



## Review article

# Colonic drug delivery: Formulating the next generation of colon-targeted therapeutics

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## ABSTRACT

Colonic drug delivery can facilitate access to unique therapeutic targets and has the potential to enhance drug bioavailability whilst reducing off-target effects. Delivering drugs to the colon requires considered formulation development, as both oral and rectal dosage forms can encounter challenges if the colon's distinct physiological environment is not appreciated. As the therapeutic opportunities surrounding colonic drug delivery multiply, the success of novel pharmaceuticals lies in their design. This review provides a modern insight into the key parameters determining the effective design and development of colon-targeted medicines. Influential physiological features governing the release, dissolution, stability, and absorption of drugs in the colon are first discussed, followed by an overview of the most reliable colon-targeted formulation strategies. Finally, the most appropriate *in vitro*, *in vivo*, and *in silico* preclinical investigations are presented, with the goal of inspiring strategic development of new colon-targeted therapeutics.

## 1. Introduction

Colonic drug delivery is experiencing a renaissance due to the multitude of associated pharmaceutical benefits and opportunities discovered in recent years [1]. Targeting drugs to the colon can enable superior treatment of local diseases, access to local therapeutic targets, reductions in systemic drug exposure and associated toxicity, and even improvements in drug bioavailability [2–4]. Traditionally, colonic drug delivery has focused primarily on local diseases, such as inflammatory bowel disease (IBD) and colorectal cancer [5,6]. Colonic drug delivery can improve the treatment of local diseases by optimising drug concentration at the target site whilst limiting systemic exposure [7,8]. Increasing characterisation of the colonic and rectal environments has led to the recognition of new local targets, such as the microbiome, enteric immune system, and lymphatic system [9,10]. These emerging targets not only allow treatment of colonic pathologies but may facilitate treatment of systemic conditions and those affecting distal organs [11–13]. For example, the intestinal microbiome has been discovered to modulate traumatic spinal cord injury, dyslipidaemia, urinary tract infections, and even neurological conditions, such as Alzheimer's and Parkinson's disease [14–18]. Moreover, the mesenteric lymphatic system could be targeted to treat insulin resistance and facilitate access to

the brain [19,20]. This expansion in potential therapeutic targets, coupled with advancements in pharmaceutical sciences, has led to heightened interest in colonic delivery of new treatment modalities, including probiotics, postbiotics, vaccines, oligonucleotides, and biologics [21–25].

The first case of colonic drug delivery was sulfasalazine, a prodrug that was introduced for the treatment of IBD in 1941 [26]. Sulfasalazine is composed of the active 5-aminosalicylic acid (5-ASA, mesalamine, mesalazine) linked to the carrier molecule sulfapyridine through an azo bond [27]. Sulfasalazine is activated in the colon by bacteria that cleave its azo bond, releasing 5-ASA for local treatment of inflammation. Whilst sulfasalazine has remained an effective IBD treatment for almost a century, around half of patients report allergic reactions or other adverse events following its use [28]. These observations have been associated with the sulfapyridine component of the prodrug [29], sparking subsequent development of other 5-ASA prodrugs, including olsalazine and balsalazide [30,31]. The prodrug strategy is still utilised for colonic delivery, for example an azo prodrug of tofacitinib was recently shown to effectively treat a mouse model of IBD [32]. Similarly, gut restriction of molecules (particularly peptides) could enable local colonic action by preventing systemic absorption [33]. However, design of prodrugs/gut-restricted therapeutics is drug-specific and can require

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lengthy regulatory approval [34]. This review will instead focus on formulation-based colonic drug delivery, as a single formulation can enable the colonic release of many diverse types of drugs. Further, modified-release formulations of approved drugs can achieve faster market authorisation as they do not need to repeat studies proving the intrinsic drug properties, such as toxicity or pharmacology [35].

Formulations that enable colonic drug delivery include orally administered dosage forms with colon-specific release and those that are administered via the rectum. The first formulation enabling targeted drug delivery to the terminal ileum/proximal colon (also known as ileocolonic delivery) via the oral route was published in 1982, constituting a pH-sensitive methacrylate polymer marketed as Eudragit® S (Evonik) [36]. Eudragit® S is a synthetic copolymer composed of poly (methacrylic acid, methyl methacrylate) at a ratio of 1:2 that dissolves when intestinal pH exceeds 7.0 [37]. As such, Eudragit® S is used as an enteric coating for oral dosage forms designed to release drugs in the terminal ileum. To date it is a prevalent technology that has been incorporated in several marketed formulations, notably those intended to deliver mesalazine to the colon for the treatment of IBD, such as Asacol® (Tillotts Pharma) and Lialda®/Mezavant® (Cosmo Pharmaceuticals). However, the reliability of pH-sensitive polymers for colonic drug delivery has been called into question for some time [1,37,38]. Human studies have found enteric coatings to show inconsistent release patterns, with coatings dissolving prematurely or remaining intact in some individuals [39,40]. This inconsistency arises from variability in intestinal pH and leads to a proportion of patients not receiving the intended dose of their prescribed drug. Further, the disease pathophysiology can increase the risk of enteric coating failure; for example, in IBD intestinal pH is often markedly lower than in healthy individuals [41].

Other mechanisms for achieving colonic drug delivery can similarly present variable release profiles in patients. For example, the time-based approach to accessing the colon via the oral route can be highly affected by variability in gastrointestinal (GI) transit time. Time-based formulation approaches utilise systems that are triggered upon ingestion (e.g., by exposure to GI fluids or low gastric pH) to begin a lag phase that should last until the dosage form enters the colon where site-specific drug release occurs [1,6,42]. However, many factors, including presence of disease, surgery, concurrent medications, or a change in diet, can alter patients' GI transit times and lead to time-based approaches failing to predictably deliver drugs to the colon [43]. Related challenges may be observed with microbiota-dependent systems, which utilise coatings that remain intact in the proximal GI tract and are digested by colonic bacterial enzymes. Upon coating digestion, site-specific drug release should occur, forming the basis for this colon-targeting strategy [44–46]. Polysaccharides are common materials used for microbiota-triggered colonic drug delivery as they are indigestible by human enzymes and degraded by the majority of the population's microbiota due to broad expression of bacterial polysaccharidases [47,48]. That said, if the colonic microbiome becomes significantly perturbed (e.g., following antibiotic administration or due to severe disease), then there is a risk that polysaccharidases will not be produced in sufficient concentrations for coating digestion [49]. Ultimately, this would lead to dosage forms being excreted intact within stool without releasing their drug cargo. In recognition of the unpredictability of relying on one physiological stimulus for colonic drug release, several advanced multi-faceted technologies have been developed in recent years [1]. Newly licensed technologies have provided dependable colonic drug delivery in clinical studies [50,51]; these advances are now revolutionising how orally administered, colon-targeted systems are developed and have opened numerous therapeutic opportunities for the field.

Rectal drug delivery encompasses different advantages and disadvantages compared to colonic delivery via the oral route. For one, rectal formulations are less affected by inter- and intra-patient physiological variability as they do not need to transit the upper GI tract before reaching the colon. In an empty state, the rectal environment is

relatively stable, thus facilitating predictable drug release and absorption [52]. Further, the rectal route can be advantageous for the delivery of drugs that have unpleasant taste profiles, cause GI irritation, are unstable in the upper GI tract or undergo significant hepatic first pass metabolism [53]. The rectal route may also be beneficial in situations when safe swallowing is impaired, for instance due to dysphagia, unconsciousness, or in patients at extremes of age [54]. Despite these advantages, rectal formulations are generally less accepted than oral formulations for reasons including cultural preconceptions, invasiveness, and ease of administration. Mechanistically, rectal formulations can also face retention challenges and generally cannot deliver drugs beyond the colon's splenic flexure [52]. For this reason, they are typically best suited for treating pathologies local to the distal colon and rectum, though systemic products such as vaccines have begun to be explored [55,56]. Rectal formulations can take several identities, most commonly suppositories, enemas, foams, and gels. Traditionally, rectal administration has focused on the delivery of small molecule drugs in simple solutions, emulsions, or suspensions for treatment of constipation, IBD, haemorrhoids, pain, or nausea and vomiting [52]. However, in recent years more varied and advanced formulations have been investigated both preclinically and in trials, which can serve as inspiration for how the potential of the delivery route may be maximised [57–60].

This review provides a timely update on the most effective strategies for developing new colon-targeted treatments. A detailed evaluation of the colonic environment will first be presented to facilitate strategic formulation design. Next, techniques underpinning multi-stimuli targeting technologies will be discussed, with specific attention cast on mechanisms with reliable *in vivo* performances. The review will conclude with an overview of the pertinent preclinical investigations for novel colon-targeted medicines, highlighting the most appropriate *in vitro*, *in silico*, and *in vivo* models to select when translating new treatments.

## 2. Appreciating the colonic landscape

### 2.1. Key anatomy

To selectively target drugs to the colon, it is vital to appreciate the colon's unique physiological characteristics in context of the complete GI tract and other associated organs. At the macroscopic scale, the colon comprises of the proximal and distal colon and is approximately 1.6 m (male average:  $1.66 \pm 0.36$  m; female average:  $1.55 \pm 0.29$  m) of the average 6 m GI tract [61,62]. The proximal colon can be subdivided into three main sections: caecum, ascending colon, and transverse colon; and the distal into four: descending colon, sigmoid colon, rectum, and the anus. The ascending colon is linked to the transverse colon via the hepatic flexure, and the splenic flexure links the transverse colon and descending colon. The diameter of the colon is significantly wider than the small intestine (mean terminal ileum diameter:  $1.87 \pm 0.36$  cm) [61]. It is widest at the caecum (males:  $4.7 \pm 0.9$  cm; females:  $4.8 \pm 0.8$  cm) and becomes progressively narrower towards the sigmoid colon (males:  $3.4 \pm 0.6$  cm; females:  $3.2 \pm 0.6$  cm) before widening at the rectum (males:  $4.0 \pm 1.0$  cm; females:  $3.5 \pm 1.0$  cm). The caecum, transverse and sigmoid colon lie within the peritoneal cavity of the abdomen, are suspended by mesentery, and are fairly mobile [63]. Comparatively, the ascending colon, descending colon, and rectum are retroperitoneal, thus are fixed in location [61]. The colon enables numerous physiological functions, with key examples including water, mineral, and vitamin absorption; faecal compaction; digestion of polysaccharides; and enteric immunoregulation [10,64]. The appendix is attached to the caecum around 1–2 cm below the ileocaecal junction [64]. Research over the last decade has debunked the long-held belief that the appendix is an evolutionary artifact with little biological function. In fact, the appendix is likely a sanctuary for colonic microbiota, providing a reserve of microorganisms should the existing colonic

microbiome be depleted [65]. Further, the appendix may constitute an important niche of the enteric immune system [66].

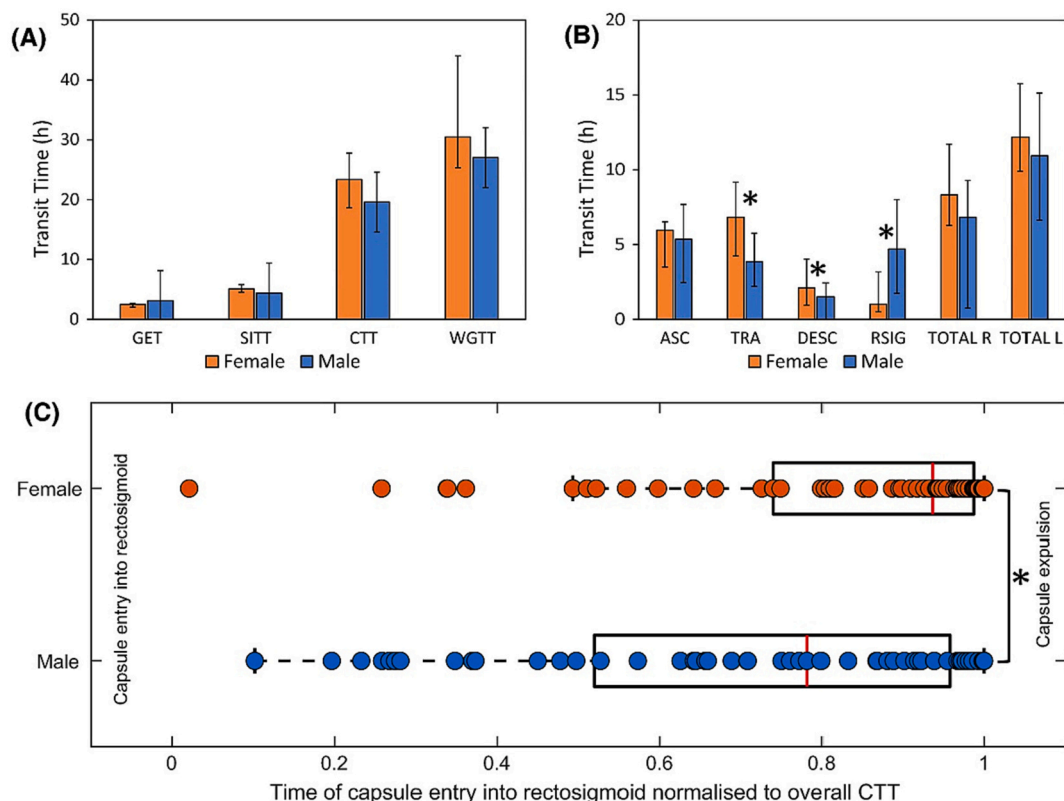
## 2.2. Transit time

The time that it takes pharmaceuticals to reach the colon is dependent on transit through the upper GI tract, which in turn is dependent upon the nature of the dosage form, phase of peristalsis, and whether food has also been ingested. In the fasted state, gastric emptying time of dissolved drugs is 10 – 20 minutes, and can be significantly longer for large solid dosage forms [62]. Food intake, especially intake of high-fat food, significantly delays gastric emptying of medicines [67]. Generally, females of reproductive age, particularly when in the luteal phase of the menstrual cycle, have significantly longer gastric emptying times than post-menopausal females and males [68,69]. The presence of disease can also lengthen gastric transit, for example patients with type 2 diabetes mellitus have been reported to have up to 300% longer gastric transit times than healthy individuals [43,70]. These sources of variability should be closely considered during the development of a colon-targeted formulation, as characteristics of the target patient population will likely guide the time that dosage forms are expected to reside in the stomach.

The average time taken for dosage forms to transit the small intestine is 3 - 4 hours however, as with gastric emptying, large variability between and within individuals exists [71]. When the stomach is empty, small intestinal motility is controlled by repetitive contractions known as the migrating motor complex (MMC). In the fed state, contractions within the small intestine are more frequent and serve to mix luminal contents to facilitate enzymatic digestion and absorption of nutrients

[72,73]. Thus, medicines transit the small intestine at increased velocity in the fed state [71].

The caecum is the gateway to the colon and is connected to the terminal ileum by the ileocaecal valve. The function of the ileocaecal valve is to control the transit of ileal contents into the colon and prevent translocation of colonic microbiota into the ileum [74]. Relaxation of the ileocaecal valve occurs in response to distension within the terminal ileum, allowing digested food and pharmaceutical formulations to pass into the caecum. In juxtaposition, distension of the caecal lumen triggers tightening of the valve to prevent backwards movement of intestinal contents [75]. Luminal contents are propelled along the colon by various types of coordinated smooth muscle contractions and relaxations [76]. Neurogenic contractions are known to occur infrequently (6 - 20 times per 24 hours in healthy individuals) at high amplitudes, causing mass transit of contents along significant distances of the colon in one movement [67,71]. These incidences of mass transport are thought to occur when distension of the colonic lumen innervates mechanosensitive nerve endings in smooth muscles [77]. In addition to neurogenic contractions, pacemaker cells in the deep circular muscle of the colonic wall drive slower, more frequent myogenic contractions (occurring 2 - 6 times/minute) that are most evident in the distal colon. These low amplitude contractions sum with the more propulsive forces to form clusters of coordinated contractions [77]. The total colonic transit time of a dosage form is dependent on numerous pharmaceutical and biological factors, including patient sex (and phase of menstrual cycle), patient age, patient health, microbiome composition, and dosage form size and phase [68,78,79]. Research suggests that liquids are propagated at higher speeds than solids through the colon as they initiate more forceful neurogenic contractions [77]. Large solid dosage forms are



**Fig. 1.** Gastrointestinal transit time data from 111 healthy human volunteers. (A) effect of biological sex on transit time through the whole gastrointestinal tract. (B) effect of biological sex on transit time through specific colonic regions. (C) time of entry of ingestible electromagnetic capsules (diameter 8.3 mm; length 21.5 mm) into the rectosigmoid region of the colon, with values normalised to sex-specific colonic transit time. Higher values demonstrate delayed entry into the rectosigmoid segment before expulsion via defecation. Values are displayed as medians. GET: gastric emptying time, SITT: small intestinal transit time, CTT: colonic transit time, WGTT: whole gut transit time, ASC: ascending colon, TRA: transverse colon, DESC: descending colon, RSIG: rectosigmoid, TOTAL R: total right colon, TOTAL L: total left colon. Errors bars: 95% CI for median; \* $p < 0.05$ . Image used with permission from reference [80].

likely to transit the colon more quickly than smaller solids, as small particles could become trapped in mucosal folds [67]. A study using electromagnetic tracking reported the median colonic transit time of a 8.3 x 21.5 mm capsule in 111 healthy adults as 21 hours, with large variation around the average (Fig. 1) [80]. In agreement with previous research, the study found that female sex was significantly correlated with longer transit time through the transverse and descending colon. Increasing age was associated with longer total colonic transit and whole gut transit. These findings provide useful information for the design of therapeutics intended for site-specific action within the colon and could aid designing bespoke formulations for specific patient groups.

### 2.3. Vascular and lymphatic network

The colon receives its main supply of oxygenated blood from the superior and inferior mesenteric arteries and their respective branches [64]. The main routes of deoxygenated blood away from the colon are the superior and inferior mesenteric veins, which unite to form the splenic vein that subsequently progresses into the hepatic portal vein. This venous architecture presents an important consideration for the development of colon-targeted therapeutics, as drugs absorbed into systemic circulation via the colon will be subject to hepatic metabolism before encountering other organs [81]. Thus, colon-targeted drugs intended for systemic action should either be resistant to, or should be activated by, hepatic metabolism, otherwise drug bioavailability will be significantly reduced. Drugs administered locally to the lower rectum are the exceptions to this feature, as the venous drainage of the distal rectum occurs through branches of the iliac vein which merge with the inferior vena cava to transport blood directly to the right atrium of the heart, thus bypassing the liver [52].

Drugs delivered to the colon may also enter systemic circulation via the mesenteric lymph nodes (MLNs), especially if delivering lipophilic drugs in highly lipidic formulations [82,83]. The network of lymph nodes around the colon is positioned similarly to the vascular network, and functions to drain interstitial fluid, chyle, antigens, and plasma cells/proteins from colonic tissue [84]. Lymph from the proximal colon is drained from colonic MLNs where it passes into the intestinal lymph trunk, then the thoracic duct, and eventually into systemic circulation via the internal jugular and left subclavian veins. MLNs play an important role in the colon's immune functions by balancing tolerogenic and inflammatory responses in the gut via the adaptive immune system, in a manner that is distinct from the small intestine [85,86]. This immunogenic dichotomy between the small intestine and colon could provide opportunities for regionally selective immune modulation.

### 2.4. The epithelium

Unlike the small intestine, the epithelium of the colon does not contain villi [1]. However, microvilli are present on the apical surface of epithelial cells. These microvilli, and the presence of irregularly folded mucosae and colonic crypts, increase the colonic surface area by 10–15 times that of a smooth tube [62]. Due to the reduced surface area, colonic drug permeability is generally lower than in the small intestine, though Biopharmaceutical Classification System (BCS) class I drugs are mostly well absorbed, with relative colonic bioavailabilities of > 70 % [87]. In comparison, BCS class III and IV drugs commonly have lower colonic permeabilities and thus bioavailabilities < 50 % [88]. Colonic epithelial cells are columnar in nature and are organised in a monolayer, with adjacent cells connected through tight junctions [89]. Due to differences in claudin expression (the cell-cell adhesion molecules that form tight junctions), the descending colon is more permeable to drugs than the ascending colon [90].

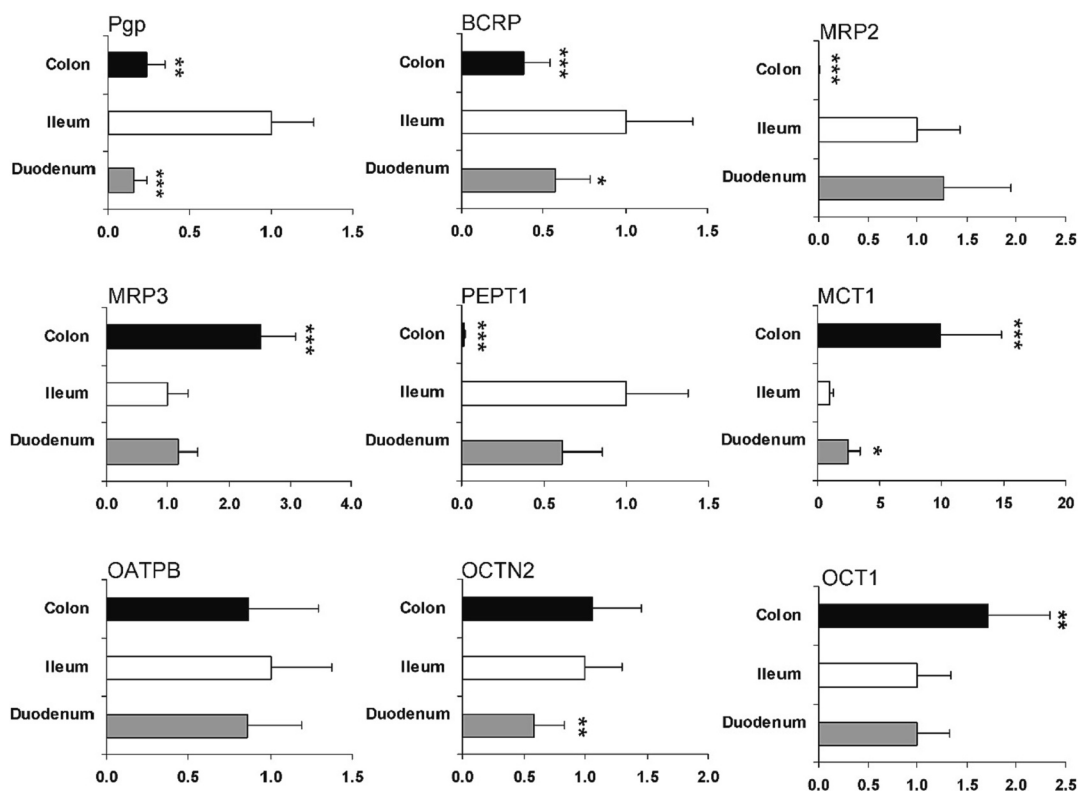
Key types of epithelial cells within the colon include colonocytes, goblet cells, neuroendocrine cells, and immunoregulatory cells; these are continuously renewed every 4–5 days as new intestinal stem cells are produced and differentiated within the crypts of colonic glands

[91,92]. Colonic immune cells can be found within gut-associated lymphoid tissues and include microfold (M) cells, and the rarer tuft cells, which play a key role in maintaining human immune tolerance to gut microbiota [93,94]. Exploiting the innate functions of M cells and other colonic immune cells could present opportunities for novel vaccines and treatments for immune-mediated diseases, such as IBD [89,95].

Compared to the small intestine, the colon has overall lower concentrations of drug uptake and efflux transporters, and cytochrome P450 (CYP450) enzymes, expressed on its epithelium [1,96]. Proteomics has elucidated that colonic drug transporters are chiefly composed of monocarboxylate transporter protein 1 (~55%), multidrug-resistance-associated protein (MRP) 3 (~14%), MRP4 (~9%), MRP2, P-glycoprotein (P-gp), organic anion transporting peptide 2B1, breast cancer resistance protein (BCRP), peptide transporter protein 1 (PEPT<sub>1</sub>), and organic cation transporter 1 [97]. Concentrations of P-gp, the BCRP ABCG<sub>2</sub>, and PEPT<sub>1</sub> are particularly lower in the colon compared to the jejunum and ileum [98]. This characteristic presents numerous opportunities for colonic drug delivery, as drugs that are significantly effluxed by apical membrane transporters or inactivated by CYP450s in the small intestine could obtain increased bioavailability by being released in the colon. This concept can be exemplified by simvastatin, which shows three-fold higher bioavailability when formulated for delayed rather than immediate GI release due to avoidance of small intestinal CYP450s [99]. Conversely, few transporters (MRP3, MCT1, and OCT1) show increased expression in the colon than in the small intestine (Fig. 2) [100]. The presence of disease can also alter transporter expression in the colon. For example, IBD patients (especially those experiencing acute inflammation) may have lower P-gp, MRP4, MCT1 and ABCG<sub>2</sub>, and increased MRP2, expression on colonocytes compared to healthy individuals [98,101]. The colonic expression of enzymes CYP3A5 and UGT2B7 is also thought to be reduced in inflammatory diseases [101].

The entire colonic epithelium is coated with a double layer of mucus that aids passage of chyme through the lumen and provides a physical barrier between microbiota and the colonic epithelium [61]. This mucus can impede drugs' contact, and thus absorption, over the epithelium, thereby presenting a significant barrier to systemic bioavailability. Colonic mucus is composed of water (~95%), the MUC2 mucin (a glycoprotein that forms mesh-like structures of oligomers at the epithelium), lipids, sloughed epithelial cells, proteins, inorganic salts, and DNA [102]. Goblet cells on the colonic epithelium secrete MUC2, facilitating total renewal of the mucus layer every 24–48 hours, as old mucus is either digested by microbiota or naturally sheared away. The inner mucus layer is anchored to goblet cells and is impermeable to bacterial cells > 0.5 µm in diameter. The outer mucus layer, situated around 200 µm from the epithelium, has a much looser structure due to partial protease digestion [103]. This outer mucus layer is colonised by mucolytic bacteria that utilise mucus as a carbon-based energy source; common species include *Akkermansia muciniphila*, *Bacteroides fragilis*, and *Bifidobacterium bifidum* [104]. The thickness of the double mucus layer progressively increases from the proximal to the distal colon, with reported thicknesses of 36.7 µm in the caecum, 39.1 µm in the ascending colon, 57.5 µm in the transverse colon, 69.6 µm in the descending colon, and 101.5 µm in the rectum [102]. In healthy individuals, the pH of the double mucus layer has been measured between 7.1–7.5, which is significantly higher than luminal fluid pH due to the epithelial secretion of bicarbonate ions [105].

The main routes of drug absorption across the colonic epithelium are passive paracellular or transcellular diffusion, and active transcellular transport. Paracellular diffusion, which occurs between epithelial cells, is more common for hydrophilic and/or high molecular weight drugs [52]. Paracellular drug diffusion may increase if epithelial integrity is compromised, for example due to the disruption of tight junctions between cells; this can occur in IBD, obesity, and type 1 diabetes mellitus [106]. Conversely, transcellular diffusion, which occurs through cells, is positively correlated with drug lipophilicity as drugs must permeate the



**Fig. 2.** The relative expression of drug transporters along different segments of the human gastrointestinal tract. Values are presented as averages with standard deviations, and were obtained from the intestinal tissues of 13 – 14 individuals undergoing diagnostic biopsy. Significance indicates where transcript levels were significantly different from levels in the ileum, with (\* $p < 0.05$ ), (\*\* $p < 0.01$ ) or (\*\*\*) $p < 0.001$ ). The image has been reused with permission from reference [100].

epithelial cell lipid bilayer. Drugs' susceptibility to active transport via cell surface proteins is determined by binding affinity, which is influenced by hydrophilicity/lipophilicity. For example, the P-gp efflux transporter has higher affinity for cationic lipophilic compounds [90,107]. Drug pKa and the pH of the GI region are important determinants of its absorption route, as they will affect the extent that a drug is ionised, and thus its hydrophilicity/lipophilicity.

### 2.5. Luminal fluid and gas

The characteristics of luminal fluid naturally change along the GI tract, providing both opportunities and challenges for oral drug delivery [1]. In the stomach, gastric fluid can vary between pH 0.4 – 4.0 in the fasted state and pH 2.0 – 4.5 in the fed state, and is significantly higher in females compared to males due to reduced rates of gastric acid secretion [68]. This acidic pH may aid drug delivery by facilitating the dissolution of basic drugs, though can also inactivate peptide therapeutics and probiotics [108,109]. Upon entering the duodenum, luminal pH increases to 5.0 – 7.0 due to bicarbonate secretion from Brunner's glands and the pancreas [62]. Luminal pH subsequently continues to increase through the jejunum (pH  $6.63 \pm 0.53$ ) and the ileum (pH  $7.49 \pm 0.46$ ), though it is important to recognise that substantial inter-individual variability does exist [110]. The pH of fluids in the ascending colon is generally lower than in the ileocaecal region due to production of lactate and short chain fatty acids (SCFAs) by colonic microbiota [1]. The pH of ascending colonic fluid is also more acidic than that in the distal colon, where production of lactate and SCFAs is considerably lower. Knowledge of luminal pH is exceptionally important for drug delivery, as it can affect the solubility of drugs and provide opportunities for site-specific GI release [40,111,112].

Colonic fluid contains water, chyme, microbiota, electrolytes, proteins (including enzymes and antibodies), bile acids, lipids, SCFAs, and other various metabolites [71,113]. The composition of colonic fluid

varies substantially between and within individuals depending on numerous factors, such as food/water intake and the colonic region sampled; the data in Table 1 presents average values sourced from human adults.

It is important to recognise that measurement of GI pH can vary significantly depending on study methodology. In general, *in situ* measurement of pH with telemetric capsules or probes can be deemed as more reliable than *ex vivo* measurement of extracted fluids. This is because loss of dissolved gases or continuing bacterial fermentation can alter the pH of fluids, especially when pH is not measured immediately after extraction; generally, the pH of colonic fluids has been reported to decrease following extraction [117]. The missing values in Table 1, and those representative of only one subject, show that the colonic environment remains under characterised in humans. Further research that expands awareness of the colonic environment could be beneficial for multiple fields, for example for understanding how disease impacts colonic physiology, and for designing new formulations that target distinct sites within the colon.

The volume of fluid present within the colon may significantly influence drugs' bioavailability. The volume of fluid within the colon shows extensive inter-individual variability, however an average total colonic fluid of around 560 mL has been measured in fasted healthy adults [119]. Colonic fluid volume significantly rises after a meal due to an ingress of liquid and food residue, and steadily decreases along the colon due to water absorption [119]. Most colonic fluid is associated with microbiota, chyme, or other biomass, and as such is not available for interaction with drugs [1]. The volume of free colonic fluid is much lower ( $2 \pm 1$  mL in fasted state;  $7 \pm 4$  mL 30 mins after glass of water) than the total fluid volume and is scattered into discrete pockets ( $11 \pm 5$  pockets in fasted state;  $17 \pm 7$  pockets 30 mins after glass of water), showing considerable inter-individual variability [120]. Therefore, the extent of drug dissolution in the colon may significantly depend on contact with free fluid pockets. Magnetic resonance imaging has shown

**Table 1**

The characteristics of luminal fluid within different colonic regions in human adults. NF: data not found; symbol  $\pm$  represents standard deviation; ranges in brackets represent the recorded ranges in cited studies. \*Study only sampled males; \*\*Result is taken from a single individual.

	Caecum	Ascending colon	Transverse colon	Descending colon	Sigmoid colon	Rectum
pH	6.4 $\pm$ 0.4 [110]	6.37 $\pm$ 0.58 [110]	6.61 $\pm$ 0.83 [110]	7.04 $\pm$ 0.67 [110]	7.38 $\pm$ 0.59 [114]	7.15 $\pm$ 0.44 [114]
Total fluid volume (fasted state)	5.0 $\pm$ 2.1* [115]	138 (114 - 208) [116]	132 (99 - 188) [116]	111 (60 - 185) [116]	NF	1 - 3 [52]
Buffer capacity (mmol/l/ $\Delta$ pH)	19.2 $\pm$ 10.2 - fasted state* [115] 33.6 $\pm$ 13.1 - fed state* [115]	21.4 - fasted state [117] 37.7 - fed state [117]	44.4 - fasted state** [112]	44.4 - fasted state** [112]	NF	NF
Surface tension (mN/m)	NF	42.7 - fasted state [117] 39.2 - fed state [117]	NF	NF	NF	NF
Osmolality (mOsmol/Kg)	144 $\pm$ 65 - fasted state* [115] 267 $\pm$ 197 - fed state* [115]	81 $\pm$ 102 - fasted state [117] 224 $\pm$ 125 - fed state [117]	NF	NF	NF	NF
Protein content (mg/mL)	10.2 $\pm$ 2.2 - fasted state* [115] 6.2 $\pm$ 3.2 - fed state* [115]	9.8(4.6) - fasted state [117] 6.9(2.3) - fed state [117]	NF	NF	NF	NF
Carbohydrate content (mg/mL)	2.3 $\pm$ 10 - fasted state* [115] 9.8 $\pm$ 7.0 - fed state* [115]	8.1 $\pm$ 8.6 - fasted state [117] 14.0 $\pm$ 7.4 - fed state [117]	NF	NF	NF	NF
Bile acid concentration ( $\mu$ M)	183 $\pm$ 221 - fasted state* [115] 280 $\pm$ 305 - fed state* [115]	115.2 $\pm$ 119.3 - fasted state [117] 587.4 $\pm$ 412.8 - fed state [117]	NF	NF	NF	NF
Cholesterol concentration ( $\mu$ M)	1004 $\pm$ 1072 - fasted state* [115] 640 $\pm$ 771 - fed state* [115]	594.2 - fasted state [117] 1501.8 - fed state [117]	NF	NF	NF	NF
Phospholipid concentration ( $\mu$ M)	166 $\pm$ 110 - fasted state* [115] 82 $\pm$ 77 - fed state* [115]	NF	NF	NF	NF	NF
Total SCFA concentration (mmol/kg), (fed state, post-mortem)	131 $\pm$ 9 [118]	123 $\pm$ 12 [118]	117 $\pm$ 9 [118]	80 $\pm$ 17 [118]	100 $\pm$ 30 [118]	100 $\pm$ 30 [118]
Free fatty acid concentration ( $\mu$ M)	143 $\pm$ 118 - fasted state* [115] 150 $\pm$ 141 - fed state* [115]	NF	NF	NF	NF	NF

that fluid pockets may predominately be found in the caecum, ascending colon, and descending colon [121]. In children, free colonic fluid volume is lower than adults and does not vary according to biological sex [122]. Clinical studies show that low solubility drugs are poorly absorbed in the colon, a finding that may be exacerbated by the colon's low availability of free fluid [88].

In addition to fluid, drug formulations can encounter gas pockets within the colon. The typical amount of gas within the colon has been reported as 100 - 300 mL, with the volume, location, and identity of gas significantly influenced by microbiome composition, diet, and transit time [123,124]. Common colonic gases include carbon dioxide, hydrogen, ammonia, nitrogen, methane, and sulphur-containing gases [123]. The lumen of the colon is close to anaerobic, with  $pO_2$  measured as 11 mm Hg ( $\sim$  2%) in the lumen of the ascending colon and 3 mm Hg ( $\sim$  0.4%) in the sigmoid colon, creating a negative oxygen gradient along the colon, which rises at the rectum [125]. Colonic epithelial cells are adapted to hypoxic conditions via altered gene expression and most colonic bacteria are obligate anaerobes [126].

## 2.6. The microbiome

The colon hosts the highest density and diversity of microorganisms of the entire body, with estimates that an individual's colonic bacteria alone enumerate  $10^{10}$  -  $10^{12}$  cells per mL colonic fluid, encode for 150 times more genes than the human genome, and can be composed from

over 1,000 possible species [127–129]. In addition to bacteria, components of the microbiome include fungi, viruses, archaea, free DNA, and many associated enzymes and metabolites. The composition of the microbiome varies along the colon due to variations in  $pO_2$ , pH, bile acid concentration, nutrient availability, immune activity, and transit rate. For example, secondary bile acids such as cholic acid are toxic to bacterial cells, especially Gram-positive species [130]. The most common bacterial phyla inhabiting the colon are the Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria [10]. Generally, bacterial diversity is thought to increase between the proximal and distal colon, and subsequently decrease at the rectum [52]. Certain species predominate in particular colonic regions (e.g., *Enterococci* are most abundant in the caecum and *Shigella* are most abundant in the sigmoid colon) [10]. Bacteria responsible for the fermentation of dietary polysaccharides to lactic acid and SCFAs are mostly located within the proximal colon. Less is known regarding the biogeography of viruses and fungi in the colon, however *Microviridae* and *Caudovirales* bacteriophages have been recorded as the most common viruses, and *Candida albicans* as the most common fungus [131,132]. Colonic microbiome composition is highly individual, and is influenced by age, sex, lifestyle, health, medication use, and diet. That said, functions of the colonic microbiome are conserved between individuals, indicating that most healthy individuals' microbiomes perform general functions (such as fibre fermentation) to a similar extent [47]. Dysregulations in colonic microbiome function have been closely linked to many disease states, as

such the colonic microbiome is an attractive and emerging therapeutic target [133–135].

The substantial metabolic capacity of the microbiome can alter the pharmacokinetics (PK) of drugs, and thus their therapeutic efficacy, via a plethora of mechanisms in a patient-specific manner [128,136,137]. The intestinal concentrations of over 150 small molecule drugs are currently known to be directly depleted by microbiota [138]. It has recently been demonstrated that gut microbiota can accumulate drugs as well as chemically transform them [139]. Therefore, researchers should consider the likelihood and potential impact of microbiome-mediated drug depletion when developing colon-targeted medicines [137,140]. Compared to the more proximal regions of the colon, microbial degradation of drugs in the rectum is generally considered to be insignificant [52]. As such the rectum may present opportunities for the local delivery of drugs that are susceptible to microbial depletion. In addition to the microbiome potentially depleting drugs in the colon, drugs may affect the composition and functioning of colonic microbiota. Antibiotics are well known to significantly impact the intestinal microbiome, with effects possibly lasting for months to years after antibiotic exposure and even impacting the efficacy and safety of other drugs and vaccines [49,141–144]. Many drugs with non-microbial targets, and even pharmaceutical excipients, have also been identified as affecting microbiota [145–150]. Such effects should be appreciated when delivering medicines to the colon; drugs with negative microbiome effects may be less suitable for colonic delivery, and those with positive microbiome effects could represent opportunities for new targeted treatments [151,152].

### 3. Advanced colonic drug delivery strategies

#### 3.1. Multi-stimuli systems for colonic drug delivery via the oral route

As discussed, formulation strategies relying on a single physiological stimulus for colonic drug delivery (i.e., pH, transit time, or microbial enzymes) can be subject to substantial inter- and intra-patient variability in targeting performance [1]. As such, the approach of combining drug release mechanisms based on more than one physiological stimulus has been proven as more reliable in recent years [51]. Depending on their design, these combination or multi-faceted systems may be classified as sequential or parallel trigger systems. A sequential trigger system is based on multiple matrices separated into different layers, wherein the drug release mechanism unfolds in a consecutive layer-by-layer order, starting from the outermost layer to the core [153]. In this manner, drug release is dependent on each layer being sequentially activated by its physiological stimulus. In juxtaposition, a parallel system involves multiple drug release mechanisms occurring simultaneously. Parallel systems typically achieve simultaneous activation of independent trigger mechanisms by incorporating the mechanisms into a single layer [51]. In this sense, if one mechanism fails due to physiological variability (e.g., failure to reach a pH-mediated dissolution threshold or be metabolised by colonic microbiota) then drug release should still occur via the other mechanism(s). Accordingly, parallel systems represent the most reliable strategy for the development of new colon-targeted medicines. To date, multi-stimuli coatings for tablets, capsules and pellets have gained the most translational success within colonic drug delivery [50,51]. However, there is potential for multi-stimuli colon-targeted oral nanomedicines, though such systems are rare and yet to show significant results in human studies [154–157]. When designing new targeted formulations it is imperative to consider the safety of materials for oral delivery. Many materials currently utilised for colonic delivery are Generally Recognised As Safe (GRAS) by the US Food and Drug Administration (FDA), including polysaccharides for microbiota-triggered release, Eudragit® polymers for pH-triggered release, and hydroxypropyl methylcellulose (HPMC) for time-triggered release [158]. The selection of colon-targeting system for new medicines will depend on numerous indication-specific criteria, namely the drug to be delivered, the disease to be treated, and any pathophysiological effects

that may alter the GI environment. Developers may wish to avoid systems that rely on physiological stimuli that are altered in specific disease states. For instance, microbiota and time-triggered combination systems may be less reliable for treating antibiotic-associated *Clostridioides difficile* infection. This is because patients will likely have colonic dysbiosis and decreased gastrointestinal transit time, therefore coatings may not be fully degraded by microbiota or undergo sufficient lag times to provide colonic drug release [159]. Colon-targeted systems are commonly developed to treat IBD, for which drug selection is based on disease severity (Fig. 3). Whilst a significant proportion of patients with IBD will require parenteral treatment at some point in their lives, colon-targeting formulation strategies could facilitate oral/rectal administration of current parenteral-only drugs, such as biologics [160]. Several clinical studies have demonstrated how modified release coatings can enable the delivery of antibodies for the treatment of IBD, these have recently been reviewed by Brayden [33]. Where systemic delivery of biologics is desired, formulations may need to incorporate permeation enhancers, such as medium chain fatty acids or salcaprozate sodium, to enable passage across the colonic epithelium [161,162]. At present there are 5 FDA-approved products that deliver peptides orally for systemic action, these include Rybelsus® (semaglutide) and Mycapssa® (octreotide) [33]. As the formulation strategy will be unique to each colonic drug delivery project, it is important to appreciate the myriad of options when designing a multi-stimuli system. Herein, an overview of advanced parallel systems is provided, as their multiple independent release mechanisms maximise the chance of successful colon-targeting despite variability in GI conditions arising from patient or disease-specific factors.

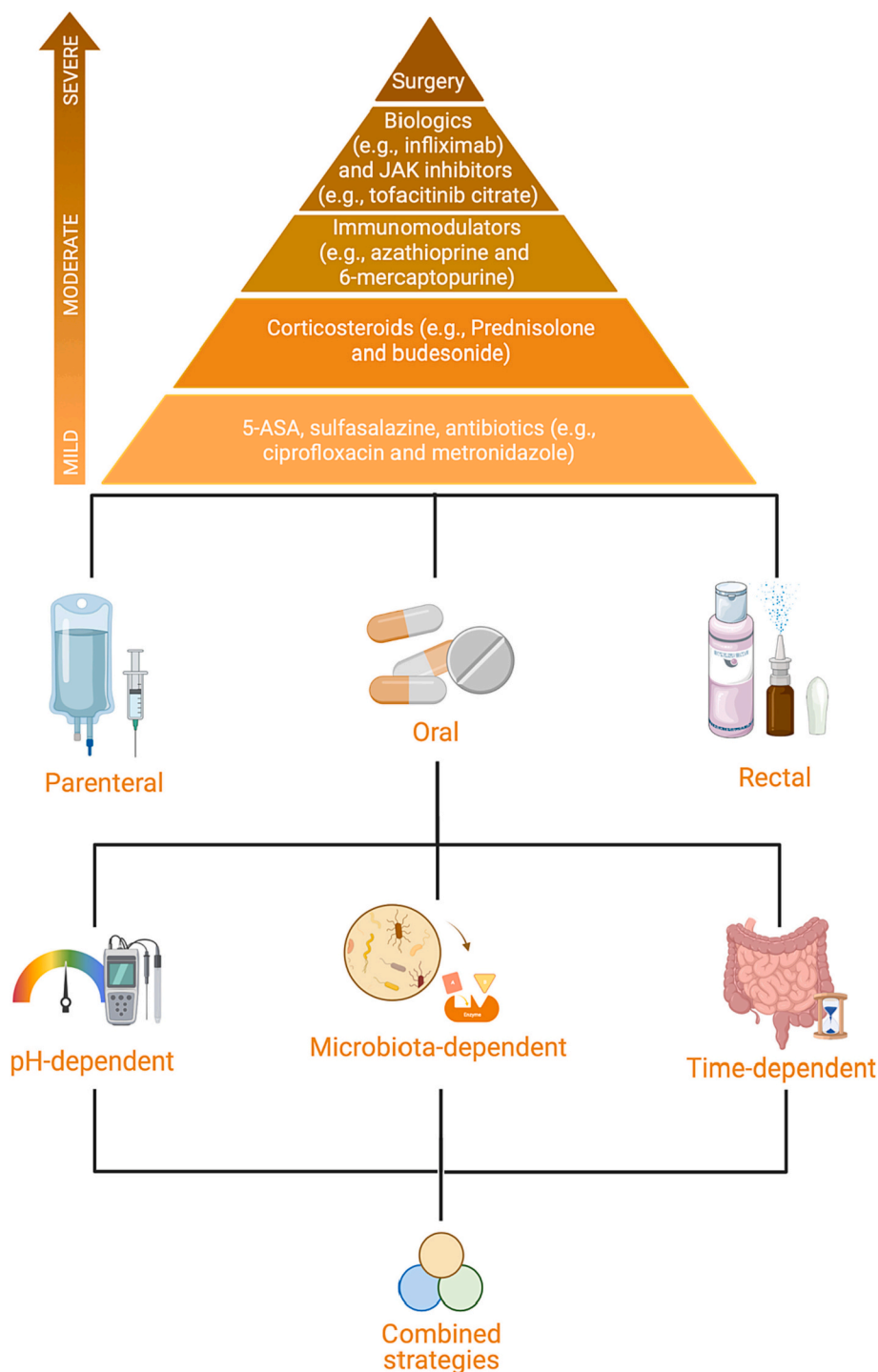
#### 3.2. pH- and microbiota-triggered combination systems

##### 3.2.1. Phloral®

The Phloral® system was the first dual-triggered technology to be successfully marketed for colonic drug delivery. It is a single layer coating system that is composed of a homogenous mixture of Eudragit® S and resistant starch (amylose and amylopectin) [163,164]. Owing to the individual trigger mechanisms, the two components act complementarily to one another and can compensate for each other's activity in the case where one fails to be activated [51]. In this system, Eudragit® S ensures the tablet's integrity is maintained as it travels through the stomach and small intestine. Additionally, it acts as a structuring agent for the starch, controlling its swelling. Resistant starch on the other hand, serves as a substrate for colonic microbiota, providing an alternative method for triggering drug release if the critical pH threshold of Eudragit® S is not attained. Indeed, the Phloral® technology has been successfully shown positive outcomes in the treatment of IBD, irrespective of patients' feeding status [7,51]. Phloral® has also been successfully applied to treat *Clostridioides difficile* infection [4] and obesity [3].

##### 3.2.2. OPTICORE™

OPTICORE™ — short for OPTimised Colonic Release — is a recently developed combination system designed to rapidly release drugs in the ileocolonic region, where fluid volumes are higher than the mid- to distal colon [116,165]. The system is based on two layers of coating; the base layer comprising a neutral enteric polymer (Eudragit® S) combined with a buffering salt. This layer is in turn surrounded by an outer Phloral® coat [165]. As the base layer is alkaline, formulation of acidic drugs may require an additional HPMC layer to isolate the acidic drug from the alkaline base layer. Rapid ileocolonic drug release from the OPTICORE™ system is obtained by accelerating dissolution of the Phloral® coating. As the formulation moves into the distal GI tract, pores form in the Phloral® coating, allowing ingress of luminal fluid and dissolution of the enteric base layer. This dissolution triggers an increase in the ionic strength, pH, and buffer capacity at the inner surface of the remaining Phloral® layer, leading to rapid ionisation and dissolution of



**Fig. 3.** A summary of inflammatory bowel disease treatment modalities, in which oral colonic drug delivery can be attained via combinations of physiological trigger mechanisms.

the Eudragit® S portion of Phloral® [166–168].

To date, the OPTICORE™ system has been effectively used for the treatment of IBD due to its ability to selectively deliver 5-ASA to the site of colonic inflammation [50]. OPTICORE™ forms the colon-targeting mechanism within Asacol™ 1600 mg, a marketed treatment for IBD licensed in Europe after successfully passing Phase III clinical trials [169]. Notably, the multi-stimuli system facilitates colonic delivery of 1.6 g 5-ASA, which is the highest drug dose ever to be licensed for oral delivery. As 5-ASA is acidic, the buffering of the base layer within OPTICORE™ acts to accelerate dissolution through the Phloral® layer

resulting in faster colonic release. The ability to deliver such large doses of 5-ASA reduces patients’ medication burden. The OPTICORE™ technology has also been investigated for the treatment of *Clostridioides difficile* infection via the ileocolonic delivery of metronidazole benzoate, another acidic drug [170].

### 3.3. pH- and time-triggered combination systems

Whilst pH- and time-dependent parallel systems are less common, several systems have been investigated. An example of such is the



combination of two enteric polymers (Eudragit® S and L) with a time-dependent polymer (Eudragit® RS) to coat 5-ASA pellets with or without inulin [171]. The combination was tested at different ratios and coating thicknesses, wherein 16:64:20 w/w ratio of Eudragit® S:L:RS at 15% coating thickness was found to be ideal for achieving colon-targeted drug delivery in *in vitro* experiments. In a rat model of UC, the coated pellets showed significantly better therapeutic outcomes over the Pentasa product (pH-only triggered). It should be noted that no significant therapeutic difference was observed between pellets with and without inulin, demonstrating that it was the combination and the pH- and time-triggered mechanisms that afforded additional therapeutic benefit.

In a different approach, flurbiprofen tablets were compress-coated with sodium alginate (SA) alone and in combination with Eudragit® S100 [172]. Tested in healthy humans, the presence of the pH-dependent polymer in the coating prevented the swelling of SA in the stomach region and hindered the drug release, resulting in a higher drug concentration in the colonic region as opposed to the use of SA in isolation. *In vivo* X-ray imaging corroborated with these findings and showed that the tablets retained their structural integrity as they transited to the colon.

### 3.4. Microbiota- and time-triggered combination systems

Whilst single or sequential microbiota- and time-dependent triggers for colonic drug delivery are well explored, their combination as parallel mechanisms is rare [6]. Common materials used as microbiota-sensitive triggers include pectin, guar gum, chitosan, and resistant starch. Time-dependent mechanisms can include reservoir, capsular or osmotic designs, with reservoir systems utilising erodible or diffusive polymers such as HPMC being the most thoroughly explored [1]. An example of a parallel system is the combination of pectin and HPMC (80:20 ratio) for tablet coating, which harnesses microbial digestion of pectin and the GI swelling of HPMC [173]. In a pilot study, the coating was shown to reliably deliver cargo to either the ascending or transverse colons of 6 healthy male humans. Elsewhere, injection moulded capsule shells combining HPMC with high-amylose starch were fabricated wherein the release of paracetamol from the capsule shells was studied using *in vitro* and *ex vivo* models [174]. Whilst the presence of HPMC provides a time-controlled drug release as it swells, the starch is efficiently metabolised by the microbiota, causing the rapid drug release in the colonic environment. It has been suggested that the ratios of the polymers, thickness of the shell and its shape could be modified to fine-tune the drug release. These findings can act as inspiration for the formulation of new products that would benefit from release in specific colonic regions, as dosage form morphology can be tailored to facilitate indication-specific drug release.

### 3.5. pH-, microbiota- and time-triggered combination systems

In a recent approach, Moutaharrik et al. suggested an advanced colon-targeted drug delivery system that combined three independent trigger mechanisms [175]. In this system, the two-layered coating is composed of an inner swellable, time-dependent cellulose derivative (i.e., HPMC or hydroxypropyl cellulose (HPC)), surrounded by a blend of a pH-dependent polymer (i.e., Eudragit® S) and a microbiota-triggered polysaccharide (i.e., high-amylose starch – Amylo N460). This first example of a triple colon-targeting mechanism was successful, as the cellulose derivative, Eudragit® S and Amylo N460 provided reliable colon-targeting performance in *in vitro* and *ex vivo* models. Following this encouraging early data, the further development of the system in *in vivo* models appears promising.

### 3.6. Rectal drug delivery

Rectal preparations (e.g., enemas, foams, gels and suppositories) are

commonly used for the treatment of IBD with 5-ASA [176,177] and corticosteroids [178–180], as well as constipation, haemorrhoids, pain, and nausea/vomiting [52]. In recent years, development of rectal preparations has expanded from traditional formulation to more advanced systems that can promote local drug retention and/or systemic absorption depending on the specific indication [55,56]. In this regard, 3 types of enemas have been distinguished: (a) strongly hypotonic enemas that induce drug uptake into tissue and circulation, (b) hypertonic enemas that induce secretion of body fluids into the lumen and cause rapid systemic drug uptake, and (c) moderately hypotonic enemas that have faeces-like ionic compositions and result in high local drug bioavailability but minimised systemic drug absorption. Herein, the osmolality of rectal formulations plays an important role in determining the bioavailability of the drug. In the colon, sodium ions are actively pumped into the lumen by epithelial tissue to promote water absorption. This intrinsic property can thus be utilised to osmotically direct drugs into the epithelium. This can be achieved by reducing the tonicity of rectal formulations, resulting in an osmotic gradient that causes hydrophilic drugs and mucus-penetrating nanoparticles to cross the epithelial tissue surface without damaging it [181,182]. As an example, a study comparing 3 types of tenofovir-loaded enemas has shown that hypotonic sodium-based enemas increased drug concentration in mice colorectal tissues as opposed to isotonic and hypertonic preparations that resulted in a prompt systemic drug absorption [183].

Despite the advantages of rectal formulations, they may elicit local irritation in the rectum, cause discomfort due to leakage or increase defecation urge, thus rendering them unacceptable by some patients [183]. Therefore, these factors should be regarded as key formulation challenges that if overcome could increase the likelihood of patient acceptability. A possible solution to rectal leakage of dosage forms is the use of thermosensitive polymers, such as poloxamers or pluronics, which transition from a liquid to gel phase at body temperature [184]. In addition to reducing rectal leakage, thermosensitive gelling may increase drug bioavailability through mucoadhesion and could reduce the potential discomfort of inserting a solid dosage form. Despite promise in numerous animal studies and a human study in 1998, a thermo-gelling rectal formulation has yet to be marketed, potentially due to difficulties in achieving cost-effective high-scale production [184–187].

## 4. Translating technologies for real patient benefits

### 4.1. *In vitro* investigations

Evaluating the disintegration, dissolution, and stability of colon-targeted systems is essential in the development of new products, though correlating *in vitro* results with expected *in vivo* outcomes can be complicated by designing methods to account for the colon's physiological idiosyncrasies. The United States Pharmacopeia (USP) dissolution methods are widely accepted in industry for investigating the behaviour of immediate release formulations, especially those containing highly soluble drugs, as demonstrated by the existence of biowaver programs [188]. However, traditional USP apparatuses, such as the multi-vessel USP 3 apparatus, are not wholly suitable for the biorelevant characterisation of colon targeted dosage forms. Reasons include the irrelevant fluid volumes and mixing rates used, the incompatibility of disintegrating dosage forms, and the assumption that dissolution is the rate-limiting aspect of absorption [189]. Absorption across the colonic epithelium is generally slower than the small intestine, as such permeability may be the rate-limiting step for many colon targeted drugs [87]. These drawbacks have led to the development of colon-specific methods, which may pair more advanced dissolution and drug stability setups (i.e., bicarbonate buffers, simulated intestinal fluids, animal fluids/tissues, human faecal slurries, and biorelevant hydrodynamics) with tailored permeability investigations [190].

#### 4.1.1. Dissolution for oral dosage forms

**4.1.1.1. Bicarbonate buffers.** USP standardised buffers, such as hydrochloric acid, phosphate, acetate, and citrate, have maintained a central role in the evaluation of drug release from pharmaceutical formulations: they cover a wide pH range and have defined ionic strength and buffer capacity [191]. However, despite their ongoing use, these buffers generally lack biological relevancy for the human intestine. The use of bicarbonate-based buffers for biorelevant dissolution testing of colon targeted drugs was first proposed in 2005 upon recognition that bicarbonate buffers more closely match the buffer capacity of intestinal fluid than commonly used phosphate buffers [192]. Fadda et al. tested the capacity of bicarbonate media to predict the *in vivo* behaviour of sustained and delayed release mesalazine preparations [193]. The products' release patterns observed in Krebs bicarbonate buffer more closely matched the disintegration and PK profiles recorded in humans, when compared to standard phosphate buffer. Indeed, this has been further demonstrated in a study using Lialda® (Mezavant® XL, 5-ASA) [194], in which *in vitro* drug release showed a strong correlation with human data collected via gamma-scintigraphy [195]. Similar observations have been made for prednisolone tablets prepared with four different Eudragit® coatings [38]. Unfortunately the main limitation of using bicarbonate media is the continuous evaporation of CO<sub>2</sub> resulting in an increasing buffer pH; in this regard, both manual and automated approaches have been developed to successfully stabilise bicarbonate buffer pH during dissolution testing [196]. A recent study reported that dissolution of Asacol® 400 mg and generic colon-targeted 5-ASA tablets in bicarbonate buffer using a novel flow-through cell system correlated well with the *in vivo* plasma PK measured in 48 healthy male adults [197].

**4.1.1.2. Addition of bile salts and enzymes.** Colonic fluid differs greatly from simple aqueous buffer systems: bile salts and enzymes are important mediators of drug release and solubilisation from targeted formulations [198]. Vertzoni et al. developed simulated colonic fluids that mimic the fasted and fed states (FaSSCoF and FeSSCoF) through the addition of biorelevant concentrations of bile salts, phospholipids, and proteins, and management of buffer capacity, osmolality, and surface tension [199]. The researchers demonstrated that FaSSCoF and FeSSCoF could more accurately predict the solubility of 3 poorly soluble drugs in human colonic fluid than standard buffers. For example, addition of bile salts to buffers increases the solubility of lipophilic drugs in a manner more relevant to the human colon [189]. Addition of colonic enzymes is important for the assessment of drug release from targeted formulations that utilise enzymatic degradation of polysaccharide-based coatings [51]. Enzymes can be actively produced within media by microbiota sourced from human/animal faeces or intestinal fluids [200]. If cell-free media are preferred, then addition of biorelevant concentrations of enzymes into buffers could mimic *in vivo* conditions, however numerous classes of enzymes would ideally be included to appreciate the colon's broad metabolic functionality. Key enzymes found in colonic fluid include the carbohydrate-active microbiota enzymes (e.g., glycoside hydrolases, polysaccharide lyases, and carbohydrate esterases) and those known to metabolise drugs (e.g., phosphorylases, reductases, and decarboxylases) [201–204]. Though not routinely considered during pharmaceutical development, the ability of colonic microbiota to alter the bioavailability of drugs (often in a patient-specific manner) is an important PK consideration that should be screened for during characterisation of new active compounds [137,160,205].

**4.1.1.3. Faecal slurries.** Faecal slurries, composed of raw faecal material and media designed to mimic colon physiology and support microbial growth, are widely used for predicting drug release, solubility, and stability in the colon [200,206]. Faeces sourced from healthy humans has an average pH of 6.64, and contains microbiota (25 – 54 % of solid faecal material), undigested polysaccharides (~ 25 %), proteins (2 – 25

%), lipids (2 – 15 %), and inorganic elements (e.g., potassium, phosphate, sodium, calcium, and magnesium) [207]. The percentage weight of faecal material per volume of support media can vary widely (with 1 – 60 % reported in the literature), though concentrations of 10 – 25 % are common as they allow effective homogenisation of faecal material with support media and accurate volume measurement [205,206,208]. The composition of support media often includes electrolytes (e.g., sodium, phosphate, magnesium, bicarbonate), bile salts, yeast extract, L-cysteine, and peptone water (the latter 3 components included to stimulate microbial growth) [200]. The exact composition of a faecal slurry may be modified to mimic variations in physiology, such as pH, bile acid concentration, and buffer capacity. However, it should be recognised that the initial pH of the slurry and available carbon substrates significantly influence microbiota composition and functionality; bacteria produce organic acids that can accumulate, decrease media pH, and result in bacterial stasis/death [209].

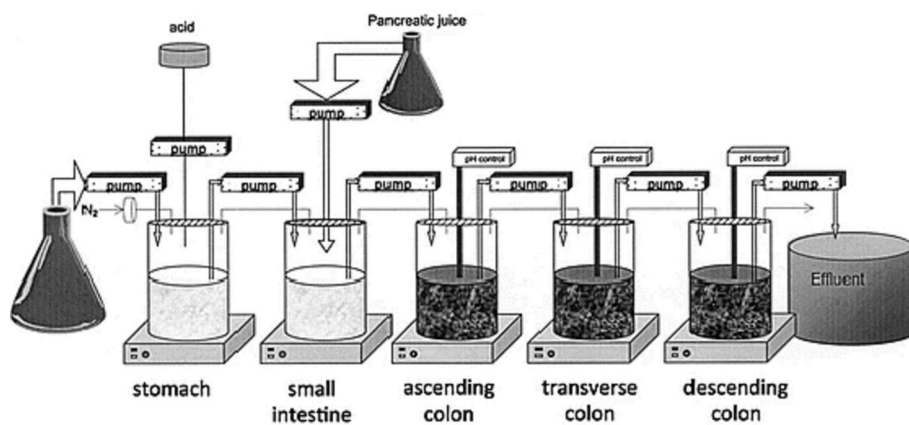
Raw drugs and/or formulations are often incubated in faecal slurry for short timeframes ( $\leq 24$  hours) with regular withdrawal of samples to analyse drug dissolution and stability; this type of setup is known as a static batch culture. Continuous culture systems, where longer incubations are required, involve the replacement of spent culture with fresh growth medium [210]. More advanced systems may include multiple compartments that simulate different regions of the colon and allow fine adjustments of parameters such as pH or transit time between segments (Fig. 4) [196,211]. One such model, known as The Mucosal Simulator of the Human Intestinal Microbial Ecosystem (M-SHIME®) includes vessels representative of the stomach, small intestine, and ascending, transverse, and descending colon that allow treatment periods up to 4 weeks [212]. The colonic regions of the M-SHIME® also contain mucin-covered microcosms that facilitate the culture of mucosal, in addition to luminal, microbiota [213]. Regardless of the complexity of faecal slurry incubations, experiments should be completed in anaerobic environments maintained at 37 °C to replicate human colonic conditions.

Though not truly representative of colonic microbiome composition, human faecal material is easily obtained and represents an effective method for predicting formulation behaviour *in vivo*, provided that faecal samples are processed correctly and repeatedly [212,214–216]. There is also the advantage that patient samples can be obtained to allow analysis of formulation behaviour in a target disease state [217]. Rodent faeces may also be used; though the mouse/rat microbiome differs in composition to the human colon, rat caecal content has comparable abundance of Bifidobacteria and Bacteroides [218]. Variability between animals may also be lessened through the standardisation of living environment and diet.

#### 4.1.2. Dissolution methods for rectal dosage forms

Rectal dosage forms are typically immediate release dosage forms, which include suppositories, rectal capsules, semisolids, and liquid preparations. There are many factors that affect *in vivo* drug release from rectal dosage forms, relating to the active ingredient (e.g., lipophilicity, solubility, particle size) and the physiological rectal environment [52,219]. The *in vivo* prediction of drug behaviour in the rectal environment requires a well-designed *in vitro* dissolution system. Unfortunately, there are currently no formally validated *in vitro* drug release characterisation methods for rectal dosage forms reported in pharmacopoeia, though a few specifications are presented in the Dissolution Methods Database recommended by the FDA [220]. For solid rectal formulations the database reports the use of the paddle apparatus in high volumes of aqueous media at various pH values [220]. The main difficulties facing the design of appropriate dissolution methods are founded on the scarcity of knowledge on the composition of the human rectal fluid. This not only complicates the development of relevant dissolution media but also impairs the development of permeability models [221].

The most utilised rectal dosage forms are suppositories, and



**Fig. 4.** Schematic of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) model. Different regions of the gastrointestinal tract are represented as insulated glass vessels connected via peristaltic pumps. The fluid pH, vessel volume, and pump flow rates can be controlled to reflect distinct patient populations. Faecal samples are inoculated into colonic vessels to simulate the microbiome. The SHIME® model can be used to predict the stability of drugs in the gastrointestinal tract and analyse the effect of drugs on colonic microbiota. Image used with permission from reference [212].

depending on their bases, exist in either hydrophilic or lipophilic forms [52]. Hydrophilic forms are composed from polyethylene glycols (PEGs) and other non-ionic surface-active ingredients chemically related to PEGs. Since these products dissolve in aqueous dissolution media, a USP apparatus 1, 2 or 4 combined with specific pH conditions that mimic the human rectum, can be used [52]. As suppositories may float on the surface of the medium a sinker device is normally required: Palmieri designed a novel basket [222], the so-called Palmieri basket, which incorporates wider vertical slots to allow a more intense interaction of the medium with the rectal formulation. This setup correctly predicted aspirin release from hydrophilic suppositories in aqueous buffer [222].

Hydrophobic suppositories are made from solid fats, at body temperature they melt and release the drug from the fatty base into the rectal fluid [221]. When determining the drug dissolution from these forms, the test conditions should be evaluated case-by-case based on the drug properties and melting pattern of the base. One method was developed by Schoonen et al. in 1976 [223], who fixed a lipophilic formulation under a circular glass plate to separate the medium into two compartments, the plate was placed inside 900 mL phosphate buffer at physiological pH. The upper compartment was kept under constant agitation for a total of 5 hours, and samples were collected from the upper compartment at specific timepoints. Klein et al. then combined this setup with a paddle apparatus [221]. In the early 2000s, the European Pharmacopoeia III and British Pharmacopoeia presented an unvalidated dissolution apparatus, which has been used to test paracetamol release from 4 different lipophilic base suppositories [224]. Phosphate buffer (pH 7.4) is pumped inside a thermostatically controlled flow-through cell where a suppository is placed. However, the dissolution system revealed some problems in its methodology, thus an accurate *in vitro-in vivo* correlation (IVIVC) cannot be evaluated from this setup [224]. In summary, an accurate bio-predictive dissolution method, especially for lipophilic suppositories, has yet to be established.

#### 4.1.3. Investigating *in vitro* colonic permeability

Distinct measurements should be employed when predicting colonic drug permeability, due to the colon's physiological differences to the small intestine. Immortalised cell lines such as Caco-2 are widely used to predict epithelial drug permeability, however they are susceptible to culture-derived variability and lack the expression of key enzymes, e.g., CYP3A4, and a mucus layer [90,225]. Interestingly, although Caco-2 cells are derived from the colonic epithelium, their expression of transporters is more similar to that of the small intestine [100]. Nonetheless, a study of 18 small molecular weight drugs found good correlation ( $R^2 = 0.74$ ) between Caco-2 permeability and colonic absorption recorded in humans, hence validating the utility of the model in defined conditions [88]. Further, it is often underappreciated that cell lines have a biological sex, indicating that drug permeability studies across cell lines alone are not sufficient to identify variability between male and

female patients [68]. The Caco-2 and C2BBel colonic epithelial cells are male, whereas the HT-29 and LS 174 T colonic cell lines are female. Thus, where cell lines are used to predict colonic drug permeability, every attempt should be made to include cells presenting both male and female sexes.

Alternative models for assessing colonic drug permeability include organoid systems, Using chambers utilising human colonic tissue, and colons-on-a-chip. The precise specifications of these models, alongside their strengths and challenges, have been extensively reviewed by Lemmens et al. [90]. Briefly, these technologies may offer advantages over traditional Caco-2 permeability experiments by facilitating more physiologically relevant epithelial landscapes that can be constructed to mimic a desired disease state [226–228]. The advancement of bio-printing also offers the opportunity to construct unique, finely-tuned colonic tissue models that move beyond traditional cell culture; such a system has been developed for the small intestine and has been shown to resemble *in vivo* intestinal tissue more closely than Caco-2 monolayers [229]. Further, emerging systems also allow the combination of drug permeability with microbiome stability studies by coculturing epithelial tissue models alongside colonic microbiota. These intestines-on-a-chip can sustain the extended coculture of human and microbial cells at tuneable oxygen gradients and relevant microbial diversities [126].

#### 4.2. Animal models for investigating colonic drug delivery

During the development of novel colon-targeted formulations, it is important to select *in vivo* animal models that best mimic the physiology of the human GI tract. In reality, the choice of animal model is often dictated by cost, availability, and ease of handling [230], frequently to a greater extent than physiological similarity. Animals have marked interspecies divergences in GI anatomy and physiology leading to pronounced diversity in drug absorption and bioavailability [231]. Unfortunately, there is no single animal that perfectly replicates the human GI tract. Due to such variability, researchers investigating colonic drug delivery routinely use various animal models: common choices are small rodents such as mice, rats, rabbits, guinea pigs, and larger animals such as dogs, pigs, and non-human primates [231]. Recognising the applicability of animal models for the assessment of colon-targeted medicines in humans is essential for optimising the efficacy of pre-clinical development. It is likely that the most relevant animal model depends on dosage form characteristics, parameters to be tested, and the indication to be treated. Further, the equal representation of both female and male animals during pre-clinical investigation is paramount for formulations intended to treat humans of both sexes [68]. The advantages and disadvantages of animals commonly used in pharmaceutical research will be discussed henceforth, in the context of colonic anatomy, fluids, transit, and microbiota.

#### 4.2.1. Animal colonic anatomy

Colonic anatomy varies greatly between species, probably due to species' specific nutrient absorption and processing that modulated gut structure and functions during evolution [232]. Omnivores and herbivores have longer colons to better absorb nutrients from fibre-rich and low protein diets, compared to carnivores [231]. Rats and mice have intestinal structures and functions similar to that of humans and are considered good models for colorectal cancer disease [233], however they have a non-sacculated colon, lack adipose tissue in the submucosa, and rats especially have much larger caecums due to higher fibre intake [233]. In comparison, dogs have small caecums, with a shorter colon than that of humans and no sacculations [234]. Dogs are also likely to have higher intraluminal pressures than humans, meaning that formulations sensitive to mechanical stress could disintegrate earlier in the dogs' GI tract [235]. The anatomy of pigs' GI tract is more similar to humans: in the relative length of the intestinal segments and in the intestinal surface area, explaining why this animal is deemed an excellent model for nutritional absorption [236]. Pressure within the pig GI tract is also comparable to that of humans [237]. Rabbits have a well-defined caecum because of their cecotrophic nature – they excrete faeces coated with bacteria that require reingestion for complete nutrient absorption [231].

#### 4.2.2. Animal colonic fluids

Knowledge of the pH and volume of animals' GI fluid is essential for an accurate prediction of drug disintegration, dissolution, and absorption in humans. In humans, intraluminal pH increases between the ileum and the colon and can be exploited by enteric coatings to enable site-specific drug delivery. Healthy guinea pigs, rabbits, and pigs have been found to have comparable colonic pH readings to humans [110]. Beagles present a pH increase between the small intestine and colon, with variable colonic pH readings (pH 5 – 8) [235]. Rodents' intraluminal pH decreases from ~ 7.0 in the distal small intestine to ~ 6.0 in the caecum, which is generally lower than the average human caecal pH [238,239]. Lower pH values can cause the precipitation of drugs that dissolve at basic pH and can also cause an inaccurate performance of pH-dependent coating polymers such as Eudragit® S and FS [238]. Therefore, the use of rodents for the evaluation of pH-triggered colonic delivery formulations should be cautioned if researchers wish to extrapolate dosage form release to humans. On the contrary, the rat, especially in the fed state, seems to be the animal model with a relative free fluid volume best resembling humans, although undigested food may interfere with drug dissolution [238].

The age of animals used in drug delivery studies should also be considered, as the ageing process can lead to significant changes in physiology, as also observed in humans [240,241]. For example, young rats (4 weeks) have smaller colons, reduced total GI fluid volumes, and higher colonic buffer capacity compared to aged rats (38 weeks) [239]. The viscosity of colonic fluid is comparable between humans, guinea pigs, rabbits, and pigs, however the relatively higher amount of water in these animals' gut may be inappropriate for predicting drug dissolution in humans [242].

#### 4.2.3. Animal colonic transit

GI motility is an essential determinant of orally administered drugs' bioavailability. The length of colonic transit varies significantly between animals. Compared to humans, with a median colonic transit of 21 hours, rats have a much shorter colonic transit (approximately 62.2 ± 21.2 minutes), that can potentially be prolonged with the administration of prokinetic agents [80,243]. That said, care should be taken if co-administering prokinetic agents with test formulations, as off-target effects could interfere with clinical measurements and/or metabolism of the test drug. The average transit of a 26 x 13 mm SmartPill telemetric capsule through the colons of male landrace pigs has been recorded as 53.77 ± 31.68 hours in the fasted state and 102.47 ± 59.54 following a high-caloric meal [244]. Pig gastric emptying times are also much longer than in humans, with averages of 68 – 233 hours (landrace pigs);

24 – 672 hours (Yorkshire pigs); > 54 hours (Yucatan pigs); and > 48 hours (Göttingen minipigs) [237]. Therefore, pigs are likely not the most appropriate model when investigating colon-targeted formulations affected by transit time. In comparison, the dog may be more comparable to humans. A study investigating 31 healthy adult dogs (various breeds, 14/31 female) found that colonic transit time varied from 7.12 – 42.88 hours and whole GI transit was 21.57 – 57.38 hours [245]. The beagle, a commonly used model in pharmaceutical research, has specifically been found to correlate well with humans, demonstrating average colonic transits of 25.4 ± 3.3 hours (fasted) and 28.2 ± 4.7 hours (fed), and whole gut transits of 27.3 ± 3.3 (fasted) and 33.0 ± 4.1 (fed) [235]. However, the researchers did suggest that beagle colonic transit could appear elevated, as capsules may move more quickly through the proximal and distal colon and subsequently remain in the rectum for extended periods.

#### 4.3. In silico prediction for colonic drug delivery

Drug development is a notoriously expensive and high-risk process, therefore the ability to expedite formulation development and predict *in vivo* behaviour whilst saving valuable resources is highly sought [246]. Several predictive technologies exist and could aid the development of novel colon-targeted medicines, namely design of experiments (DoE), molecular dynamics (MD), mechanistic modelling (MM), machine learning, and other less common techniques such as finite element analysis and computational fluid dynamics (Fig. 5) [247].

DoE is a widely utilised approach within pharmaceutical research and can be applied to model how several process parameters affect selected process outcomes. DoE has been used in multiple studies to optimise the composition of colon-targeted coatings comprised of enteric polymers for successful *in vivo* administration [248,249]. It is most useful when researchers wish to investigate how a limited number of process parameters (for example, the proportion of enteric polymers in a coating) influence defined quality attributes (such as coating drug release rate). In addition to the optimisation of coating composition, DoE could be used to model and predict how other formulation parameters (e.g., dosage form size, shape, or production method) affects the reliability of colonic drug release. DoE is less useful when working with large datasets in which many ( $\geq 10$ ) independent variables exist or where complicated non-linear relationships exist between independent and dependent variables. In these cases, alternative predictive technologies may be more appropriate [250].

Molecular modelling is used for computationally simulating interactions between molecules by applying equations relating to classical and quantum physics [251]. Molecular systems can be represented at varying detail, including full atomistic and course-grained models, with the choice dependent on the experimental requirements and available resources [252]. Due to its ability to accurately predict dynamic interactions at the atomic level, MD has become an established *in silico* tool for the discovery of new drugs, for example by enabling the analysis of drug-target binding [253]. From a drug discovery perspective, MD could be employed to discover compounds with activity at emerging therapeutic targets in the colon, such as the microbiome [254]. For instance, MD has recently predicted the inhibition of gut bacterial  $\beta$ -glucuronidases by the biflavanoid, amentoflavone; these findings could aid the prevention of microbiome-led drug metabolism, as many drugs are known to be biotransformed by bacterial  $\beta$ -glucuronidases [255]. Elsewhere, molecular modelling (including MD) has discovered the influence of microbial metabolites on longevity-associated metabolic pathways; this could lead to the identification of novel therapies to support healthy ageing through the gut [256]. From a drug development perspective, MD has many potential applications for formulation design. MD could be used to definitively map the drug loading and release mechanisms of stimuli-responsive formulations and as such design targeted dosage forms with highly explainable *in vivo* behaviours [161,252]. MD has also been used to understand the formation of

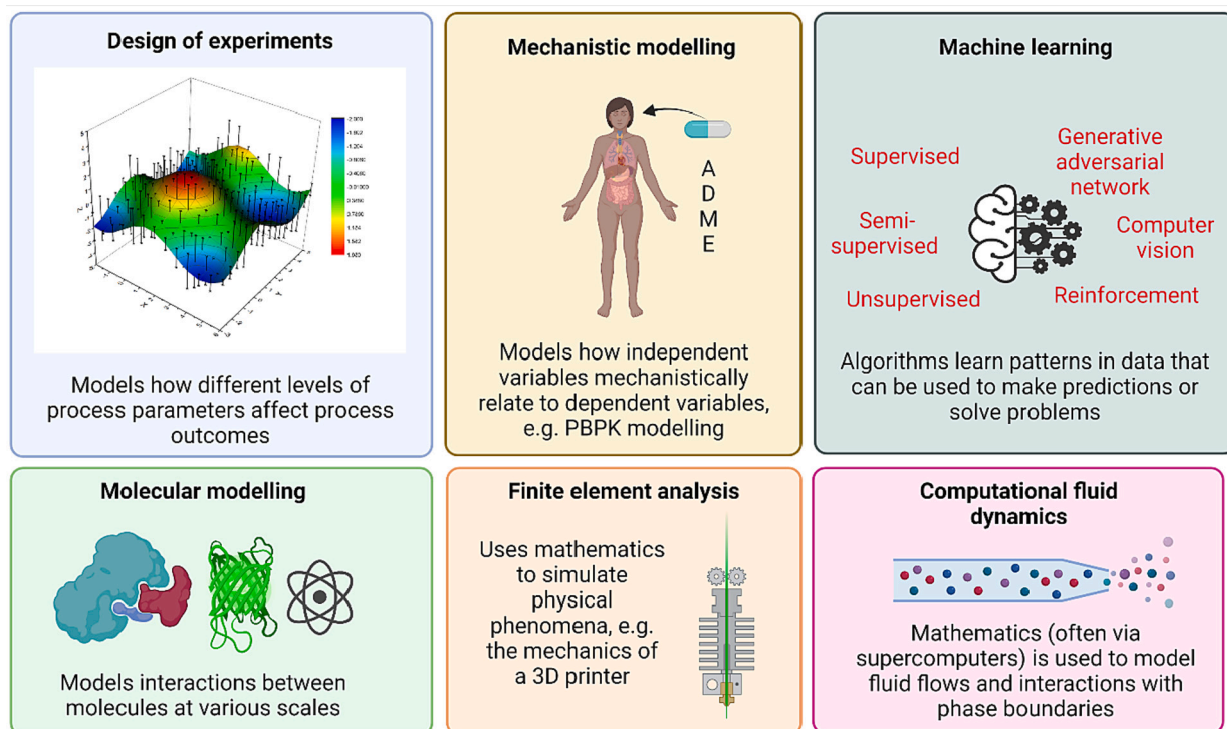


Fig. 5. *In silico* technologies with the potential to be applied in developing colon-targeted medicines, ranging from analysing drug-target interactions to optimising formulation manufacture.

polymer coated nanoparticles produced by flow nanoprecipitation, exemplifying how atom-level insight could be used to optimise colon-targeted nanoformulations [257,258].

MM and ML are distinctly useful when working with many types of data, from the atomic level to much larger scales. MM involves developing mathematical formulae that represent how independent variables mechanistically relate to dependent variables [250]. The formulae are simplified representations, as they cannot feasibly include every possible factor that could affect interactions and are constructed based on hypotheses generated from existing experimental observations. MM includes the physiologically based pharmacokinetic (PBPK) models that are commonly used in drug development, which simulate how drug absorption, distribution, metabolism, and excretion (ADME) influence drug concentration in plasma and/or tissues [259]. As key ADME parameters can be adjusted, PBPK models can accurately predict how physiological variability affects pharmacokinetics in different patient groups [260]. Research utilising the common PBPK software GI-Sim and GastroPlus has shown that both tools could predict the colonic absorption of 14 drugs, with performances acceptably high as to promote their replacement of *in vivo* regional absorption in dogs during preclinical drug development [261]. Advantages of MM include the ability to work with both small and large datasets, uncover how variables mechanistically interact within a model, and importantly, understand the mathematical basis on which predictions are made. ML may be more suitable in cases when the mechanisms behind interactions are not yet known or are very complex, for example when assessing how physiological variability (involving many biological variables) affects the efficacy of pharmaceutical formulations.

In recent years, ML has been increasingly explored for its ability to reduce costs and increase success throughout the entire drug development pipeline, from drug discovery to clinical trials [262,263]. ML encompasses a plethora of technologies that utilise various types of mathematical algorithms to learn patterns in data, which can be leveraged to predict experimental outcomes for untested data [247]. Two key

types of ML are supervised and unsupervised learning [264]. In supervised learning, algorithms are trained on datasets with labelled samples, such as a large dataset of drugs with known solubilities in colonic fluid. Supervised ML would involve the algorithm learning how sample characteristics may predict their label; in the example this would involve the algorithm learning how drug characteristics affect their solubilities in colonic fluid. If sample characteristics are sufficiently predictive of the label, then the resultant ML model could be used to predict labels for untested samples (e.g., drugs with unknown colonic fluid solubilities). Unsupervised learning involves unlabelled datasets, whereby patterns within data can be elucidated using techniques such as clustering and dimension reduction [265]. Both types of learning may be combined to form semi-supervised learning, in which unlabelled data is labelled using unsupervised methods, and subsequently utilised for supervised ML tasks [266]. Emerging forms of ML, such as reinforcement learning, active learning, multi-task learning, and generative models offer further opportunities to exploit available experimental data and maximise the efficiency of future experimental efforts [267–270].

ML has begun to be used extensively for formulation design [247]. For example, a supervised artificial neural network (ANN) trained on over 950 formulations has predicted the drug release times of 3D printed medicines with a mean error of just 24.29 minutes [271]. Elsewhere, an ANN predicted the size and drug loading efficiency of nanoparticles based on excipient composition, resulting in more accurate predictions than a DoE model applied to the same task [272]. ML has also been leveraged to predict drug-microbiome interactions [136,138,140]. As the amount of data available to research departments continues to increase, quantum computing may supersede classical computing for ML and other *in silico* techniques, due to its capacity to process datasets containing hundreds of thousands of datapoints much more efficiently [273,274]. It is also likely that multiple *in silico* predictive technologies will be increasingly combined within tasks to exploit their individual advantages. Such hybrid approaches could afford researchers the largescale data exploration facilitated by ML, mechanistic insight

provided by MM, and atomic visualisation enabled by MD [275]. For example, Reker et al. used ML combined with MD to discover 100 novel co-aggregated solid drug nanoparticles composed of self-assembled drug-excipient combinations, two of which were successfully characterised *ex vivo* and *in vivo* [276]. This study exemplifies how the unification of advanced *in silico* technologies can be employed to accelerate and optimise formulation development by exploiting pre-existing big data.

## 5. Conclusions and future perspectives

Colonic drug delivery is at an exciting point in time, whereby modern knowledge underlying the colon's unique physiology, effective formulation strategies, and advanced *in vitro*, *in vivo*, and *in silico* development tools can be harnessed to develop novel and efficacious medicines. This review has outlined how conditions in the GI tract should inform every stage of colon-targeted medicines' development; from the identification of colon-specific targets, to the design of formulations that reliably deliver drug to the intended site of action, to the preclinical assessment of formulations. Parallel-trigger formulation technologies have enabled heightened reliability of colonic drug delivery via the oral route, opening opportunities for selective modulation of emerging systemic targets or better treatment of local diseases. Future research could expand on these formulation strategies to develop indication-specific formulations, whereby drug release triggers may be based on pathophysiology or tuned to special patient populations. Further, there is marked potential for innovation within rectal drug delivery, where advanced techniques (such as precision formulation or *in silico* design) could be utilised to overcome the current challenges facing the administration route. Another area for opportunity is the expansion of physiologically relevant tools for the assessment of rectal formulations' PK, as well as standardisation of *in vitro* and *in vivo* models used for PK assessment of orally delivered colon-targeted medicines. Finally, more studies that demonstrate how advanced *in silico* tools can be applied to reduce the resources and time required to develop colon-targeted medicines would be beneficial for inspiring the adoption of these tools by others. Recent advances in how colon-targeted medicines are designed and developed presents substantial possibilities for translating safer and more effective treatments to the clinic.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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## References

- [1] A. Awad, C.M. Madla, L.E. McCoubrey, F. Ferraro, F.K.H. Gavins, A. Buanz, S. Gaisford, M. Orlu, F. Siepman, J. Siepman, A.W. Basit, Clinical translation of advanced colonic drug delivery technologies, *Adv. Drug Deliv. Rev.* 181 (2022), 114076.
- [2] C. Campbell, P.T. McKenney, D. Konstantinovskiy, O.I. Isaeva, M. Schizas, J. Verter, C. Mai, W.-B. Jin, C.-J. Guo, S. Violante, R.J. Ramos, J.R. Cross, K. Kadaveru, J. Hambor, A.Y. Rudensky, Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells, *Nature* 581 (2020) 475–479.
- [3] M. Peiris, R. Aktar, D. Reed, V. Cibert-Goton, A. Zdanaviciene, W. Halder, A. Robinow, S. Corke, H. Dogra, C.H. Knowles, A. Blackshaw, Decoy bypass for appetite suppression in obese adults: role of synergistic nutrient sensing receptors GPR84 and FFAR4 on colonic endocrine cells, *Gut* 71 (2022) 928–937.
- [4] J.R. Allegretti, M. Fischer, S.V. Sagi, M.E. Bohm, H.M. Fadda, S.R. Ranmal, S. Budree, A.W. Basit, D.L. Glettig, E.L. de la Serna, A. Gentile, Y. Gerardin, S. Timberlake, R. Sadovsky, M. Smith, Z. Kassam, Fecal microbiota transplantation capsules with targeted colonic versus gastric delivery in recurrent clostridium difficile infection: a comparative cohort analysis of high and low dose, *Dig. Dis. Sci.* 64 (2019) 1672–1678.
- [5] K. Ciftci, M.J. Groves, Delivery of antitumor compounds to the rat colon: *in vitro* and *in vivo* evaluation, *Int. J. Pharm.* 145 (1996) 157–164.
- [6] A. Foppoli, A. Maroni, S. Moutaharrik, A. Melocchi, L. Zema, L. Palugan, M. Cerea, A. Gazzaniga, *In vitro* and human pharmacocintigraphic evaluation of an oral 5-ASA delivery system for colonic release, *Int. J. Pharm.* 572 (2019), 118723.
- [7] V. Yadav, A. House, S. Matiz, L.E. McCoubrey, K.A. Bettano, L. Bhawe, M. Wang, P. Fan, S. Zhou, J.D. Woodhouse, E. Poimenidou, L. Dou, A.W. Basit, L.Y. Moy, R. Saklatvala, L.G. Hegde, H. Yu, Ileocolonic-targeted JAK inhibitor: a safer and more effective treatment for inflammatory bowel disease, *Pharmaceutics* 14 (2022).
- [8] B. Verstockt, D. Alsoud, J.V. Oostrom, J. Smith, J. Styli, S. Singh, S.V. Genep, P. Rahimian, J. Sabino, M. Ferrante, S. Singh, G. D'Haens, S. Vermeire, Tofacitinib tissue exposure correlates with endoscopic outcome (poster), in: *Progenity (Ed.)*, 17th Congress of the European Crohn's and Colitis Organisation, 2022.
- [9] F. Vuijk, J. Dicksved, S.Y. Lam, G.M. Fuhler, L. van der Laan, A. van de Winkel, S. R. Konstantinov, M. Spaander, M.P. Peppelenbosch, L. Engstrand, E.J. Kuipers, Composition of the mucosa-associated microbiota along the entire gastrointestinal tract of human individuals, *United European Gastroenterol. J.* 7 (2019) 897–907.
- [10] K.R. James, T. Gomes, R. Elmentaite, N. Kumar, E.L. Gulliver, H.W. King, M. D. Stares, B.R. Bareham, J.R. Ferdinand, V.N. Petrova, K. Polanski, S.C. Forster, L. B. Jarvis, O. Suchanek, S. Howlett, L.K. James, J.L. Jones, K.B. Meyer, M. R. Clatworthy, K. Saeb-Parsy, T.D. Lawley, S.A. Teichmann, Distinct microbial and immune niches of the human colon, *Nat. Immunol.* 21 (2020) 343–353.
- [11] E.S. Winkler, S. Shrihari, B.L. Hykes, S.A. Handley, P.S. Andhey, Y.-J.S. Huang, A. Swain, L. Droit, K.K. Chebrolu, M. Mack, D.L. Vanlandingham, L.B. Thackray, M. Cella, M. Colonna, M.N. Artyomov, T.S. Stappenbeck, M.S. Diamond, The intestinal microbiome restricts alphavirus infection and dissemination through a bile acid-type I IFN signaling axis, *Cell* 182 (2020) 901–918.e918.
- [12] I. Ferrocino, V. Ponzio, M. Pellegrini, I. Goitre, M. Papurello, I. Franciosa, C. D'Eusebio, E. Ghigo, L. Cocolin, S. Bo, Mycobiota composition and changes across pregnancy in patients with gestational diabetes mellitus (GDM), *Sci. Rep.* 12 (2022).
- [13] E. van Tilburg Bernardes, V.K. Pettersen, M.W. Gutierrez, I. Laforest-Lapointe, N. G. Jendzjowsky, J.B. Cavin, F.A. Vicentini, C.M. Keenan, H.R. Ramay, J. Samara, W.K. MacNaughton, R.J.A. Wilson, M.M. Kelly, K.D. McCoy, K.A. Sharkey, M. C. Arrieta, Intestinal fungi are causally implicated in microbiome assembly and immune development in mice, *Nat. Commun.* 11 (2020) 2577.
- [14] K.A. Kigerl, K. Mostacada, P.G. Popovich, Gut microbiota are disease-modifying factors after traumatic spinal cord injury, *Neurotherapeutics* 15 (2018) 60–67.
- [15] J. Jin, R. Cheng, Y. Ren, X. Shen, J. Wang, Y. Xue, H. Zhang, X. Jia, T. Li, F. He, H. Tian, Distinctive gut microbiota in patients with overweight and obesity with dyslipidemia and its responses to long-term orlistat and ezetimibe intervention: a randomized controlled open-label trial, *Front. Pharmacol.* 12 (2021).
- [16] C.J. Worby, H.L.T. Schreiber, T.J. Straub, L.R. van Dijk, R.A. Bronson, B.S. Olson, J.S. Pinkner, C.L.P. Obernuefemann, V.L. Munoz, A.E. Paharik, P.N. Azimzadeh, B.J. Walker, C.A. Desjardins, W.C. Chou, K. Bergeron, S.B. Chapman, A. Klim, A. L. Manson, T.J. Hannan, T.M. Hooton, A.L. Kau, H.H. Lai, K.W. Dodson, S. J. Hultgren, A.M. Earl, Longitudinal multi-omics analyses link gut microbiome dysbiosis with recurrent urinary tract infections in women, *Nat. Microbiol.* 7 (2022) 630–639.
- [17] Y. Yan, S. Ren, Y. Duan, C. Lu, Y. Niu, Z. Wang, B. Inglis, W. Ji, Y. Zheng, W. Si, Gut microbiota and metabolites of alpha-synuclein transgenic monkey models with early stage of Parkinson's disease, *NPJ Biofilms Microbiomes* 7 (2021) 69.
- [18] A. Kaiyrylkyzy, S. Kozhakhmetov, D. Babenko, G. Zholdasbekova, D. Alzhanova, F. Olzhayev, A. Baibulatova, A.R. Kushugulova, S. Askarova, Study of gut microbiota alterations in Alzheimer's dementia patients from Kazakhstan, *Sci. Rep.* 12 (2022).
- [19] E. Cao, M.J. Watt, C.J. Nowell, T. Quach, J.S. Simpson, V. De Melo Ferreira, S. Agarwal, H. Chu, A. Srivastava, D. Anderson, G. Gracia, A. Lam, G. Segal, J. Hong, L. Hu, K.L. Phang, A.B.J. Escott, J.A. Windsor, A.R.J. Phillips, D.J. Creech, N.L. Harvey, C.J.H. Porter, N.L. Trevisakis, Mesenteric lymphatic dysfunction

- promotes insulin resistance and represents a potential treatment target in obesity, *Nat. Metab.* 3 (2021) 1175–1188.
- [20] A. Brookes, L. Ji, T.D. Bradshaw, M. Stocks, D. Gray, J. Butler, P. Gershkovich, Is oral lipid-based delivery for drug targeting to the brain feasible? *Eur. J. Pharm. Biopharm.* 172 (2022) 112–122.
- [21] S.H. Lee, R. Bajracharya, J.Y. Min, J.W. Han, B.J. Park, H.K. Han, Strategic approaches for colon targeted drug delivery: an overview of recent advancements, *Pharmaceutics* 12 (2020).
- [22] Y. Tao, R. Wang, Q. Lai, M. Wu, Y. Wang, X. Jiang, L. Zeng, S. Zhou, Z. Li, T. Yang, Y. Yao, Y. Wu, L. Yu, Y. Fu, W. Lai, Y. Peng, Y. Lu, Z. Zhang, C. Guo, G. Zhang, L. Gou, J. Yang, Targeting of DDR1 with antibody-drug conjugates has antitumor effects in a mouse model of colon carcinoma, *Mol. Oncol.* 13 (2019) 1855–1873.
- [23] K.K. Sahu, R.S. Pandey, Development and characterization of HBsAg-loaded Eudragit nanoparticles for effective colonic immunization, *Pharm. Dev. Technol.* 24 (2019) 166–175.
- [24] T. Zhang, W. Zhang, C. Feng, L.-Y. Kwok, Q. He, Z. Sun, Stronger gut microbiome modulatory effects by postbiotics than probiotics in a mouse colitis model, *NPJ Sci. Food* 6 (2022).
- [25] Y. Luo, C. De Souza, M. Ramachandran, S. Wang, H. Yi, Z. Ma, L. Zhang, K. Lin, Precise oral delivery systems for probiotics: A review, *J. Control. Release* 352 (2022) 371–384.
- [26] N. Svartz, Behandlung der ulzerösen Kolitis mit Salazopyrin, *Digestion* 66 (1941) 312–322.
- [27] A.A. Khan, J. Piris, S. Truelove, An experiment to determine the active therapeutic moiety of sulphasalazine, *Lancet* 310 (1977) 892–895.
- [28] S. Riley, What dose of 5-aminosalicylic acid (mesalazine) in ulcerative colitis? *Gut* 42 (1998) 761–763.
- [29] U. Klotz, Clinical pharmacokinetics of sulphasalazine, its metabolites and other prodrugs of 5-aminosalicylic acid, *Clin. Pharmacokinet.* 10 (1985) 285–302.
- [30] A.N. Wadworth, A. Fitton, Olsalazine, *Drugs* 41 (1991) 647–664.
- [31] R.P. Chan, D.J. Pope, A.P. Gilbert, P.J. Sacra, J. Baron, J.E. Lennard-Jones, Studies of two novel sulfasalazine analogs, ipsalazide and balsalazide, *Dig. Dis. Sci.* 28 (1983) 609–615.
- [32] J. Zhao, B. Zhang, Q. Mao, K. Ping, P. Zhang, F. Lin, D. Liu, Y. Feng, M. Sun, Y. Zhang, Q.H. Li, T. Zhang, Y. Mou, S. Wang, Discovery of a colon-targeted azo prodrug of tofacitinib through the establishment of colon-specific delivery systems constructed by 5-ASA-PABA-MAC and 5-ASA-PABA-diamine for the treatment of ulcerative colitis, *J. Med. Chem.* 65 (2022) 4926–4948.
- [33] D.J. Brayden, Localised delivery of macromolecules to the large intestine: translation to clinical trials, *BioDrugs* 36 (2022) 687–700.
- [34] A. Najjar, R. Karaman, The prodrug approach in the era of drug design, *Expert Opin. Drug Deliv.* 16 (2019) 1–5.
- [35] Committee for Medicinal Products for Human Use, Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms, London, UK, 2014.
- [36] M.J. Dew, P.-J. Hughes, M.G. Lee, B.K. Evans, J. Rhodes, An oral preparation to release drugs in the human colon, *Br. J. Clin. Pharmacol.* 14 (1982) 405–408.
- [37] S. Thakral, N.K. Thakral, D.K. Majumdar, Eudragit: a technology evaluation, *Expert Opin. Drug Deliv.* 10 (2013) 131–149.
- [38] V.C. Ibeke, H.M. Fadda, G.E. Parsons, A.W. Basit, A comparative in vitro assessment of the drug release performance of pH-responsive polymers for ileo-colonic delivery, *Int. J. Pharm.* 308 (2006) 52–60.
- [39] V.C. Ibeke, F. Liu, H.M. Fadda, M.K. Khela, D.F. Evans, G.E. Parsons, A.W. Basit, An investigation into the in vivo performance variability of pH responsive polymers for ileo-colonic drug delivery using gamma scintigraphy in humans, *J. Pharm. Sci.* 95 (2006) 2760–2766.
- [40] V.C. Ibeke, H.M. Fadda, E.L. McConnell, M.K. Khela, D.F. Evans, A.W. Basit, Interplay between intestinal pH, transit time and feed status on the in vivo performance of pH responsive ileo-colonic release systems, *Pharm. Res.* 25 (2008) 1828–1835.
- [41] G.B. Hatton, C.M. Madla, S.C. Rabbie, A.W. Basit, All disease begins in the gut: Influence of gastrointestinal disorders and surgery on oral drug performance, *Int. J. Pharm.* 548 (2018) 408–422.
- [42] A. Melocchi, M. Uboldi, F. Briatico-Vangosa, S. Moutaharrik, M. Cerea, A. Foppoli, A. Maroni, L. Palugan, L. Zema, A. Gazzaniga, The Chronotopic™ System for Pulsatile and Colonic Delivery of Active Molecules in the Era of Precision Medicine: Feasibility by 3D Printing via Fused Deposition Modeling (FDM), *Pharmaceutics*, 2021, p. 759.
- [43] G.B. Hatton, C.M. Madla, S.C. Rabbie, A.W. Basit, Gut reaction: impact of systemic diseases on gastrointestinal physiology and drug absorption, *Drug Discov. Today* 24 (2019) 417–427.
- [44] M. Ashford, J. Fell, D. Attwood, H. Sharma, P. Woodhead, An evaluation of pectin as a carrier for drug targeting to the colon, *J. Control. Release* 26 (1993) 213–220.
- [45] Y.S.R. Krishnaiah, V. Satyanarayana, B. Dinesh Kumar, R.S. Karthikeyan, P. Bhaskar, In vivo evaluation of guar gum-based colon-targeted oral drug delivery systems of celecoxib in human volunteers, *European Journal of Drug Metab. Pharm.* 27 (2002) 273–280.
- [46] A.W. Basit, M.D. Short, E.L. McConnell, Microbiota-triggered colonic delivery: Robustness of the polysaccharide approach in the fed state in man, *J. Drug Target.* 17 (2009) 64–71.
- [47] L. Tian, X.W. Wang, A.K. Wu, Y. Fan, J. Friedman, A. Dahlin, M.K. Waldor, G. M. Weinstock, S.T. Weiss, Y.Y. Liu, Deciphering functional redundancy in the human microbiome, *Nat. Commun.* 11 (2020) 6217.
- [48] L.F. Siew, S.-M. Man, J.M. Newton, A.W. Basit, Amylose formulations for drug delivery to the colon: a comparison of two fermentation models to assess colonic targeting performance in vitro, *Int. J. Pharm.* 273 (2004) 129–134.
- [49] L. Maier, C.V. Goemans, J. Wirbel, M. Kuhn, C. Eberl, M. Pruteanu, P. Muller, S. Garcia-Santamarina, E. Caecae, B. Zhang, C. Gekeler, T. Banerjee, E. E. Anderson, A. Milanese, U. Lober, S.K. Forslund, K.R. Patil, M. Zimmermann, B. Stecher, G. Zeller, P. Bork, A. Typas, Unravelling the collateral damage of antibiotics on gut bacteria, *Nature* 599 (2021) 120–124.
- [50] F. Varum, A.C. Freire, R. Bravo, A.W. Basit, OPTIGORE™, an innovative and accurate colonic targeting technology, *Int. J. Pharm.* 583 (2020), 119372.
- [51] F. Varum, A.C. Freire, H.M. Fadda, R. Bravo, A.W. Basit, A dual pH and microbiota-triggered coating (Phloral(TM)) for fail-safe colonic drug release, *Int. J. Pharm.* 583 (2020), 119379.
- [52] S. Hua, Physiological and pharmaceutical considerations for rectal drug formulations, *Front. Pharmacol.* 10 (2019) 1196.
- [53] T.J. Purohit, S.M. Hanning, Z. Wu, Advances in rectal drug delivery systems, *Pharm. Dev. Technol.* 23 (2018) 942–952.
- [54] Y.H. Jani, F. Liu, M. Orlu, N. Desai, F. du Chayla, F. Ruiz, T. Vallet, Medicine acceptability for older people in hospital and care home: the influence of setting, *Int. J. Pharm. Pract.* 30 (2022) 67–74.
- [55] E.L. McConnell, A.W. Basit, S. Murdan, Colonic antigen administration induces significantly higher humoral levels of colonic and vaginal IgA, and serum IgG compared to oral administration, *Vaccine* 26 (2008) 639–646.
- [56] C.-S. Cho, S.-K. Hwang, M.-J. Gu, C.-G. Kim, S.-K. Kim, D.-B. Ju, C.-H. Yun, H.-J. Kim, Mucosal vaccine delivery using mucoadhesive polymer particulate systems, *Tissue Eng. Regen. Med.* 18 (2021) 693–712.
- [57] T. Moqejwa, T. Marimuthu, P.P.D. Kondiah, Y.E. Choonara, Development of stable nano-sized transfersomes as a rectal colloid for enhanced delivery of cannabidiol, *Pharmaceutics* 14 (2022).
- [58] J.O. Awoloke, B.T. Adeyanju, A. Adeniyi, O.P. Aduloju, B.A. Olofinbiyi, Randomised controlled trial of sublingual and rectal misoprostol in the prevention of primary postpartum haemorrhage in a resource-limited community, *J. Obstet. Gynecol. India* 70 (2020) 462–470.
- [59] D. Zetner, L.P.K. Andersen, R. Alder, M.L. Jessen, A. Tolstrup, J. Rosenberg, Pharmacokinetics and safety of intravenous, intravesical, rectal, transdermal, and vaginal melatonin in healthy female volunteers: a cross-over study, *Pharmacology* 106 (2021) 169–176.
- [60] T. Takagi, Y. Naito, Y. Higashimura, K. Uchiyama, T. Okayama, K. Mizushima, K. Katada, K. Kamada, T. Ishikawa, Y. Itoh, Rectal administration of carbon monoxide inhibits the development of intestinal inflammation and promotes intestinal wound healing via the activation of the Rho-kinase pathway in rats, *Nitric Oxide Biol. Chem.* 107 (2021) 19–30.
- [61] L. Barducci, J.C. Norton, S. Sarker, S. Mohammed, R. Jones, P. Valdastri, B. S. Terry, Fundamentals of the gut for capsule engineers, *Progr. Biomed. Eng.* 2 (2020).
- [62] K. Taylor, M.E. Aulton, *Aulton's Pharmaceutics, The Design and Manufacture of Medicines*, 6th ed., Elsevier, Amsterdam, Netherlands, 2021.
- [63] A. Kalra, C.J. Wehrle, F. Tuma, in: *StatPearls* (Ed.), *Anatomy, Abdomen and Pelvis, Peritoneum*, StatPearls Publishing, Online, 2021.
- [64] P. Kahai, P. Mandiga, C.J. Wehrle, S. Lobo, in: *StatPearls* (Ed.), *Anatomy, Abdomen and Pelvis, Large Intestine*, StatPearls Publishing, Online, 2021.
- [65] S. Cai, Y. Fan, B. Zhang, J. Lin, X. Yang, Y. Liu, J. Liu, J. Ren, H. Xu, Appendectomy is associated with alteration of human gut bacterial and fungal communities, *Front. Microbiol.* 12 (2021).
- [66] L. Vitetta, J. Chen, S. Clarke, The vermiform appendix: an immunological organ sustaining a microbiome inoculum, *Clin. Sci. (Lond.)* 133 (2019) 1–8.
- [67] W. Weitschies, H. Blume, H. Monnikes, Magnetic marker monitoring: high resolution real-time tracking of oral solid dosage forms in the gastrointestinal tract, *Eur. J. Pharm. Biopharm.* 74 (2010) 93–101.
- [68] C.M. Madla, F.K.H. Gavins, H.A. Merchant, M. Orlu, S. Murdan, A.W. Basit, Let's talk about sex: differences in drug therapy in males and females, *Adv. Drug Deliv. Rev.* 113804 (2021).
- [69] P. Vasavid, T. Chaiwatanarat, P. Pusuwan, C. Sritara, K. Roysri, S. Namwongprom, P. Kuanrakcharoen, T. Premprabha, K. Chunlertrith, S. Thongsawat, S. Sirthornpunya, B. Ovartlarnporn, U. Kachintorn, S. Leelakusolvong, C. Kositchaiwat, S. Chakkaphak, S. Gonlachanvit, Normal solid gastric emptying values measured by scintigraphy using Asian-style meal: a multicenter study in healthy volunteers, *J. Neurogastroenterol. Motil.* 20 (2014) 371–378.
- [70] K. Triantafyllou, C. Kalantzis, A.A. Papadopoulos, P. Apostolopoulos, T. Rokkas, N. Kalantzis, S.D. Ladas, Video-capsule endoscopy gastric and small bowel transit time and completeness of the examination in patients with diabetes mellitus, *Dig. Liver Dis.* 39 (2007) 575–580.
- [71] E.L. McConnell, H.M. Fadda, A.W. Basit, Gut instincts: explorations in intestinal physiology and drug delivery, *Int. J. Pharm.* 364 (2008) 213–226.
- [72] M. Müller, E.E. Canfora, E.E. Blaak, Gastrointestinal transit time, glucose homeostasis and metabolic health: modulation by dietary fibers, *Nutrients* 10 (2018) 275.
- [73] J. Worsøe, L. Fynne, T. Gregersen, V. Schlageter, L.A. Christensen, J.F. Dahlerup, N.J.M. Rijkhoff, S. Laurberg, K. Krogh, Gastric transit and small intestinal transit time and motility assessed by a magnet tracking system, *BMC Gastroenterol.* 11 (2011) 145.
- [74] M. Feldman, S. Seng and Fordtran's Gastrointestinal and Liver Disease, 11th ed., Elsevier, 2021.

- [75] J. Campbell, J. Berry, Y. Liang, Chapter 71 - Anatomy and physiology of the small intestine, in: C.J. Yeo (Ed.), *Shackelford's Surgery of the Alimentary Tract*, 2 Volume Set (Eighth Edition), Elsevier, Philadelphia, 2019, pp. 817–841.
- [76] M. Corsetti, M. Costa, G. Bassotti, A.E. Bharucha, O. Borrelli, P. Dinning, C. Di Lorenzo, J.D. Huizinga, M. Jimenez, S. Rao, R. Spiller, N.J. Spencer, R. Lentle, J. Pannemans, A. Thys, M. Benninga, J. Tack, First translational consensus on terminology and definitions of colonic motility in animals and humans studied by manometric and other techniques, *Nat. Rev. Gastroenterol. Hepatol.* 16 (2019) 559–579.
- [77] N.J. Spencer, P.G. Dinning, S.J. Brookes, M. Costa, Insights into the mechanisms underlying colonic motor patterns, *J. Physiol.* 594 (2016) 4099–4116.
- [78] H. Tian, Q. Chen, B. Yang, H. Qin, N. Li, Analysis of gut microbiome and metabolite characteristics in patients with slow transit constipation, *Dig. Dis. Sci.* 66 (2020) 3026–3035.
- [79] T. Córdova-Fraga, R. Huerta-Franco, G. Gutiérrez-Juárez, M. Sosa-Aquino, M. Vargas-Luna, The colon transit time in different phases of the menstrual cycle: assessed with biomagnetic technique, *Neurol. Clin. Neurophysiol.* 2004 (2004) 31.
- [80] G.K. Nandhra, E.B. Mark, G.L. Di Tanna, A.-M. Haase, J. Poulsen, S. Christodoulides, V. Kung, M.W. Klinge, K. Knudsen, P. Borghammer, K. O. Andersen, L. Fynne, N. Sutter, V. Schlageter, K. Krogh, A.M. Drewes, M. Birch, S.M. Scott, Normative values for region-specific colonic and gastrointestinal transit times in 111 healthy volunteers using the 3D-Transit electromagnet tracking system: Influence of age, gender, and body mass index, *Neurogastroenterol. Motil.* 32 (2020), e13734.
- [81] J.W. Pelley, 20 - Tissue biochemistry, in: J.W. Pelley (Ed.), *Elsevier's Integrated Biochemistry*, Mosby, Philadelphia, 2007, pp. 179–190.
- [82] Y. Chu, C. Qin, W. Feng, C. Sheriston, Y. Jane Khor, C. Medrano-Padial, B. E. Watson, T. Chan, B. Ling, M.J. Stocks, P.M. Fischer, P. Gershkovich, Oral administration of tipranavir with long-chain triglyceride results in moderate intestinal lymph targeting but no efficient delivery to HIV-1 reservoir in mesenteric lymph nodes, *Int. J. Pharm.* 602 (2021), 120621.
- [83] A. Jewell, A. Brookes, W. Feng, M. Ashford, P. Gellert, J. Butler, P.M. Fischer, D. J. Scurr, M.J. Stocks, P. Gershkovich, Distribution of a highly lipophilic drug cannabidiol into different lymph nodes following oral administration in lipidic vehicle, *Eur. J. Pharm. Biopharm.* 174 (2022) 29–34.
- [84] M. Mikhael, Y.S. Khan, in: *Statpearls* (Ed.), *Anatomy, Abdomen and Pelvis, Lymphatic Drainage*, StatPearls Publishing, Online, 2021.
- [85] S.A. Houston, V. Cerovic, C. Thomson, J. Brewer, A.M. Mowat, S. Milling, The lymph nodes draining the small intestine and colon are anatomically separate and immunologically distinct, *Mucosal Immunol.* 9 (2016) 468–478.
- [86] D. Esterházy, M.C.C. Canesso, L. Mesin, P.A. Müller, T.B.R. de Castro, A. Lockhart, M. ElJalby, A.M.C. Faria, D. Mucida, Compartmentalized gut lymph node drainage dictates adaptive immune responses, *Nature* 569 (2019) 126–130.
- [87] Y. Yang, Y. Zhao, A. Yu, D. Sun, L.X. Yu, Chapter 12 - oral drug absorption: evaluation and prediction, in: Y. Qiu, Y. Chen, G.G.Z. Zhang, L. Yu, R.V. Mantri (Eds.), *Developing Solid Oral Dosage Forms (Second Edition)*, Academic Press, Boston, 2017, pp. 331–354.
- [88] C. Tannergren, A. Bergendal, H. Lennernäs, B. Abrahamsson, Toward an increased understanding of the barriers to colonic drug absorption in humans: implications for early controlled release candidate assessment, *Mol. Pharm.* 6 (2009) 60–73.
- [89] E. Martini, S.M. Krug, B. Siegmund, M.F. Neurath, C. Becker, Mend Your Fences: the epithelial barrier and its relationship with mucosal immunity in inflammatory bowel disease, cellular and molecular, *Gastroenterol. Hepatol.* 4 (2017) 33–46.
- [90] G. Lemmens, A. Van Camp, S. Kourula, T. Vanuytsel, P. Augustijns, Drug disposition in the lower gastrointestinal tract: targeting and monitoring, *Pharmaceutics* 13 (2021).
- [91] A.J.M. Santos, Y.-H. Lo, A.T. Mah, C.K. Juo, The intestinal stem cell niche: homeostasis and adaptations, *Trends Cell Biol.* 28 (2018) 1062–1078.
- [92] S. Kong, Y.H. Zhang, W. Zhang, Regulation of intestinal epithelial cells properties and functions by amino acids, *Biomed. Res. Int.* 2018 (2018) 2819154.
- [93] U.M. Mörbe, P.B. Jørgensen, T.M. Fenton, N. von Burg, L.B. Riis, J. Spencer, W. W. Agace, Human gut-associated lymphoid tissues (GALT); diversity, structure, and function, *Mucosal Immunol.* 14 (2021) 793–802.
- [94] S.K. Hendel, L. Kellermann, A. Hausmann, N. Bindslev, K.B. Jensen, O.H. Nielsen, Tuft cells and their role in intestinal diseases, *Front. Immunol.* 13 (2022).
- [95] S.C. Corr, C.C.G.M. Gahan, C. Hill, M-cells: origin, morphology and role in mucosal immunity and microbial pathogenesis, *FEMS Immunol. Med. Microbiol.* 52 (2008) 2–12.
- [96] E.L. McConnell, F. Liu, A.W. Basit, Colonic treatments and targets: issues and opportunities, *J. Drug Target.* 17 (2009) 335–363.
- [97] M. Drozdziak, D. Busch, J. Lapczuk, J. Müller, M. Ostrowski, M. Kurzawski, S. Oswald, Protein abundance of clinically relevant drug transporters in the human liver and intestine: a comparative analysis in paired tissue specimens, *Clin. Pharmacol. Ther.* 105 (2019) 1204–1212.
- [98] M. Drozdziak, I. Czekawski, S. Oswald, A. Drozdziak, Intestinal drug transporters in pathological states: an overview, *Pharmacol. Rep.* 72 (2020) 1173–1194.
- [99] M. Tubic-Grozdanis, J.M. Hilfinger, G.L. Amidon, J.S. Kim, P. Kijek, P. Staubach, P. Langguth, Pharmacokinetics of the CYP 3A substrate simvastatin following administration of delayed versus immediate release oral dosage forms, *Pharm. Res.* 25 (2008) 1591–1600.
- [100] G. Englund, F. Rorsman, A. Rönnblom, U. Karlbom, L. Lazorova, J. Gråsjö, A. Kindmark, P. Artursson, Regional levels of drug transporters along the human intestinal tract: co-expression of ABC and SLC transporters and comparison with Caco-2 cells, *Eur. J. Pharm. Sci.* 29 (2006) 269–277.
- [101] T. de Waal, N. Handin, J. Brouwers, M. Ferrante, S. Vermeire, T. Vanuytsel, P. Artursson, P. Augustijns, The impact of inflammation on the expression of drug transporters and metabolic enzymes in colonic tissue from ulcerative colitis patients, *Int. J. Pharm.* 122282 (2022).
- [102] F. Taherali, F. Varum, A.W. Basit, A slippery slope: on the origin, role and physiology of mucus, *Adv. Drug Deliv. Rev.* 124 (2018) 16–33.
- [103] G.M.H. Birchenough, M.E. Johansson, J.K. Gustafsson, J.H. Bergström, G. C. Hansson, New developments in goblet cell mucus secretion and function, *Mucosal Immunol.* 8 (2015) 712–719.
- [104] M. Herath, S. Hosie, J.C. Bornstein, A.E. Franks, E.L. Hill-Yardin, The role of the gastrointestinal mucus system in intestinal homeostasis: implications for neurological disorders, *Front. Cell. Infect. Microbiol.* 10 (2020).
- [105] S.G. Nugent, D. Kumar, D.S. Rampton, D.F. Evans, Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosulicylates and other drugs, *Gut* 48 (2001).
- [106] B. Lee, K.M. Moon, C.Y. Kim, Tight junction in the intestinal epithelium: its association with diseases and regulation by phytochemicals, *J Immunol Res* 2018 (2018) 2645465.
- [107] L. Dou, F.K.H. Gavins, Y. Mai, C.M. Madla, F. Taherali, M. Orlu, S. Murdan, A. W. Basit, Effect of food and an animal's sex on p-glycoprotein expression and luminal fluids in the gastrointestinal tract of wistar rats, *Pharmaceutics* 12 (2020).
- [108] J. Wang, V. Yadav, A.L. Smart, S. Tajiri, A.W. Basit, Toward oral delivery of biopharmaceuticals: an assessment of the gastrointestinal stability of 17 peptide drugs, *Mol. Pharm.* 12 (2015) 966–973.
- [109] M. Fredua-Agyeman, S. Gaisford, Comparative survival of commercial probiotic formulations: tests in bioequivalent gastric fluids and real-time measurements using microcalorimetry, *Benefic. Microbes* 6 (2015) 141–151.
- [110] D.F. Evans, G. Pye, R. Bramley, A.G. Clark, T.J. Dyson, J.D. Hardcastle, Measurement of gastrointestinal pH profiles in normal ambulant human subjects, *Gut* 29 (1988) 1035–1041.
- [111] A.W. Basit, F. Podczek, J.M. Newton, W.A. Waddington, P.J. Ell, L.F. Lacey, The use of formulation technology to assess regional gastrointestinal drug absorption in humans, *Eur. J. Pharm. Sci.* 21 (2004) 179–189.
- [112] H.M. Fadda, T. Sousa, A.S. Carlsson, B. Abrahamsson, J.G. Williams, D. Kumar, A. W. Basit, Drug solubility in luminal fluids from different regions of the small and large intestine of humans, *Mol. Pharm.* 7 (2010) 1527–1532.
- [113] K. Pyper, J. Brouwers, P. Augustijns, I. Khadra, C. Dunn, C.G. Wilson, G. W. Halbert, Multidimensional analysis of human intestinal fluid composition, *Eur. J. Pharm. Biopharm.* 153 (2020) 226–240.
- [114] C.J. McDougall, R. Wong, P. Scudera, M. Lesser, J.J. DeCosse, Colonic mucosal pH in humans, *Dig. Dis. Sci.* 38 (1993) 542–545.
- [115] C. Reppas, E. Karatza, C. Goumas, C. Markopoulos, M. Vertzoni, Characterization of contents of distal ileum and cecum to which drugs/drug products are exposed during bioavailability/bioequivalence studies in healthy adults, *Pharm. Res.* 32 (2015) 3338–3349.
- [116] G. Major, K. Murray, G. Singh, A. Nowak, C.L. Hoad, L. Marciani, A. Silos-Santiago, C.B. Kurtz, J.M. Johnston, P. Gowland, R. Spiller, Demonstration of differences in colonic volumes, transit, chyme consistency, and response to psyllium between healthy and constipated subjects using magnetic resonance imaging, *Neurogastroenterol. Motil.* 30 (2018), e13400.
- [117] A. Diakidou, M. Vertzoni, K. Goumas, E. Söderlind, B. Abrahamsson, J. Dressman, C. Reppas, Characterization of the contents of ascending colon to which drugs are exposed after oral administration to healthy adults, *Pharm. Res.* 26 (2009) 2141–2151.
- [118] J.H. Cummings, E.W. Pomare, W.J. Branch, C.P. Naylor, G.T. Macfarlane, Short chain fatty acids in human large intestine, portal, hepatic and venous blood, *Gut* 28 (1987) 1221–1227.
- [119] S.E. Pritchard, L. Marciani, K.C. Garsed, C.L. Hoad, W. Thongborisute, E. Roberts, P.A. Gowland, R.C. Spiller, Fasting and postprandial volumes of the undisturbed colon: normal values and changes in diarrhea-predominant irritable bowel syndrome measured using serial MRI, *Neurogastroenterol. Motil.* 26 (2014) 124–130.
- [120] K. Murray, C.L. Hoad, D.M. Mudie, J. Wright, K. Heissam, N. Abrehart, S. E. Pritchard, S. Al Atwah, P.A. Gowland, M.C. Garnett, G.E. Amidon, R.C. Spiller, G.L. Amidon, L. Marciani, Magnetic resonance imaging quantification of fasted state colonic liquid pockets in healthy humans, *Mol. Pharm.* 14 (2017) 2629–2638.
- [121] C. Schiller, C.P. Fröhlich, T. Giessmann, W. Siegmund, H. Mönnikes, N. Hosten, W. Weitschies, Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging, *Aliment. Pharmacol. Ther.* 22 (2005) 971–979.
- [122] J. Goelen, B. Alexander, H.E. Wijesinghe, E. Evans, G. Pawar, R.D. Horniblow, H. K. Batchelor, Quantification of fluid volume and distribution in the paediatric colon via magnetic resonance imaging, *Pharmaceutics* 13 (2021).
- [123] P. Iovino, C. Bucci, F. Tremolaterra, A. Santonicola, G. Chiarioni, Bloating and functional gastro-intestinal disorders: where are we and where are we going? *World J. Gastroenterol.* 20 (2014) 14407–14419.
- [124] C. Barber, M. Mezo, C. Sabater, F. Vallejo, R.A. Bendezu, M. Masihi, F. Guarner, J.C. Espín, A. Margolles, F. Azpiroz, Differential effects of western and mediterranean-type diets on gut microbiota: A metagenomics and metabolomics approach, *Nutrients* 13 (2021).
- [125] R. Singhal, Y.M. Shah, Oxygen battle in the gut: Hypoxia and hypoxia-inducible factors in metabolic and inflammatory responses in the intestine, *J. Biol. Chem.* 295 (2020) 10493–10505.
- [126] S. Jalili-Firoozinezhad, F.S. Gazzaniga, E.L. Calamari, D.M. Camacho, C.W. Fadel, A. Bein, B. Swenor, B. Nestor, M.J. Cronce, A. Tovaglieri, O. Levy, K.E. Gregory,



- D.T. Breault, J.M.S. Cabral, D.L. Kasper, R. Novak, D.E. Ingber, A complex human gut microbiome cultured in an anaerobic intestine-on-a-chip, *Nature, Biomed. Eng.* 3 (2019) 520–531.
- [127] J. Qin, R. Li, J. Raes, M. Arumugam, K.S. Burgdorf, C. Manichanh, T. Nielsen, N. Pons, F. Levenez, T. Yamada, D.R. Mende, J. Li, J. Xu, S. Li, D. Li, J. Cao, B. Wang, H. Liang, H. Zheng, Y. Xie, J. Tap, P. Lepage, M. Bertalan, J.-M. Batto, T. Hansen, D. Le Paslier, A. Linneberg, H.B. Nielsen, E. Pelletier, P. Renault, T. Sicheritz-Ponten, K. Turner, H. Zhu, C. Yu, S. Li, M. Jian, Y. Zhou, Y. Li, X. Zhang, S. Li, N. Qin, H. Yang, J. Wang, S. Brunak, J. Doré, F. Guarnier, K. Kristiansen, O. Pedersen, J. Parkhill, J. Weissenbach, M. Antolin, F. Artiguenave, H. Blottiere, N. Borrueil, T. Bruls, F. Casellas, C. Chervaux, A. Cultrone, C. Delorme, G. Denariar, R. Dervyn, M. Forte, C. Friss, M. van de Guchte, E. Guedon, F. Haimet, A. Jamet, C. Juste, G. Kaci, M. Kleerebezem, J. Knol, M. Kristensen, S. Layec, K. Le Roux, M. Leclerc, E. Maguin, R. Melo Minardi, R. Oozeer, M. Rescigno, N. Sanchez, S. Tims, T. Torrejon, E. Varela, W. de Vos, Y. Winogradsky, E. Zoetendal, P. Bork, S.D. Ehrlich, J. Wang, H.I.T. C. Meta, A human gut microbial gene catalogue established by metagenomic sequencing, *Nature* 464 (2010) 59–65.
- [128] L.E. McCoubrey, A.W. Basit, Addressing drug–microbiome interactions: the role of healthcare professionals, *Pharm. J.* 308 (2022) 7958.
- [129] K. Martinez-Guryn, V. Leone, E.B. Chang, Regional diversity of the gastrointestinal microbiome, *Cell Host Microbe* 26 (2019) 314–324.
- [130] Y. Tian, W. Gui, I. Koo, P.B. Smith, E.L. Allman, R.G. Nichols, B. Rimal, J. Cai, Q. Liu, A.D. Patterson, The microbiome modulating activity of bile acids, *Gut Microbes* 11 (2020) 979–996.
- [131] G. Liang, F.D. Bushman, The human virome: assembly, composition and host interactions, *Nat. Rev. Microbiol.* 19 (2021) 514–527.
- [132] F. Strati, M. Di Paola, I. Stefanini, D. Albanese, L. Rizzetto, P. Lionetti, A. Calabro, O. Jousson, C. Donati, D. Cavalieri, C. De Filippo, Age and gender affect the composition of fungal population of the human gastrointestinal tract, *Front. Microbiol.* 7 (2016) 1227.
- [133] L.E. McCoubrey, M. Elbadawi, A.W. Basit, Current clinical translation of microbiome medicines, *Trends Pharmacol. Sci.* 43 (2022) 281–292.
- [134] L.E. McCoubrey, M. Elbadawi, M. Orlu, S. Gaisford, A.W. Basit, Harnessing machine learning for development of microbiome therapeutics, *Gut Microbes* 13 (2021) 1–20.
- [135] Q. Su, Q. Liu, R.I. Lau, J. Zhang, Z. Xu, Y.K. Yeoh, T.W.H. Leung, W. Tang, L. Zhang, J.Q.Y. Liang, Y.K. Yau, J. Zheng, C. Liu, M. Zhang, C.P. Cheung, J.Y. L. Ching, H.M. Tun, J. Yu, F.K.L. Chan, S.C. Ng, Faecal microbiome-based machine learning for multi-class disease diagnosis, *Nat. Commun.* 13 (2022) 6818.
- [136] L.E. McCoubrey, S. Gaisford, M. Orlu, A.W. Basit, Predicting drug–microbiome interactions with machine learning, *Biotechnol. Adv.* 54 (2021), 107797.
- [137] Z. Vinarov, M. Abdallah, J.A.G. Agundez, K. Allegaert, A.W. Basit, M. Braeckmans, J. Ceulemans, M. Corsetti, B.T. Griffin, M. Grimm, D. Keszthelyi, M. Kozioliek, C.M. Madia, C. Matthys, L.E. McCoubrey, A. Mitra, C. Reppas, J. Stappaerts, N. Steenackers, N.L. Trevasakis, T. Vanuytsel, M. Vertzoni, W. Weitschies, C. Wilson, P. Augustijns, Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: An UNGAP review, *Eur. J. Pharm. Sci.* 162 (2021), 105812.
- [138] L.E. McCoubrey, S. Thomaidou, M. Elbadawi, S. Gaisford, M. Orlu, A.W. Basit, Machine learning predicts drug metabolism and bioaccumulation by intestinal microbiota, *Pharmaceutics* 13 (2021).
- [139] M. Klünemann, S. Andrejev, S. Blasche, A. Mateus, P. Phapale, S. Devendran, J. Vappiani, B. Simon, T.A. Scott, E. Kafkia, D. Konstantinidis, K. Zirngibl, E. Mastroianni, M. Banzhaf, M.-T. Mackmull, F. Hövelmann, L. Nesme, A. R. Brochado, L. Maier, T. Bock, V. Periwal, M. Kumar, Y. Kim, M. Tramontano, C. Schultz, M. Beck, J. Hennig, M. Zimmermann, D.C. Sévin, F. Cabreiro, M. M. Savitski, P. Bork, A. Typas, K.R. Patil, Bioaccumulation of therapeutic drugs by human gut bacteria, *Nature* 597 (2021) 533–538.
- [140] L.E. McCoubrey, M. Elbadawi, M. Orlu, S. Gaisford, A.W. Basit, Machine learning uncovers adverse drug effects on intestinal bacteria, *Pharmaceutics* 13 (2021).
- [141] M. Mulder, D. Radjabzadeh, J.C. Kieft-de Jong, A.G. Uitterlinden, R. Kraaij, B. H. Stricker, A. Verbon, Long-term effects of antimicrobial drugs on the composition of the human gut microbiota, *Gut Microbes* 12 (2020) 1795492.
- [142] T. Hagan, M. Cortese, N. Roupahel, C. Boudreau, C. Linde, M.S. Maddur, J. Das, H. Wang, J. Guthmiller, N.Y. Zheng, M. Huang, A.A. Uphadhyay, L. Gardinassi, C. Petitdemange, M.P. McCullough, S.J. Johnson, K. Gill, B. Cervasi, J. Zou, A. Bretin, M. Hahn, A.T. Gewirtz, S.E. Bosinger, P.C. Wilson, S. Li, G. Alter, S. Khurana, H. Golding, B. Pulendran, Antibiotics-driven gut microbiome perturbation alters immunity to vaccines in humans, *Cell* 178 (2019) 1313–1328 e1313.
- [143] J. Baillargeon, H.M. Holmes, Y.-L. Lin, M.A. Raji, G. Sharma, Y.-F. Kuo, Concurrent use of warfarin and antibiotics and the risk of bleeding in older adults, *Am. J. Med.* 125 (2012) 183–189.
- [144] T. Hakozaki, C. Richard, A. Elkrif, Y. Hosomi, M. Benlaifaoui, I. Mimpfen, S. Terrisse, L. Derosa, L. Zitvogel, B. Routy, Y. Okuma, The gut microbiome associates with immune checkpoint inhibition outcomes in patients with advanced non-small cell lung cancer, *Cancer Immunol. Res.* 8 (2020) 1243.
- [145] L. Maier, M. Pruteanu, M. Kuhn, G. Zeller, A. Telzerow, E.E. Anderson, A. R. Brochado, K.C. Fernandez, H. Dose, H. Mori, K.R. Patil, P. Bork, A. Typas, Extensive impact of non-antibiotic drugs on human gut bacteria, *Nature* 555 (2018) 623–628.
- [146] J.A. Blakeley-Ruiz, C.S. McClintock, H.K. Shrestha, S. Poudel, Z.K. Yang, R. J. Giannone, J.J. Choo, M. Podar, H.A. Baghdoyan, R. Lydic, R.L. Hettich, Morphine and high-fat diet differentially alter the gut microbiota composition and metabolic function in lean versus obese mice, *ISME Commun.* 2 (2022).
- [147] C.N. Brocker, T. Velenosi, H.K. Flaten, G. McWilliams, K. McDaniel, S.K. Shelton, J. Saben, K.W. Krausz, F.J. Gonzalez, A.A. Monte, Metabolomic profiling of metoprolol hypertension treatment reveals altered gut microbiota-derived urinary metabolites, *Hum. Genom.* 14 (2020) 10.
- [148] K.F. Dekkers, S. Sayols-Baixeras, G. Baldanzi, C. Nowak, U. Hammar, D. Nguyen, G. Varotsis, L. Brunkwall, N. Nielsen, A.C. Eklund, J. Bak Holm, H.B. Nielsen, F. Ottosson, Y.T. Lin, S. Ahmad, L. Lind, J. Sundstrom, G. Engstrom, J.G. Smith, J. Arnlov, M. Orho-Melander, T. Fall, An online atlas of human plasma metabolite signatures of gut microbiome composition, *Nat. Commun.* 13 (2022) 5370.
- [149] Z.E. Ilhan, V. Brochard, N. Lapaque, S. Auvin, P. Lepage, Exposure to anti-seizure medications impact growth of gut bacterial species and subsequent host response, *Neurobiol. Dis.* 167 (2022), 105664.
- [150] A. Vich Vila, V. Collij, S. Sanna, T. Sinha, F. Imhann, A.R. Bourgonje, Z. Mujagic, D. Jonkers, A.A.M. Masclee, J. Fu, A. Kurilshikov, C. Wijmenga, A. Zernakova, R. K. Weersma, Impact of commonly used drugs on the composition and metabolic function of the gut microbiota, *Nat. Commun.* 11 (2020) 362.
- [151] H. Wu, E. Esteve, V. Tremaroli, M.T. Khan, R. Caesar, L. Mannerås-Holm, M. Ståhlman, L.M. Olsson, M. Serino, M. Planas-Félix, G. Xifra, J.M. Mercader, D. Torrents, R. Burcelin, W. Ricart, R. Perkins, J.M. Fernández-Real, F. Bäckhed, Metformin alters the gut microbiome of individuals with treatment-naïve type 2 diabetes, contributing to the therapeutic effects of the drug, *Nat. Med.* 23 (2017) 850–858.
- [152] R.R. Nayak, M. Alexander, I. Deshpande, K. Stapleton-Gray, B. Rimal, A. D. Patterson, C. Ubeda, J.U. Scher, P.J. Turnbaugh, Methotrexate impacts conserved pathways in diverse human gut bacteria leading to decreased host immune activation, *Cell Host Microbe* 29 (2021) 362–377 e311.
- [153] M.A. Kamm, W.J. Sandborn, M. Gassull, S. Schreiber, L. Jackowski, T. Butler, A. Lyne, D. Stephenson, M. Palmen, R.E. Joseph, Once-daily, high-concentration MMX mesalazine in active ulcerative colitis, *Gastroenterology* 132 (2007) 66–75.
- [154] B. Brar, K. Ranjan, A. Palria, R. Kumar, M. Ghosh, S. Sihag, P. Minakshi, Nanotechnology in colorectal cancer for precision diagnosis and therapy, *Front. Nanotechnol.* 3 (2021).
- [155] P. Chaubey, M. Momin, S. Sawarkar, Significance of ligand-anchored polymers for drug targeting in the treatment of colonic disorders, *Front. Pharmacol.* 10 (2020).
- [156] Y. Hou, J. Jin, H. Duan, C. Liu, L. Chen, W. Huang, Z. Gao, M. Jin, Targeted therapeutic effects of oral inulin-modified double-layered nanoparticles containing chemotherapeutics on orthotopic colon cancer, *Biomaterials* 283 (2022).
- [157] H.T. Hoang, S.H. Jo, Q.T. Phan, H. Park, S.H. Park, C.W. Oh, K.T. Lim, Dual pH-/thermo-responsive chitosan-based hydrogels prepared using “click” chemistry for colon-targeted drug delivery applications, *Carbohydr. Polym.* 260 (2021).
- [158] Ramboll Environ, Generally recognized as safe (GRAS) notification for basic methacrylate copolymer, Arlington, Virginia, United States, 2017.
- [159] A.L. Gregory, D.A. Pensinger, A.J. Hryckowian, A short chain fatty acid-centric view of *Clostridioides difficile* pathogenesis, *PLoS Pathog.* 17 (2021), e1009959.
- [160] V. Yadav, F. Varum, R. Bravo, E. Furrer, A.W. Basit, Gastrointestinal stability of therapeutic anti-TNF alpha IgG1 monoclonal antibodies, *Int. J. Pharm.* 502 (2016) 181–187.
- [161] R. Kneiszl, S. Hossain, P. Larsson, In silico-based experiments on mechanistic interactions between several intestinal permeation enhancers with a lipid bilayer model, *Mol. Pharm.* 19 (2022) 124–137.
- [162] C. Twarog, S. Fattah, J. Heade, S. Maher, E. Fattal, D.J. Brayden, Intestinal Permeation Enhancers for Oral Delivery of Macromolecules: A Comparison between Salcaprozate Sodium (SNAC) and Sodium Caprate (C10), *Pharmaceutics*, 2019.
- [163] C.C. Dodo, J. Wang, A.W. Basit, P. Stapleton, S. Gaisford, Targeted delivery of probiotics to enhance gastrointestinal stability and intestinal colonisation, *Int. J. Pharm.* 530 (2017) 224–229.
- [164] V.C. Ibekwe, M.K. Khela, D.F. Evans, A.W. Basit, A new concept in colonic drug targeting: a combined pH-responsive and bacterially-triggered drug delivery technology, *Aliment. Pharmacol. Ther.* 28 (2008) 911–916.
- [165] F. Varum, R. Bravo, A. Basit, OPTICORE™: a First-in-Class Colonic Targeting Technology, in: *ONdrugDelivery Magazine*, Frederick Furness Publishing Ltd, Lewes, United Kingdom, 2020, pp. 40–44.
- [166] T.T. Kararli, C.F. Kirchoff, J.E. Truelove, Ionic strength dependence of dissolution for Eudragit S-100 coated pellets, *Pharm. Res.* 12 (1995) 1813–1816.
- [167] F.J.O. Varum, F. Veiga, J.S. Sousa, A.W. Basit, Mucoadhesive platforms for targeted delivery to the colon, *Int. J. Pharm.* 420 (2011) 11–19.
- [168] F.J. Varum, G.B. Hatton, A.C. Freire, A.W. Basit, A novel coating concept for ileo-colonic drug targeting: proof of concept in humans using scintigraphy, *Eur. J. Pharm. Biopharm.* 84 (2013) 573–577.
- [169] G.R. D’Haens, W.J. Sandborn, G. Zou, L.W. Stitt, P.J. Rutgeerts, D. Gilgen, V. Jairath, P. Hindryckx, L.M. Shackelton, M.K. Vandervoort, C.E. Parker, C. Muller, R.K. Pai, O. Levchenko, Y. Marakhouksi, M. Horynski, E. Mikhailova, N. Kharchenko, S. Pimanov, B.G. Feagan, Randomised non-inferiority trial: 1600 mg versus 400 mg tablets of mesalazine for the treatment of mild-to-moderate ulcerative colitis, *Aliment. Pharmacol. Ther.* 46 (2017) 292–302.
- [170] D. Preisig, F. Varum, R. Bravo, C. Hartig, J. Speis, S. Abbes, F. Caobelli, D. Wild, M. Puchkov, J. Huwyler, M. Haschke, Colonic delivery of metronidazole-loaded capsules for local treatment of bacterial infections: a clinical pharmacoscintigraphy study, *Eur. J. Pharm. Biopharm.* 165 (2021) 22–30.
- [171] H. Shahdadi Sardou, A. Akhgari, A.H. Mohammadpour, A. Beheshti Namdar, H. Kamali, A.H. Jafarian, H. Afrasiabi Garekani, F. Sadeghi, Optimization study of combined enteric and time-dependent polymethacrylates as a coating for colon

- targeted delivery of 5-ASA pellets in rats with ulcerative colitis, *Eur. J. Pharm. Sci.* 168 (2022), 106072.
- [172] S.K. Vemula, P.R. Veerareddy, V.R. Devadasu, Pharmacokinetics of colon-specific pH and time-dependent flurbiprofen tablets, *Eur. J. Drug Metab. Pharmacokinet.* 40 (2015) 301–311.
- [173] L.A. Hodges, S.M. Connolly, J. Band, B. O'Mahony, T. Ugurlu, M. Turkoglu, C. G. Wilson, H.N.E. Stevens, Scintigraphic evaluation of colon targeting pectin-HPMC tablets in healthy volunteers, *Int. J. Pharm.* 370 (2009) 144–150.
- [174] F. Casati, A. Melocchi, S. Moutaharrik, M. Uboldi, A. Foppoli, A. Maroni, L. Zema, C. Neut, F. Siepman, J. Siepman, A. Gazzaniga, Injection molded capsules for colon delivery combining time-controlled and enzyme-triggered approaches, *Int. J. Mol. Sci.* 21 (2020) 1917.
- [175] S. Moutaharrik, A. Maroni, A. Melocchi, L. Zema, A. Foppoli, M. Cerea, L. Palugan, C. Neut, F. Siepman, J. Siepman, A. Gazzaniga, Oral colon delivery platform based on a novel combination approach: Design concept and preliminary evaluation, *J. Drug Deliv. Sci. Technol.* 66 (2021), 102919.
- [176] R.D. Cohen, D.M. Woseth, R.A. Thisted, S.B. Hanauer, A meta-analysis and overview of the literature on treatment options for left-sided ulcerative colitis and ulcerative proctitis, *Am. J. Gastroenterol.* 95 (2000) 1263–1276.
- [177] G.P. Christophi, A. Renegarajan, M.A. Giorba, Rectal budesonide and mesalamine formulations in active ulcerative proctosigmoiditis: efficacy, tolerance, and treatment approach, *Clin. Exp. Gastroenterol.* 9 (2016) 125–130.
- [178] C. Mulder, G. Tytgat, Topical corticosteroids in inflammatory bowel disease, *Aliment. Pharmacol. Ther.* 7 (1993) 125–130.
- [179] W. Kruijs, V. Neshita, M. Pesegova, A. Alekseeva, P. Andreev, O. Datsenko, O. Levchenko, S. Abdulkhakov, Y. Lozynskyy, Y. Mostovoy, Budesonide suppositories are effective and safe for treating acute ulcerative proctitis, *Clin. Gastroenterol. Hepatol.* 17 (2019) 98–106, e104.
- [180] M. Naganuma, N. Aoyama, T. Tada, K. Kobayashi, F. Hirai, K. Watanabe, M. Watanabe, T. Hibi, Complete mucosal healing of distal lesions induced by twice-daily budesonide 2-mg foam promoted clinical remission of mild-to-moderate ulcerative colitis with distal active inflammation: double-blind, randomized study, *J. Gastroenterol.* 53 (2018) 494–506.
- [181] P. Xiao, S. Gumber, M.A. Marzinke, A.A. Date, T. Hoang, J. Hanes, L.M. Ensign, L. Wang, L. Rohan, E.J. Fuchs, C. Hendrix, F. Villinger, Hypo-osmolar formulation of tenofovir (TFV) enema promotes uptake and metabolism of TFV in tissues, leading to prevention of SHIV/SIV infection, *Antimicrob. Agents Chemother.* 62 (2018).
- [182] T. Hoang, A.A. Date, J.O. Ortiz, T.W. Young, S. Bensouda, P. Xiao, M. Marzinke, L. Rohan, E.J. Fuchs, C. Hendrix, S. Gumber, F. Villinger, R.A. Cone, J. Hanes, L. M. Ensign, Development of rectal enema as microbicide (DREAM): preclinical progressive selection of a tenofovir prodrug enema, *Eur. J. Pharm. Biopharm.* 138 (2019) 23–29.
- [183] K. Maisel, S. Chattopadhyay, T. Moench, C. Hendrix, R. Cone, L.M. Ensign, J. Hanes, Enema ion compositions for enhancing colorectal drug delivery, *J. Control. Release* 209 (2015) 280–287.
- [184] M. Bialik, M. Kuras, M. Sobczak, E. Oledzka, Achievements in thermosensitive gelling systems for rectal administration, *Int. J. Mol. Sci.* 22 (2021).
- [185] C.-K. Kim, S.-W. Lee, H.-G. Choi, M.-K. Lee, Z.-G. Gao, I.-S. Kim, K.-M. Park, Trials of in situ-gelling and mucoadhesive acetaminophen liquid suppository in human subjects, *Int. J. Pharm.* 174 (1998) 201–207.
- [186] A.M. Abd El Razek, A.A. Hasan, S.A. Sabry, M.A. Mahdy, E.E. Hamed, Metoclopramide hydrochloride thermally sensitive rectal in situ gelling system, a novel out-patient treatment for vomiting in pediatric age, *J. Drug Deliv. Sci. Technol.* 50 (2019) 9–17.
- [187] M.M. El-Sonbaty, H.R. Ismail, A.A. Kassem, A.M. Samy, M.A. Akl, Mucoadhesive thermoreversible formulation of metoclopramide for rectal administration: a promising strategy for potential management of chemotherapy-induced nausea and vomiting, *Pharm. Dev. Technol.* 25 (2020) 535–546.
- [188] U.S. Food and Drug Administration, The dissolution procedure: development and validation, FDA, Rockville, Maryland, United States, 2021.
- [189] H. Batchelor, J. Butler, *Dissolution*, Biopharmaceutics, 2022, pp. 73–98.
- [190] K. Stamatopoulos, H.K. Batchelor, M.J.H. Simmons, Dissolution profile of theophylline modified release tablets, using a biorelevant Dynamic Colon Model (DCM), *Eur. J. Pharm. Biopharm.* 108 (2016) 9–17.
- [191] M. Wahlgren, M. Axenstrand, Å. Håkansson, A. Marefati, B. Lomstein Pedersen, In Vitro Methods to Study Colon Release: State of the Art and An Outlook on New Strategies for Better In-Vitro Biorelevant Release Media, *Pharmaceutics*, 2019, p. 11.
- [192] H.M. Fadda, A.W. Basit, Dissolution of pH responsive formulations in media resembling intestinal fluids: bicarbonate versus phosphate buffers, *J. Drug Deliv. Sci. Technol.* 15 (2005) 273–279.
- [193] H.M. Fadda, H.A. Merchant, B.T. Arafat, A.W. Basit, Physiological bicarbonate buffers: stabilisation and use as dissolution media for modified release systems, *Int. J. Pharm.* 382 (2009) 56–60.
- [194] A. Goyanes, G.B. Hatton, H.A. Merchant, A.W. Basit, Gastrointestinal release behaviour of modified-release drug products: dynamic dissolution testing of mesalazine formulations, *Int. J. Pharm.* 484 (2015) 103–108.
- [195] H. Wray, R. Joseph, M. Palmen, D. Pierce, Combined pharmacokinetic and scintigraphic analyses for the comparison of 5-ASA release profiles from MMXT<sup>™</sup> mesalazine and another delayed-release mesalazine formulation: P-0030, *Inflamm. Bowel Dis.* 14 (2008) S19–S20.
- [196] M.A. García, F. Varum, J. Al-Gousous, M. Hofmann, S. Page, P. Langguth, In vitro methodologies for evaluating colon-targeted pharmaceutical products and industry perspectives for their applications, *Pharmaceutics* 14 (2022).
- [197] S. Ikuta, H. Nakagawa, T. Kai, K. Sugano, Development of bicarbonate buffer flow-through cell dissolution test and its application in prediction of in vivo performance of colon targeting tablets, *Eur. J. Pharm. Sci.* 180 (2023).
- [198] C. Reppas, M. Vertzoni, Biorelevant in-vitro performance testing of orally administered dosage forms, *J. Pharm. Pharmacol.* 64 (2012) 919–930.
- [199] M. Vertzoni, A. Diakidou, M. Chatziliadis, E. Soderlind, B. Abrahamsson, J. B. Dressman, C. Reppas, Biorelevant media to simulate fluids in the ascending colon of humans and their usefulness in predicting intracolonic drug solubility, *Pharm. Res.* 27 (2010) 2187–2196.
- [200] V. Yadav, Y. Mai, L.E. McCoubrey, Y. Wada, M. Tomioka, S. Kawata, S. Charde, A. W. Basit, 5-Aminolevulinic acid as a novel therapeutic for inflammatory bowel disease, *Biomedicines* 9 (2021).
- [201] A. Froidurot, V. Julliard, Cellulolytic bacteria in the large intestine of mammals, *Gut Microbes* 14 (2022) 2031694.
- [202] B. Javdan, J.G. Lopez, P. Chankhamjon, Y.J. Lee, R. Hull, Q. Wu, X. Wang, S. Chatterjee, M.S. Donia, Personalized mapping of drug metabolism by the human gut microbiome, *Cell* 181 (2020) 1661–1679 e1622.
- [203] S.P. van Kessel, A.K. Frye, A.O. El-Gendy, M. Castejon, A. Keshavarzian, G. van Dijk, S. El Aidy, Gut bacterial tyrosine decarboxylases restrict levels of levodopa in the treatment of Parkinson's disease, *Nat. Commun.* 10 (2019) 310.
- [204] M. Zimmermann, M. Zimmermann-Kogadeeva, R. Wegman, A.L. Goodman, Mapping human microbiome drug metabolism by gut bacteria and their genes, *Nature* 570 (2019) 462–467.
- [205] Z. Coombes, V. Yadav, L.E. McCoubrey, C. Freire, A.W. Basit, R.S. Conlan, D. Gonzalez, Progestogens are metabolized by the gut microbiota: implications for colonic drug delivery, *Pharmaceutics* 12 (2020) 760.
- [206] E. Karatza, M. Vertzoni, U. Muenster, C. Reppas, The impact of handling and storage of human fecal material on bacterial activity, *J. Pharm. Sci.* 105 (2016) 3458–3461.
- [207] C. Rose, A. Parker, B. Jefferson, E. Cartmell, The characterization of feces and urine: a review of the literature to inform advanced treatment technology, *Crit. Rev. Environ. Sci. Technol.* 45 (2015) 1827–1879.
- [208] P. Day-Walsh, E. Shehata, S. Saha, G.M. Savva, B. Nemeckova, J. Speranza, L. Kellingray, A. Narbad, P.A. Kroon, The use of an in-vitro batch fermentation (human colon) model for investigating mechanisms of TMA production from choline, L-carnitine and related precursors by the human gut microbiota, *Eur. J. Nutr.* 60 (2021) 3987–3999.
- [209] Z.E. Ilhan, A.K. Marcus, D.-W. Kang, B.E. Rittmann, R. Krajmalnik-Brown, G. Suen, pH-Mediated microbial and metabolic interactions in fecal enrichment cultures, *mSphere* 2 (2017) e00047-00017.
- [210] D. Fera-Gervasio, W. Tottey, N. Gaci, M. Alric, J.M. Cardot, P. Peyret, J.F. Martin, E. Pujos, J.L. Sébédio, J.F. Brugère, Three-stage continuous culture system with a self-generated anaerobiosis to study the regionalized metabolism of the human gut microbiota, *J. Microbiol. Methods* 96 (2014) 111–118.
- [211] K. Venema, P. van den Abbeele, Experimental models of the gut microbiome, *Best Pract. Res. Clin. Gastroenterol.* 27 (2013) 115–126.
- [212] T. Van de Wiele, P. Van den Abbeele, W. Ossieur, S. Possemiers, M. Marzorati, The simulator of the human intestinal microbial ecosystem (SHIME®), in: K. Verhoeckx, P. Cotter, I. López-Expósito, C. Kleiveland, T. Lea, A. Mackie, T. Requena, D. Swiatecka, H. Wichers (Eds.), *The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models*, Springer International Publishing, Cham, 2015, pp. 305–317.
- [213] J. Ghyselincx, L. Verstrepen, F. Moens, P. Van Den Abbeele, A. Bruggeman, J. Said, B. Smith, L.A. Barker, C. Jordan, V. Leta, K.R. Chaudhuri, A.W. Basit, S. Gaisford, Influence of probiotic bacteria on gut microbiota composition and gut wall function in an in-vitro model in patients with Parkinson's disease, *Int. J. Pharm.* X 3 (2021).
- [214] C. Tannergren, A. Borde, C. Borestrom, B. Abrahamsson, A. Lindahl, Evaluation of an in vitro faecal degradation method for early assessment of the impact of colonic degradation on colonic absorption in humans, *Eur. J. Pharm. Sci.* 57 (2014) 200–206.
- [215] C. Wu, T. Chen, W. Xu, T. Zhang, Y. Pei, Y. Yang, F. Zhang, H. Guo, Q. Wang, L. Wang, B. Zhao, The maintenance of microbial community in human fecal samples by a cost effective preservation buffer, *Sci. Rep.* 11 (2021).
- [216] J. Jones, S.N. Reinke, A. Ali, D.J. Palmer, C.T. Christophersen, Fecal sample collection methods and time of day impact microbiome composition and short chain fatty acid concentrations, *Sci. Rep.* 11 (2021) 13964.
- [217] J. Ghyselincx, L. Verstrepen, F. Moens, P. Van den Abbeele, J. Said, B. Smith, I. Bjarnason, A.W. Basit, S. Gaisford, A 4-strain probiotic supplement influences gut microbiota composition and gut wall function in patients with ulcerative colitis, *Int. J. Pharm.* 587 (2020), 119648.
- [218] G. Hawksworth, B.S. Drasar, M.J. Hill, Intestinal bacteria and the hydrolysis of glycosidic bonds, *J. Med. Microbiol.* 4 (1971) 451–459.
- [219] T.J. Purohit, S.M. Hanning, Z. Wu, Advances in rectal drug delivery systems, *Pharm. Dev. Technol.* 23 (2018) 942–952.
- [220] FDA, FDA-Recommended Dissolution Methods Database, CDER, Rockville, MD, 2018.
- [221] S. Klein, Rectal dosage forms, in: *In Vitro Drug Release Testing of Special Dosage Forms*, 2019, pp. 211–233.

- [222] A. Palmieri, Suppository dissolution testing: apparatus design and release of aspirin, *Drug Dev. Ind. Pharm.* 7 (1981) 247–259.
- [223] A. Schoonen, F. Moolenaar, C. Haverschmidt, T. Huizinga, The interphase transport of drugs from fatty suppository bases, *Pharm. Weekblad* 111 (1976) 585–589.
- [224] S. Janicki, M. Sznitowska, W. Zebrowska, H. Gabiga, M. Kupiec, Evaluation of paracetamol suppositories by a pharmacopoeial dissolution test – comments on methodology, *Eur. J. Pharm. Biopharm.* 52 (2001) 249–254.
- [225] I. Hubatsch, E.G.E. Ragnarsson, P. Artursson, Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers, *Nat. Protoc.* 2 (2007) 2111–2119.
- [226] M. Vancamelbeke, T. Laeremans, W. Vanhove, K. Arnauts, A.S. Ramalho, R. Farré, I. Cleynen, M. Ferrante, S. Vermeire, Butyrate does not protect against inflammation-induced loss of epithelial barrier function and cytokine production in primary cell monolayers from patients with ulcerative colitis, *J. Crohn's Colitis* 13 (2019) 1351–1361.
- [227] Å. Sjöberg, M. Lutz, C. Tannergren, C. Wingolf, A. Borde, A.-L. Ungell, Comprehensive study on regional human intestinal permeability and prediction of fraction absorbed of drugs using the Ussing chamber technique, *Eur. J. Pharm. Sci.* 48 (2013) 166–180.
- [228] I. Dotti, R. Mora-Buch, E. Ferrer-Picón, N. Planell, P. Jung, M.C. Masamunt, R. F. Leal, J. Martín de Carpi, J. Llach, I. Ordás, E. Batlle, J. Panés, A. Salas, Alterations in the epithelial stem cell compartment could contribute to permanent changes in the mucosa of patients with ulcerative colitis, *Gut* 66 (2017) 2069.
- [229] L.R. Madden, T.V. Nguyen, S. Garcia-Mojica, V. Shah, A.V. Le, A. Peier, R. Visconti, E.M. Parker, S.C. Presnell, D.G. Nguyen, K.N. Retting, Bioprinted 3D primary human intestinal tissues model aspects of native physiology and ADME/Tox functions, *iScience* 2 (2018) 156–167.
- [230] H. Graham, P. Åman, The pig as a model in dietary fibre digestion studies, *Scand. J. Gastroenterol.* 22 (1987) 55–61.
- [231] G.B. Hattton, V. Yadav, A.W. Basit, H.A. Merchant, Animal farm: considerations in animal gastrointestinal physiology and relevance to drug delivery in humans, *J. Pharm. Sci.* 104 (2015) 2747–2776.
- [232] R.N. Hughes, in: R.N. Hughes (Ed.), *Diet selection: an interdisciplinary approach to foraging behaviour*, Blackwell Scientific Publications, Oxford, 1993.
- [233] E. Nascimento-Gonçalves, B.A.L. Mendes, R. Silva-Reis, A.I. Faustino-Rocha, A. Gama, P.A. Oliveira, Animal models of colorectal cancer: from spontaneous to genetically engineered models and their applications, *Vet. Sci.* 8 (2021) 59.
- [234] S.C. Sutton, Companion animal physiology and dosage form performance, *Adv. Drug Deliv. Rev.* 56 (2004) 1383–1398.
- [235] M. Koziolok, M. Grimm, T. Bollmann, K.J. Schafer, S.M. Blattner, R. Lotz, G. Boeck, W. Weitschies, Characterization of the GI transit conditions in Beagle dogs with a telemetric motility capsule, *Eur. J. Pharm. Biopharm.* 136 (2019) 221–230.
- [236] F. Musial, M.D. Crowell, A.W. French, N. Guiv, Effect of prolonged, continuous rectal distention on mouth-to-cecum and colonic transit time in pigs, *Physiol. Behav.* 52 (1992) 1021–1024.
- [237] C. Suenderhauf, N. Parrott, A physiologically based pharmacokinetic model of the minipig: data compilation and model implementation, *Pharm. Res.* 30 (2013) 1–15.
- [238] E.L. McConnell, A.W. Basit, S. Murdan, Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments, *J. Pharm. Pharmacol.* 60 (2008) 63–70.
- [239] H.A. Merchant, S.C. Rabbie, F.J.O. Varum, F. Afonso-Pereira, A.W. Basit, Influence of ageing on the gastrointestinal environment of the rat and its implications for drug delivery, *Eur. J. Pharm. Sci.* 62 (2014) 76–85.
- [240] C. Stillhart, K. Vučićević, P. Augstjins, A.W. Basit, H. Batchelor, T.R. Flanagan, I. Gesquiere, R. Greupink, D. Keszthelyi, M. Koskinen, C.M. Madla, C. Matthys, G. Miljus, M.G. Mooij, N. Parrott, A.-L. Ungell, S.N. de Wildt, M. Orlu, S. Klein, A. Müllertz, Impact of gastrointestinal physiology on drug absorption in special populations—An UNGAP review, *Eur. J. Pharm. Sci.* 147 (2020), 105280.
- [241] H.A. Merchant, F. Liu, M. Orlu Gul, A.W. Basit, Age-mediated changes in the gastrointestinal tract, *Int. J. Pharm.* 512 (2016) 382–395.
- [242] H.A. Merchant, E.L. McConnell, F. Liu, C. Ramaswamy, R.P. Kulkarni, A.W. Basit, S. Murdan, Assessment of gastrointestinal pH, fluid and lymphoid tissue in the guinea pig, rabbit and pig, and implications for their use in drug development, *Eur. J. Pharm. Sci.* 42 (2011) 3–10.
- [243] J.E. Dalziel, W. Young, P. Bercik, N.J. Spencer, L.J. Ryan, K.E. Dunstan, C. M. Lloyd-West, P.K. Gopal, N.W. Haggarty, N.C. Roy, Tracking gastrointestinal transit of solids in aged rats as pharmacological models of chronic dysmotility, *Neurogastroenterol. Motil.* 28 (2016) 1241–1251.
- [244] L.J. Henze, N.J. Koehl, H. Bennett-Lenane, R. Holm, M. Grimm, F. Schneider, W. Weitschies, M. Koziolok, B.T. Griffin, Characterization of gastrointestinal transit and luminal conditions in pigs using a telemetric motility capsule, *Eur. J. Pharm. Sci.* 156 (2021), 105627.
- [245] C.S. Boillat, F.P. Gaschen, G.L. Hosgood, Assessment of the relationship between body weight and gastrointestinal transit times measured by use of a wireless motility capsule system in dogs, *Am. J. Vet. Res.* 71 (2010) 898–902.
- [246] C.H. Wong, K.W. Siah, A.W. Lo, Estimation of clinical trial success rates and related parameters, *Biostatistics* 20 (2019) 273–286.
- [247] M. Elbadawi, L.E. McCoubrey, F.K.H. Gavins, J.J. Ong, A. Goyanes, S. Gaisford, A. W. Basit, Harnessing artificial intelligence for the next generation of 3D printed medicines, *Adv. Drug Deliv. Rev.* 175 (2021), 113805.
- [248] N. Shah, O.P. Sharma, T. Mehta, A. Amin, Design of experiment approach for formulating multi-unit colon-targeted drug delivery system: in vitro and in vivo studies, *Drug Dev. Ind. Pharm.* 42 (2016) 825–835.
- [249] D. Hales, L.R. Tefas, I. Tomuță, C. Moldovan, D. Gulei, R. Munteanu, A. Porfire, Development of a curcumin-loaded polymeric microparticulate oral drug delivery system for colon targeting by quality-by-design approach, *Pharmaceutics* 12 (2020).
- [250] R.E. Baker, J.-M. Peña, J. Jayamohan, A. Jérusalem, Mechanistic models versus machine learning, a fight worth fighting for the biological community? *Biol. Lett.* 14 (2018) 20170660.
- [251] Y. Gu, M. Li, Chapter 3 - Molecular modeling, in: H. Ishida, T. Agag (Eds.), *Handbook of Benzoxazine Resins*, Elsevier, Amsterdam, 2011, pp. 103–110.
- [252] T. Casalini, Not only in silico drug discovery: molecular modeling towards in silico drug delivery formulations, *J. Control. Release* 332 (2021) 390–417.
- [253] I. Bera, P.V. Payghan, Use of molecular dynamics simulations in structure-based drug discovery, *Curr. Pharm. Des.* 25 (2019) 3339–3349.
- [254] Z. Dvorak, F. Kopp, C.M. Costello, J.S. Kemp, H. Li, A. Vrzalova, M. Stepankova, I. Bartonkova, E. Jiskrova, K. Poulikova, B. Vyhidalova, L.U. Nordstrom, C. V. Karunaratne, H.S. Ranhotra, K.S. Mun, A.P. Naren, I.A. Murray, G.H. Perdeu, J. Brtko, L. Toporova, A. Schon, B.D. Wallace, W.G. Walton, M.R. Redinbo, K. Sun, A. Beck, S. Kortagere, M.C. Neary, A. Chandran, S. Vishveshwara, M. M. Cavalluzzi, G. Lentini, J.Y. Cui, H. Gu, J.C. March, S. Chatterjee, A. Matson, D. Wright, K.L. Flannigan, S.A. Hirota, R.B. Sartor, S. Mani, Targeting the pregnane X receptor using microbial metabolite mimicry, *EMBO Mol. Med.* 12 (2020), e11621.
- [255] X.-G. Tian, J.-K. Yan, C.-P. Sun, J.-X. Li, J. Ning, C. Wang, X.-K. Huo, W.-Y. Zhao, Z.-L. Yu, L. Feng, X. Lv, X.-C. Ma, Amentoflavone from *Selaginella tamariscina* as a potent inhibitor of gut bacterial  $\beta$ -glucuronidase: Inhibition kinetics and molecular dynamics simulation, *Chem. Biol. Interact.* 340 (2021), 109453.
- [256] R. Salekeen, M.H.B. Siam, D.I. Sharif, M.S. Lustgarten, M.M. Billah, K.M.D. Islam, In silico insights into potential gut microbial modulation of NAD<sup>+</sup> metabolism and longevity, *J. Biochem. Mol. Toxicol.* 35 (2021).
- [257] I.D. Styliari, V. Taresco, A. Theophilus, C. Alexander, M. Garnett, C. Laughton, Nanoformulation-by-design: an experimental and molecular dynamics study for polymer coated drug nanoparticles, *RSC Adv.* 10 (2020) 19521–19533.
- [258] N. Salah, L. Dubuquoy, R. Carpentier, D. Betbeder, Starch nanoparticles improve curcumin-induced production of anti-inflammatory cytokines in intestinal epithelial cells, *Int. J. Pharm.* X 4 (2022).
- [259] Committee for Medicinal Products for Human Use, Guideline on the reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation, European Medicines Agency, London, UK, 2018.
- [260] S. Basu, Y.T. Lien, V. Vozmediano, J.-F. Schlender, T. Eissing, S. Schmidt, C. Niederalt, Physiologically based pharmacokinetic modeling of monoclonal antibodies in pediatric populations using PK-Sim, *Front. Pharmacol.* 11 (2020).
- [261] E. Eckernas, C. Tannergren, Physiologically based biopharmaceutics modeling of regional and colon absorption in dogs, *Mol. Pharm.* 18 (2021) 1699–1710.
- [262] S.J. Trenfield, A. Awad, L.E. McCoubrey, M. Elbadawi, A. Goyanes, S. Gaisford, A. W. Basit, Advancing pharmacy and healthcare with virtual digital technologies, *Adv. Drug Deliv. Rev.* 182 (2022), 114098.
- [263] S. Harrer, P. Shah, B. Antony, J. Hu, Artificial intelligence for clinical trial design, *Trends Pharmacol. Sci.* 40 (2019) 577–591.
- [264] D.M. Camacho, K.M. Collins, R.K. Powers, J.C. Costello, J.J. Collins, Next-generation machine learning for biological networks, *Cell* 173 (2018) 1581–1592.
- [265] S. Badillo, B. Banfai, F. Birzele, L. Davydov II, T. Hutchinson, J. Kam-Thong, B. Siebourg-Polster, J.D. Zhang Steiert, An introduction to machine learning, *Clin. Pharmacol. Ther.* 107 (2020) 871–885.
- [266] N. Liu, C.B. Chen, S. Kumara, Semi-supervised learning algorithm for identifying high-priority drug-drug interactions through adverse event reports, *IEEE J. Biomed. Health Inform.* 24 (2020) 57–68.
- [267] C.-T. Chen, G.X. Gu, Generative deep neural networks for inverse materials design using backpropagation and active learning, *Adv. Sci.* 7 (2020) 1902607.
- [268] L.E. McCoubrey, N. Seegobin, M. Elbadawi, Y. Hu, M. Orlu, S. Gaisford, A. W. Basit, Active machine learning for formulation of precision probiotics, *Int. J. Pharm.* 121568 (2022).
- [269] P. Bannigan, M. Aldeghi, Z. Bao, F. Hase, A. Aspuru-Guzik, C. Allen, Machine learning directed drug formulation development, *Adv. Drug Deliv. Rev.* 175 (2021), 113806.
- [270] J.J. Ong, B.M. Castro, S. Gaisford, P. Cabalar, A.W. Basit, G. Pérez, A. Goyanes, Accelerating 3D printing of pharmaceutical products using machine learning, *Int. J. Pharm.* X 4 (2022), 100120.
- [271] B.M. Castro, M. Elbadawi, J.J. Ong, T. Pollard, Z. Song, S. Gaisford, G. Perez, A. W. Basit, P. Cabalar, A. Goyanes, Machine learning applied to over 900 3D printed drug delivery systems, *J. Control. Release.* 337 (2021) 530–545.
- [272] Y. Li, M.R. Abbaspour, P.V. Grootendorst, A.M. Rauth, X.Y. Wu, Optimization of controlled release nanoparticle formulation of verapamil hydrochloride using artificial neural networks with genetic algorithm and response surface methodology, *Eur. J. Pharm. Biopharm.* 94 (2015) 170–179.

- [273] K. Batra, K.M. Zorn, D.H. Foil, E. Minerali, V.O. Gawriljuk, T.R. Lane, S. Ekins, Quantum machine learning algorithms for drug discovery applications, *J. Chem. Inf. Model.* 61 (2021) 2641–2647.
- [274] S.S. Gill, A. Kumar, H. Singh, M. Singh, K. Kaur, M. Usman, R. Buyya, Quantum computing: a taxonomy, systematic review and future directions, *Softw. Pract. Exp.* 52 (2022) 66–114.
- [275] J. Zhang, S.D. Petersen, T. Radivojevic, A. Ramirez, A. Pérez-Manríquez, E. Abeliuk, B.J. Sánchez, Z. Costello, Y. Chen, M.J. Fero, H.G. Martin, J. Nielsen, J.D. Keasling, M.K. Jensen, Combining mechanistic and machine learning models for predictive engineering and optimization of tryptophan metabolism, *Nat. Commun.* 11 (2020).
- [276] D. Reker, Y. Rybakova, A.R. Kirtane, R. Cao, J.W. Yang, N. Navamajiti, A. Gardner, R.M. Zhang, T. Esfandiary, J. L'Heureux, T. von Erlach, E. M. Smekalova, D. Leboeuf, K. Hess, A. Lopes, J. Rogner, J. Collins, S.M. Tamang, K. Ishida, P. Chamberlain, D. Yun, A. Lytton-Jean, C.K. Soule, J.H. Cheah, A. M. Hayward, R. Langer, G. Traverso, Computationally guided high-throughput design of self-assembling drug nanoparticles, *Nat. Nanotechnol.* 16 (2021) 725–733.