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Targeted colonic release formulations of mesalazine – A clinical pharmaco-scintigraphic proof-of-concept study in healthy subjects and patients with mildly active ulcerative colitis

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ABSTRACT

Colonic targeting of orally applied therapeutic drugs remains a challenge. Tablet coatings relying on gastrointestinal pH and colonic bacterial enzymes as triggers in association with an inner alkaline layer are expected to improve targeting efficiency. Mesalazine release from three differently coated tablets labelled with 1 MBq ^{153}Sm was characterised in a single centre, open-label, parallel group study in nineteen healthy subjects and seven patients with mildly active ulcerative colitis. Two semi-organic and one aqueous-based outer coating with different ratios of enteric polymer and resistant starch were tested. All coatings showed comparable release lagtimes in biorelevant dissolution media and were not affected by neutron-activation of the samarium tracer. Mesalazine pharmacokinetics and gamma scintigraphy were used to characterise drug release, anatomical site of tablet disintegration and gastrointestinal transit. Initial tablet disintegration occurred at the ileo-caecal junction or beyond in 92 % of the subjects. Time to initial tablet disintegration was inversely correlated with maximal plasma concentrations and systemic mesalazine exposure. Although high inter-subject variability precluded detection of differences between solvent types and different enteric polymer to polysaccharide ratios, the dual pH and enzymatic triggered release system in combination with an inner alkaline layer promoted mesalazine release at the target site with high accuracy.

1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease with relapsing inflammation of the mucosal layer of the colon. Mesalazine (mesalamine, 5-aminosalicylic acid, 5-ASA) (Wang et al., 2016a; Wang et al., 2016b) is recommended as first-line therapy for the treatment of patients with mild-to-moderate UC in the guidelines of the European Crohn's and Colitis Organisation (Raine et al., 2022) and the American Gastroenterology Association (Ko et al., 2019). However, high doses recommended for the induction and maintenance of remission of UC require intake of multiple oral tablets each day.

Non-adherence to maintenance therapy with mesalazine in patients with mild-to-moderate UC in remission is common and is associated with an increased risk of symptomatic relapse (Kane et al., 2003; Khan et al., 2012), decreased quality of life (Kane, 2006), loss of potential chemoprevention benefit (Kane, 2006) and increased cost of care due to disease relapse (Kane and Shaya, 2008). Thus, the introduction of high strength mesalazine products, with more convenient dosing regimens, may help UC patients remain adherent to their medication schedule.

Treatment of UC relies mostly on enteric-coated formulations, which prevent the release of the active substance until after the passage through the acidic environment of the stomach, leading to high local drug availability in the duodenum and beyond. Commonly used enteric polymer coatings include Eudragit® L and Eudragit® S, which dissolve in a pH dependent manner at pH 6 and pH $\geq$ 7, respectively (Fadda and Basit, 2005; Khan et al., 2000). Enteric coating dissolution in the

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gastrointestinal tract is based on the pH gradient along the gut. Due to the production of short chain fatty acids by colonic bacteria this pH gradient changes at the ileocecal junction (ICJ) (Evans et al., 1988), resulting in a lower pH in the ascending colon compared to the small intestine and the more distal colonic regions. However, the pH gradient of the gastro-intestinal tract can show significant inter- and intra-individual variability (Fallingborg et al., 1989), particularly in patients with inflammatory bowel disease, where the pH in the colon is often lower than in healthy subjects (Fallingborg et al., 1993; Hatton et al., 2018; Nugent et al., 2001). In combination with an accelerated transit time through the large intestine, particularly through inflamed regions (Haase et al., 2016; Hebden et al., 2000), this low pH poses a significant challenge for accurate colonic delivery triggered by pH alone.

Mesalazine 1600 mg is the highest strength oral mesalazine tablet developed and commercialised to date. It was designed with a new colonic delivery system (D'Haens et al., 2017) to overcome the aforementioned physiological variables and limitations. It consists of a multi-layered formulation with modified drug release designed to deliver mesalazine to the ileocolonic region for the induction and maintenance of remission in patients with mild-to-moderate UC. The coating technology consists of an outer layer of enteric polymer (Eudragit® S/resistant starch (high amylose starch, Amylo N-400) and a middle layer of partially neutralized Eudragit® S with drug release accelerating properties (Varum et al., 2020a; Varum et al., 2020b). Additionally, a hypromellose isolation layer separates the mildly acidic tablet core from the alkaline middle coating layer. The middle coating layer is designed to accelerate tablet disintegration once intestinal fluid has penetrated the outer coating after reaching the trigger pH 7 (Liu et al., 2010; Varum et al., 2013) or after pore formation due to digestion of the starch component of the outer coating layer by bacterial enzymes in the colon. Both triggers (pH and bacterial enzymes) (Allegretti et al., 2019; Dodoo et al., 2017; Ibekwe et al., 2008) in combination with the release-accelerating middle layer are designed to improve drug release, even under challenging conditions of low colonic pH and/or faster transit times at the terminal ileum and beyond.

Gamma-scintigraphy is one of the most common visualisation techniques to track gastrointestinal transit and disintegration of oral dosage forms *in vivo*, but it requires incorporation of a radioactive marker, such as Technetium (^{99m}Tc) or Indium (^{111}Indium), which have short half-lives. These nuclides are frequently added using a drill and fill technique, which limits a uniform distribution within the dosage form and may result in an unsynchronised release of the marker and the drug. Additionally, these radiomarkers require facilities authorised to handle radioactive compounds. Using neutron-activation, on the other hand, non-radioactive isotopes such as Samarium (^{152}Sm) can be handled in a standard GMP manufacturing facility, where the marker can be uniformly incorporated into the dosage form during a standard unit operation (e.g. granulation) (Sakkinen et al., 2006; Sakkinen et al., 2004). Afterwards, the non-radioactive ^{152}Sm isotope is converted into the radioactive ^{153}Sm isotope by neutron-activation. The uniform distribution of the marker is expected to improve visualisation of tablet disintegration, drug release and drug distribution within the colon.

The aim of this proof-of-concept study in healthy subjects and patients with mildly active UC was to characterize the release of mesalazine from formulations with three different and optimised outer layer coating compositions using pharmacokinetics and gamma-scintigraphy with neutron-activated ^{153}Sm to support the selection of a marketable formulation with optimized colonic release.

2. Materials and methods

2.1. Materials

Samarium oxide (99.99 % purity) was obtained from Alfa Aeasar, Kandel, Germany, mesalazine from Pharmazell, Raubling, Germany, hypromellose (Pharmacoat® 603 and Pharmacoat 606) from ShinEtsu, Tokyo, Japan. Microcrystalline cellulose and sodium starch glycolate (Explotab®) were obtained from JRS Pharma, Rosenberg, Germany.

Resistant starch (Amylo N-400) was sourced from Roquette-Fréres, Beinheim, France. Polysorbate 80 (Tween® 80), polyethyleneglycol 6000, magnesium stearate and 1-Butanol were supplied by Merck, Darmstadt, Germany. Ethanol 96 % was purchased from Thermofisher Scientific, Loughborough, UK. Eudragit® S100 and colloidal silica (Aerosil® 200) were obtained from Evonik, Darmstadt, Germany. Triethyl citrate was supplied by Jungbunzlauer, Ladenburg, Germany. Glyceryl monostearate was purchased from Hänseler AG, Herisau Switzerland. Sodium hydroxide was obtained from VWR International, Dietikon, Switzerland, buffer salts used for the preparation of dissolution buffers from Sigma-Aldrich; Buchs, Switzerland.

2.2. Manufacture of coated tablets

Tablet cores containing 1600 mg mesalazine and (in case of tablets used in the scintigraphy study) 5 mg of naturally abundant samarium oxide (Sm_2O_3, 99.9 % purity) were manufactured by a standard high-shear wet granulation process followed by compression into biconvex tablets. Samarium oxide was blended with mesalazine and wet granulated (high-shear granulation) by addition of a hypromellose (Pharmacoat® 603) binding solution. The wet granules were sieved and thereafter dried in a fluid bed drier until product temperature reached 42 °C. The dried granules were then blended with microcrystalline cellulose (Avicel® PH102) as filler, sodium starch glycolate (Explotab®) as disintegrant, colloidal silica (Aerosil® 200) as glidant and magnesium stearate as lubricant. Colloidal silica and magnesium stearate were blended with a portion of the compression blend and passed through a 1 mm sieve. The final blend was compressed into oblong tablets using a Fette P1200 rotary press. The resultant tablet cores were first coated with an isolation layer made of hypromellose and macrogol 6000 (as plasticizer); an inner coating of partially neutralised Eudragit® S adjusted to pH 8 containing a buffer salt (KH_2PO_4), triethyl citrate as plasticizer and glycerylomonostearate as glidant. The outer layer is composed of a mixture of a resistant starch and Eudragit® S, known as Phloral™ (Fig. 1), including also triethyl citrate (as plasticizer) and glycerylmonostearate (as glidant). Red and yellow iron oxides are used as pigments. Three different compositions of the outer layer were investigated: two semi-organic coatings were prepared with a ratio of Eudragit® S and resistant starch of 50:50 (formulation A) and 70:30 (formulation B), respectively. A third aqueous-based formulation was prepared with a ratio of Eudragit® S and resistant starch of 70:30 (formulation H). All coating steps were conducted using a perforated pan coater (Glatt, Binzen Germany) as previously described (Varum et al., 2020a; Varum et al., 2020b). Briefly, the isolation layer was coated to 3 mg/cm^2 with the following process parameters: spray rate 3.2 g/min, airflow 30 m3/h, atomizing air pressure 0.4 bar, outlet air temperature 41.3–43.5 °C. The middle layer was coated to 5 mg/cm^2 with the following process parameters: spray rate 3.25 g/min, airflow 40 m^3/h, atomizing air pressure 0.4 bar, outlet air temperature 40.3–42.7 °C. The outer layer was coated to 5 mg/cm^2 with the following process parameters:, airflow 40 m^3/h, atomizing air pressure 0.4 bar, outlet air temperature 33.1–35.8 °C.

2.3. In vitro drug release using biorelevant static and dynamic dissolution

Drug release from coated tablets (formulation A, B, H) was assessed by dissolution using a static or dynamic dissolution setup based on bicarbonate buffer and pH titration to simulate pH gradients found in the small and large intestine (Garbacz et al., 2014; Goyanes et al., 2015; Merchant et al., 2014).

The gastro resistance step was performed in a disintegration apparatus. The tablets were agitated for 2 h at 37 °C in 0.1 N HCl. Subsequently, the tablets were placed in vessels of a USP II dissolution system containing 900 mL of pH 7.4 Krebs buffer (static pH control) or modified

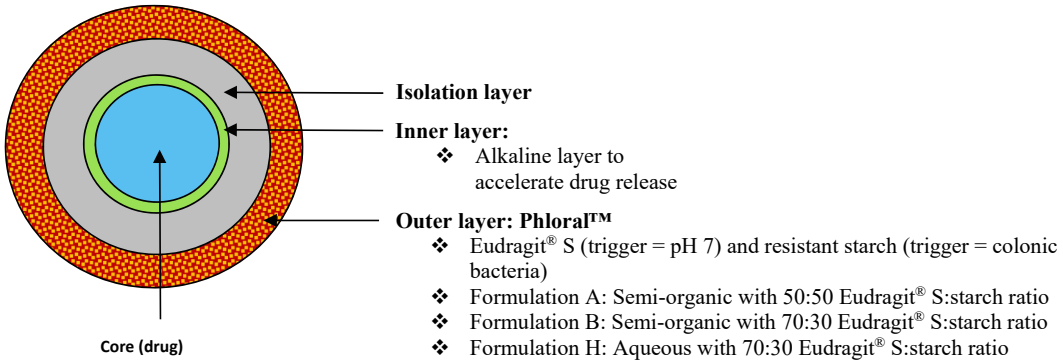

Fig. 1. Schematic representation of the OPTICORE™ coating technology displaying three alternative outer layer formulations (Formulation A, B, H) designed for colonic targeting.

Hanks buffer solution (with dynamic pH control) as described elsewhere (Fadda and Basit, 2005; Merchant et al., 2014). Temperature was kept constant at 37 °C and stirrer speed was set to 50 rpm. Released active ingredient was quantified online every 15–30 min by UV detection (λ = 310 nm). Each vessel was equipped with a separate pH electrode and two gas-inlet cannulas. By dynamic pH regulation, a biorelevant pH gradient was applied, mimicking the transit through the gastrointestinal tract (Goyanes et al., 2015). After 35 min, the media composition was changed by adding 50 mL of modified Krebs buffer solution. For a dissolution window of interest from 0 to 180 min, f2 values were calculated using a bootstrap approach (random seed 100, 5000 cycles), including only values >1 % release and not more than 1 value >85 %.

2.4. In vitro verification of tablet coating quality after neutron activation

Previous prototype coated tablets as used in an earlier clinical study NCT02306772 (semi-organic outer layer, 70 % Eudragit S: 30 % resistant starch, equivalent to formulation B) were used to evaluate the robustness of the coated tablets upon neutron-activation. Drug release from prototype coated tablets in simulated distal small intestinal fluid was assessed by dissolution tests in pH 7.4 Krebs buffer after pre-exposure to 0.1 N HCl for 2 h, as described previously (static pH setup) (Fadda and Basit, 2005; Varum et al., 2013). The lag time was determined as the first time point for which drug release was above 5 %. Tablets were tested after manufacture prior to neutron-activation and four weeks after neutron-activation (i.e. after sufficient decay of radioactivity).

2.5. Neutron-activation

Mesalazine coated tablets (formulation A, B, H) were irradiated for 75 s to achieve a final radioactivity of 1.4 GBq (MBq) of ^{153}Sm at the Reactor Institute Delft (Netherlands). This activity is sufficient to provide an activity dose of around 1 MBq of ^{153}Sm at the time of administration to the study participants. This activity dose corresponds to a radiation effective dose of approx. 0.8 mSv, which is below the 1 mSv yearly exposure limit for maximum yearly exposure to radioactivity according to Swiss legislation (Yeong et al., 2011). Upon irradiation, tablets were transported in a protective casing to the University Hospital Basel, Division of Nuclear Medicine, Switzerland where radioactivity was measured prior to administration to the study participants.

Prior to the clinical study, the influence of the neutron-activation process on mesalazine purity and drug release from coated tablets was assessed using tablets of a technical batch of the same composition as the clinical batch. After completion of radioactive decay, i.e. four weeks after neutron activation, coated tablets were analysed for mesalazine impurities and compared to initial impurity profiles. Samples were analysed using a reversed-phase high-performance liquid chromatography (RP-HPLC) method with a C18 column (Symmetry®, Waters Corporation, Milford, MA, USA) and a phosphate buffer/acetonitrile gradient containing tetrabutylammonium hydrogen sulphate as ion pairing reagent. Similarly, four weeks after neutron activation, dissolution experiments were performed as described above and dissolution profiles were compared with those of non-irradiated coated tablets.

2.6. Clinical study

The three different mesalazine tablet formulations were evaluated in an open-label, single-site pharmaco-scintigraphic clinical phase I study in healthy subjects and in patients with mildly active UC. Inclusion criteria for patients with mild UC activity were a stool frequency of 1–2 times per day above the normal frequency with or without occasional streaks of blood in the stool during the past week, whose clinical disease activity was considered as mild by the treating gastroenterologist. No formal power calculations were performed for this proof-of-concept study.

Formulations A and B correspond to the designations Coating D and E in the NCT02306785 study registration, respectively. The study (NCT02306785) was approved by the local ethics committee (Ethikkommission beider Basel, EKBB-Nr 126/12 and 163/13) and the Swiss Agency for Therapeutic Products (Swissmedic, Nrs 2012DR1136 and 2013DR1136). All participants provided written informed consent prior to the study. The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice Guidelines.

Healthy subjects were instructed to abstain from over-the-counter and prescription medication (including laxatives, vitamins and natural and herbal remedies) between the screening visit (visit 1) and completion of the study. Occasional paracetamol (maximum 1 g over 24 h) or acetyl-salicylic acid was allowed. Patients were allowed to take other medications as long as they did not have a direct pharmacological impact on GI motility and stool consistency. Oral or rectal mesalazine was not permitted on the treatment day.

Healthy subjects and patients with mildly active UC were instructed to fast from midnight before day 1. The study medication (radio-labelled mesalazine 1600 mg) was taken orally, unbroken and unchewed with 200 mL of water. Study participants remained fasted until 4 h post-dose and then were provided standardised meals (light lunch, snack and dinner at 4, 8, and 10 h post-morning dose). Healthy subjects and UC patients were alternately allocated to formulations A and B. After approval of a study amendment, another 9 study participants were allocated to formulation H. To keep the number of patients low it was planned to recruit at least 3 patients and 6 healthy volunteers per formulation.

2.7. Gamma scintigraphic image analysis

Planar scintigraphic images (anterior and posterior) were acquired

on a Siemens Symbia Intevo SPECT/CT device (Siemens, Erlangen, Germany) equipped with a low-energy, high-resolution collimator. A static acquisition before the tablet administration was used to assess background activity. At the same timepoints as for PK sampling (see below), static images (5 min) were acquired. Two ^{57}Co-labelled markers were placed on both iliac crests as anatomic reference points. The photopeak emission energy window for ^{153}Sm was set to 103 ± 10 %. Images were reconstructed using the proprietary iterative conjugate gradient algorithm on a Siemens Syngo workstation. No CT-based attenuation correction was applied. The localisation, transit time, location of and time to disintegration of each tablet were tracked periodically by planar scintigraphy. The recorded time of movement of the coated tablet from the stomach to the small intestine (gastric emptying) was taken as the mid-time between the times recorded for the last image of the tablet in the stomach and the first image of the tablet in the small intestine. Initial disintegration was defined as the initial spread of activity (as opposed to the focal, homogeneous activity on preceding timepoints) within the abdomen, confirmed at two consecutive time points to exclude artefacts by peristaltic movement.

2.8. Pharmacokinetic analysis

Concentrations of mesalazine and *N*-Acetyl-5-aminosalicylic acid were measured using a validated LC-MS/MS method (Tillotts Pharma AG, Rheinfelden, Switzerland) with a calibration range of 2.00–2′000 ng/mL (data on file). The plasma samples were fortified with the stable

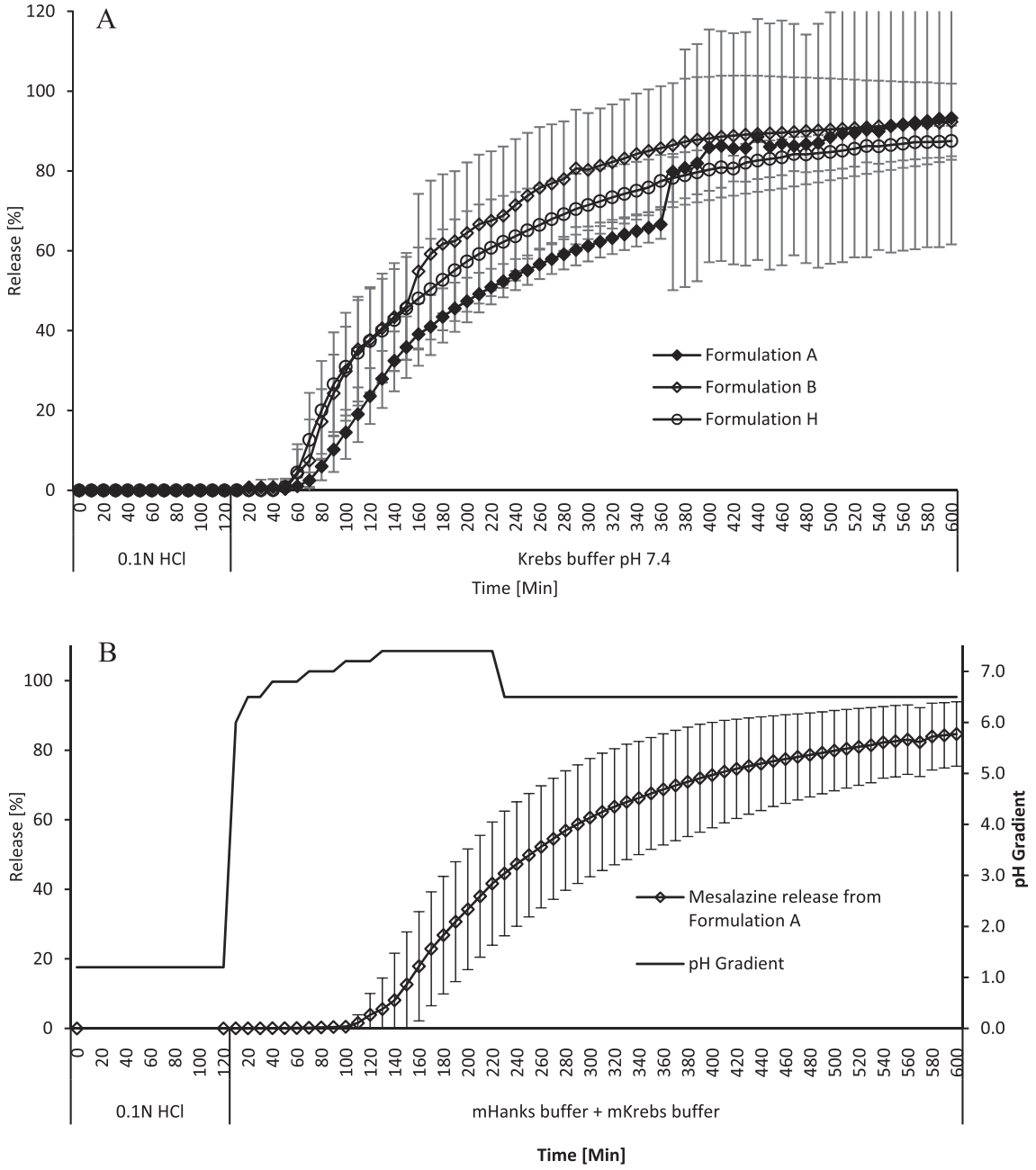

Fig. 2. Drug release from Formulation A, B and H coated tablets in pH 0.1 N HCl followed by Krebs buffer pH 7.4 (A) and drug release from Formulation A coated tablets in 0.1 N HCl followed by bicarbonate buffer with dynamic pH control (B). Release lagtimes for Formulations A, B, and H (50–110 min, 60–80 min, and 60–80 min, respectively) were overlapping. Considering a dissolution window of interest until 180 min, f2 values for the comparison of Formulation A vs B, A vs H, and B vs H were 44, 48, and 61, respectively.

labelled internal standards mesalamine-d3 and *N*-Acetyl-5-aminosalicylic acid-d3. Samples were precipitated with methanol and mesalazine was derivatised with propionic anhydride. After centrifugation, the supernatant was diluted with water containing 1 % formic acid. An aliquot of 20 µL of the sample was injected onto the HPLC system. Both analytes were quantified in the selected reaction monitoring mode using heated electrospray ionisation in negative ion mode.

Pharmacokinetic sampling was conducted pre-dose (0 h), then every hour for the first 16 h, and at 24 and 48 h. Between hours 4–8, sampling took place every 30 min. Urine was collected from all study subjects during the first 24-hours post-dose (three sampling periods: 0–8 h, 8–16 h, 16–24 h) to estimate total intestinal absorption of mesalazine. The maximum observed drug concentration (C_{max}), the time to reach C_{max} (t_{max}), and the time to the first sample with a quantifiable drug concentration (t_{first}) were obtained directly from the plasma concentration–time data. Plasma concentration–time profiles of mesalazine and *N*-Acetyl-5-aminosalicylic acid were analysed with non-compartmental analysis in WinNonlin (Version 5.2.1, Pharsight Corporation, Mountain View, CA, USA) and obtained pharmacokinetic parameters were summarised using descriptive statistics, including geometric mean, standard deviation (SD), minimum, maximum, median, and coefficient of variation (CV%) of geometric means. For mean value calculations, all below limit of quantification values were set to zero.

3. Results

3.1. In vitro drug release

Drug release from the three different formulations (A, B, H) in static pH 7.4 bicarbonate buffer showed that the three different tablet formulations were fully robust to simulated fasted gastric fluid and started to release under the conditions mimicking the distal ileum with a pH above 7 with comparable release lagtime and release rates (Fig. 2A). Drug release from Formulation A in biorelevant dissolution media with a pH gradient resembling human gastrointestinal pH is presented (Fig. 2B). With a dynamic pH control, drug release from coated tablets (Formulation A) is only initiated in conditions resembling the distal ileum (highest pH), showing good robustness to simulated gastric and upper small intestinal conditions.

3.2. Effect of neutron-activation

Inclusion of samarium oxide in the tablet core and subsequent exposure to the neutron-activation process did not have any significant impact on the quality of the produced tablets. The impurity profile after irradiation revealed only one single impurity above the disregard limit (0.02 %). 3-aminosalicylic acid was detected at a concentration of 0.037 %. This is far below the specified limit for any individual related substance (not more than 0.1 %). Therefore, the impurity profile shows the safety of the tablets after irradiation. Similarly, no detrimental effect was seen in gastric robustness and in drug release lag time in pH 7.4 Krebs buffer (Fig. 3).

3.3. Clinical study

Nineteen healthy subjects (median age 26.5 years, range 21.5–50.6 years, 12 male, 7 female) and seven patients (median age 32.2 years, range 26.4–52.8 years, 3 male, 4 female) with mildly active UC were enrolled, assessed and completed the study. Six healthy subjects and three patients received formulation A (50:50 ratio of Eudragit® S and resistant starch, semi-organic coating), six healthy subjects and two patients formulation B (70:30 ratio of Eudragit® S and resistant starch, semi-organic coating), and seven healthy subjects and two patients formulation H (70:30 ratio of Eudragit® S and resistant starch, aqueous coating). Due to difficulties recruiting eligible patients with mildly active UC for this study, only 2 instead of the planned 3 patients were allocated to each of the two formulations B and H, respectively. Mean (±SD) age for healthy subjects administered formulations A, B and H, was 27.10 (±4.91), 33.34 (±12.25) and 26.00 (±2.35) years, respectively, and ranged from 21.5 to 50.6 years. For patients administered formulations A, B and H, mean (±SD) age was 43.65 (±10.48), 37.85 (±13.49) and 28.21 (±2.61) years, respectively, and ranged from 26.4 to 52.8 years. The three formulations were well tolerated and no drug-related serious adverse events occurred. The most frequently reported

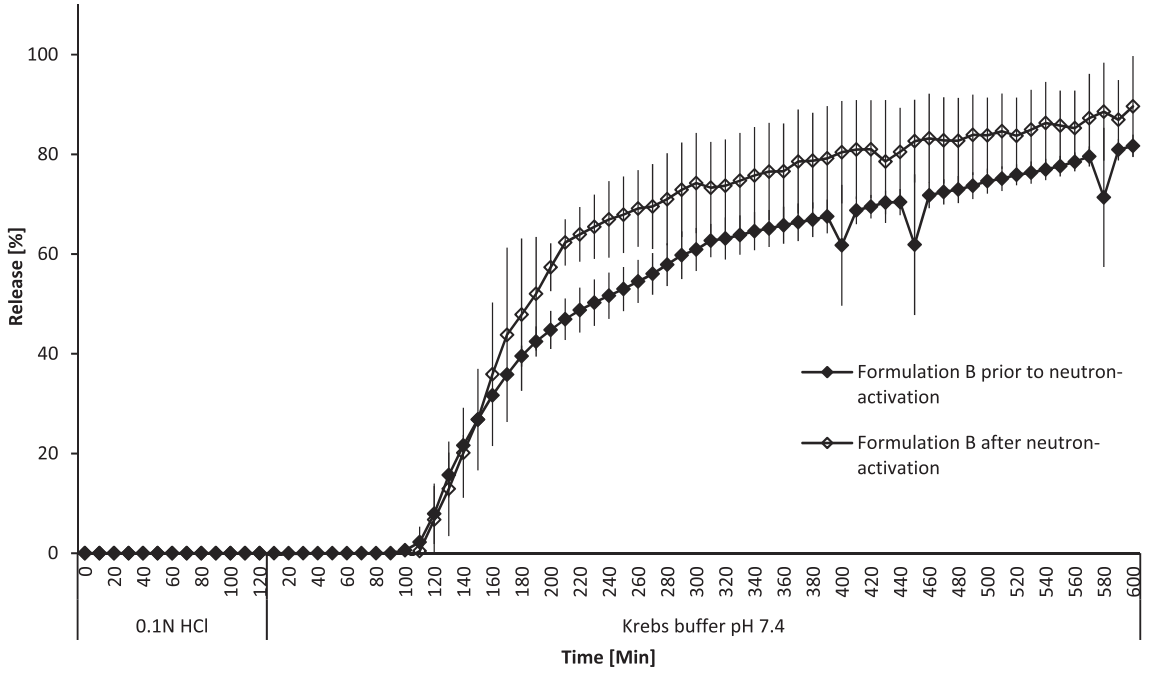

Fig. 3. The effect of the neutron-activation process on drug release from coated tablets (prototype equivalent to formulation B) in pH 7.4 Krebs buffer upon pre-exposure to 0.1 N HCl for 2 h. Tablets were tested before (release lagtime range: 120–140 min) and 4 weeks after neutron activation (release lagtime range: 110–130 min). f2 similarity factor (considering dissolution window of interest until 180 min: 64.2. Data are shown as mean ± standard deviation.

adverse event was headache of mild or moderate severity (54 %).

3.4. Time and location of tablet disintegration

Time and location of disintegration as well as residence and transit times of the three different tablet formulations obtained by gamma scintigraphic imaging are listed in Table 1. Initial tablet disintegration occurred at the ICJ or beyond in 24 of the 26 study participants (92 %). One tablet (formulation H, healthy volunteer) started to disintegrate in the small intestine and in one study patient (formulation B), no tablet disintegration was observed. Complete tablet disintegration (CTD) occurred in the right colon (either ICJ or AC) in 19 of 26 (73 %) study participants, with the highest rate for the aqueous formulation H (89 %) and lower rates for the two semi-organic formulations A and B (78 % and 50.0 %, respectively). In five subjects (19 %), CTD occurred in the transverse or descending colon and in two subjects (8 %) no CTD was observed (one subject with incomplete and one subject without tablet disintegration during entire observation period). An illustration of tablet disintegration and transit of radiolabelled tablet fragments along the colon in a study participant is shown in Fig. 4.

No significant differences regarding anatomical site of initial ($p = 0.7$) or complete ($p = 0.12$) tablet disintegration were found for the three different formulations (healthy subjects and patients combined). The median (interquartile range) time until initial tablet disintegration for formulations A, B, and H was 270 (240, 450), 300 (270, 540) and 270 (240, 330) minutes and for complete tablet disintegration 400 (270, 600), 540 (390, 720) and 390 (300, 480) minutes, respectively. Intra-individual variability was high and no statistically significant differences were observed between the three formulations ($p = 0.47$ for time to ITD, $p = 0.41$ for time to CTD). Residence and transit times were also highly variable, without significant differences between the three formulations (Table 1).

3.5. Pharmacokinetics

The concentration–time profiles for mesalazine and its main metabolite *N*-Acetyl-5-aminosalicylic acid after a single oral dose of mesalazine 1600 mg in the three tablet formulations A, B and H in fasted study subjects are shown in Fig. 5 and pharmacokinetic parameters are presented in Table 2. Data from one study patient (subject 502122, formulation H) with high mesalazine and *N*-Acetyl-5-aminosalicylic acid concentrations at baseline was excluded from the analysis.

The concentration–time profiles of mesalazine for all three tested formulations were characterised by a large inter-subject variability, which was lower for formulation H compared to formulations A and B. A median lag time between oral drug intake to the first measurable plasma concentration between 3.0 and 4.2 h was observed in all but one subject who had received formulation H (subj 502124). Maximal plasma concentrations (C_{max}) were reached after 5.0 to 8.3 h, with the highest C_{max} after administration of formulation H. Systemic mesalazine exposure ($AUC_{0\text{-}48h}$) was comparable for all three formulations.

There was a positive correlation of time to ITD with t_{first} and t_{max} (Fig. 6 A and B, Spearman $r = 0.48$ and 0.72, respectively, p less than 0.02 for both) and a significant negative correlation of time to ITD with C_{max} and $AUC_{0\text{-}48h}$ (Fig. 6 C and D, $r = -0.72$ and -0.68, respectively, p less than 0.001). For the main metabolite *N*-Ac-5-ASA maximal plasma concentrations were reached later, and C_{max} as well as AUC were higher compared to the parent compound. There were no statistically significant differences for any of the PK parameters between the tested formulations.

Table 1
Residence and transit times as well as time and location of initial and complete disintegration of the three coated tablet formulations in healthy subjects and patients with mildly active UC determined by gamma-scintigraphy.

Formulation	Study participants	Gastric retention time	Small intestinal transit time	Ileocecal junction residence time	Colonic arrival time	Initial tablet disintegration time	Initial site of disintegration (n subjects)	Complete tablet disintegration time	Site of complete tablet disintegration (n subjects)
A	Healthy (n = 6)	30 (30, 30)	195 (120, 210)	45 (30, 90)	315 (240, 390)	330 (240, 450)	ICJ (2)AC (3)TC (1)	500 (330, 660)	AC (4)TC (1)DC (1)
	Patient (n = 3)	30 (30, 90)	150 (90, 150)	30 (30, 60)	270 (240, 270)	240 (240, 540)	ICJ (1)AC (2)	270 (270, 540)	AC (3)
	All (n = 9)	30 (30, 30)	150 (120, 210)	30 (30, 60)	270 (240, 360)	270 (240, 450)		400 (270, 600)	
B	Healthy (n = 6)	30 (30, 60)	180 (120, 180)	45 (30, 60)	285 (240, 315)	390 (270, 540)	ICJ (1)AC (2)TC (2)DC (1)	570 (390, 720)	AC (3)DC (3)
	Patient (n = 2)	60 (30, 90)	105 (90, 120)	75 (30, 120)	240 (240, 240)	270 (270, 270)[1]	AC (1)	300 (300, 300)[1]	AC (1)no TD (1)
	All (n = 8)	30 (30, 75)	150 (120, 180)	45 (30, 90)	255 (240, 310)	300 (270, 540)		540 (390, 720)	
H	Healthy (n = 7)	30 (30, 60)	150 (90, 180)	30 (30, 90)	240 (240, 330)	270 (240, 330)	SI (1)ICJ (1)AC (5)	360 (300, 480)	AC (7)
	Patient (n = 2)	105 (30, 180)	105 (60, 150)	30 (30, 30)[1]	320 (240, 400)	420 (240, 600)	AC (1)TC (1)	420 (420, 420)[1]	AC (1)[2]
	All (n = 9)	30 (30, 60)	150 (90, 150)	30 (30, 80)	240 (240, 330)	270 (240, 330)		390 (300, 480)	
p-value for within-group comparison[3]		0.905	0.527	0.902	0.688	0.473		0.412	

Data are given as median (Q1, Q3) in minutes. AC; ascending colon, DC; descending colon, ICJ; ileocecal junction, SI: small intestine; TD, tablet disintegration; TC; transverse colon. [1]Result from 1 patient, [2]one patient with incomplete release, [3]healthy volunteers and patients combined.

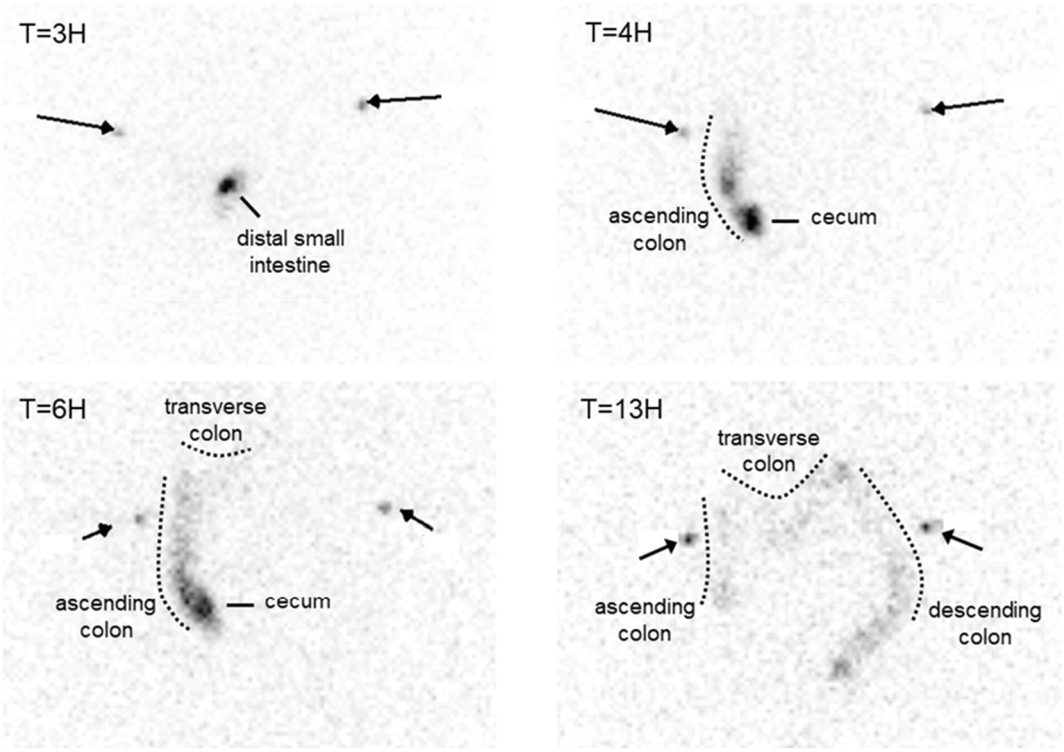

Fig. 4. Visualization of tablet disintegration and intra-intestinal distribution of labelled tablet content using gamma-scintigrapic imaging. Images of one study participant (subject 5) after intake of formulation A are shown at 4 different timepoins. T = 3H (3 h after tablet intake); intact tablet in the distal small intestine, T = 4H; initial tablet disintegration in the cecum with release and transport of radiolabelled tablet particles in the ascending colon, T = 6H; transport of labelled tablet particles from the acending colon into the right transverse colon, T = 13H; labelled tablet particles spread along the transverse and descending colon. Arrows indicate ^{57}Co-labelled markers on iliac crests as anatomic reference points.

Within the first 24 h, approx. 20 % of the administered dose was excreted in the urine, mainly in form of *N*-Acetyl-5-aminosalicylic acid and less than 3 % as unchanged mesalazine. While excretion of 5-ASA was almost complete within the first 24 h after drug administration, this was not the case for the acetylated metabolite, and therefore the absorbed amount could not be reliably estimated. There was a high correlation between the cumulative amount of renally excreted 5-ASA and *N*-Ac-5-ASA with total systemic exposure of 5-ASA (R = 0.93, Supplementary Figure 1). Intersubject variability was high and there were no significant differences in the excretion profiles between the three formulations (Supplementary Figure 2).

4. Discussion

In this study, we used pharmaco-scintigraphic imaging to characterise the release of ^{153}Sm-labelled mesalazine tablets from three different high-dose formulations designed for colonic drug delivery in healthy volunteers and patients with mildly active ulcerative colitis.

Overall, colonic targeting was successfully achieved with initial tablet disintegration at the ileocecal junction or beyond in more than 90 % and complete tablet disintegration in the right colon in more than 75 % of the study participants for two of the three formulations. Although inflammation in UC patients typically starts in the distal parts of the colon, inflammation progresses to more proximal parts of the colon in many patients, and some patients also have patches of cecal inflammation (Ordás et al., 2012). As mesalazine is not well absorbed from the colon it remains topically available and proximal release in the colon is thus of advantage to cover various stages of disease extension throughout the colon.

Although the combination of two release triggers in the tested formulations provided more reliable targeting compared to other formulations using e.g. pH-change as sole trigger, the lower rate of complete tablet disintegration in the target region and the two cases without complete disintegration illustrate the difficulties of targeting the colon with oral formulations, particularly with single-unit dosage forms. Gastrointestinal pH drops between the ileum and the colon and in some gastrointestinal diseases, such as in UC (Hatton et al., 2018), the pH in the colon is often lower than in healthy individuals (Fallingborg et al., 1993; Nugent et al., 2001). Additionally, transit time through the colon may be accelerated, particularly through inflamed regions (Haase et al., 2016; Hebden et al., 2000). This presents a challenge for the drug release from enteric-coated dosage forms in the colon, especially for large single-dose units, which typically have faster transit times than multiparticulates (Varum et al., 2010) (Abrahamsson et al., 1996). Some studies have reported an occasional excretion of intact Eudragit® S coated tablets (McConnell et al., 2008). Another high-strength mesalazine formulation (1200 mg, MMX®, Lialda™/Mezavant™) designed to achieve sustained release throughout the colon, showed an initial tablet disintegration time of 4.8 ± 1.3 h and a complete disintegration time 17.4 ± 8.6 h (Wray et al., 2008a; Wray et al., 2008b). This initial tablet disintegration time is comparable to the values observed for Formulation A, B and H. However, time to complete disintegration was significantly shorter in the present study which may allow for a more proximal colonic release and better mesalazine dissolution due to the higher volume of fluid available in proximal compared to more distal parts of the colon (Schiller et al., 2005). In another pharmaco-scintigraphy study using ^{153}Sm as radiomarker, the OPTICORE™ coated capsules, containing immediate release metronidazole benzoate granules, showed a high-colon targeting accuracy (8 out 9 healthy subjects). This was despite the considerable variability in colonic arrival times, ranging between 3.5 and 9 h and with initial disintegration between 3 and 12 h. The colonic release of metronidazole benzoate resulted in significant reduction in systemic exposure in comparison to a reference immediate release formulation (Preisig et al., 2021). In the fasted state, mesalazine

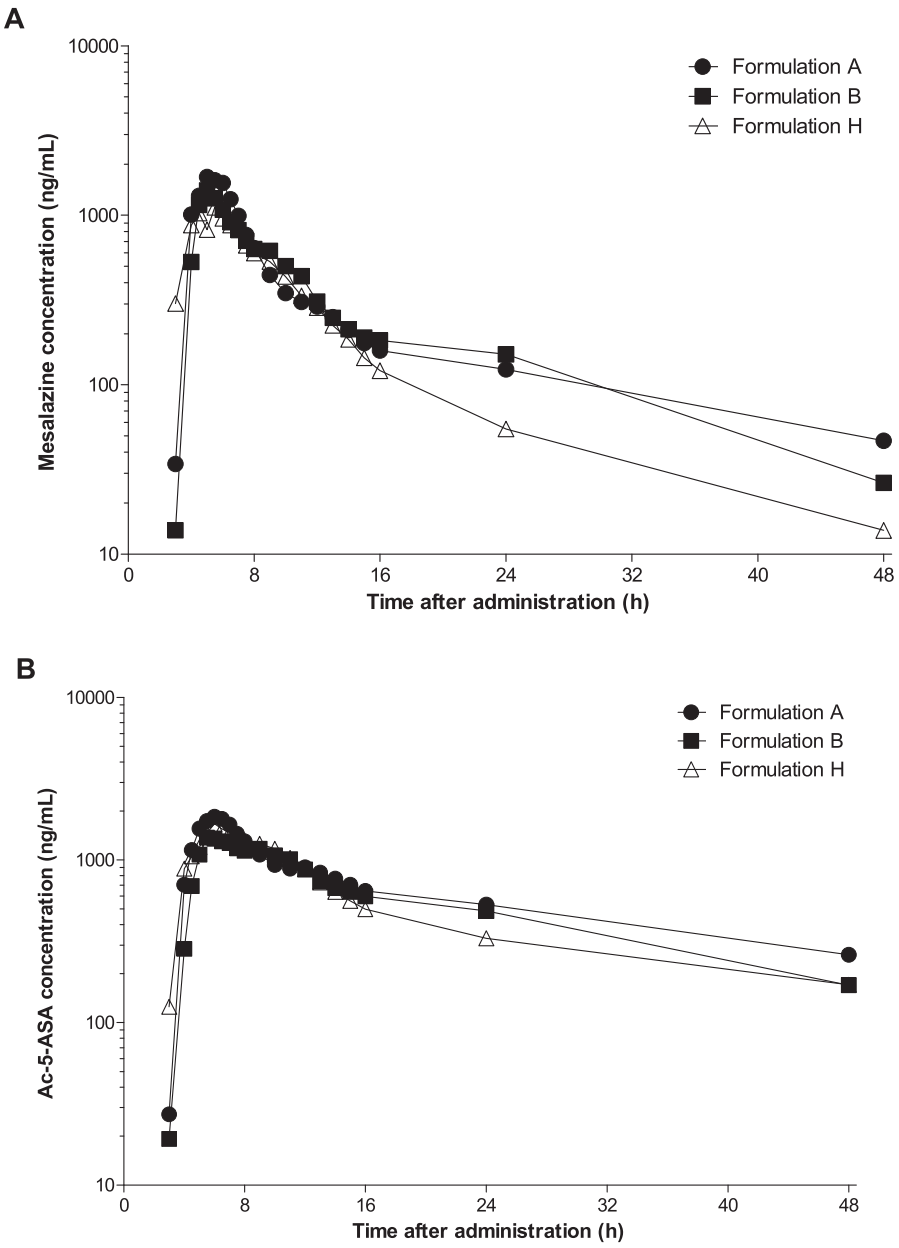

Fig. 5. Mean plasma concentration versus time profiles of mesalazine (A) and *N*-Acetyl-5-aminosalicylic acid (*N*-Ac-5-ASA) (B) after administration of a single dose of 1600 mg coated tablet from formulation A, B and H.

tablets coated with low-viscosity HPMC (time-dependent release) and Eudragit® L (pH-dependent release) showed a colonic arrival time of 3.96 h ± 1.49 h (n = 6) with tablet disintegration occurring at approximately 5 h later (9.46 ± 3.19), significantly later that disintegration times observed in our study (Foppoli et al., 2019).

The three formulations differed regarding the ratio of enteric polymer (Eudragit® S) to polysaccharide (resistant starch) and the solvent used in the outer layer preparation (semi-organic vs aqueous). Independent of the coating composition, formulations were fully robust to simulated gastric fluid and drug release in biorelevant pH 7.4 bicarbonate buffer, simulating the terminal ileum (Fadda and Basit, 2005). Therefore, *in vivo* disintegration times would be expected to be similar when pH is the main release trigger. Several factors such as differences in the content of resistant starch in the coating, the film formation mechanism of the coating (influenced by the solvent composition) and variations in microbiome composition on the other hand may affect *in vivo* disintegration triggered by bacterial enzymes. In the case of semi-organic coatings, the enteric polymer is fully solubilised in ethanol, therefore, film formation occurs due to the solvent evaporation during the coating process. This results in the polymer chains moving closer together until they enter into contact. The solvent and added plasticiser contribute to the film elasticity. In the case of aqueous based coatings, an aqueous polymer dispersion (polymer not dissolved), also known as polymer latex, is used. After water evaporation, the individual polymer particles move closer together and with the contribution of higher plasticiser quantity, they coalesce upon contact to form a homogeneous and flexible film. Formulation B with the lowest target rate and the longest median time to initial and complete disintegration had a lower proportion of starch in the outer coating (30 %, based on polymer) than formulation A (50 %). Accordingly, a lower surface area is available for degradation by bacterial enzymes, as more enteric polymer surrounds the polysaccharide. This potentially decreases the effectiveness of the enzymatically triggered release and, together with microbiome variations (especially in UC patients), may explain the higher variability and

Table 2
Pharmacokinetic parameters of mesalazine (5-ASA) and *N*-Acetyl-5-amino-salicylic acid (*N*-Ac-5-ASA) in plasma after the administration of one tablet of Formulation A, B and H to fasted healthy subjects or patients with mildly active UC.

Formulation	Subject	5-ASA			
		t_{first} (h)	t_{max} (h)	c_{max} (ng/ml)	AUC_{0-48h} (ng·h/ml)
A	Healthy (n = 6)	4.0 (3.0/4.5)	5.7 (4.0/24.0)	1075 (118)	8327 (73)
	Patient (n = 3)	3.0 (3.0/4.0)	9.9 (4.0/13.9)	833 (145)	8376 (64)
	all (n = 9)	4.0 (3.0/4.5)	6.0 (4.0/24.0)	987 (339)	8340 (106)
B	Healthy (n = 6)	4.2 (3.0/5.5)	11.5 (5.0/15.9)	697 (188)	8616 (104)
	Patient (n = 2)*	4.3 (4.0/4.5)	6.0 (4.5/7.5)	317 (112)	1584 (138)
	all (n = 8)	4.2 (3.00/5.5)	8.3 (4.5/15.9)	572 (227)	5640 (319)
H	Healthy (n = 7)	3.0 (3.0/5.0)	5.5 (4.0/8.0)	1895 (53)	7294 (25)
	Patient (n = 2)**	1.0	4.1	1530	13,200
	all (n = 9)	3.0 (1.0/5.0)	5.0 (4.0/8.0)	1850 (54)	7860 (35)
Formulation	Subject	*N*-Ac-5-ASA			
		t_{first} (h)	t_{max} (h)	c_{max} (ng/ml)	AUC_{0-48h} (ng·h/ml)
A	Healthy (n=6)	3.98 (3.00/4.50)	9.9 (6.00/48.00)	1419 (171)	22,359 (53)
	Patient (n=3)	3.03 (2.97/4.00)	11.0 (4.55/13.90)	1952 (116)	31,316 (15)
	all (n=9)	3.97 (2.97/4.50)	11.0 (4.55/48.0)	1580 (140.6)	25,000 (54.6)
B	Healthy (n=6)	4.24 (3.00/5.53)	12.44 (5.50/15.90)	1393 (192)	24,508 (52)
	Patient (n=2)	4.25 (4.00/4.50)	6.98 (6.00/7.97)	399 (153)	5052 (250)
	all (n=8)	4.24 (3.00/5.53)	9.44 (5.50/15.9)	1020 (174.2)	16,500 (183.7)
H	Healthy (n=7)	3.00 (3.00/5.03)	6.02 (4.00/9.87)	2166 (33)	22,908 (25)
	Patient (n=2) **	1.03	4.08	1120	13,600
	all (n=9)	3.00 (1.03/5.03)	6.01 (4.00/9.87)	1990 (42.9)	21,500 (31.6)

Results are presented as geometric mean (CV%) for C_{max}, AUC_{0-48h} and median (min/max) for t_{first}, t_{max}.
*1 patient with no scintigraphic evidence of tablet disintegration and very low concentrations.
**1 patient excluded from PK analysis due to high predose mesalazine concentrations (subj502122, formulation H).

later disintegration times. On the other hand, formulation H, which also had a lower proportion of starch (30 % based on polymer), showed a less variable and more proximal tablet disintegration. This could be explained by facilitated enzymatic degradation of the starch in the aqueous based formulation since aqueous enteric coatings are typically less robust than organic coatings (Bando and McGinity, 2006; Thoma and Bechtold, 1999).

Using neutron-activation to convert non-radioactive samarium oxide into a radioactive label allowed a uniform incorporation of the marker into the tablet core in a standard GMP manufacturing facility. The radioactive marker was granulated together with the active molecule mesalazine, resulting in a uniform distribution within the tablet core, which is a prerequisite for optimal visualisation of mesalazine distribution within the colon after tablet disintegration. However, neutron-activation can potentially induce changes in a drug product (Ahrabi et al., 1999a; Watts et al., 1993). The coatings tested in this study comprise different polymers, such as Eudragit® S and resistant starch, which could be sensitive to the neutron-activation process with possible breakdown of the polymer chains and alteration of the properties of these excipients with potential impact on drug product performance. Detrimental effects after exposure to neutron-activation have been described, such as accelerated drug release from chitosan granules (Sakkinen et al., 2004) or other polymeric excipients (Ahrabi et al., 2000; Ahrabi et al., 1999a,b; Watts et al., 1993). In our study, no significant impact on drug purity or tablet quality was observed for the tested formulation (Formulation B), indicating that the neutron-activation process did not alter the properties of the coating and thus was a suitable method for marker activation. The lack of impact of the neutron-activation process on this coating technology (equivalent for Formulation A), applied on capsules, has also recently been described (Preisig et al., 2021). Since the only difference between Formulation B and H is the solvent composition, no differences in terms of neutron irradiation process would be expected in the aqueous-based coated tablets (Formulation H).

Neutron-activation of the marker within a few hours before application of the study drug to study participants allowed near real-time visualisation of the gastro-intestinal transit of the intact tablet and subsequently of the released labelled mesalazine-containing granules. Inter-individual retention times varied significantly, with gastric retention ranging from less than 60 min to more than 3 h. In the fasted state, gastric motility is under the influence of the migrating myoelectric complex (MMC) (Varum et al., 2010). Gastric emptying of single-units typically occurs during Phase III ("housekeeper wave", higher magnitude contractions) of the MMC cycle. However, some MMC cycles bypass the stomach and originate in the small intestine, which may contribute to a longer gastric retention of modified dosage forms, particularly, single-units in the fasted state (Kellow et al., 1986). The small intestinal transit, which is less affected by food or dosage formulation type (Davis et al., 1986) lasted for a median of 150 min and showed lower variability than gastric emptying, which is in line with the literature (Davis et al., 1986; Fischer et al., 2017).

Coupled with gamma-scintigraphic imaging, pharmacokinetic assessment can provide additional insights into the *in vivo* performance of different dosage forms. Time to first quantifiable and maximal drug plasma concentration correlated with the time to initial tablet disintegration obtained from scintigraphic imaging. Interestingly, in most study subjects, quantifiable mesalazine plasma concentrations appeared before tablet disintegration could be observed, indicating that a small amount of drug was already released from the intact tablet, possibly due to partial disruption of the coating prior to scintigraphically observable tablet disintegration. On the other hand, maximal plasma concentrations and systemic exposure were inversely correlated with time to initial tablet disintegration. This indicates lower drug absorption from more distal parts of the gastrointestinal tract, a prominent advantage of colonic drug delivery, resulting in higher local drug concentrations and reduced risk for adverse concentration-dependent systemic effects.

The main limitation of this proof-of-concept study is the small number of study participants with either normal or only minimally altered gastrointestinal physiology. Therefore, no conclusions can be drawn regarding potential differences between the different formulations and no extrapolations can be made regarding the performance of the formulations in patients with more active gastrointestinal disease. More frequent sampling and imaging, particularly beyond 8 h after study drug administration would have improved the detection of potential differences between the tested formulations during the complete

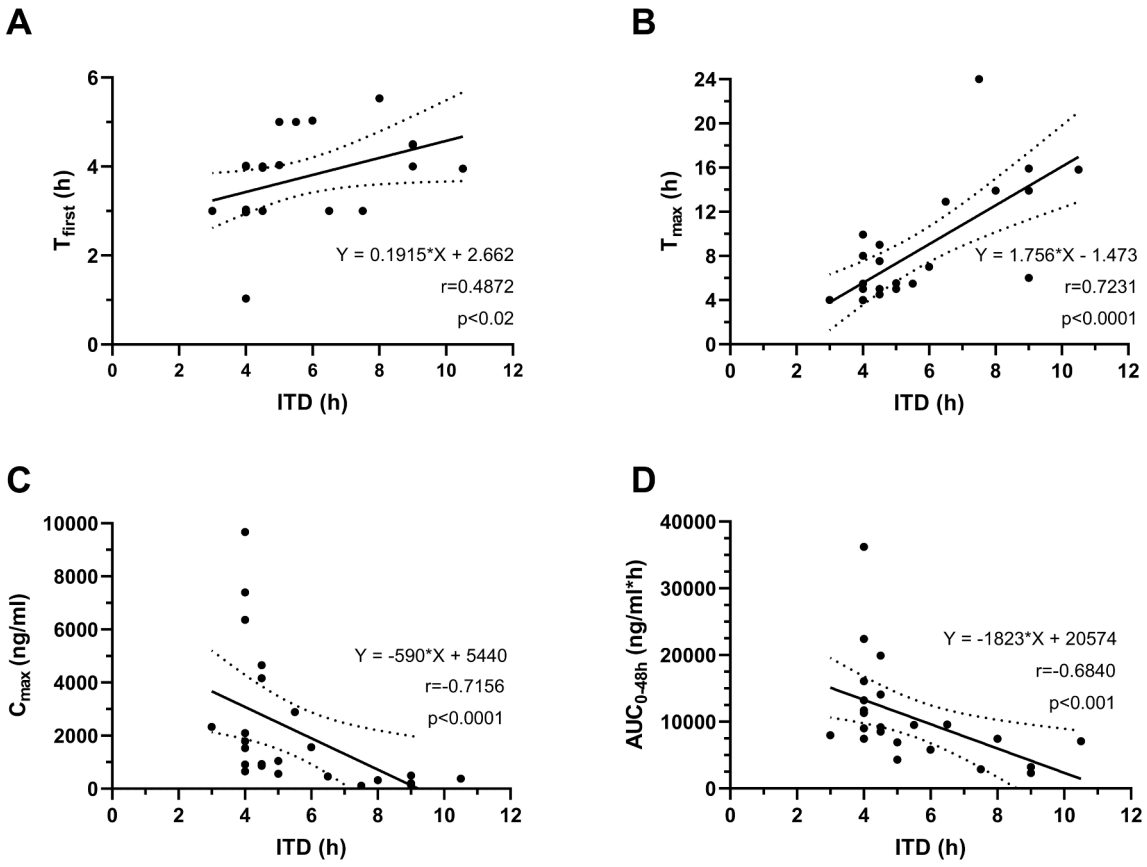

Fig. 6. Correlation between initial disintegration time (ITD) and time (hours) to first quantifiable mesalazine concentration Tfirst (A), time to maximal mesalazine concentration Tmax (B), maximal mesalazine concentration Cmax (C), and area under the plasma concentration time courve AUC0-48 h (D). Solid line, linear regression; dotted lines, 95 % confidence interval; r, Spearman coefficient of correlation.

tabled disintegration phase. However, the trial provided important information concerning robustness of the formulations during transit in the upper gastrointestinal tract and allowed for the planning of subsequent patient studies (D'Haens et al., 2017).

5. Conclusions

In summary, the three tested coatings were robust to upper gastrointestinal conditions both *in vitro* and *in vivo*. The neutron-activation process allowed manufacturing in a standard GMP facility and did not change properties of the coating or induce degradation of the active ingredient. In subjects with normal or minimally altered gastrointestinal physiology, accurate colonic targeting with large high-strength tablets could be achieved in more than 75 % of the cases either with a semi-organic coating containing 50 % starch or with an aqueous based coating containing 30 % starch. Scintigraphic imaging using neutron-activated, samarium-labelled tablets in combination with pharmacokinetic analysis were useful tools for this proof-of concept study.

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Trademark statement

The rights to the OPTICORE™ technology including the rights to the trademark, are owned by Tillotts Pharma AG in various countries. The rights to the Phloral™ technology, including the rights to the trademark, are owned by UCL School of Pharmacy in various countries.

CRediT authorship contribution statement

F. Varum: Conceptualization, Formal analysis, Writing – review & editing, Visualization. **H. Thorne:** Writing – review & editing. **R. Bravo:** Conceptualization, Writing – review & editing, Supervision. **D. Gilgen:** Project administration. **C. Hartig:** Investigation, Writing – review & editing, Visualization. **G.P. Nicolas:** Investigation, Visualization, Formal analysis. **D. Wild:** Methodology, Formal analysis, Writing – review & editing. **E. Liakoni:** Investigation, Writing – original draft. **M. Haschke:** Conceptualization, Formal analysis, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Manuel Haschke reports financial support was provided by Tillotts Pharma AG. Felipe Varum, Helen Thorne, Roberto Bravo, Denise Gilgen, Claudia Hartig reports a relationship with Tillotts Pharma AG that includes: employment. Felipe Varum, Roberto Bravo has patent issued to Tillotts Pharma.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2022.122055.

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