 5.5[a]	513± 7.3[a]	1.05± 0.03[a]	5.28± 0.03[a], 5.12± 0.04[b]	49.6± 1.7[a], 47.2± 1.5[b]	713± 10	49± 1[a], 45± 1[b]		[43]
Milk (48.1% fat*)			1.03± 0.005[a]	6.72± 0.02[a], 6.63± 0.01[b]	14.4± 0.2[a], 13.9± 0.2[b]	285± 2.7	54.2± 0.4[a], 49.8± 0.6[b]	1.9± 0.04[a],	[43]

								1.5± 0.04[b]	
Partially digested milk (i. 3.5% fat milk, ii. milk + HCl + pepsin, iii. Milk + HCl + pepsin + lipase)				i. 6.5, ii. 4.7-2.6 (in 6 h), iii. 5.1-4 (in 6h)	i. 13-19 ii. 19-38 iii. 47-69	i. 260, ii. 338-462, iii. 475-540			[44]
Ensure® (30.1% fat)			1.04± 0.016[a]	6.68± 0.01[a], 6.58± 0.1[b]	15.4± 0.1[a], 16.4[b]	375± 3.5	50.5± 0.2[a], 47.8± 0.1[b]	6.3± 0.09[a], 4.4± 0.07[b]	[43]
Ensure Plus® (29.1% fat)			1.08± 0.003[a]	6.62± 0.03[a], 6.45± 0.02[b]	20± 0.7[a], 21± 0.3[b]	730± 10	53.2± 0.2[a], 48.4± 0.1[b]	19.1± 0.1[a], 12.3± 0.1[b]	[43]
Early FeSSGF (milk based)				6.4	21.33	559			[37]

Middle FeSSGF (milk based)				5	25	400			[37]
Late FeSSGF (milk based)				3	25	300			[37]
Early FeSSGF (Lipofundin® based)				6.4	21	559			[66]
Middle FeSSGF (Lipofundin® based)				5	25	400			[66]
Late FeSSGF (Lipofundin® based)				3	25	300			[66]
Nutrison®				5.4		420			[5]
FSGES				5					[125]

Intralipid® 30%						320		2.7± 0.06	[126]

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Table 5. Composition of nutrient drinks/parenteral emulsions used as fed state gastric media in *vitro*.

Nutrient drink	Composition (per Litre)[†]
Scandishake® mix	598 kcal, 30.4 g fat, 11.7 g protein, 69.5 g carbohydrate[††]
Ensure®	930 kcal, 25 g fat, 38 g protein, 135 g carbohydrate
Ensure Plus®	1500 kcal, 46 g fat, 55 g protein, 210 g carbohydrate
Nutrison®	1000 kcal, 39 g fat, 40 g protein, 123 g carbohydrate
Intralipid® 30	3000 kcal, 300 g fat

[†] according to the manufacturer [63] (Ensure® and Ensure Plus® vanilla flavour)

[††] 85 g powder in 240 mL whole milk.

Figure captions

Figure 1: mean pH values from aspirates of patients after administration of 500 mL Ensure Plus® containing 10 mg/mL PEG 4000. (data extracted from [13])

Figure 2. Schematic representation demonstrating the lipid digestion, formation of micelles and drug absorption in the small intestine after administration of a meal; processes taking place in the stomach in the highlighted rectangle. (modified from [22])

Figure 3. Pharmacokinetic parameters after administration of two 100 mg itraconazole capsules under fasted and fed (standard breakfast) state conditions. * denotes statistically significant difference ($p < 0.001$, multivariate ANOVA). (data extracted from [68])

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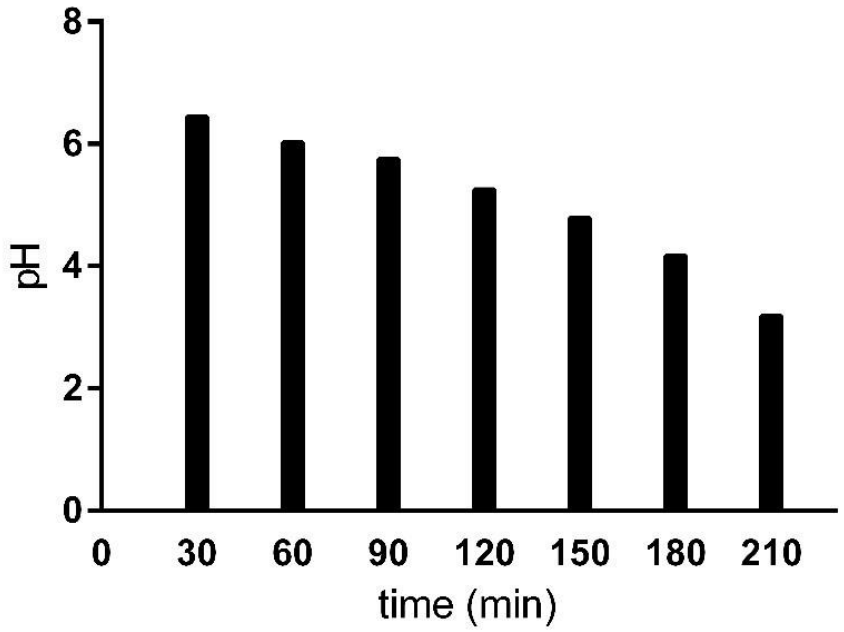

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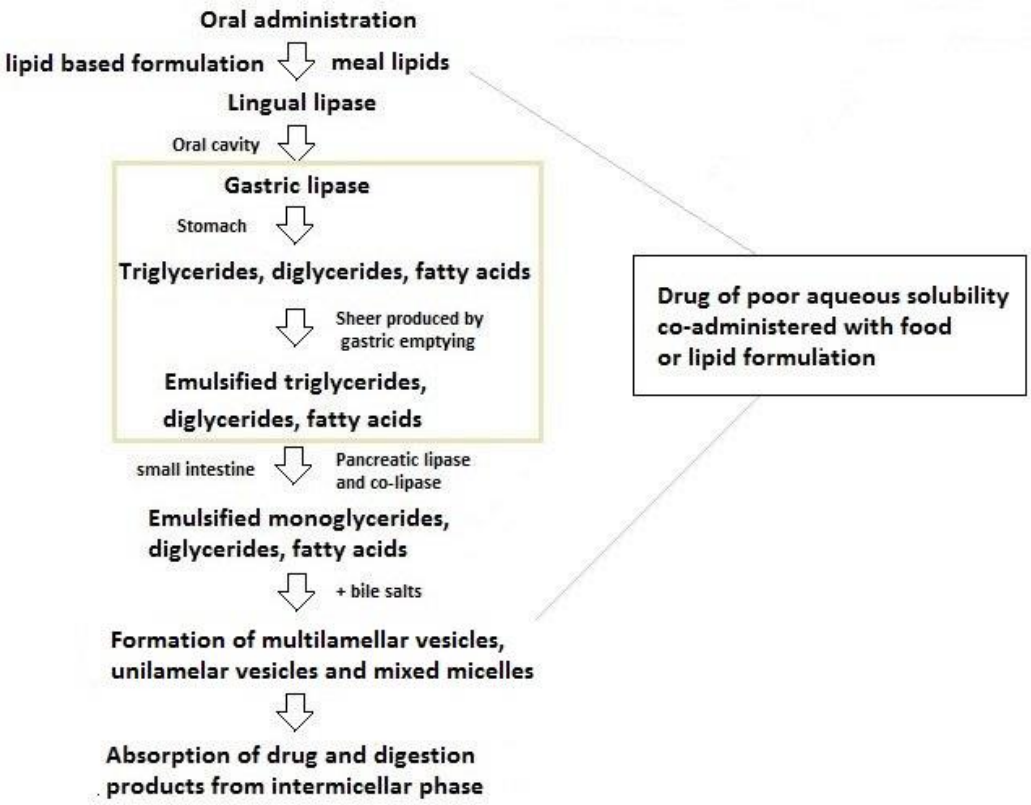

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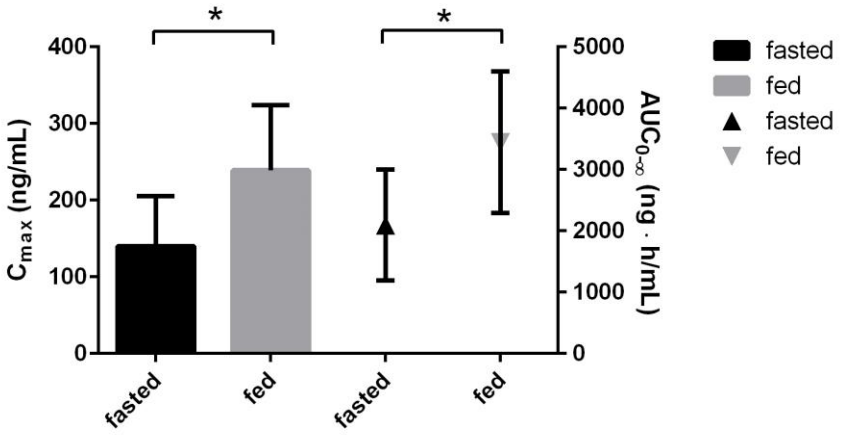
*
*
400
300
200
100
0
5000
4000
3000
2000
1000
0
C_{max} (ng/mL)
$AUC_{0-\infty}$ (ng · h/mL)
fasted
fed
fasted
fed
fasted
fed
fasted
fed

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