The colon targeting efficacies of mesalazine medications and their impacts on the gut microbiome

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ABSTRACT

Successful treatment of ulcerative colitis (UC) is highly dependent on several parameters, including dosing regimen and the ability to deliver drugs to the disease site. In this study two strategies for delivering mesalazine (5-aminosalicylic acid, 5-ASA) to the colon were compared in an advanced in vitro model of the human gastrointestinal (GI) tract, the SHIME® system. Herein, a prodrug strategy employing bacteria-mediated drug release (sulfasalazine, Azulfidine®) was evaluated alongside a formulation strategy that utilised pH and bacteria-mediated release (5-ASA, Octasa® 1600 mg). SHIME® experiments were performed simulating both the GI physiology and colonic microbiota under healthy and inflammatory bowel disease (IBD) conditions, to study the impact of the disease state and ileal pH variability on colonic 5-ASA delivery. In addition, the effects of the products on the colonic microbiome were investigated by monitoring bacterial growth and metabolites. Results demonstrated that both the prodrug and formulation approaches resulted in a similar percentage of 5-ASA recovery under healthy conditions. On the contrary, during experiments simulating the GI physiology and microbiome of IBD patients (the target population) the formulation strategy resulted in a higher proportion of 5-ASA delivery to the colonic region as compared to the prodrug approach ($P < 0.0001$). Interestingly, the two products had distinct effects on the synthesis of key bacterial metabolites, such as lactate and short chain fatty acids, which varied according to disease state and ileal pH variability. Further, both 5-ASA and sulfasalazine significantly reduced the growth of the faecal microbiota sourced from six healthy humans. The findings support that the approach selected for colonic drug delivery could significantly influence the effectiveness of UC treatment, and highlight that drugs licensed for UC may differentially impact the growth and functioning of the colonic microbiota.

1. Introduction

Ulcerative colitis (UC) is a chronic condition that causes inflammation and ulceration within the colon and rectum. As a type of inflammatory bowel disease (IBD), the prevalence of UC is increasing, particularly in high income countries. A systematic analysis reported an 85.1% increase in global IBD from 1990 to 2017, with >6.8 million cases recorded worldwide in 2017 [1]. The symptoms of UC include abdominal pain, bowel urgency, fatigue, rectal bleeding, and diarrhoea, which commonly lead to a reduction in patients' quality of life [2]. Acute flares of UC are first treated with a topical aminosalicylate via suppositories, enemas, or rectal foams [3]. If topical aminosalicylates do not achieve

remission within four weeks, then treatment with an oral aminosalicylate should be considered. Both rectal and oral aminosalicylates, used alone or in combination, are recommended as first line therapies for maintenance of UC remission [3].

Aminosalicylates exert their anti-inflammatory actions locally by activating peroxisome proliferator-activated γ receptors on colonocytes and preventing the recruitment of leucocytes to the intestinal epithelium [4,5]. Mesalazine (5-aminosalicylic acid, 5-ASA) is the active molecule, and can be delivered in its unconjugated form or within an azo-bonded prodrug, e.g., sulfasalazine, balsalazide, or olsalazine, which liberate 5-ASA upon metabolism by colonic microbiota [6]. The azo-bonded prodrugs are commonly formulated within standard release oral dosage

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forms, as they are not absorbed within the upper gastrointestinal (GI) tract. For sulfasalazine, its poor small intestinal absorption is thought to be due to significant efflux by membrane MRP2 and BCRP transporters [7]. Following transit through the small intestine, the azo-bonded prodrugs effectively deliver 5-ASA to the colon upon bacterial cleavage of their azo bond. However, 5-ASA liberation may be affected by inter-individual variability in microbial metabolism and, in the case of sulfasalazine, can lead to systemic side effects due to colonic absorption of the cleaved sulfapyridine moiety [4,6]. In comparison, 5-ASA cannot be delivered within an immediate release formulation as it is readily absorbed in the proximal jejunum, leading to insufficient drug concentrations in the colon [8]. As such, 5-ASA must be formulated for targeted colonic release.

Site-specific colonic release of 5-ASA can be achieved by coating oral dosage forms with materials that resist degradation in the upper GI tract and selectively release their cargo upon entering the colon [9,10]. Most formulations on the market rely on pH-sensitive materials, such as the methacrylate-based Eudragit® L and S copolymers, to delay 5-ASA release. Such products include Asacol® 400 and 800, Lialda®/Mesavant®, Salofalk®, and Mesasal®, and depend on the pH of the GI tract reaching at least 6.0 (Eudragit® L) or 7.0 (Eudragit® S). Once the critical pH is reached dissolution of the enteric polymer is triggered, facilitating drug release in the lower GI tract.

Despite their prominence on the market, there have been numerous reports of pH-mediated coatings releasing drugs prematurely and even failing to disintegrate due to pH variability within patients' GI tracts [11–14]. Clearly, the failure of targeted dosage forms to successfully release 5-ASA close to, or within, the colon could significantly impair the effective treatment of UC. In response to these findings, two novel colon-targeting technologies have been launched onto to the market in recent years. The first, Phloral®, utilises a coating composed of blended Eudragit® S and resistant starch that acts as a dual triggered enabler of colonic drug delivery; the Eudragit® S is designed to dissolve at pH > 7.0 within the ileocolonic region, but should pH not reach 7.0, then the colonic microbiota will metabolise the resistant starch and still achieve local drug release [9,15–17]. The second technology, OPTICORE®, employs an inner alkaline layer underneath a Phloral® coating. This inner alkaline layer creates a microenvironment of elevated pH and buffer capacity when the Phloral® coating begins to disintegrate upon contact with colonic fluid. This leads to rapid ionisation of the Eudragit® S within the Phloral® coating, thus expediting the rate of drug release [18]. The OPTICORE® technology is utilised in Octasa® 1600 mg (Asacol 1600 mg), a colon targeted tablet that delivers a high dose of 5-ASA for induction and maintenance of remission in UC [19].

In this study two strategies utilised for 5-ASA delivery to the colon were compared in a realistic in vitro model of the human GI tract based on the established SHIME® technology [20,21]. The semi-dynamic SHIME® system was used since it simultaneously simulates both the physiological (pH, media composition, digestive enzymes, and transit time) and microbiota-dependent factors (colonic microbiota) that can have a significant impact on the colon-targeting performance of pH-, time-, and colonic microbiota-dependent drug delivery systems during passage through the complete GI tract. The semi-dynamic SHIME® was operated under conditions simulating both the GI physiology and colonic microbiota of healthy individuals and IBD patients to generate mechanistic insights into how the disease state itself could impact the GI performance of colon-targeting delivery strategies. The SHIME® technology has previously been utilised to simulate both healthy and diseased states, though this is the first published example of the semi-dynamic SHIME® system being employed to model GI physiology in the IBD state [21–24].

The azo-bonded prodrug approach was directly compared to the formulation approach by measuring the amount of 5-ASA reaching the colon following administration of Azulfidine® (an enteric coated tablet designed to release sulfasalazine in the small intestine) and Octasa® 1600 mg (the OPTICORE® coated tablet containing 5-ASA). The

Azulfidine® and Octasa® 1600 mg products were selected for investigation as Azulfidine® represents the traditional and common approach of delivering 5-ASA to the colon via the sulfasalazine prodrug, and Octasa® 1600 mg represents the only marketed product that utilises a dual-trigger formulation strategy for colonic drug delivery. As this marks the first time that these prodrug and formulation colonic delivery strategies have been compared, it was unknown which technique would be most efficient: the enzymatic conversion of solubilised sulfasalazine to 5-ASA, or the digestion/dissolution of the OPTICORE® coating. As both mechanisms employ bacteria-mediated 5-ASA release, it was also unknown how variability in microbiome composition would affect colonic delivery. Hence, the main goals of this study were to ascertain: 1.) the relative colon-targeting efficacies of the two colonic drug delivery strategies in the healthy and IBD states; 2.) the colon-targeting efficacy of Octasa® 1600 mg when pH-mediated 5-ASA release was inhibited by a low ileal pH; 3.) the impact of the two products on the growth and metabolic activity of the colonic microbiota. The ultimate goal of this work is to support healthcare providers to choose the most effective treatments for patients with UC.

2. Materials and methods

2.1. Materials

Azulfidine® tablets (500 mg sulfasalazine, coated with gastro-resistant cellulose acetate phthalate (CAP)) were purchased at a community pharmacy in Ghent, Belgium. Octasa® 1600 mg tablets (1600 mg 5-ASA, coated with OPTICORE®) were provided by University College London. Oxgall was obtained from BD Bioscience (Aalst, Belgium) and lecithin was ordered from Carl Roth (Karlsruhe, Germany). Sodium chloride (NaCl), pepsin, sodium hydrogen carbonate (NaHCO$_3$), magnesium sulfate hepta-hydrate (MgSO$_4$.7H$_2$O), di-potassium hydrogen phosphate (K$_2$HPO$_4$), and potassium di-hydrogen phosphate (KH$_2$PO$_4$) were acquired from Chem-lab analytical BVBA (Zedelgem, Belgium). Pancreatin, gastric lipase, hemin, menadione, mucin type II, L-cysteine HCl, zinc sulfate hepta-hydrate (ZnSO$_4$.7H$_2$O), iron sulfate hepta-hydrate (FeSO$_4$.7H$_2$O), sodium dithionite, sodium thioglycolate, and 5-ASA were ordered from Merck Life Science B.V. (Overijse, Belgium). Sodium hydroxide (NaOH), calcium di-chloride di-hydrate (CaCl$_2$.2H$_2$O), manganese di-chloride tetra-hydrate (MnCl$_2$.4H$_2$O), methanol (HPLC-gradient grade), acetonitrile (ACN, LC-MS grade), and high purity water for HPLC were all purchased from VWR International Europe BVBA (Leuven, Belgium). Yeast extract, agar powder, and special peptone were ordered from Thermo Fisher Scientific BVBA (Merelbeke, Belgium). Starch was purchased from Soubry (Roeselare, Belgium) and sodium thioglycolate from Applichem (Darmstadt, Germany). Water for media and experimental runs was purified using an Elix Advantage 10 water purification system (Merck Millipore, Darmstadt, Germany) while water for UHPLC analysis and sample preparation was purified using a Milli-Q® IQ 7010 system (Merck Millipore) equipped with a Milli-Q® Q-pod (0.22 μM filter).

2.2. Methods

2.2.1. Production of simulated gastrointestinal fluids

2.2.1.1. Simulated gastric fluid. The simulated fasted state gastric juice was prepared by dissolving 0.055 g/L oxgall, 0.003 g/L lecithin, 1.999 g/L NaCl, 1.576 g/L pepsin, and 9.600 g/L gastric lipase in pure water and adjusting the pH to 1.60.

2.2.1.2. Simulated duodenal and jejunal fluids. The simulated duodenal and jejunal fluids were compositionally the same, constituting 12.25 g/L KH$_2$PO$_4$, 2.06 g/L oxgall, 1.39 g/L NaOH, 4.01 g/L NaCl, and 2.67 g/L pancreatin in pure water. The pH of both fluids was adjusted to 6.50.

2.2.1.3. Simulated ileal fluid. Simulated ileal fluid consisted of 12.25 g/L KH_2PO_4 dissolved in pure water. The fluid was adjusted to pH 6.90 for the IBD and healthy donors with low ileal pH experiments, and pH 7.40 for the healthy donors with higher ileal pH experiments.

2.2.1.4. Simulated colonic fluid. The simulated colonic fluid was prepared by dissolving 7.891 g/L K_2HPO_4, 24.743 g/L KH_2PO_4, 2.834 g/L $NaHCO_3$, 3.034 g/L yeast extract, 3.034 g/L special peptone, 11.806 g/L starch, 1.520 g/L mucin, 0.760 g/L L-cysteine HCl, 0.366 g/L oxgall, 3.034 mL/L Tween 80, 0.152 g/L $MgSO_4.7H_2O$, 0.152 g/L $CaCl_2.2H_2O$, 0.099 g/L $MnCl_2.4H_2O$, 0.009 g/L $ZnSO_4.7H_2O$, 0.009 g/L $FeSO_4.7H_2O$, 0.009 g/L hemin, and 0.009 g/L menadione in pure water. Afterwards, the pH of the colonic fluid was adjusted to pH 5.00 for the experiments using the IBD donors or to pH 5.80 for the experiments using healthy donors.

2.2.2. Processing of human faecal slurry

To study the impact of the gut microbiome on drug product performance, faecal samples were sourced from one healthy volunteer and three volunteers diagnosed with IBD. Only one healthy volunteer was recruited as previous research has shown that the faecal microbiota sourced from five healthy humans has the metabolic capacity to metabolise sulfasalazine to 5-ASA at a reliably rapid rate (half-lives of 54.7–84.4 min), that should be complete by 24 h of colonic incubation [6]. Further, many different bacterial species in healthy humans' guts possess the genes required for carbohydrate metabolism [25]. As such, significant inter-individual variability in healthy microbiome composition was not judged to be a risk for the conversion of sulfasalazine or the metabolism of the starch in the Octasa® coating.

Collection of faecal samples from the four humans was completed with informed consent according to ethical approval obtained from Ghent University Hospital (reference: B670201836585). Upon donation of the faecal samples, a faecal slurry was prepared by homogenising faecal material in an anaerobic phosphate buffer (15 *w/v* %), composed of 8.8 g/L K_2HPO_4, 6.8 g/L KH_2PO_4, 0.1 g/L sodium thioglycolate, and 0.015 g/L sodium dithionite in pure water with pH adjusted to 7.0. Separate faecal slurries were prepared for each of the three IBD donors and the one healthy donor. Faecal slurry was diluted (50:50) with an in-house cryoprotectant solution, which is a modified version of the cryoprotectant developed by Hoefman et al. [26]. The obtained suspensions were aliquoted, flash frozen and then preserved at −80 °C. Just before the initiation of the colonic phase of the experiments, selected aliquots were thawed and immediately added to the reactors. This ensured that the microbiota would be metabolically active for the colonic phase of the experiment, as they would be actively utilising the nutrients in the simulated colonic fluid and proliferating.
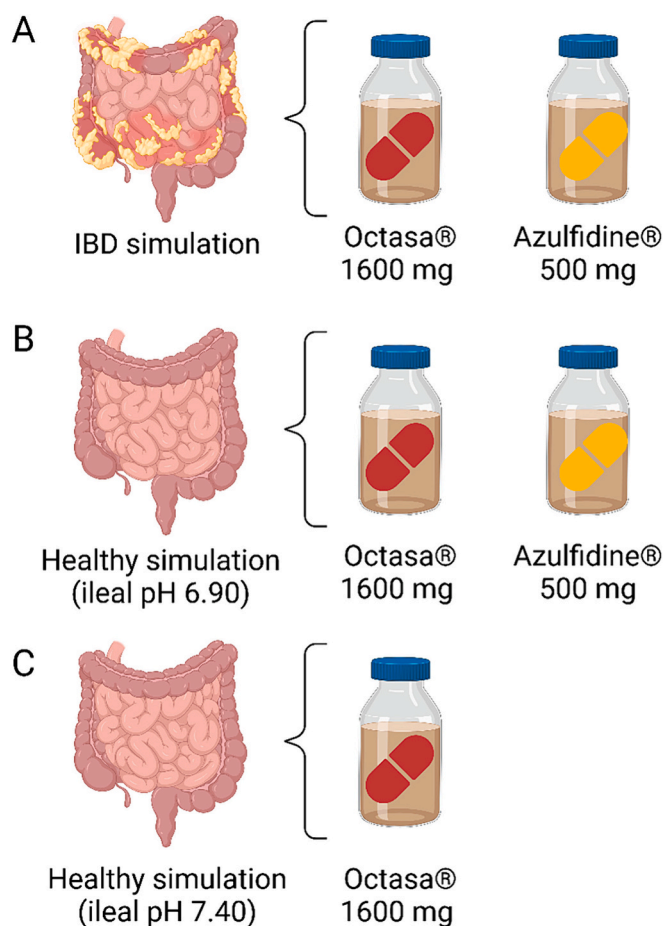
2.2.3. The SHIME® semi-dynamic gastrointestinal model

The SHIME® semi-dynamic GIl release model is a fully automated computer-controlled system harboring nine separate bioreactors allowing to test three conditions in biological triplicate per experimental run. Each bioreactor consists of a custom-made double jacket (ProDigest B·V) glass reactor that allows the control of the temperature of the bioreactor through a circulating water bath. Each reactor is sealed at the top with a custom-made lid (ProDigest B.V.) containing multiple passageways for inserting a pH electrode, tubing for active pH control, and sampling ports. Anaerobic conditions are maintained by active flushing of the vessels with nitrogen. The pH of the bioreactors is actively controlled by the software at a specified pH by the addition of 2 M NaOH and 0.5 M HCl. All automated pump actions are controlled by the software and executed with peristaltic precision pumps. Each vessel is placed on top of a magnetic stirrer that drives a stirrer bar inside the vessel. In each bioreactor, the transit of an oral dosage form through the complete GI tract is studied by sequentially simulating the passage through the stomach, small intestine, and colon.

2.2.4. Experimental design

In this study, five experimental conditions comprised of three experimental arms were tested in biological triplicate (Fig. 1). In two experimental arms (Fig. 1A and B), Octasa® 1600 mg and Azulfidine® 500 mg tablets were compared in an IBD and a healthy simulation with an ileal pH of 6.90 (referred to as low ileal pH). The pH of the ileal phase of the two simulations was matched at pH 6.90 to investigate the behaviour of the tablets in a low ileal pH scenario, arising due to disease or healthy variability [27]. At an ileal pH of 6.90, it was known that the Eudragit® S in Octasa® 1600 mg would not dissolve, hence 5-ASA release from the tablet would depend on the colonic microbiota fermenting the resistant starch in the coating [15]. In juxtaposition, the enteric coating of Azulfidine® is designed to dissolve at pH 5.50, hence sulfasalazine release from the tablets was expected in the duodenal phase. As such, this experimental design allowed assessment of the products' abilities to deliver drug to the colon in IBD and healthy low ileal pH conditions. In a third experimental arm (Fig. 1C), 5-ASA release from Octasa® 1600 mg tablets was assessed in a healthy simulation with an ileal pH of 7.40 (named high ileal pH), to assess the product's colon-targeting efficacy in a setting where Eudragit® S dissolution from the tablet coating was expected.

2.2.5. Simulation of gastrointestinal transit

The impact of GI physiology and the colonic microbiota associated with IBD and healthy individuals on drug product performance was

Fig. 1. The three experimental arms (A – C) and 5 conditions tested in the semi-dynamic SHIME® model to simulate the impact of inflammatory bowel disease (IBD) and variability in gastrointestinal pH on drug release from Octasa® 1600 mg or Azulfidine® 500 mg tablets. Each of the five experimental conditions were tested in triplicate. Tablets were placed in basket sinkers within the vessels to ensure constant submersion in the simulated GI fluids.

elucidated by varying fluid pH, transit time between GI segments, and the colonic microbiota composition in the SHIME® vessels. Fig. 2 presents the pH and transit time profiles for each of the three experimental arms in this study. Further detail of the methods used within each arm are provided within the following sections. All vessels were stirred at 450 rpm during the experiments.

2.2.5.1. IBD simulation. In this experiment conditions within the SHIME® model were modulated to reflect average conditions within the GI tract in an IBD state [28]. For each vessel, a tablet (Octasa® 1600 mg or Azulfidine® 500 mg) was submerged in 55 mL simulated gastric fluid (pH 1.6) in combination with 250 mL deionised water, to mimic tablet ingestion with a glass of water. Vessels were stirred for 45 min under these conditions, as 45 min reflects the average emptying time of a solid monolithic dosage form under fasted state conditions [29]. After this period the simulated gastric fluid was drained from the vessels and filled with 53.3 mL simulated duodenal fluid (pH 6.5). After a further 20 min, 46.7 mL simulated jejunal fluid was added to the reactor and pH was linearly increased from 6.5 to 6.9 over 120 min. At 140 min after the jejunal state was initiated, 46.7 mL of jejunal fluid was removed from the reactor and replaced with 146.7 mL simulated ileal fluid (pH 6.9). Ileal conditions were maintained for 100 min, leading to a total small intestinal transit time of 240 min. The small intestinal transit time under IBD conditions was longer as compared to healthy conditions (see Section 2.2.5.2) as demonstrated in vivo [30–32].

Following completion of the small intestinal phase, the colonic phase was initiated by adding 332 mL of simulated colonic fluid and 28 mL resuspended faecal inoculum to the reactors. For each experimental product (Octasa® 1600 mg or Azulfidine® 500 mg) the faecal inocula from each of the three IBD patients were tested, resulting in triplicate runs for each experimental condition. For the first 9 h of the colonic

simulation pH was maintained at 5.0, reflecting the low pH of the proximal colon in IBD as compared to healthy individuals [28]. Next, the pH was increased to 6.3 and maintained for 4 h to simulate the transverse colon. Finally, pH was increased to 6.8 and held constant for 11 h to reflect the distal portion of the colon. Upon completion of this phase the colonic incubation was concluded (total 24 h). The pH and transit times were selected based on the fact that lower proximal colonic pH [28] and longer proximal colonic transit times are reported for UC patients as compared to healthy individuals whereas the transversal and distal colonic pH and overall total colonic transit time are similar [31,32].

2.2.5.2. Healthy simulations – Low and high ileal pH. Conditions within the healthy simulations were identical to that of the IBD simulation up until the jejunal phase. The jejunal phase was conducted with the same volume and pH as the IBD simulation (6.5 to 6.9 over 120 min), however it was not extended to 140 min as in the IBD experiments. Instead, at 120 min of jejunal incubation, 46.7 mL of jejunal fluid was removed and replaced with 146.7 mL simulated ileal fluid. This shorter transit time reflects that observed in a healthy state [27]. In the healthy simulation with low ileal pH, the ileal pH was maintained at pH 6.9, as in the IBD experiments. Contrastingly, in the healthy simulation with high ileal pH, the ileal pH was maintained at pH 7.4. These two experimental arms were included to capture the interindividual differences in GI pH that can occur in healthy humans, that can significantly impact the dissolution of colon-targeting materials, such as the Eudragit® S in Octasa® 1600 mg [13].

The ileal phase in both healthy simulations (low and high ileal pH) lasted 40 min to attain a total small intestine transit time of 180 min; this was 60 min shorter than the IBD small intestine simulation, based on clinical measurements in both healthy and diseased individuals
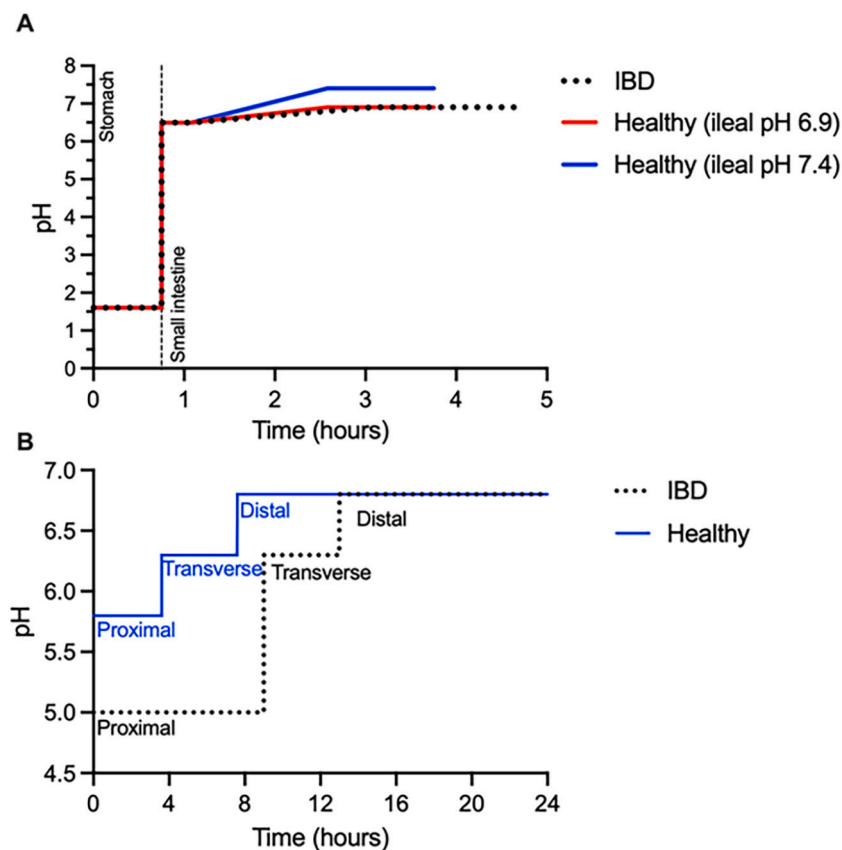
Fig. 2. The pH and transit time profiles used in the semi-dynamic SHIME® model to simulate GI conditions found in (A) the upper GI tract of IBD and healthy states with low and high ileal pH and in (B) the colonic environment of IBD and healthy states. The pH was increased linearly between colonic segments.

[27,31–34].

As in the IBD experiments, the colonic phase was initiated by adding 332 mL of simulated colonic fluid and 28 mL of resuspended faecal inoculum to the vessels. In the healthy simulations the faecal inoculum was derived from the one healthy volunteer, hence simulating a healthy colonic microbiome composition. The initial colonic pH was higher in the healthy simulations compared to the IBD simulation, as has been observed through clinical measurements [28]. In the healthy simulations initial colonic pH was maintained at pH 5.8 for 3 h 36 min to reflect transit through the healthy proximal colon. Subsequently, pH was increased to 6.3 for a further 4 h and then increased again to pH 6.8 for the remaining 11 h (total colonic transit: 24 h). Transit times were based on evidence that transit through the proximal colon is faster in healthy individuals as compared to diseased individuals, whereas total colonic transit times are comparable between these groups [31,32].

2.2.6. Sampling of simulated gastrointestinal fluid

The fluid within reactors was sampled at distinct timepoints over the course of the experiments. In the gastric and small intestinal environments 1.50 mL fluid from each vessel was extracted for 5-ASA quantification, and subsequently replaced with the same volume of simulated GI fluid. Under healthy conditions samples were taken at the start and the end of the stomach phase (ST0 and ST45), and after 20 (SI20), 80 (SI80), 145 (SI145), and 180 min (SI180) in the small intestinal phase. Under IBD conditions samples were taken at the start and the end of the stomach phase (ST0 and ST45), and after 20 (SI20), 80 (SI80), 190 (SI190), and 240 min (SI240) in the small intestinal phase. For colonic sampling, 10 mL fluid was extracted for 5-ASA, lactate, short chain fatty acids (SCFAs), and branched short chain fatty acids (bSCFA) quantification after 0 (C0), 1, (C60), 2 (C120), 3 (C180), and 24 h (C1140) of colonic incubation. In addition, samples for only 5-ASA quantification (1.5 mL) were taken after 15 (C15), 30 (C30), 90 (C90), and 150 min (C150) of colonic incubation. Samples for SCFA and bSCFA were stored at −20 °C prior to extraction (see Section 2.2.8). In parallel to this, part of the samples was centrifuged for 5 min at 7690 x*g* and the supernatant was used for lactate quantification (See section 2.2.8). For 5-ASA quantification, the samples were diluted using a 50:50 *v*/v water-methanol mixture according to Supplementary Table 1. Dilutions were centrifuged at 20,817 x*g* for 7 min at 37 °C and the supernatant was recovered for 5-ASA quantification (see Section 2.2.7).

2.2.7. Quantification of 5-ASA concentration

The concentration of 5-ASA was monitored using an ultra-high performance liquid chromatography (RP-UHPLC) system, consisting of an Acquity®Arc™ equipped with a 2998 PDA detector from Waters (Milford, MA, USA). The chromatographic separation was conducted utilizing an XSelect HSS C18 XP Column (100 Å pore size, 2.5 μm particle size, 2.1 mm i.d. x 100 mm dimensions, Waters, Milford, MA, USA), with an XSelect HSS T3 XP Vanguard Cartridge pre-column (100 Å pore size, 2.5 μm particle size, 2.1 mm i.d. x 5 mm, Waters N.V., Antwerp, Belgium). The analysis was conducted using an isocratic method with a mobile phase consisting of 90% eluent A (0.1% trifluoroacetic acid in water; *v*/v %) and 10% eluent B (100% methanol) over a total runtime of 6 min. All analyses were carried out with a 10 μL sample volume, using a 0.4 mL/min flow rate, and whilst maintaining a 30 °C column temperature. 5-ASA was detected at a wavelength of 210 nm, and peak integration was performed using Empower Pro software. The 5-ASA concentrations were quantified using external standards.

2.2.8. Measurement of microbial metabolites

The concentrations of three SCFAs (acetate, propionate, butyrate) and three branched SCFAs (isobutyrate, isovalerate, and isocaproate) were monitored with gas chromatography (GC) coupled with flame ionisation detection (FID). Samples (generated as described in Section 2.2.6) were combined with 2-methyl hexanoic acid as an internal standard and 2.0 mL of this mixture was extracted. Extraction involved

adding the 2.0 mL sample to 500 μL sulphuric acid (diluted 1:1 with water), adding 0.2 g sodium chloride and 400 μL of internal standard solution (1.5 mL 2-methylhexanoic acid +98.5 mL 0.1 M NaOH +100 mL water), and 2.0 mL diethyl ether. This mixture was then rotated for 2 min on a Hulashaker (Thermo Fisher Scientific, Waltham, Massachusetts, United States) at 20 rpm before centrifugation for 3 min at 1000 x*g*. The supernatant was analysed using a GC-2014 gas chromatograph (Shimadzu, 's-Hertogenbosch, the Netherlands), equipped with a GC SGE capillary column, 30 m × 0.32 mm ID-BP21x 0.25 μm (Achrom, Machelen, Belgium), an FID and a split injector. The injection volume was 1.0 μL and the column temperature profile was set from 110 to 160 °C, rising at 6 °C min^{-1}. The carrier gas was nitrogen (flow rate 95.6 mL/min) and the temperatures of the injector and detector were both 200 °C.

Lactate quantification within samples was performed using a commercially available enzymatic assay kit (R-Biopharm,Darmstadt, Germany) according to the manufacturer's instructions.

2.2.9. Assessment of the impact of 5-ASA and sulfasalazine on gut microbial growth

2.2.9.1. Collection and culture of human faecal microbiota.
Collection of faecal samples from six healthy humans (*n* = 3 males; n = 3 females; aged 27–35), with informed consent and who had not taken antibiotics in the preceding 12 months, was approved by the UCL Biobank Ethical Review Committee at Royal Free London NHS Foundation Trust (reference no. NC2017.010). The volunteers deposited fresh faecal samples into plastic receptacles containing an AnaeroGen sachet (Thermo Fisher Scientific, Loughborough, UK) to generate anaerobic conditions for maximal microbial viability. Samples were stored at −20 °C overnight and moved into an anaerobic chamber (Electrotek 500TG workstation, Electrotek, West Yorkshire, UK) maintained at 37 °C and 70% relative humidity the next morning. The faecal samples were homogenised and combined with a basal media (as used by [35]) at a ratio of 1:3 to create a faecal slurry. An aliquot (0.5 mL) of faecal slurry from each donor was then inoculated into separate 100 mL bottles of sterile and anaerobic Bryant and Burkey growth medium (BBM), which has been shown to preserve the microbial richness of faecal samples [36,37]. The microbiota within each bottle was cultured for 24 h with gentle agitation at 100 rpm to establish stable microbiota communities. Following this, a further 1.0 mL of each culture was aliquoted into a second 100 mL sterile BBM and incubated for a second 24 h within the anaerobic chamber. These final cultures were subsequently combined with 30% glycerol in ¼ Ringer's solution to obtain 15% *v*/v glycerol stock suspensions for each human donor, which were frozen in 1.0 mL aliquots at −80 °C. The microbial abundances of the aliquots were enumerated by serially diluting one thawed aliquot per donor (using dilution factors of $10^2–10^{11}$) in phosphate buffered saline and plating on solid media composed of BBM and 1.20% agar powder. Plates were incubated for 72 h under anaerobic conditions at 37 °C, and colonies were then visually counted, allowing the calculation of the colony forming units (CFU) present in each millilitre of sample (Supplementary Table 2). For experimentation the aliquots were thawed only once and immediately prior to use, to preserve microbial viability [38].

2.2.9.2. Measuring microbial growth.
The impacts of 5-ASA and sulfasalazine on the growth of the human faecal microbiota was measured via optical density (OD). 20 mL of sterile BBM was equilibrated for over eight hours within an anaerobic chamber (A25 Sleeved Anaerobic Workstation, Don Whitley Scientific, Bingley, UK; containing 5% CO_2, 5% H_2, 90% nitrogen) set at 37 °C, to allow all oxygen to diffuse from the medium. Then, the anaerobic BBM was inoculated with 0.20 mL of thawed human faecal microbiota (see Section 2.2.9.1). The microbiota was allowed to proliferate for 16 h under mild agitation (100 rpm). The next day, stock solutions of sulfasalazine and 5-ASA were prepared in

sterile BBM. Aliquots of these stock solutions were then combined with the faecal microbiota cultures from each donor ($n = 6$ donors, $n = 3$ incubations per donor) at concentrations of 1.39 mg/mL sulfasalazine and 0.53 mg/mL 5-ASA and agitated gently at 100 rpm.

The sulfasalazine concentration (1.39 mg/mL) was selected to reflect that used in the SHIME® experiments. As sulfasalazine is 38.44% 5-ASA by molar weight, the 5-ASA incubation concentration was set to 0.53 mg/mL, to achieve an equal representation of 5-ASA in both experiments. As 5-ASA would be liberated from sulfasalazine by the faecal bacteria this would allow investigation of the effects of both drugs on microbial growth. Drug-free control incubations ($n = 3$ per donor) were also employed to facilitate comparison of the microbiota's growth in the absence of drug. At 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 h, 1.0 mL incubation medium was extracted and diluted with BBM at a ratio of 1:3. The OD of each incubation was then recorded at 600 nm using a cell density meter designed for measurement of microbial growth (CO 8000 Biowave, VWR International, Pennsylvania, USA). The growth of the microbiota in the absence of drug, and in the presence of sulfasalazine and 5-ASA, were compared once all OD timepoints had been collected by inspecting the microbial growth curves.

2.2.10. Data analysis

Data were analysed and plotted within GraphPad Prism (Version 10.0.2 (232)). Unless otherwise stated, all datapoints on plots represent means and error bars represent standard deviations. A two-way ANOVA with a Tukey's multiple comparisons test was utilised to compare the difference between groups at multiple timepoints. In all cases, a P value of <0.05 was considered significant.

3. Results and discussion

3.1. Colonic delivery of 5-ASA via the prodrug and formulation approaches

3.1.1. 5-ASA recovery from prodrug conversion in healthy and IBD models

Fig. 3 demonstrates the colon targeting ability of the prodrug strategy (via Azulfidine®) in both healthy and IBD conditions, as represented by 5-ASA recovery in the SHIME® colonic environment. The gastro-resistant enteric coated tablets remained intact in the stomach and began disintegrating in the duodenal fluid. Sulfasalazine was not converted into 5-ASA during transit through the small intestine. Upon exposure to the colonic environment, dissolved sulfasalazine began to be converted into 5-ASA via a reduction of the diazo-bond mediated by the colonic microbiota.

Under healthy conditions, approximately 20% of 5-ASA was recovered during the initial 180 min of colonic incubation. Afterwards, sulfasalazine was almost completely converted from 3 to 24 h of colonic incubation resulting in a final 5-ASA recovery of $97 \pm 4\%$. These results are in line with previous studies reported in literature that studied the

conversion of sulfasalazine into 5-ASA during in vitro incubations inoculated with the faecal samples of healthy individuals. During these studies, 70–100% of sulfasalazine was reported to be converted within 2–7 h [6,39,40]. Further, published pharmacokinetic information for Azulfidine® tablets has described that the lag time for 5-ASA to enter systemic circulation following tablet administration is 6.1 ± 2.3 h [41]. This corresponds with the SHIME® results in Fig. 3 in which 5-ASA began to be detectable in the healthy and IBD simulations from 5 to 5.75 h.

In comparison, lower conversion of sulfasalazine into 5-ASA was observed during the experiments under IBD conditions for all three donors tested. Indeed, the 5-ASA recovery was $46 \pm 2\%$, $42 \pm 2\%$, and $36 \pm 5\%$ after 24 h for IBD Donors 1, 2, and 3, respectively. Microbial variation between donors' faecal samples may explain the differences in sulfasalazine degradation and 5-ASA recovery. Indeed, this is confirmed by the reduced conversion of sulfasalazine into 5-ASA by IBD Donor 3 when compared to the IBD Donors 1 ($P = 0.0003$) and IBD Donor 2 ($P = 0.0483$). As such, the healthy donor showed increased 5-ASA recovery compared to all three IBD donors ($P < 0.0001$). This indicates that a healthy colonic microbiota, with higher bacterial diversity, could more efficiently convert sulfasalazine to 5-ASA compared to the microbiota of the IBD patients with reduced diversity [42]. The Clostridium and Eubacterium genera are the main gut bacteria capable of producing the azoreductases that enable sulfasalazine metabolism to 5-ASA [43]. Interestingly, particular species with significant azoreductase activity, i.e., *Clostridioides leptum*, *Eubacterium rectale,* and *Eubacterium hallii*, have been shown to be depleted in IBD and enriched in the healthy gut [6,44]. This evidence could explain why the IBD donors converted sulfasalazine less efficiently than the healthy donor in this study. Furthermore, the initial low pH of the proximal colon under IBD conditions could further result into a slower bacterial metabolism and hence a lower conversion rate of sulfasalazine into 5-ASA. However, it is worth considering that only one healthy donor was used in this study, and that some variability among healthy individuals is expected. Nonetheless, these results highlight the importance of using a disease model when assessing colonic drug delivery for IBD medications.

3.1.2. 5-ASA release from the targeted formulation in healthy and IBD models

Fig. 4 shows the colonic targeting capability of the formulation strategy (via Octasa® 1600 mg) in healthy conditions, with higher and lower ileal pH, and in IBD conditions. In the healthy model with an ileal pH of 7.4, in which the pH-mediated release would trigger, 5-ASA release began gradually in between 65 min (pH 6.95) and 110 min (pH 7.4) in the small intestine and continued until 24 h in the colon. In IBD and healthy conditions with an ileal pH of 6.9, in which pH-mediated release would not trigger, no release of 5-ASA was observed during passage through the stomach and small intestine. On the contrary, digestion of the tablet coating's starch component by the colonic
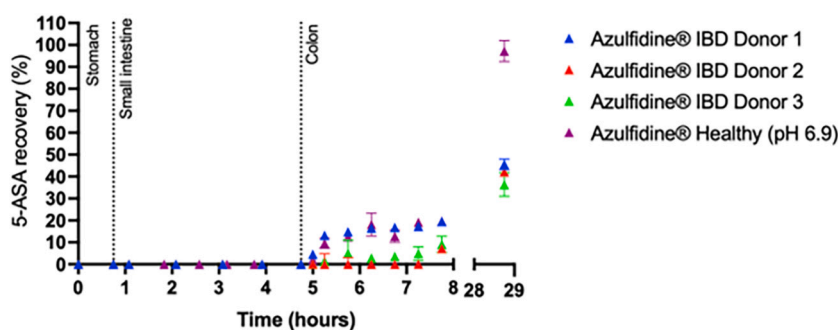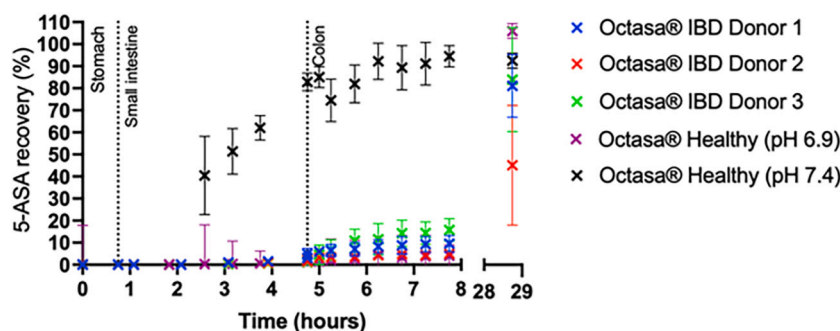

Fig. 3. The total recovery of 5-ASA from Azulfidine® 500 mg tablets (the prodrug strategy) in the inflammatory bowel disease (IBD) and healthy state SHIME® simulations. Each datapoint reflects the mean and standard deviation of three experimental replicates. Total time in each compartment was 45 min (stomach); 180/240 min (healthy/IBD simulations, small intestine); 24 h (colon).

Fig. 4. The total release of 5-ASA from Octasa® 1600 mg tablets (the formulation strategy) in the inflammatory bowel disease (IBD) and healthy state SHIME® simulations. Each datapoint reflects the mean and standard deviation of three experimental replicates. Total time in each compartment was 45 min (stomach); 180/240 min (healthy/IBD simulations, small intestine); 24 h (colon).

microbiota was evident by the gradual increase in 5-ASA occurring only in the colon.

5-ASA release was significantly faster in the healthy high ileal pH simulation compared to the simulations with the lower ileal pH, in which the pH formulation trigger would not be activated ($P < 0.0001$). However, after 24 h 5-ASA release in both healthy simulations, regardless of ileal pH, was statistically similar. This demonstrates that the formulation could still enable full drug release even when relying solely on microbial digestion of the coating. Bacteria-mediated drug release in the IBD simulations showed inter-individual variability, with the microbiota from Donors 1 and 3 enabling significantly more 5-ASA release than Donor 2 ($P < 0.0001$). This could be attributed to varying extents of dysbiosis between patients. In addition, the healthy condition with low ileal pH enabled higher 5-ASA release than all the IBD conditions, exemplifying that a healthy microbiome was more efficient than an IBD state microbiome in digesting the OPTICORE® coating ($P < 0.0001$).

Altogether, these findings confirm that the dual pH and bacteria-mediated formulation triggers were effective for colonic delivery of 5-ASA under diseased and healthy small intestinal pH conditions, and in dysbiotic and healthy microbiome states. This is in line with previously reported clinical studies where the Phloral® coating enabled colonic delivery in humans [45,46] and where targeted colonic 5-ASA release was achieved using the OPTICORE® formulation strategy [47]. Similarly, the Phloral® coating has been shown to enable 100% colonic release of 5-ASA in human faecal slurry after 7 h to 24 h [9].

3.1.3. Comparing 5-ASA release between the prodrug and formulation strategies

Fig. 5 compares 5-ASA release between the two colon-targeting strategies, by overlaying the average 5-ASA recoveries in the SHIME®

experiments discussed in Sections 3.1.1 and 3.1.2. Under healthy conditions both the prodrug and the formulation strategy resulted in similar 5-ASA recoveries with Azulfidine® reaching $97 \pm 2\%$ and Octasa® reaching $104 \pm 6\%$ and $92 \pm 2\%$ under low and high ileal pH conditions, respectively. However, it should be noted that full recovery of 5-ASA using the Octasa® formulation resulted in the delivery of 1600 mg of 5-ASA to the target site whereas only 192 mg of 5-ASA is released using the prodrug. On the other hand, during the experiments simulating the physiology and colonic microbiota of the drug's target population (IBD) substantial lower 5-ASA recoveries were obtained using the prodrug as compared to the formulation strategy. After 24 h of colonic incubation, the Octasa® 1600 mg tablets (formulation strategy) released around 2 times more 5-ASA into the colonic environment in IBD and healthy conditions, respectively, than Azuldifine® (prodrug strategy) ($P < 0.0001$). This indicates that the formulation strategy was more effective than the sulfasalazine prodrug strategy for colonic 5-ASA delivery. These findings are important, as they highlight that formulation-mediated colonic drug delivery could be more effective than prodrug-mediated colonic drug delivery in patients. In UC, the amount of 5-ASA delivered to the colon is paramount for therapeutic success. Indeed, a clinical study of 50 UC patients has shown that concentrations of 5-ASA in the colonic mucosa are higher in patients achieving endoscopic disease remission [48]. As such, selecting a drug product that maximises the colon targeting of 5-ASA could increase patients' therapeutic outcomes.

3.2. Impact of Octasa® 1600 mg and Azulfidine® 500 mg on colonic microbiome metabolism

3.2.1. Lactate

Fig. 6 shows the impacts of Octasa® 1600 mg and Azulfidine® 500
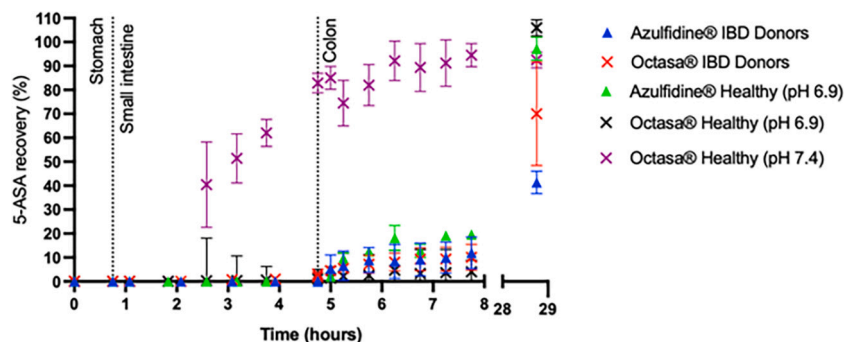
Fig. 5. The total recovery of 5-ASA from Octasa® 1600 mg (formulation strategy) and Azulfidine® 500 mg tablets (prodrug strategy) in the inflammatory bowel disease (IBD) and healthy state SHIME® simulations. Healthy donor datapoints reflect the mean and standard deviation of three experimental replicates from one individual; IBD donor datapoints reflect the mean and standard deviations of three experimental replicates, each from three different donors. Total time in each compartment was 45 min (stomach); 180/240 min (healthy/IBD simulations, small intestine); 24 h (colon).
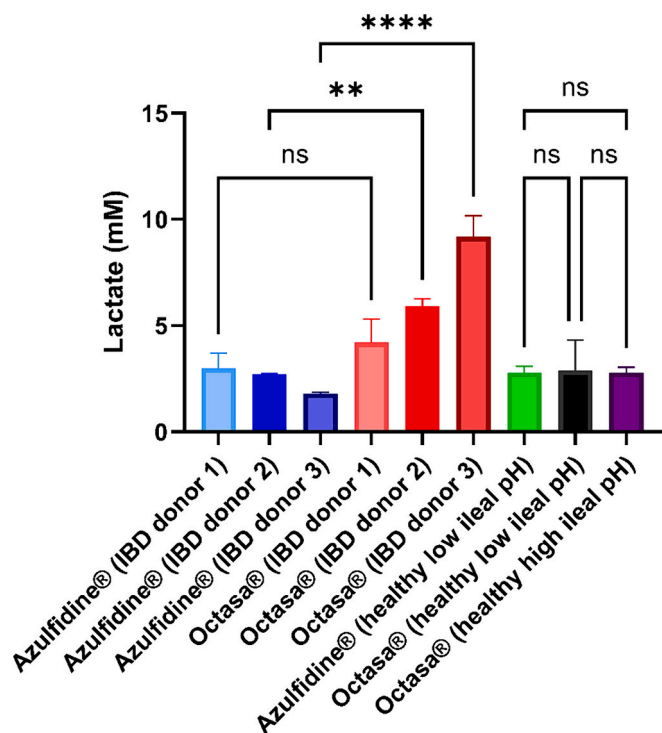
Fig. 6. The impacts of Octasa® 1600 mg and Azulfidine® 500 mg tablets on the concentration of lactate in the inflammatory bowel disease (IBD) and healthy state SHIME® simulations at the 24 h colonic timepoint. Each datapoint reflects the mean and standard deviation of three experimental replicates.

mg tablets on the concentrations of lactate in the healthy and IBD simulations at 24 h. Lactate concentrations were generally very low until the 24 h colonic timepoint, signifying that it was the microbiota in the colonic phase that contributed towards its production. As such, only the 24 h concentrations have been presented here to afford better differentiation between the groups. This is the same for the SCFA and branched SCFA results shown in Section 3.2.2. Plots showing the metabolite concentrations from 0 to 24 h are presented in Supplementary Figs. 1 and 2.

The amount of lactate produced in the three healthy experiments was similar, showing that neither tablet nor the ileal pH affected bacterial lactate synthesis relative to another. In the healthy human colon lactate concentration is usually <4 mM, therefore the lactate concentrations in the healthy simulations were biorelevant [49].

Lactate concentrations in the three Octasa® IBD simulations were significantly higher than those in the healthy Octasa® simulations ($P \leq$ 0.0002). This was expected, as lactate accumulation is associated with IBD and positively correlated with disease severity. In a study of 23 patients with moderate IBD, faecal lactate concentration was measured as 8.9 $\pm$ 7.0 mM, which corresponds with the lactate concentrations observed in the Octasa® IBD vessels in this study [50]. Based on the lactate concentrations, IBD donor 3 had the most severe IBD, followed by donor 2, and then donor 1. Interestingly, the donor 2 and 3 Azulfidine® IBD vessels had significantly lower concentrations of lactate than the Octasa® IBD vessels ($P \leq 0.001$). This suggests that the sulfasalazine in Azulfidine® may have altered microbial activity, and resulted in lower lactate accumulation, to a greater extent than the 5-ASA in Octasa® in these vessels. When contemplating this, it is pertinent to consider that the dose of sulfasalazine in Azulfidine® is lower than the 5-ASA dose in Octasa® (500 mg vs. 1600 mg). It is also possible that the excipients in these products played a role in this microbiome effect. For example, the starch in the Octasa® 1600 mg coating may have encouraged microbial synthesis of lactate and SCFAs, however, this is expected to be a minor effect due to the low overall amount of starch in

the tablet.

Reducing lactate may be a beneficial therapeutic strategy in IBD, as lactate accumulation within the colon can result in toxic acidification of the colonic microbiota from pH < 5.50, leading to perturbations in microbiome composition and SCFA synthesis [51]. As such, the ability of Azulfidine® to reduce accumulation of lactate in the SHIME® system could highlight an important facet of its therapeutic action in vivo.

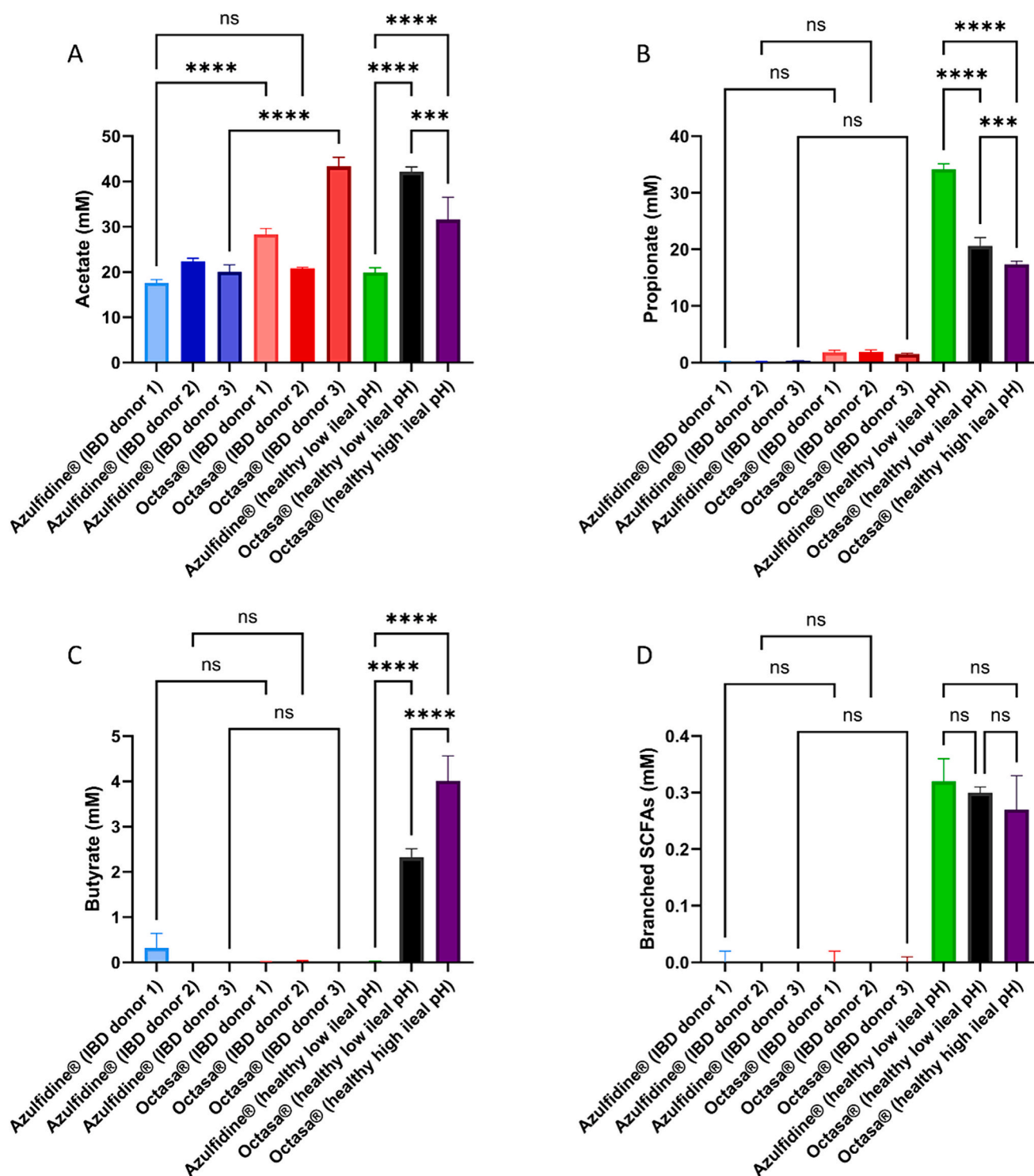### 3.2.2. Short chain fatty acids and branched short chain fatty acids

Fig. 7 shows the impacts of Octasa® 1600 mg and Azulfidine® 500 mg tablets on the concentrations of SCFAs and branched SCFAs in the healthy and IBD simulations. In the healthy state vessels, the concentrations of the SCFAs acetate and butyrate were significantly higher after 24 h treatment with the Octasa® 1600 mg tablets, compared to the Azulfidine® tablets ($P < 0.0001$) (Fig. 7A and C). Contrastingly, Azulfidine® increased propionate concentrations in the healthy simulations relative to Octasa® ($P < 0.0001$) (Fig. 7B). There was also a pH effect found in the healthy simulations, whereby the Ocatasa® low ileal pH vessels reached significantly higher acetate and propionate, but lower butyrate, concentrations than the Octasa® high ileal pH vessels ($P <$ 0.0001). There was no significant difference between the concentrations of branched SCFAs of the healthy state vessels at 24 h (Fig. 7D). These results are interesting, as they highlight the sensitivity of the healthy state colonic microbiota to the dosage forms and GI pH variation.

In the IBD state, Octasa® 1600 mg tablets achieved higher acetate concentrations in 2/3 donors and higher propionate concentrations in 3/3 donors than the Azulfidine® tablets at 24 h (P < 0.0001) (Fig. 7A and B). In terms of butyrate, the Azulfidine® tablets achieved higher 24 h concentrations than the Octasa® tablets in 1/3 IBD donors ($P =$ 0.0096) (Fig. 7C). SCFAs are produced by colonic bacteria during polysaccharide fermentation and are generally regarded as highly beneficial for human health [52]. Acetate, propionate, and butyrate concentrations may be protective against IBD via a number of mechanisms, including local immunomodulation, and are decreased in the faeces of IBD patients compared to healthy controls [53,54]. As such, the ability of medications to increase colonic SCFAs concentrations could be an important therapeutic asset. These findings support two interesting hypotheses: the first, that the two investigated products differentially modulate microbiome functioning; the second, that an IBD state microbiome may respond differently to the medications than a healthy state microbiome. Whilst these hypotheses would require confirmation in head-to-head human studies, there is data in animals and humans that supports the impacts of 5-ASA and sulfasalazine on the gut microbiome [55–58].

Focusing on IBD, sulfasalazine has previously been found to alter faecal microbiome composition in a male IBD rat model, leading to reduced relative abundances of Proteobacteria (Pseudomonadota) and Bacteroidetes (Bacteroidota), and increased relative abundance of Firmicutes (Bacillota) compared to an untreated control [55]. These changes were observed to transition the microbiome composition towards an IBD-free state. However, sulfasalazine did decrease the biodiversity of the faecal microbiome below that of the IBD-free rats. In a separate study with mice of both sexes, 5-ASA reduced the relative abundance of faecal Bacteroidota and increased the relative abundances of Bacillota and Actinobacteria (Actinomycetota), most significantly in females [56]. 5-ASA also decreased the Shannon diversity index in faeces in both sexes compared to untreated controls. Though these two studies were conducted in different species of rodent, they do point to commonalities between the impacts of 5-ASA and sulfasalazine on an IBD microbiome composition; namely, reduced relative abundances of Bacteroidota, increased relative abundances of Bacillota, and potentially reduced bacterial diversity.

There were no differences in 24 h branched SCFA concentrations between the Octasa® and Azulfidine® vessels in any of the IBD simulations (Fig. 7D). However, it was interesting to note that 24 h concentrations of branched SCFAs were markedly lower in the IBD

Fig. 7. The impacts of Octasa® 1600 mg and Azulfidine® 500 mg tablets on the concentration of short chain fatty acids (SCFAs): acetate (A), propionate (B), and butyrate (C); and branched SCFAs (D) in the inflammatory bowel disease (IBD) and healthy state SHIME® simulations at the 24 h colonic timepoint. Each datapoint reflects the mean and standard deviation of three experimental replicates.

simulations compared to the healthy simulations ($P < 0.0001$). As with the SCFAs, the concentrations of branched SCFAs are known to be significantly reduced in the faeces of IBD patients compared to healthy individuals [54]. As such, these results have good biorelevance and suggest that neither tablets increased branched SCFA concentrations to healthy levels.

3.3. Impact of 5-ASA and sulfasalazine on gut microbial growth

As Octasa® and Azulfidine® tablets were found to differentially impact SCFA concentrations in the SHIME® simulations, and there is evidence from the literature that 5-ASA and sulfasalazine may reduce bacterial abundance, the impact of the drugs on the growth of the gut microbiota was assessed. Fig. 8 shows the effects of 5-ASA and sulfasalazine on the 24 h growth of the faecal microbiota sourced from 6
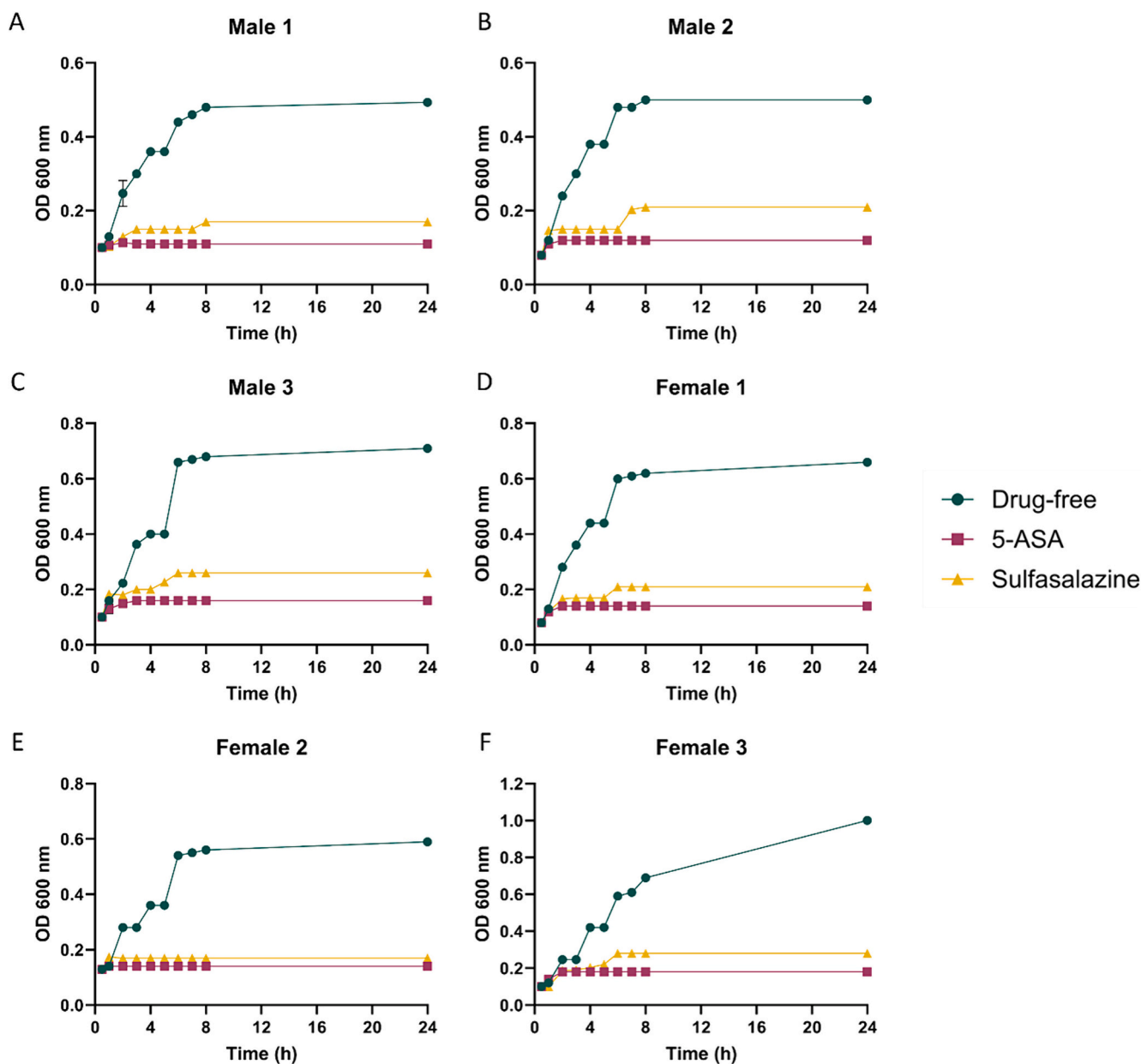
Fig. 8. The impact of 5-ASA and sulfasalazine on the growth of the faecal microbiota sourced from 6 healthy humans. A - C: male volunteers 1–3; D - F: female volunteers 1–3. Experiments for each donor-treatment pairing were conducted in triplicate.

healthy humans. Clearly, both drugs exerted antimicrobial effects; the cell density of the microbiota cultures exposed to the drugs was significantly lower than those left untreated (P < 0.0001 from 2 to 24 h for all donor-drug pairings**).**

Interestingly, the antimicrobial impact of 5-ASA was significantly greater than sulfasalazine, with all donors' microbial abundance significantly lower in the presence of 5-ASA at 2 h onwards (*P* < 0.05). This is surprising, as both drug incubations contained equal moles of 5-ASA (considering all sulfasalazine was cleaved by bacteria) and the sulfasalazine incubations contained an additional 0.86 mg/mL of sulfapyridine, a known antibacterial compound. Unexpectedly, the combination of 5-ASA and sulfapyridine within sulfasalazine did not increase antimicrobial activity compared to 5-ASA incubated alone. One explanation for this could be that the cleaving of sulfasalazine by azoreductases provided survival advantages to the microbiota, for example by sulfasalazine acting as an electron acceptor and aiding anaerobic respiration [59].

There is supporting evidence for 5-ASA exerting anti-gut bacterial effects in the literature; a study by Griffiths et al. reported that oral administration of 5-ASA to 12 females with irritable bowel syndrome led to a 46% reduction in faecal bacteria after 4 weeks [60]. Separate work suggests that 5-ASA may sensitise bacteria to oxidative stress by inhibiting bacterial polyP kinase [58]. In juxtaposition, the present study is the first to show the anti-gut microbiota effects of sulfasalazine, and importantly to demonstrate that these may be lesser than those occurring with 5-ASA.

The clinical impacts of 5-ASA and sulfasalazine's antimicrobial effects are yet to be proven. However, there are suggestions that inhibition of polyP kinase by 5-ASA could reduce the ability of bacteria to persist in chronically inflamed environments, as seen in UC [58]. There is also evidence that the antibacterial activity of another anti-inflammatory drug, methotrexate, aids its anti-inflammatory action in the colon [61]. Given these findings, further research delineating the relationships between the effects of 5-ASA and sulfasalazine on the colonic microbiome and their therapeutic action in IBD is warranted. Uncovering such relationships could identify new microbiome-mediated targets for the

treatment of colitis.

4. Conclusions

In this study, the efficacies of two colonic drug delivery strategies for UC were compared by measuring 5-ASA release from Azulfidine® (sulfasalazine, prodrug strategy) and Octasa® 1600 mg (5-ASA, formulation strategy) in the SHIME® system. With both targeting strategies, variation in 5-ASA release was noted between IBD donors, a healthy individual, and when ileal pH was modulated. Importantly, the formulation strategy, which employed a dual-trigger drug release mechanism, was still effective when ileal pH was lowered to prevent one of the trigger mechanisms from activating. The formulation strategy also facilitated significantly higher 5-ASA release in the colon compared to the sulfasalazine prodrug approach under conditions simulating IBD, which could suggest superior colonic drug delivery action in vivo. As well as examining the drug products' colon targeting efficacies, their impact on the microbiome was investigated. Here, both sulfasalazine and 5-ASA were observed to differentially modulate the growth and metabolism of the human faecal microbiota. In the IBD SHIME® simulations, sulfasalazine released from Azulfidine® tablets led to significantly lower microbial lactate, acetate, and propionate synthesis than that observed following 5-ASA release from Octasa® 1600 mg tablets. Concentrations of branched SCFAs were similar following dosing of the two drug products, however they were significantly lower than those recorded in the healthy simulations, and as such reflected the IBD state in the human colon. When incubated with the healthy faecal microbiota sourced from six human volunteers, both drugs significantly reduced microbial growth compared to an untreated control, with 5-ASA showing the strongest antimicrobial action. As such, this study demonstrated that the use of advanced in vitro models that accurately simulate the GI physiology and colonic microbiota of the patient population is necessary, as in vitro models that simulate healthy individuals could result in biased conclusions about the GI performance of medications. Finally, these results have revealed new interactions between the UC medicines and the human gut microbiota and promote further investigation of how these interactions influence disease treatment.

CRediT authorship contribution statement

Laura E. McCoubrey: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Nidhi Seegobin:** Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Nannapat Sangfuang:** Data curation, Investigation, Methodology. **Frédéric Moens:** Conceptualization, Formal analysis, Writing – review & editing. **Hans Duyvejonck:** Investigation, Data curation, Formal analysis. **Eline Declerck:** Investigation, Data curation. **Arno Dierick:** Investigation, Data curation. **Massimo Marzorati:** Writing – review & editing. **Abdul W. Basit:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

Abdul W. Basit is the inventor of the Phloral® Technology and holds its patent [US20070243253A1]. OPTICORE® is licensed by Tillots Pharma Ltd. as part of the Octasa® 1600 mg product.

Data availability

Data will be made available on request.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jconrel.2024.04.016.

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