Unraveling the behavior of oral drug products inside the human gastrointestinal tract using the aspiration technique: History, methodology and applications

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ABSTRACT

Fluid sampling from the gastrointestinal (GI) tract has been applied as a valuable tool to gain more insight into the fluids present in the human GI tract and to explore the dynamic interplay of drug release, dissolution, precipitation and absorption after drug product administration to healthy subjects. In the last twenty years, collaborative initiatives have led to a plethora of clinical aspiration studies that aimed to unravel the luminal drug behavior of an orally administered drug product. The obtained drug concentration-time profiles from different segments in the GI tract were a valuable source of information to optimize and/or validate predictive in vitro and in silico tools, frequently applied in the non-clinical stage of drug product development. Sampling techniques are presently not only being considered as a stand-alone technique but are also used in combination with other in vivo techniques (e.g., gastric motility recording, magnetic resonance imaging (MRI)). By doing so, various physiological variables can be mapped simultaneously and evaluated for their impact on luminal drug and formulation behavior. This comprehensive review aims to describe the history, challenges and opportunities of the aspiration technique with a specific focus on how this technique can unravel the luminal behavior of drug products inside the human GI tract by providing a summary of studies performed over the last 20 years. A section ‘Best practices’ on how to perform the studies and how to treat the aspirated samples is described. In the conclusion, we focus on future perspectives concerning this technique.

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1. Introduction

Unraveling the complex gastrointestinal (GI) processes that a drug product needs to face after oral administration remains extremely challenging. However, there are numerous techniques described in the literature that can contribute to a better understanding of how GI physiology may impact oral drug behavior and vice versa (Hens et al., 2016c, 2020). Gaining knowledge on how these underlying mechanisms influence the systemic exposure of a drug is extremely valuable and may explain the differences in fraction absorbed of a drug between-and within-subjects. The underlying variables that play a major role in determining the fraction absorbed of a drug are subject of interest to be implemented in biopredictive in vitro and in silico tools to adequately reflect the in vivo behavior of a drug product during the non-clinical stage of drug development (LennerNäs et al., 2014). When optimized and validated, these models will allow for a more rational selection of formulation strategies in the pharmaceutical industry, resulting in time- and cost-effective research which is a benefit for pharmaceutical companies.

The different techniques that are commonly used in daily practice to investigate the luminal behavior of a drug can be classified into two categories: invasive and non-invasive methodologies. It should be noted that some of these methodologies are more historical than operational; however, they have been of paramount importance for innovations in drug and formulation development; besides, they have created the basis for several novel applications, leading to an in-depth comprehension of different GI variables such as gastric emptying (e.g., scintigraphy, tracer assessment), motility (e.g., gastric/small intestinal/colononic manometry), GI volume changes (e.g., MRI), temperature (e.g., telemetric capsules) and GI pH (e.g., intraluminal sampling technique, telemetric capsules) (Díaz Tartera et al., 2017; Hens et al., 2016c; Näslund et al., 2000). Based on pioneer work of Armstrong et al. and Spiller et al., novel insights were created to explore luminal concentrations of an orally administered drug product in combination with the measurement of surrounding physiology as, for example, the influence of motility on intestinal absorption (Armstrong et al., 1993; Braeckmans et al., 2020; Farmer et al., 2018; Hens et al., 2017, 2020; Spiller et al., 1988; Van Den Abeele et al., 2017b).

Direct measurement of luminal concentrations of a drug after oral administration of the drug product is a valuable tool to assess the luminal behavior of a drug product in the human GI tract. Moreover, the observed differences between subjects can be an important indicator to explain inter-subject differences in systemic exposure, where the underlying GI variability can be a potential source of bio-inequivalence. Measuring luminal and systemic concentrations simultaneously creates a valuable data source concerning optimization of in vitro and in silico models; both approaches can be directly validated with GI concentration-time profiles; in case of in silico tools, predicted systemic concentration-time profiles can be directly compared with the observed systemic exposure in humans. However, caution should be taken as the assumption of well-stirred compartment in systemic sampling may not apply in sampling from the GI lumen (Mudie et al., 2014; Murray et al., 2017; Schiller et al., 2005).

Throughout the years, the aspiration technique has demonstrated - for various drug formulations - how the drug formulation behaves in a complex GI environment and, consequently, what the potential impact of this environment may be on the obtained luminal and systemic concentration-time profiles of the drug. Additionally, the aspirated fluids have been characterized for their specific content (e.g., bile salts, phospholipids, lipid degradation products, pH and enzymatic activity) and the effect of these variables on luminal drug concentrations were thoroughly explored (Lindahl et al., 1997; Rietorst et al., 2018; 2016b, 2016a). A wide variety of applications has been described related to this technique, dating back from the late 19th century when acidic content from the more easily accessible stomach was aspirated (Lewis, 1999).

Currently, the intestinal sampling technique allows aspirating fluids in more distal parts of the GI tract, which enabled the determination of the permeability of the membrane for a series of compounds by the Loc-I-Gut® technique, thoroughly explored in the nineties (Amidon et al., 1995; LennerNäs, 1998, 1997). From 2000 onwards, the sampling technique was further explored to unravel the formulation behavior for numerous compounds that are commercially available on the market (Brouwers and Augustijns, 2014; Butler et al., 2019). An intra-intestinal infusion technique to administer drugs - with narrow therapeutic index and with an absorption process mainly occurring in the small intestine (absorption windows) - has been developed and approved, as seen for levodopa (used in the later stage of Parkinson’s disease) (Nyholt et al., 2012, 2003).

This review describes the history, challenges and opportunities of the aspiration technique with a specific focus on how this technique can unravel the luminal behavior of drug products inside the human GI tract by providing a comprehensive summary of studies performed over the last 20 years. A section ‘Best practices’ on how to perform the studies and how to treat the aspirated samples is included. In conclusion, the future perspectives of this technique are addressed.

2. History of the sampling technique

Although GI sampling is perceived as a relatively new technique to investigate drug and formulation behavior in the human GI tract, numerous manuscripts reporting on GI intubation can be found in the ‘older’ literature. The sampling of GI fluids for drug absorption research has been adopted by pharmaceutical scientists from (digestive) physiology studies, of which the early literature has already been reviewed by Sladen in 1968 (Sladen, 1968). Indeed, GI intubation has served many purposes, including the characterization of GI physiology (e.g., pH, motility, digestion), diagnosis (e.g., microbiota studies) and the infusion of liquids for diagnostic or therapeutic purposes along the GI tract.

Direct in vivo GI physiological and digestion investigations were already initiated in the early 19th century (between 1825 and 1834) with the sampling of acidic gastric content. One of the pioneers in direct research on GI physiology and digestion was Dr. Beaumont and his patient St. Martin. Dr. Beaumont, although situated on the Michigan frontier and lacking both formal medical education and scientific training, treated a gunshot wound but expected the patient to die from his injuries (Tanner, 2000). However, St. Martin survived, but with a fistula in his stomach that never fully healed. Most of the direct in vivo experiments were conducted, as a blessing in disguise, by tying a piece of food to a string and inserting it through the hole (remaining from the gunshot) into St. Martin’s stomach. At regular time points, Dr. Beaumont visually examined gastric digestion by removing and observing the remaining food particle (Beaumont, 1838).

The early GI intubation experiments considered the pylorus as a difficult hindrance for access to the small intestine. To the best of our knowledge, the first intestinal intubation in humans was described in 1908 by Scheltema, a pediatrician from the Netherlands (Scheltema, 1908). The idea of positioning a tube in the intestinal tract came from the observation of a chicken which had swallowed a long horsehair, one end protruding from the beak, and the other end from the cloacal aperture. During his intubation studies, Scheltema introduced a tube through the nose into the pharynx under local anesthesia by insufflation of cocaine powder or a cocaine solution. The end of the tube was taken out of the pharynx into the mouth, and a bulb was attached to the tube; subsequently, the tube was drawn back into the pharynx, while, after the next swallowing movement, the tube continued its passive movement along the GI tract, facilitated by the presence of the bulbous end. Introducing the tube allowed to locally administer fluids. While the tube was in position, Scheltema also collected the contents of the small intestine for examination. The passage of the tube from the mouth to the anus was referred to as permeation, illustrating that terminology has evolved since then.
A major contribution to the field of GI fluid aspiration was made by Miller and Abbott who introduced a double-lumen catheter with a collapsible balloon at the end of the tube in 1934 (Miller and Abbott, 1934), as summarized in 1949 (Miller, 1949). Abbott was investigating the effect of drugs on the motility in the duodenum using a balloon fixed at the end of a duodenal tube. He got frustrated as the balloon easily moved down the intestinal tract, impeding him from doing the intended measurements. This “slippery” effect of the distended balloon rapidly turned into an advantage to have a tube moving along the GI tract, reaching the ileum in only a few hours. In a next step, a second catheter was attached to the first one, immediately after the inflatable balloon, allowing the local administration of fluid or the collection of intestinal contents.

Subsequently, a double-lumen was created with one lumen for inflating/deflating the balloon to control the progress of the tube, and one lumen for the sampling of fluid or injection of liquid. The balloon eventually served three purposes: (1) facilitating tube movement along the GI tract, (2) creating an intestinal obstruction enabling the collection of contents above the inflated balloon, and (3) creating the possibility to measure contractions along the intestinal tract.

Later on, Miller and Abbott further adapted the tube into a triple-lumen tube of which two lumens were connected to an inflatable balloon, while the third one was used for fluid collection. The two balloons allowed the isolation of a segment of the small intestine for different experimental purposes (e.g., studies of secretory and absorptive functions of the intestine, measurement of intra-intestinal pressure changes at the level of each balloon) (Abbott and Miller, 1936).

In 1957, Abbott published a lively story on the selection of volunteers for his GI catheter studies. He also commented on the compensation “the tube swallowers” received. When doing a perfusion experiment using a dog, one would have to sacrifice the dog at the end of the day. As a dog was worth 2 US $ these days, two dollars a day became the compensation for the volunteers (Abbott, 1957).

In an important in vivo GI intubation study in humans, performed by Hofmann and Borgström in 1964, the intraluminal phase of dietary fat digestion and absorption was investigated in detail (Hofmann and Borgström, 1964). Following the oral feeding of a test meal containing corn oil, human small intestinal content was collected and investigated by GI intubation (Borgström, 1960; Hofmann and Borgström, 1964). The direct in vivo study showed that intestinal lipid, during fat digestion, is selectively partitioned between a micellar and an oil phase and that intestinal absorption of dietary lipid takes place from a micellar solution containing mainly free fatty acid and 2-monoglyceride (Borgström, 1960; Hofmann and Borgström, 1964).

In 1966, Fordtran and Locklear published a study in which gastric and small intestinal fluids were collected after the intake of a meal to assess their ionic constituents and osmolality (Fordtran and Locklear, 1966). The impact of two different meals on the assessment of ionic constituents and osmolality was compared, i.e. a 6 oz (170 g) sirloin steak-based meal versus a doughnuts-based meal. The volunteers were intubated with a single-lumen polyvinyl catheter (1.8 mm internal diameter), at the tip of which a 4 cm piece of polyvinyl tubing with multiple holes was attached to facilitate the collection of the fluids. In separate experiments, they managed to collect gastric, duodenal, jejunal and ileal fluids. Based on free flow through the catheter by siphonage, fluids were collected under oil.

The intubation technique appeared to be useful to explore site-dependent drug absorption in man. Jobin et al. were one of the first to apply the intubation technique in drug absorption studies (Jobin et al., 1985). They reported in 1985 on the site-dependent absorption of metoprolol. Introducing a triple-lumen catheter in the intestine and a double-lumen catheter in the stomach, samples were collected from the stomach, duodenum and jejunum after perfusion of a homogenized meal containing metoprolol (between 93.2 and 95.5 mg) into the stomach.

This short review of the existing literature illustrates that GI intubation studies span over more than a century. In parallel to advancing the possibilities of local treatment, the intubation approach contributed tremendously to our understanding of GI physiology, digestion along the GI tract, and the behavior of drugs and formulations in the GI lumen.

3. Anatomy, physiology and contents characterization of the upper and lower GI tract

3.1. Upper GI tract

3.1.1. Stomach

The stomach is a highly complex organ with a critical role in the initial steps of digestion before the processed food particles are transferred into the small intestine where further digestion and absorption take place. Anatomically, the stomach is subdivided into the fundus which contains the cardia or esophageal orifice, the corpus and the more distally located antrum.

Functionally, the stomach is traditionally divided between a proximal storage compartment and a distal compartment involved in mechanical digestion and propulsion. The proximal stomach will actively relax upon food intake in the process of adaptive relaxation or gastric accommodation (Cannon and Lieb, 1911; Kindt and Tack, 2006). The temporary storage of the food in the fundus is followed by a tonic contraction of the proximal stomach and repetitive contractions of the distal stomach with the propulsion of the gastric contents against a closed pylorus, resulting in mixing and grinding until the particle size is small enough (1–2 mm) to pass through the pylorus (Kelly, 1980; Meyer et al., 1981). This initial phase reflects the lag time of gastric emptying after which emptying occurs in a linear fashion (Camilleri, 2006; Camilleri et al., 1985). In contrast to solids and caloric liquids, emptying of low- or non-caloric liquids is exponential (i.e., a first-order process) with a half-emptying time of approximately 15 min and is mainly determined by an antroduodenal pressure gradient, largely independent of antral peristaltic activity (Camilleri et al., 1985; Hausken et al., 2002; Indireshkumar et al., 2000; Kelly, 1980). The rate of gastric emptying is regulated by a duodenogastric feedback mechanism and is slowed down by higher caloric content, lipids and acid and is modulated by gut hormones (Camilleri, 2019; Dooley et al., 1984; Heidell et al., 1988).

Motility in the fasted state is characterized by the migrating motor complex (MMC), a cyclic pattern of contractions in which 4 phases have been defined. Phase 3 is the most active one with high-amplitude contractions originating from the antrum (70%) or the duodenum (30%) and migrating distally to the ileum in approximately 1.5 to 2 h (Deloose et al., 2012). Non-digestible, larger (food) remnants empty from the stomach during phase 3 of the MMC in the fasting state (Oberle et al., 1990). The parietal cells from the fundus and the corpus secrete hydrochloric acid (HCl) to maintain an acidic environment with a pH of 2–2.5 in the fasted state which rises to 4.5–6.0 immediately after the meal (Camilleri, 2006).

3.1.2. Small intestine

The small intestine functions as a vital organ for digestion and absorption of nutrients and drugs. In its latter role, small bowel motility patterns may modulate pharmacokinetics of ingested drugs. The small intestine is a large organ, ranging up to 6 m with a narrow diameter of approximately 2.5 cm, composed of a complex network of blood vessels, nerves, and muscles located between the stomach and proximal colon (Kiela and Ghishan, 2016). The small intestine is comprised of three different regions:

1. The duodenum is the shortest of the three sections, approximately 30 cm in length, and has the greatest diameter (Jayaraman et al., 2001). It connects to the stomach at the pylorus and extends to the ligament of Treitz or duodenojejunal flexure. The ampulla duodeni
major or the ampulla of Vater is located in the descending part of the duodenum and is the location where the common bile duct and main pancreatic duct join together to access the duodenal lumen. The sphincter of Oddi within the ampulla regulates release of bile and pancreatic fluids into the duodenum.

2 The jejunum is approximately 2.5 m in length extending from the distal duodenum to the proximal ileum (Collins and Badireddy, 2019). The primary function of the jejunum is the absorption of nutrients digested in the duodenum. The jejunal surface contains circular folds and a mucous membrane covered in villi and microvilli, 0.5 – 1.6 mm in length, increasing the surface area by 30 – 600 fold (Kiela and Ghishan, 2016). Jejunal villi are the longest within the small intestine.

3 The ileum is the longest small intestine segment and extends from the distal jejunum to the ileocecal sphincter. Its length is approximately 3.5 m (Collins and Badireddy, 2019). The primary function includes absorption of lipid degradation products as well as vitamin B₁₂ and bile salts (Jung et al., 2010; Tappenden, 2014).

To summarize, the small intestine is an organ with diverse physiologic roles including digestion, fluid and nutrient absorption, fluid secretion, and transit and dispersion of luminal contents. A more recent MRI study sheds light on how luminal water appears as a population of discontinuous liquid pockets of varying size rather than as a single, more static pocket (Mudie et al., 2014). All of these activities are important to modify luminal drug behavior throughout the GI tract (Fig. 1). Disruption of small intestine anatomy and/or physiology can have a significant impact on the overall function of the human body and may affect drug absorption, distribution and clearance for orally administered drug products.

3.2. Lower GI tract

The colon is the distal part of the GI tract with the important function of absorbing water and electrolytes, vitamins, fermenting nutrients and store fecal content (Phillips, 1984). It consists of five parts: the right or ascending colon, the transverse colon, the left or descending colon, the sigmoid colon and the rectum. Because of the difficulties in accessing the entire length of the colon normally filled with feces, the colon has been less studied than other parts of the gut like the esophagus and the stomach. However, it has been always clear that the transit time of the colon is physiologically slow as compared to the gastric emptying and the small bowel transit time and this is likely to be related to the fact that this long transit time is required to accommodate absorption, fermentation and storage. Indeed, the mean colonic transit time in health is about 35 h as compared to up to 3 h of the small bowel transit time and of up to 4 h of the gastric emptying time (Camilleri et al., 2008). Using scintigraphy, Abrahamsson and co-workers demonstrated that a colonic transit time may vary between 10 h and 3 days, clearly showing the high (intersubject) variability

Fig. 1. Illustrative overview of anatomical and physiological variables that affect luminal drug behavior throughout the gastrointestinal (GI) tract.
Physicochemical characteristics of fluids in the lower intestine, based on data collected with direct sampling five hours after the administration of a glass of water to fasted healthy adults (fasted state) and five hours after the consumption of the high-fat, high-calorie meal (Food and Drug Administration 2002; European Medicines Agency 2010) by fasted healthy adults (simulating fed state conditions) (Diakidou et al., 2009; Reppas et al., 2015) can be summarized as follows: in the distal ileum, the pH is approx. 8.0, regardless of the dosing conditions. In the proximal colon (cecum and ascending colon) the pH in the fasted state is about 7.8 whereas in the fed state a small drop in pH can be observed (pH 6.0). Buffer capacity does not differ significantly between prandial states in the distal ileum; in the fasted state values are similar to those reported for the upper intestine. Compared to cecal and ascending colon contents, ileal contents have significantly lower buffer capacity only in the fed state.

4. Aspiration of human GI fluids: Methodology

4.1. Upper GI tract

To sample contents from the upper GI lumen one or more catheters are introduced through the mouth or nose of a human volunteer (Fig. 2). Following the positioning of the tube(s), volunteers can ingest a drug formulation sometimes in combination with a solid or liquid meal (Rubbens et al., 2019), or, if the study requires, they can be administered through the gastric port of the catheter directly into the stomach (Hens et al., 2016a; Kourentas et al., 2016). Gastric and intestinal fluids can subsequently be collected as a function of time, resulting in a detailed GI drug concentration profile (Hens et al., 2014). Besides, these fluids can also be analyzed for physicochemical properties, enzyme activity and/or bile salts (Rietherst et al., 2016b). Intestinal sampling is often combined with concomitant blood sampling to relate gastrointestinal drug and formulation behavior to systemic drug disposition (Browers and Augustijns, 2014). In this case, as intraluminal sampling inevitably includes removing a certain amount of drug from the intraluminal environment, the volume of aspirated samples should be limited (Hens et al., 2016a).

Throughout the years, different types of catheters have been used. In particular, two different approaches can be applied for sampling studies: (i) a single catheter with multiple sampling ports (Fig. 3A) and (ii) multiple catheters to aspirate fluids from different segments in the GI tract (Fig. 3B). Depending on the nature of the study, a rational assessment can be made to choose the most suitable catheter. For example, when interested in luminal concentrations at more than two segments of the GI tract, it can be advised to apply a single aspiration catheter with multiple aspiration ports which makes it less invasive for the volunteer and more convenient for the researcher. From a practical point of view, the aspiration of GI fluids occurs by placing a syringe on the top of the aspiration catheter and gently pulling the syringe. To facilitate the aspiration procedure, air can be inflated via the air channels. A small amount of air will be inflated via the aspiration catheter to remove residual fluid residing in the aspiration channel.

Concerning gastric aspiration studies, numerous small-scale (typically 5 to 10 healthy adult volunteers) clinical studies have been published in which gastric drug disposition has been linked to systemic concentrations. Gastric sampling studies can be expanded with simultaneous measurements of GI motility. Intraluminal pressure waves can simultaneously be monitored in real-time using HRM (Meyer-Gerspach et al., 2018). Studies in which gastric aspirates have been collected without the administration of a drug product have also been performed and reported, even under conditions simulating the dosing conditions applied in oral drug absorption studies (e.g., fasted versus fed state) to evaluate drug dissolution or drug solubility ex vivo under standardized guidelines as promulgated by regulatory authorities. These obtained data in combination with the characterization of the aspirated fluids have been extremely useful for the development of biorelevant media (e.g., fasted/ fed state) to evaluate the impact of intragastric drug performance on the entire absorption process (Dressman et al., 1998).

Regarding intestinal aspiration studies, fluids can be aspirated as such after administration of a glass of water (i.e., fasted state conditions) or after intake of a liquid/solid meal, representing the fed state conditions. These aspirated fluids can, subsequently, be analyzed for endogenous constituents that may have a major responsibility in the differences in luminal drug behavior between- and within-subjects, explaining inter- and intra-subject differences in systemic exposure of a drug, respectively. Concerning drug formulation behavior in the intestinal tract, catheters can be positioned in the more proximal part of the small intestine (i.e., duodenum as depicted in Fig. 2) or more distally (e.g., jejunum) as depicted in Fig. 3.

Exploring luminal drug behavior in the more distal parts of the human GI tract is of utmost interest when dealing with a delayed- or controlled-release drug formulation. For instance, the delayed-release...
properties of the amorphous solid dispersion tablet of posaconazole required sampling from more distal parts of the human GI tract rather than in the upper part (Hens et al., 2016b). It should be noted that the aspiration of intestinal fluids is not as easy as compared to the aspiration of gastric fluids due to the limited amount of fluid volume and the fact that fluid has to be aspirated from the more distal parts.

4.2. Lower GI tract

The lower intestinal lumen cannot be reached from the mouth or the nose to collect intestinal contents; therefore, aspirations to date have been achieved during colonoscopy (Fig. 4).

For systemically absorbed drugs, the region of interest is the distal ileum and ascending colon; in other regions of the colon luminal conditions do not allow for adequate drug amounts to be dissolved and therefore transported through the mucosa. For locally acting drugs, however, distal regions of the large intestine are equally important. As mentioned before, the residual volumes in the colon are rather scarce which makes it difficult to adequately aspirate fluids from the different regions of the large intestine. In a clinical aspiration study in which different locally acting formulations of mesalamine were explored, authors aspirated fluids in the stomach, duodenum and jejunum (Yu et al., 2017). Fecal samples were collected to estimate the amount of drug molecules that reached the colon.

5. Exploring drug product behavior in the human GI tract

Throughout the years, numerous aspiration studies have been conducted to explore the luminal behavior of orally administered drug products. Thanks to various bilateral collaborations (e.g., Pschoulia et al., 2011; Walravens et al., 2011) and multinational initiatives (e.g., OrBiTo (IMI/EFPIA-funded by EU - https://www.imi.europa.eu/projects-results/project-factsheets/orbito), PEARL (Marie Curie Action – funded by EU) and the in vivo predictive dissolution (IPD) project (funded by U.S. FDA)) (Hens et al., 2018; 2016b; Pentafragka et al., 2020), the sampling technique has gained a lot of momentum throughout the last two decades. Table 1 gives a comprehensive overview of different studies on how the aspiration technique unraveled the luminal behavior of drug products under different test conditions. Also, the goal of each study is highlighted.

6. Composition of GI fluids and underlying GI physiology as a source of intra- & intersubject variability

It is generally acknowledged that GI variables (e.g., motility, pH, fluid volumes, bile salts) affect oral formulation performance and consequently drug plasma levels (Abuhelwa et al., 2017). The variability of some of these parameters, as GI transit times and GI pH have recently been reviewed in two comprehensive meta-analyses (Abuhelwa et al., 2016a; 2016b). Knowing the main GI determinants of $C_{\text{max}}$ and AUC variability will lead to the improvement of BE trial protocols and will guide the design of $in vitro$ predictive dissolution tests incorporating the expected range of the physiological variables (Hens et al., 2016c).

Boyd and colleagues recently reviewed several human intubation studies that attempted to characterize the $in vivo$ dissolution process for poor solubility drugs (Boyd et al., 2019). As the authors stated intraluminal drug concentrations (i.e., dissolution rate) can be related to the fluid characteristics as pH, volume or composition, as a further step, the simultaneous monitoring of intestinal motility offers a more complete picture of the product-GI environment interactions. The combined measurement of luminal fluid characteristics, motility patterns and luminal and plasma drug concentrations is illustrated in several recent intubation studies performed in human volunteers, after the administration of a solid immediate-release form of ibuprofen (Bermejo et al., 2018; Koenigsknecht et al., 2017; Paixão et al., 2018a, 2018b), paracetamol (Van Den Abeele et al., 2017a), fosamprenavir (Braeckmans et al., 2020) and atazanavir (Hens et al., 2020). The ibuprofen study was performed in fasted and fed state conditions. In the fasted study, the glass of water for dosage administration also contained phenol red as a non-absorbable marker to estimate the net fluid volume changes. Other relevant GI co-variables as luminal pH, motility and buffer strength were measured. Data analysis is still ongoing but several outcomes have been already published. Koenigsknecht et al. described thoroughly the clinical study and the results were qualitatively described, mainly pointing out the high variability of pH values across subjects and the effect of food on reducing ibuprofen plasma $C_{\text{max}}$ values. Interestingly, ibuprofen concentrations were still measured in the stomach and small intestine after 7 h which required further analysis. The work of Hens et al. mainly focused on the analysis of the buffer strength of luminal samples pointing out the much lower buffer capacity of luminal fluids in comparison with the common dissolution media and even with the so-called biorelevant media. Bermejo et al. and Paixao et al. applied multiple linear regression analyses to quantify the contribution of pH and motility indexes on plasma $C_{\text{max}}$ variability (Bermejo et al., 2018; Paixão et al., 2018a). With a basic mechanistic approach based on deconvolution from plasma levels, the relationship between $in vivo$ dissolution and $in vitro$ systemic input was explored. It was shown, using experimental luminal data, that $in vivo$ luminal concentrations are the driving force for the intestinal permeation and, thus, the systemic exposure of the drug. Absorption rates estimated from plasma levels by deconvolution data analysis showed a good correlation with the $in vivo$ dissolution, i.e., maximal absorption rates corresponded with the maximal luminal concentrations of ibuprofen. On the other
hand, besides considering ibuprofen as an acidic drug (pKa ~ 4.85) with limited dissolution kinetics in the stomach but increased solubility in the small intestine, the impact of the phase III contractions post-dose on C_{max} value was also highlighted. The longer the time to the next phase III after dosing the lower C_{max} observed in the volunteers. Later on, a compartmental (stomach-duodenum-jejunum-plasma) mass transport analysis incorporating time to the next phase III contraction post-dose in each individual and their luminal pH-time profiles showed a well-combined fitting of luminal and plasma concentrations in several subjects indicating that the intersubject variability in luminal dissolution and plasma levels was highly dependent on those independent variables (Tsune et al., 2018).

Related to inter-subject differences in the composition of GI fluids, Riethorst and co-workers characterized the duodenal aspirates of ten healthy subjects in fasted and fed state conditions (Riethorst et al., 2016b). This comprehensive work highlighted the immense inter-subject variability in the endogenous constituents (e.g., bile salts, enzymes, lipid digestion products) present in the aspirated duodenal fluids. In a follow-up study, the impact of these different constituents on drug permeation was investigated using a fractional design of experiment (DoE) approach, including 16 different fluid compositions (Riethorst et al., 2018). Evaluating the impact of each constituent towards the permeation of a drug warns us that making use of the ‘average’ simulated biorelevant media in dissolution, solubility or transport experiments is not reflecting the entire picture of these dynamic processes in vivo.

Unlike with gastric emptying of contents which occurs continuously during and after meal consumption accompanied by the so-called postprandial glucose fluctuations in the blood (Edinburgh et al., 2018; Koziolek et al., 2014; Malagelada et al., 1976), in the fed state, conventional tablets and capsules must disintegrate for any undissolved drug to start emptying from the stomach. Aspiration studies, after the standard meal (Rubbens et al., 2019) confirmed earlier imaging data in humans (Kelly et al., 2003; Weitschies et al., 2008), according to which disintegration times of conventional tablets can be significantly extended when administered after the standard meal. Recent aspiration studies indicate that, after the disintegration of conventional dosage forms to solid particles, non-ionizable BCS Class I drugs empty from the stomach with an apparent first-order rate constant of about 40 min (Pentafragka et al., 2020). For non-ionizable BCS Class II drugs the estimated half-life for gastric emptying seems to be slightly longer, about 50 min (Pentafragka et al., 2020).

The physiologic changes induced by food in the GI tract are quite well understood and initiate an increase in the retention time in the stomach, the changes in pH in the initial part of the GI tract and the increase of secretions that can influence solubility of drugs (Koziolek et al., 2019). Although the influence of these changes in the drug absorption process can be mechanistically interpreted, only a piece of direct evidence can be delivered by collecting data of the luminal contents and simultaneous determination of the plasma profiles. This has allowed to effectively connecting the GI concentration-time profiles to the observed plasma profiles, as well as to elucidate some of the underlying physiologic sources of the observed intra- and intersubject variability. In a study from the University Hospital of Michigan (Paixão et al., 2018a), the weak acid ibuprofen (immediate-release 800 mg RLD tablet) was administered with a liquid meal to healthy subjects. After oral administration, GI fluids were aspirated and analyzed for drug content, pH and buffer capacity. In parallel, blood samples were also collected. Pressure events along the GI tract were measured to evaluate the impact of GI motility on drug product performance. The authors were able to directly relate the amount of soluble ibuprofen in the duodenum and proximal jejunum to the rate of absorption of the drug as measured from the plasma profile by deconvolution. There was a strong correlation between the amount of ingested calories and the duration of the fed pattern, described by the interval between meal administration and the return of a burst of rhythmic activity propagating distally, defined as a phase III event (tMMC-III) of the MMC. The characteristic increase of gastric pH, as observed by several other studies on gastric luminal physiologic behavior, was confounded. Increased pH values return to fasting pH values over time, in particular after the tMMC-III event was observed. Inter- and intra-subject variability was approximately 30% for the plasma C_{max} and 11 and 34% for the plasma AUC, respectively. When trying to explain this variability, it was observed that the stomach pH and an interaction between the stomach pH and the tMMC-III parameter were able to explain 63% of the observed variability in ibuprofen plasma

**Fig. 4.** Schematic overview of the technique for sampling from the lumen of the lower intestine. The original picture can be accessed online in color (https://www.mayoclinic.org/tests-procedures/colonoscopy/about/pac-20393569, last accessed on June 3rd, 2020).
Table 1
Overview of aspiration studies that have been conducted over the years to unravel the luminal behavior of an orally administered drug. Information about the model drug, dosage form/strength, brand name of the administered product, region of aspiration, dosing condition and goal of the study are provided. ‘S’, ‘D’, ‘J’, and ‘P’ stands for ‘stomach’, ‘duodenum’, ‘jejunum’ and ‘plasma’, respectively.

| Model drug               | Dosage form and dose strength | Brand name         | S  | D  | J  | P  | Dosing condition                                        | Goal of the study                                                                                     | Reference                           |
|--------------------------|-------------------------------|--------------------|----|----|----|----|--------------------------------------------------------|-------------------------------------------------------------------------------------------------------|--------------------------------------|
| Abiraterone acetate      | Tablet (250 mg)               | Zytiga*            | X  |    |    |    | Fasted state (250 mL of water)                         | To determine the in vivo intraluminal concentrations of abiraterone and abiraterone acetate and to explore the intraluminal hydrolysis of the prodrug abiraterone acetate. | (Stappaerts et al., 2015)            |
| Abiraterone acetate      | Tablet (250 mg)               | Zytiga*            | X  |    |    |    | Fasted state (250 mL of water) versus Fed state (400 mL of Ensure Plus Vanilla® 20 min before intake of the abiraterone acetate tablet with 250 mL of water) | To relate the reported positive effect of food on the oral bioavailability of abiraterone to the intraluminal behavior of abiraterone acetate. | (Gebbers et al., 2016a)             |
| Albendazole              | Suspension of 50 mg, dispersed in 240 mL of table water | Compounded suspension | X  |    |    |    | Fasted state; 240 mL: (i) albendazole suspension in water, (ii) albendazole suspension in HPMC ES aqueous solution, (iii) Type IIA lipid-based albendazole suspension in water, (iv) Type IV lipid-based albendazole suspension in water; administration was through the gastric port of a two-lumen naso-gastro-intestinal tube | To explore the effectiveness of supersaturation promoting excipients on albendazole concentrations in the upper GI lumen. | (Kourentas et al., 2016)            |
| Amprenavir               | Eight soft gelatin capsules containing 150 mg of amprenavir each | Agenerase®         | X  |    |    |    | Fasted state (180 mL of water)                         | To determine intraluminal concentrations of amprenavir, and the excipient α-tocopherol polyethylene glycol 1000 succinate (TPGS). | (Brouwers et al., 2006)             |
| Atazanavir               | Capsule (150 mg of atazanavir, present as the sulfate salt) | Reyataz®           | X  |    |    |    | Fasted state (i) 240 mL of water, (ii) 240 mL of Coca-Cola® and (iii) a glass of water under hypochlorhydric conditions (PPI-condition). | To explore the impact of GI pH and motility on intraluminal and systemic concentrations of atazanavir | (Hens et al., 2020)                 |
| Danazol                  | 150 mg pre-dissolved in the olive oil portion of the meal | Compounded solution | X  |    |    |    | Fed state (liquid, heterogenous meal having physicochemical characteristics and composition similar to the standard meal suggested by regulatory agencies; administration was through the gastric port of a two-lumen naso-gastro-intestinal tube) | To characterize the micellar phase and to evaluate the impact of micellar lipids and coarse lipid particles on danazol flux through intestinal monolayers. | (Vertzoni et al., 2012)             |
| Danazol                  | Aqueous suspension (100 mg) Sunflower oil solution (100 mg) | Prepared extemporaneously | X  |    |    |    | Fed state: 30 min after initiation of standard high-calorie, high-fat meal® administration with a glass of water | To evaluate the disposition of a BCS Class II drug in the upper gastrointestinal lumen of healthy adults in the fed state* under dosing conditions simulating the situation after disintegration of orally administered IR dosage forms in BA/BE studies. | (Pentafragka et al, 2020)           |
| Diclofenac               | Tablet of potassium salt (50 mg) predissolved in 240 mL of water | Cataflam®          | X  |    |    |    | Administration of the 240 mL solution in fasted and fed state conditions (liquid meal, 400 mL of Ensure Plus) with or without concomitant use of a proton-pump inhibitor (PPI) | To investigate GI supersaturation and precipitation behavior of a weakly acidic drug | (Van Den Abeele et al., 2016)       |
| Diclofenac               | Tablet of potassium salt (50 mg) | Cataflam®          | X  |    |    |    | Fasted (240 mL of tap water) and fed state conditions (liquid meal, 400 mL of EnsurePlus®) | To gain further insight into the gastrointestinal GI disposition of diclofenac and the implications for systemic drug exposure in humans under fasted and fed state conditions. | (Van Den Abeele et al., 2017c)      |
| Diclofenac               | Tablet of potassium salt (50 mg) | Cataflam®          | X  |    |    |    | Fed state (FDA standard meal)³ | To investigate GI drug concentrations of the weakly acidic drug diclofenac when dosed to healthy volunteers after intake of the FDA standard meal. | (Rubbens et al., 2019)              |
| Dipyridamole             | Solution containing 30 or 90 mg of dipyridamole | Compounded solution | X  |    |    |    | 240 mL aqueous solution administered through the gastric port of a two lumen naso-gastro-intestinal tube | To evaluate intestinal supersaturation and precipitated dose fraction of a weak base. | (Psachoulias et al., 2011)          |
| Fenofibrate              | One capsule of 200 mg micronized fenofibrate or one tablet of 145 mg nanosized fenofibrate | Lipanthyl® (micronized) and Lipanthyl nano® (nanosized) | X  |    |    |    | Fasted (250 mL of water) versus Fed state (400 mL of Fortimel Extra® nutrient shake) | To explore the GI behavior and systemic exposure of micro- and nanosized fenofibrate in fasting and fed conditions | (Hens et al., 2015)                 |
| Fosamprenavir            | Tablet (700 mg of fosamprenavir calcium) | Telzir®            | X  |    |    |    | Fasted (180 mL of water) versus Fed state (300 mL of Scandinah Fashion®) | To explore the feasibility of linking the pharmacokinetic profile of a drug with its GI behavior in fasted and fed state conditions. | (Brouwers et al., 2007)             |

(continued on next page)
| Model drug | Dosage form and dose-strength | Brand name | S | D | J | P | Dosing condition | Goal of the study |
|------------|-------------------------------|------------|---|---|---|---|-------------------|------------------|
| Fosamprenavir | Tablet (700 mg fosamprenavir) | Fosamtic® | X | X | X | X | Fasted state (240 mL of water) | To investigate the impact of variations in upper gastrointestinal conditions on the (in)homogeneous distribution of an orally administered cyclosporin A-based solution of the weak base. |
| Ibuprofen | Tablet (800 mg) | Diflunisal Tablets, USP, 800 mg, Dr. Reddy's Laboratories Limited | X | X | X | X | Fasted state (240 mL of water) and fed state (250 mL of Ensure Plus®) | To evaluate the effect of pressure events on the drug dissolution, supersaturation and precipitation behavior of the weakly basic drug. |
| Indinavir | Capsule (400 mg) | Crixivan® | X | X | X | X | Fasted state (240 mL of water) | To evaluate the absorption of indinavir under fed and fasted conditions. |
| Itraconazole | Solid dispersion and Cyclodextrin-based solution (each 200 mg) | Sporanox® | X | X | X | X | Administration with or without a glass of water | To investigate the influence of intraluminal dilution on the behavior and performance of an orally administered cyclodextrin-based solution of the poorly soluble drug itraconazole. |
| Ketoconazole | Solution containing 100 and 300 mg | Compounded solution | X | X | X | X | Fasted state (240 mL of water) and fed state (400 mL of Ensure Plus®, fed state in the presence of the transit-stimulating agent domperidone (2 tablets of Motilium®; 20 mg) and fed state in the presence of the transit-inhibiting agent loperamide HCl (2 tablets of Imodium®; 4 mg)) | To investigate the effect of formulation pH and type on these processes. |
| Paracetamol | Aqueous suspension (500 mg) | Dafalgan® | X | X | X | X | Intake with either tap or sparkling water | To investigate the effect of pressure events on intraluminal and systemic drug disposition. |
| Paromomycin | Aqueous solution prepared by dissolving one tablet of paromomycin (220 mg) in 250 mL of water | Gabroral® | X | X | X | X | Fasted state; 240 mL of suspensions and solution | To explore the link between fasted state gastric motility and (in)homogeneous drug distribution in the stomach (corpus and antrum) and fed state in the presence of the transit-stimulating agent domperidone (2 tablets of Motilium®; 20 mg) and fed state in the presence of the transit-inhibiting agent loperamide HCl (2 tablets of Imodium®; 4 mg). |
| Posaconazole | Two suspensions (40 mg of posaconazole/mL and 60 mg of posaconazole/mL) | Noxafil® | X | X | X | X | Administered at 4-hour intervals | To evaluate intestinal supersaturation and precipitated dose fraction of a weak base. |
| Sertraline | Tablets (125 mg) | Zoloft® | X | X | X | X | Fasted state (240 mL of water) | To investigate the absorption of sertraline under fed and fasted conditions. |

(continued on next page)
| Model drug | Dosage form and dose strength | Brand name | S | D | J | P | Reference |
|------------|------------------------------|------------|---|---|---|---|-----------|
| Quercetin capsule (500 mg) | Compounded HPMC capsule | X | X | X | X | Fasted state (240 ml of water) | To investigate the intestinal metabolism of quercetin. (Chalet et al., 2018) |
| Ritonavir tablet (amorphous solid dispersion, 100 mg) | Zocor® | X | X | X | X | Fasted state (240 ml of water) and fasted state condition after prior intake of a proton-pump inhibitor (PPI) once-daily for three days | To elucidate the GI behavior of the ritonavir amorphous solid dispersion. (Van Den Abeele et al., 2020) |
| Simvastatin tablet (40 mg) | Zocor® | X | X | X | X | Fasted state (250 ml of water) and fed state | To gain more insight into the intraluminal behavior of the cyclic ester simvastatin. (Geboers et al., 2016b) |
| Tenofovir-disoproxil fumarate tablet (300 mg prodrug) | Viread® | X | X | X | X | Fasted state (250 ml of water) and fed state condition after prior intake of a proton-pump inhibitor (PPI) once-daily for three days | To monitor the luminal concentrations of theophylline after oral intake of an immediate-release versus a slow-release dosage form. (Brouwers et al., 2005) |
| Theophylline immediate- and slow-release formulation (100 mg) | Xanthium 200 (slow-release) | X | X | X | X | Fasted state (180 mL of water) | To monitor the luminal concentrations of theophylline in situ in the human small intestine. (Brouwers et al., 2005) |

* The FDA breakfast meal contains two eggs fried in butter, two strips of bacon, two slices of toast with butter, four ounces of hash brown potatoes and eight ounces of whole milk.

C_{max}. This motility and pH effect was also observed in other intestinal aspiration studies under fed conditions. In a study of Brouwers and coworkers, after the oral administration of tablet of fosamprenavir (a phosphate ester prodrug of the poorly water-soluble HIV-inhibitor amprenavir), food induced a delay in the absorption of amprenavir, with an increase in the mean T_{max} and a similar delay in the duodenal appearance of fosamprenavir (Brouwers et al., 2007). Although a relationship between gastric pH and solubility is also expected for this pro-drug, the authors also proposed a formulation effect due to food-induced difficulties in the in vitro tablet disintegration that could also explain the observed delay in the duodenal appearance. This food delayed disintegration was also proposed to explain the delayed luminal gastric appearance of the solid posaconazole available as a function of time after the oral administration of a delayed-release formulation with food (Hens et al., 2016b), or a later appearance of a large amount of solid drug in the stomach after administration of an immediate release oral formulation of the weak base diclofenac (Van Den Abeele et al., 2017c).

Overall, these studies illustrated the importance of delayed gastric emptying and possible delayed disintegration, as well as the effect of gastric pH changes that occur with food that can eventually influence the dissolution of weak acids and bases. In bioavailability and bioequivalence studies, food effects are usually studied under high-caloric, high-lipid content because these are considered to be the most discriminatory conditions. However, and regarding the gastric emptying process, the administration of approx. 800–1000 calories (e.g., the FDA breakfast meal) results in a long presence of the fed state motility and the associated disruption of the MMC, the time point of gastric emptying can be dependent on further meals and liquid intake of the subjects (Kozolek et al., 2015). Since the luminal pH, secretions and colloidal structure changes are also observed with lower amounts of ingested calories, it could be questioned if the high caloric meal is indeed the best one to compare the performance of different formulations for fed state conditions.

7. Challenges and issues

Aspirated samples could be used for (i) quantifying drug concentrations, (ii) for testing the performance of active pharmaceutical ingredients (API) ex vivo or (iii) for developing biorelevant media. However, one difficulty when aiming at quantifying drug concentrations is that, in contrast with the situation in the upper GI lumen, collection of numerous colonic samples over time is problematic, due to the limited and viscous volumes available in the region (Lemmens et al., 2020; Murray et al., 2017) and the difficulty in maintaining the colonoscope in place for a long period. Also, in the field of gut microbiota, it is extremely hard to develop a sampling method that can accurately quantify gut microbiota in contents collected from the lower intestine (Tang et al., 2020). As a result, no studies in which drug concentrations have been quantified in the region have been performed to date, except for the study performed by Lemmens and co-workers, where celecibex concentrations were measured in aspirated caecal fluids after oral administration of a 200 mg Celebrex® capsule to healthy subjects (Lemmens et al., 2020). However, it was not feasible to aspirate numerous fluids as a function of time due to the limited volume present in the lower GI tract. Table 2 and 3 are summarizing the potential hurdles that can be faced when applying the GI sampling technique to sample aspirates from the upper and lower GI tract, respectively. Suggestions towards tackling these hurdles are also provided.

8. The collection of luminal contents from the human GI tract: “Best practice”

As summarized in Table 1, GI sampling within the field of drug product evaluation may serve many purposes, including the exploration of:
The non-exhaustive list of challenges of the GI aspiration sampling technique concerning the aspiration of fluids in the upper GI tract (i.e., stomach and small intestine).

| Issue/challenge | Suggested approach, technology or cooperation |
|-----------------|-----------------------------------------------|
| The presence of a catheter may affect gastrointestinal (GI) physiology | Reduce the diameter to the extent that aspiration of samples is still possible. Process samples immediately after collection, for example: Measurement of pH and buffer capacity (limit exposure to atmospheric oxygen). |
| The composition of GI fluids may change during storage. | (i) Removal of solid material (precipitated/non-dissolved drug) by filtration or centrifugation. (ii) Blocking in-sample drug precipitation by dilution in the organic phase. (iii) Inclusion of enzymatic inhibitors (including lipase inhibitors) in case digestion influences drug and formulation behavior (especially in fed state conditions). |
| Solubility and dissolution may be affected by the presence of lipids in the gastrointestinal samples. | Explore solubility and dissolution before and after separation of the lipid phase (centrifugation). Another possibility is to control the food intake before and during the study (e.g., controlled amount of lipids). |
| GI sampling may reduce the amount of drug available for absorption | Limit the volume and number of collected GI fluids. Combine GI sampling with MRI experiments to assess fluid volume. Another possibility is to make use of a non-absorbable marker that accounts for GI secretion, transit and dilution. Combine GI sampling with pressure measurements (water perfused catheter or high-resolution manometry). Different scenarios can be hypothesized: Acidic drugs may benefit from a fast phase 3 cycle Basic low-solubility compounds may benefit from a longer residence time in the stomach, allowing sufficient time to dissolve, possibly resulting in (higher) supersaturation upon transfer into the small intestine. Soluble compounds may be less dependent on the motility cycle (possibly traveling with the co-ingested water). |
| Collected gastrointestinal samples allow assessing drug concentration, not the amount available for absorption | Limit the volume and number of collected GI fluids. Combine GI sampling with MRI experiments to assess fluid volume. Another possibility is to make use of a non-absorbable marker that accounts for GI secretion, transit and dilution. Combine GI sampling with pressure measurements (water perfused catheter or high-resolution manometry). Different scenarios can be hypothesized: Acidic drugs may benefit from a fast phase 3 cycle Basic low-solubility compounds may benefit from a longer residence time in the stomach, allowing sufficient time to dissolve, possibly resulting in (higher) supersaturation upon transfer into the small intestine. Soluble compounds may be less dependent on the motility cycle (possibly traveling with the co-ingested water). |
| GI motility may affect drug disposition | Explore solubility and dissolution before and after separation of the lipid phase (centrifugation). Another possibility is to control the food intake before and during the study (e.g., controlled amount of lipids). Combine GI sampling with pressure measurements (water perfused catheter or high-resolution manometry). Different scenarios can be hypothesized: Acidic drugs may benefit from a fast phase 3 cycle Basic low-solubility compounds may benefit from a longer residence time in the stomach, allowing sufficient time to dissolve, possibly resulting in (higher) supersaturation upon transfer into the small intestine. Soluble compounds may be less dependent on the motility cycle (possibly traveling with the co-ingested water). |
| GI physiology may be different in specific patient populations (e.g., pediatric patients, geriatric patients). | Collect GI samples through feeding tubes or when performing endoscopic examinations. As an alternative, the collection and characterization of stoma fluids can be considered. Drug behavior can be assessed ex vivo in the collected fluids. Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. Combine with: Dialysis experiments, cell-based or cell-free permeation assays. Explore the influence of lying versus supine position versus walking on GI drug distribution. Standardize position/activity as much as possible. Measure initial and equilibrium pH to evaluate the importance. |
| Luminal concentrations may not be the driving force for absorption due to interaction with intraluminal compounds. | Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. The combination can be made with dialysis experiments, cell-based or cell-free permeation assays. Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. The combination can be made with dialysis experiments, cell-based or cell-free permeation assays. |
| The position and activity status of the volunteer/patient may affect gastrointestinal drug disposition (also applicable to MRI studies). | Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. The combination can be made with dialysis experiments, cell-based or cell-free permeation assays. Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. The combination can be made with dialysis experiments, cell-based or cell-free permeation assays. |
| Equilibrium solubility studies are performed in the absence of bicarbonates (eliminated during equilibration). | Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. The combination can be made with dialysis experiments, cell-based or cell-free permeation assays. Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. The combination can be made with dialysis experiments, cell-based or cell-free permeation assays. |

It is obvious that the design of these studies is not straightforward and that multiple factors need to be taken into account to allow justified conclusions. A careful selection of the different aspects related to the study design would include, among others, the selection of the catheter, the positioning process (possible effect on GI physiology, e.g., induction of secretions), the time of drug administration, the time of food intake in case food effects are being explored, the sampling duration and frequency, the schedule of plasma sampling, and the type of meal (liquid versus solid, high fat versus low fat, high calorie versus low calorie). As explained in the methodology section, one can make use of two different approaches for sampling studies, i.e., using a single catheter or multiple catheters. The catheters used can have one or more aspiration ports. In addition to sampling, pressure measurements could be performed to monitor GI motility, either using a high-resolution manometry catheter or a water-perfused motility catheter; the latter can be integrated in the sampling catheter. The selection of the catheter type is an important factor given the study design, but also since a catheter may affect local GI physiology. Therefore, the following catheter-related factors need to be taken into account:

(1) the behavior of controlled drug release delivery systems,
(2) intestinal solubilization, supersaturation, and precipitation
(3) food and beverages effects,
(4) excipient(s) effects,
(5) drug interactions (e.g., PPI),
(6) real-life dosing conditions,
(7) intraluminal drug stability.

Table 3
Non-exhaustive list of challenges of the GI aspiration sampling technique concerning the aspiration of fluids in the lower GI tract (i.e., large intestine).

| Issue/challenge | Suggested approach, technology or cooperation |
|-----------------|-----------------------------------------------|
| Colonic fluids are difficult to collect and are viscous, two factors impeding time-dependent assessment of luminal drug concentration in the colon. | Assess drug concentrations in biopsies of colonic tissue collected as a function of time as an indirect reflection of luminal concentrations (equilibrium versus non-equilibrium conditions). |
| GI physiology may be affected by the disease state (e.g., Crohn’s disease, ulcerative colitis) | Assess drug concentrations in biopsies of colonic tissue as an indirect reflection of luminal concentrations. Compare concentrations in diseased with those in non-diseased tissue. Include information on the inflammation status. |
| The cleaning procedure (enema, bisacodyl, rinsing of the lens) before colonic sample collection may alter the composition and drug concentration of luminal samples. | Limit enema to left hemicolon and sample from the undisturbed right hemicolon. Apply a validated, colon preparation protocol which involves the administration of light laxative (bisacodyl) at least approximately 2 days before colonoscopy (Dziakidou et al., 2009). Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. The combination can be made with dialysis experiments, cell-based or cell-free permeation assays. Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. The combination can be made with dialysis experiments, cell-based or cell-free permeation assays. |
| Luminal concentrations may not be the driving force for absorption due to interaction with intraluminal compounds. | Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. The combination can be made with dialysis experiments, cell-based or cell-free permeation assays. Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. The combination can be made with dialysis experiments, cell-based or cell-free permeation assays. |
| There is no certain measure to evaluate locally acting drug products. | Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. The combination can be made with dialysis experiments, cell-based or cell-free permeation assays. Use of intestinal fluids in vitro assays to evaluate the bio-accessible fraction. The combination can be made with dialysis experiments, cell-based or cell-free permeation assays. |
8.1. Fasted and fed state studies

It should be noted that studies using catheters placed inside the GI tract are inherently invasive and can, thus, potentially affect normal GI physiology. Several studies indicate minor or non-significant effects of thin and pliable transpyloric tubes on gastric emptying, gastric secretions and duodenal-gastric reflux (Müller-Lissner et al., 1983, 1982). However, comparative studies should be performed in parallel to confirm the same results for other types of catheters. It is thus important to carefully consider the selection of the catheter, always keeping in mind that a simple design will have fewer effects on local physiology. One can partly compensate for this effect on physiology by performing comparative studies using the same catheter. Related to the diameter of the catheter used, numerous studies have been performed. Longstreth et al. have shown that a 4 mm outside diameter (OD) transpyloric tube does not significantly affect gastric emptying of contents after administration of a meal (Longstreth et al., 1975). Mueller-Lissner et al. (Müller-Lissner et al., 1982) showed similar data for a 5 mm OD, after administration of liquid meals, unlike the situation where more than one tube is passing through the pylorus (Read et al., 1983).

All aspects mentioned so far need to be carefully considered as they may all have an impact on the overall outcome. It is therefore obvious that a single standard procedure for the collection of intestinal fluids is not possible. Careful consideration of all conditions is required, so that justified decisions can be made depending on the study objectives. As an example of an intestinal sampling technique, a protocol is presented in line with the bioavailability (BA) and bioequivalence (BE) guidelines as promulgated by the US FDA, entitled ‘Food-Effect Bioavailability and Fed Bioequivalence Studies’ (U.S. Food and Drug Administration, 2017). Related to exploring food effects, regulatory guidelines that provide a protocol to perform clinical pharmacokinetic studies in fasted and fed state conditions can be considered.

The reasoning so far especially relates to the collection of samples from the upper GI tract; given the limited amount of fluids in the colon, it is presently not feasible to collect colonic fluids as a function of time to monitor formulation performance.

8.1. Upper GI tract

8.1.1. Fasted and fed state studies

Numerous actions should be considered to perform state-of-the-art aspiration studies in fasted and fed state conditions.

1. Before the start of the study, an overnight fast of at least 12 h should be respected. Water can be allowed as desired except for one hour before drug administration.
2. After the overnight fast, subjects come to the hospital in the morning and are intubated with the GI catheter(s). The positioning of the catheter(s) in the region of interest should be checked and confirmed (e.g., fluoroscopy). Note that female participants need to be screened for pregnancy (i.e., exclusion criteria).
3. After positioning of the catheter(s), the subject needs to be located in an upright supine position to make it feasible for the researcher to aspirate the fluids and, if intended, to take blood samples.
4. After a stabilization period of 30 min (to assure that excessive secretions in the stomach during the insertion of the tube and during the stabilization period have been eliminated), the subject will be asked to ingest the formulation product with 240 mL of water. Depending on the goal of the study, the co-administered beverage can change in terms of (i) the nature of the drink and (ii) the volume. In the case of fed state studies, subjects should start with the recommended meal 30 min before the administration of the drug product. The volunteers should consume this meal in 30 min or less; however, the drug product should be administered 30 min after the start of the meal. The subject will be asked to ingest the formulation product with 240 mL of water 30 min after the start of the intake of the meal. No food should be allowed for at least 4 h post-dose. Water can be allowed as desired except for one hour after drug administration.

5. For both fasted and fed state treatment periods, timed samples of biological fluid (i.e., GI fluids and/or plasma), should be collected from the subjects to permit characterization of the complete shape of the GI/ plasma concentration-time profile for the parent drug. It may be advisable to measure other moieties in the biological fluid, including active metabolites. Fluid samples will be aspirated by using a syringe. After the collection of the aspirated fluid, fluids will be further handled as discussed in the next section. Note that only a limited volume of fluid should be collected to safeguard the dynamic environment as good as possible. Afterwards, a small amount of air will be inflated via the aspiration catheter to remove residual fluid residing in the aspiration channel.

6. When the complete shape of the GI concentration-time profile(s) is captured, the GI catheters can be removed.

8.2. Lower GI tract

For the aspiration of fluids from the lower GI tract, we still face a hurdle concerning the limited and viscous amounts available for aspiration as mentioned in the section ‘Challenges and issues’. MRI studies highlighted the low amount of freely mobile water in the fasted human colon which was in the order of only a few milliliters (Murray et al., 2017; Schiller et al., 2005). Collection of samples of colonic contents, is feasible (Diakidou et al., 2009; Reppas et al., 2015; Vertzoni et al., 2010b) and useful for measuring drug solubility and preparing biorelevant media (Vertzoni et al., 2010a). In those studies, subject work-up included the administration of 10 mg of bisacodyl 50 h before colonoscopy plus 10 mg of bisacodyl 44 h before colonoscopy. With this regimen, bisacodyl effects on the intracolonic environment at the time of colonoscopy are eliminated (Diakidou et al., 2009) and collected volumes were similar to those previously observed with MRI studies (Schiller et al., 2005). A recent study demonstrated the colonic disposition of celecoxib in healthy subjects after intake of a 200 mg dose (Lemmens et al., 2020). In this study, the colon preparation procedure existed in a one-time enema of 250 mL of water to cleanse the left hemicolon, while avoiding spill over to the right hemicolon where then physiologically relevant samples could be collected. During two separate moments (approx. 2.5 h and 7.5 h after intake of the drug product), caecal content was aspirated and analyzed for celecoxib and its carboxy metabolite. Celecoxib was only extensively present in the samples collected during the second aspiration moment. However, it should be noted that it was not feasible to aspirate samples as a function of time in the context of formulation evaluation in the lower intestine.

8.3. Sample handling of the GI fluids upon collection

Following the collection of GI fluids, samples should be handled properly to generate reliable results that reflect the local intraluminal...
situation. This is usually very challenging and difficult to perform given the rapidly changing conditions upon sample collection: the sample is disconnected from its physiological environment, biochemical processes proceed, and the drug present in the sample may continue to dissolve, precipitate (in case of supersaturation), or degrade. All these challenges need to be considered when performing intestinal sampling experiments.

An important factor which relates to physiological conditions is the pH of the aspirated fluids: because of the loss of endogenous CO₂ present in the sample, the pH should be measured immediately upon collection to guarantee a representative pH value; the same applies if buffer capacity is being explored: in fasted state conditions, it should be determined immediately (Litou et al., 2020), while it is less critical in fed state conditions, as, in these conditions, pH may be governed by food compounds (or its degradation) products. In case pH may affect the solubility of a drug (pKa within pH range interval), immediate processing of the sample is required when product performance is being assessed to exclude a time dependent pH effect. Prompt sample handling is also required when intestinal samples are collected when supersaturated concentrations are expected in the intestinal environment, either following the GI pH shift (e.g., transfer from a basic drug dissolved in the acidic environment to the neutral environment of the small intestine) or based on an enabling formulation approach. It is obvious that, in case of supersaturation, samples should be centrifuged or filtered immediately upon collection, and that the supernatant should be diluted in a medium which prevents further precipitation (e.g., containing a sufficient amount of organic solvent). To determine solution concentrations of the drug, aspirates need to be centrifuged/filtrated immediately. Subsequently, the supernatant/filtrate needs to be diluted with, for example, an organic solvent that has a high solubilizing capacity concerning the drug compound (to avoid any precipitation/stability issues). Total concentrations can be determined by immediately (i.e., no centrifugation/filtration step) diluting a withdrawn sample with an organic solvent to capture dissolved and undissolved drug. The thermodynamic solubility of the drug can be determined by the shake-flask method, incubating the intestinal fluid samples for a minimal time (i.e., until the equilibrium solubility is reached) with an excess amount of the drug API at 37 °C. After a minimum period of shaking (to be determined), the fluids need be centrifuged/filtrated and the supernatant/filtrate should be diluted with an organic solvent, before bioanalysis.

In case the drug is prone to enzymatic or chemical degradation, stabilizing conditions need to be created, for instance by a reduced temperature (put the sample immediately on ice until further processing), the inclusion of chemically stabilizing agents (e.g., antioxidants), or by the inclusion of (a) specific enzyme-inhibitor(s) in the case of prodrugs or enzymatically-sensitive drug molecules are involved. Based on the reasoning so far, it is obvious that a single standard procedure of sample handling cannot be developed; the multifaceted issues necessitate a careful design of collection conditions and processing of samples to produce reliable/reproducible results when using the intestinal sampling technique.

9. Conclusion and future perspectives

In conclusion, this comprehensive review aimed to highlight the aspiration technique as an important in vivo tool to evaluate luminal drug and formulation behavior in the human GI tract. With a history of more than a century, the technique has evolved over the years and the combination with additional techniques is being considered (e.g., luminal sampling in combination with recording gastric motility). Since not all physiological variables can be measured during a clinical aspiration study at the same time, researchers are intensively collaborating to explore which techniques can be combined to measure multiple GI variables simultaneously.

Not only can the aspiration technique assist people in academics and the pharmaceutical industry to use the obtained data as a reference to optimize their in-house and in silico models, provide new knowledge for improved oral dosage forms and drug delivery innovations, the aspiration technique can also be a valuable tool to assist physicians in unraveling clinical issues in specific patient populations. Concerning the upper GI tract, it remains unclear how drugs will be presented to the intestinal membrane in patients after bariatric surgery (Roux-en-Y gastric bypass or sleeve gasterctomy) or an upper GI stoma (ileostomy or jejunostomy). As important physiological variables can be altered (e.g., transit times, secretion rates, pH) compared to healthy subjects, the knowledge of how and to what extent orally administered drugs will be absorbed in this population is largely lacking. The aspiration technique can be used to aspirate GI fluids in these populations to monitor drug and formulation behavior in the regions of interest.

Concerning the lower GI tract, it is of great importance that the anti-inflammatory drugs (e.g., mesalazine) reach the site of inflammation in the case of patients suffering from, for example, ulcerative colitis. Different formulations are available but it is unclear whether they all deliver sufficient drug levels at the desired site consistently, as they may not disperse appropriately under both active and quiescent periods of the disease. Nevertheless, safety and efficacy should be guaranteed during clinical phase studies prior to the marketing authorization of the drug product. These insights can assist in future drug development. However, it is questionable if the aspiration technique can fully resolve these specific questions as it is extremely difficult to position the catheter in a non-cleaned/prepared colon, containing almost no residual fluids to aspirate. Thus, ‘formulation evaluation’ in the lower intestine remains extremely challenging when applying the sampling technique. However, ex vivo evaluation of drug solubility in colonic aspirates is feasible (Verzonti et al., 2010a) be pushed forward where colonic content can be applied concerning solubility and permeability experiments. On the other hand, the aspiration technique could be applied to deliver drugs in specific regions (conform to the Loc-I-Gut® Loc-I-Col methods of the colon and see how this is linked to a pharmacodynamic effect (PK/PD study) (Lennerås et al., 1995; Tannergren et al., 2009; Zhang et al., 2015).

As most of the studies described in this review were based on the recruitment of healthy subjects, there is a shift towards exploring the sampling technique in patient populations (e.g., UC (Verzonti et al., 2010b), Crohn’s disease (Verzonti et al., 2020), bariatric surgery nowadays (Carlson et al., 2002). There is, indeed, definitely a need to use the aspiration technique in specific patient populations to better understand the impact of altered GI physiology on the luminal and systemic behavior of a drug and make the comparison between healthy and patient populations. When these differences can be mapped, formulation scientist can develop drug products for specific patient populations with a more rational and scientific approach, instead of hoping-for-the-best. In the end, this will result in time- and cost-effective research which is a benefit for pharmaceutical companies and, in the end, patients.

Authors’ contributions

P.A., M.V., C.R., P.L., H.L., W.L.H., J.R.B., J.T., T.V., M.C., M.B., P.P., G.L.A. and B.H. all jointly co-wrote this comprehensive review, each of which took care of a specific section. B.H. and G.L.A. were responsible for the introduction of this review. P.A. was responsible for the section ‘History of the sampling technique’; M.V., C.R., and P.A. took care of the sections ‘Aspiration of human GI fluids: methodology’ and ‘Challenges and issues’; P.L., H.L. and B.A. co-wrote the section ‘Anatomy, physiology and contents characterization’ for the upper GI tract with additional notes/comments of W.L.H., J.T., T.V. and J.R.B. Further, M.C. was responsible for describing the anatomy and physiology of the lower GI tract. M.B. and P.P. co-wrote the section ‘composition of GI fluids and underlying GI physiology as a source of intra- and intersubject variability’. P.A., C.R., M.V. and B.H.
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