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A dual pH and microbiota-triggered coating (Phloral™) for fail-safe colonic drug release

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Abstract

Enteric-coated dosage forms are widely used for targeting the ileo-colonic region of the gastrointestinal (GI) tract. However, accurate targeting is challenging due to intra- and inter-individual variability in intestinal paramaters such as fluid pH and transit times which occasionally lead to enteric coating failure. As such, a unique coating technology (Phloral™), which combines two independent release mechanisms - a pH trigger (Eudragit® S; dissolving at pH 7) and a microbiota-trigger (resistant starch), has been developed, offering a fail-safe approach to colonic targeting. Here, we demonstrate that the inclusion of resistant starch in the coating does not affect the pH mediated drug release mechanism or the robustness of the coating in the upper GI tract. In order to make the resistant starch more digestible by bacterial enzymes, heat treatment of the starch in the presence of butanol was required to allow disruption of the crystalline structure of the starch granules. Under challenging conditions of limited exposure to high pH in the distal small intestine and rapid transit through the colon, often observed in patients with inflammatory bowel disease, particularly in ulcerative colitis, this dual-trigger pH-enzymatic coating offers a revolutionary approach for site specific drug delivery to the large intestine.

Keywords

Mesalazine, Gastro resistant film coatings, Intestinal microbiota triggered drug release, Oral colon targeted drug products, Colonic targeting and drug delivery systems, Gut microbiome

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1 Introduction

Accurate colonic targeting is of paramount importance for the treatment of inflammatory bowel disease (IBD), such as ulcerative colitis (UC) and Crohn's disease (Klotz and Schwab 2005, Cohen 2006). Furthermore, this concept is also applicable for the treatment of colonic cancers, vaccination (McConnell *et al.* 2008a) or for the systemic delivery of drug molecules, such as peptides or proteins (Mackay *et al.* 1997, Rubinstein *et al.* 1997, Luppi *et al.* 2008, Wang *et al.* 2015a, Yadav *et al.* 2016), which could benefit from a lower proteolytic activity in the colon (Yadav *et al.* 2016, Bak *et al.* 2018, Lee *et al.* 2020). Current approved drug products mainly exploit a single variation in GI physiology from pH, transit time or the increase in bacterial concentrations in the distal gut to trigger drug release (McConnell *et al.* 2009, Goyanes *et al.* 2015a, Goyanes *et al.* 2015b). The former approach uses a pH responsive polymer coating, such as Eudragit® S, which dissolves at pH 7.0 to trigger drug release as the intestinal pH increases distally to a maximum at the ileocaecal junction (Evans *et al.* 1988, Fallingborg *et al.* 1989, Liu *et al.* 2010, Varum *et al.* 2013). Due to this approach, a range of products including Asacol™ and Mezavant/Lialda® are available as first-line treatments of mild to moderate IBD conditions, such as UC, Crohn's disease and collagenous colitis (Goyanes *et al.* 2015c, Ma *et al.* 2019).

Despite the clinical success achieved by pH-responsive drug delivery systems in the treatment of UC, occasionally intact tablets can be found in the stools of patients (Schroeder *et al.* 1987, McConnell *et al.* 2008c). This negative effect may be explained by an inadequate exposure to sufficient fluid volumes of pH 7 or higher for the required period of time during distal gut transit. The luminal colonic pH is reported to be lower in patients with UC when compared to healthy subjects (Fallingborg *et al.* 1993, Nugent *et al.* 2001). However, inter- and intra-individual variability also plays a role in GI pH under healthy and disease conditions (Fallingborg *et al.* 1989, Fallingborg *et al.* 1993, Nugent *et al.* 2001, Ibekwe *et al.* 2008a, Hatton *et al.* 2015, Hatton *et al.* 2018). Furthermore, the IBD patient population is diverse and some

subjects with active UC have been found to have faster small intestinal transit and colonic transit compared to healthy volunteers, at least through the inflamed region. However, other UC patient populations have been shown to have prolonged small intestinal and/or colonic transit times (Rao *et al.* 1987, Hebden *et al.* 2000, Haase *et al.* 2016, Fischer *et al.* 2017). Further complicating the scenario is the fact that the fluid available in the distal small intestine and large intestine is scarce and often found in pockets (Schiller *et al.* 2005), which hinders the process of coating and drug dissolution (Fadda *et al.* 2010). Therefore, promoting drug release using alternative triggers, such as a bacterial trigger (Basit *et al.* 2002, Tuleu *et al.* 2002, Siew *et al.* 2004, McConnell *et al.* 2008b, Karrout *et al.* 2010, Karrout *et al.* 2015), or with a combination of physiological triggers can be beneficial. Ibekwe and co-workers proposed the combination of a pH and enzymatic trigger in a single coating system, marketed as Phloral™ (Ibekwe *et al.* 2008b). The enzymatic trigger, provided by the presence of a polysaccharide in the coating (resistant high amylose starch) acts as a fail-safe mechanism in case the pH is not high enough over a sufficient period of time to allow dissolution of the enteric polymer (Eudragit® S), which is designed to dissolve at pH 7 (Ibekwe *et al.* 2008b, Dodoo *et al.* 2017).

In order to inhibit drug release during transit in the upper GI tract, allowing delivery to the ileo-colonic region, the starch embedded in the film must resist digestion by salivary and pancreatic amylases. Resistant starch is generally considered the fraction that escapes digestion in the small intestine due to pancreatic amylase action (Topping and Clifton 2001). Resistant starch is currently classified as 4 different types, namely RS_1 (physically inaccessible), RS_2 (resistant granules, such as high amylose starches), RS_3 (retrograded starch) and RS_4 (chemically modified starch) (Topping and Clifton 2001). RS_2 starches, such as those with high content of amylose (Amylo N-400, Roquette), require a much higher temperature to gelatinize and often do not completely gelatinize, being less digestible by amylases in the upper GI tract (Topping and Clifton 2001).

In the stomach the levels of bacteria are less than 10^2 CFU/ml due in part to low gastric pH (Simon and Gorbach 1984). The number of microorganisms increases gradually along the small intestine, but rises by several orders of magnitude beyond the ileocaecal junction. It has been reported that the colon contains over 400 distinct species of bacteria with a population of 10^{11}-10^{12} CFU/ml (Simon and Gorbach 1984, Eckburg *et al.* 2005). Others have postulated that the number of bacteria species may go up to 36000, considering the inter-individual variability (Frank *et al.* 2007, Sekirov *et al.* 2010). These bacteria are mainly anaerobes or facultative anaerobes; *Bacteroides*, *Clostridium groups IV and XIV*, *and Bifidobacteria* are the predominating regulatory species (Abu-Ghazaleh *et al.* 2020, Fadda 2020, Mishima and Sartor 2020). Colonic bacteria use undigested polysaccharides as their main source of fermentable carbohydrate (Hamer *et al.* 2011) and play a significant role in the metabolism of orally administered drugs (Clarke *et al.* 2019, Zimmermann *et al.* 2019a, Zimmermann *et al.* 2019b). In terms of treatment of IBD, drug products which rely on colonic bacteria to render the active drug at the site of disease are also available. These are prodrugs, such as sulfasalazine, balsalaside and olsalazine, which are enzymatically-activated by bacteria releasing the active moiety 5-aminosalicylic acid (5-ASA) (Sousa *et al.* 2008). The pro-drug approach is highly drug specific. Therefore, more flexible drug delivery systems relying on gut microbiota have been developed (Basit *et al.* 2002, Tuleu *et al.* 2002, Siew *et al.* 2004, McConnell *et al.* 2008b, Karrout *et al.* 2010, Karrout *et al.* 2015).

Here we aim to investigate the relationships between the changes in the physical properties of resistant starch that occur upon processing and their impact on the performance of Phloral™ coated tablets. A further objective is to demonstrate that these novel compositions comprising an enteric polymer and polysaccharide can provide more accurate and fail-safe colonic targeting, in comparison with tablets relying solely on standard enteric coatings.

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2 Material and methods

2.1 Materials

Lactose monohydrate was obtained from Meggle, Wasserburg Germany. Povidone (K25) was acquired from Boai NKY Pharmaceuticals Ltd, Jiaozuo, China, and sodium starch glycolate was obtained from JRS Pharma, Rosenberg, Germany. Magnesium stearate was provided by Peter Greven GmbH & Co. KG, Bad Münstereifel, Germany. Talc was obtained from Ferdinand Kreuzter Sabamühle GmbH, Nürnberg, Germany. Eudragit® S was purchased from Evonik, Darmstadt, Germany. Maize starch (Amylo N-400 formerly known as Eurylon 6) was purchased from Roquette, Lestrem, France. Polysorbate 80 (tween 80), butan-1-ol, sodium hydroxide were all purchased from Sigma-Aldrich, Buchs, Switzerland. Potassium dihydrogen phosphate, glyceryl monostearate (GMS) and triethyl citrate (TEC) were all purchased from VWR international LTD, Poole, UK. Trifluoroacetic acid and buffer salts used for dissolution buffers preparation were obtained from Sigma-Aldrich, UK. Sodium chloride and di-potassium hydrogen phosphate were obtained from Fisher Chemical. Magnesium sulphate heptahydrate and calcium chloride hexahydrate were obtained from VWR, UK. Sodium bicarbonate was obtained from Sigma Aldrich, UK, while haemin, L-cysteine HCl, vitamin K and resazurin were obtained from Sigma Life Sciences, UK. Bile salts were from Fluka Analytical, UK and FlukaChemika, UK.

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2.2 Methods

2.2.1 Starch suspension characterisation

2.2.1.1 Starch heat treatment

Aqueous starch dispersion was prepared by dispersing maize starch in butanol, followed by water, while stirring magnetically. The ratio of maize starch : butanol : water was 1 : 2 : 22. The resulting dispersion was heated to a temperature ranging between 50°C and 92°C (boiling) and left at this temperature for 90 minutes (Table 1). The dispersions were then cooled at room temperature while stirring overnight. The % solids content of the cooled preparation was calculated based on the final weight of the dispersion (considering the evaporation during heating).

2.2.1.2 Polarised light microscopy (PLM)

The effect of gelatinisation on the morphology of starch granules and birefringence can be assessed qualitatively by PLM. Raw starch granules show a characteristic maltese cross pattern and refract polarised light through their intact crystalline regions giving rise to one of the most unique characteristics of starches i.e. its birefringence. Starch gelatinisation results in starch granule swelling and amylose leaching out, with resulting loss of birefringence. Starch suspensions containing butanol, processed at different temperatures and for increasing periods of time, were analysed upon cooling. A drop of starch suspension was placed on a glass slide (with cover slip) and analysed using an optical microscope, under polarized light, connected to a camera (Nikon Microphot – FXA) and image acquisition software (Infinite 2) at 10x lens magnification.

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2.2.1.3 X-ray Powder Diffraction (XRPD) analysis

Starch (Amylo N-400) aqueous suspensions were prepared and allowed to cool overnight, while stirring. The following morning, stirring was stopped to allow the starch to sediment to the bottom of the conical flask. Liquid was carefully removed and the sediment collected and spread onto petri-dishes. After drying for 3 days at room temperature, the powder was pulverised in a mortar by a pestle to allow filling of the capillary tubes for the XRPD experiments. X-ray diffractometry experiments were performed on an Oxford Diffraction, Xcalibur microfocus NovaT X-ray diffractometer, using Cu Kα radiation. Powder X-ray diffraction experiments were performed using transmission geometry at room temperature with samples sealed in capillary tubes and rotated about Phi over 360 ° at 0.75°/s. Data processing was conducted using Xcalibur software.

2.2.1.4 Particle size measurements

Particle size analysis of the starch suspension prior and after heat treatment was performed using the X-Flow module of the CAMSIZER XT. 200 mL of Purified water (Millipore filtered) was poured into the dispersion bath and 15 µL of sample was added. Maximum (XFemax) and minimum (Xcmin) Feret diameter were determined for each sample. Each sample was analysed in triplicate. Data is represented as Q10, Q50 and Q90 (% particles below given size).

2.3 Phloral coated tablets manufacture and characterisation

2.3.1 Tablet manufacture

Tablet cores (weight = 520 mg) containing 400 mg mesalazine (5-aminosalicylic acid; 5-ASA) were kindly provided by Tillotts Pharma AG. Besides 5-ASA, tablet cores contain lactose

monohydrate, povidone, sodium starch glycolate, micronized talc and magnesium stearate. Quantitative composition of the tablet cores isnot disclosed.

2.3.2 Coating suspension preparation

Aqueous starch dispersion was prepared as described above in section 2.2.1.1. The resulting dispersion was processed at different temperatures (see Table 1) and then cooled while stirring overnight. The % solids content of the cooled preparation was calculated based on the final weight of the dispersion (considering the evaporation during heating). Separately, a Eudragit® S organic solution was prepared by dissolution of Eudragit® S 100 in 96% ethanol. The final solution contained about 6% polymer solids. The starch dispersion was then added dropwise to the Eudragit® S 100 solution to obtain a ratio of starch : Eudragit® S of 30 : 70. The coating is prepared by slowly adding the starch suspension to the organic Eudragit® S solution in order to avoid precipitation of Eudragit® S, which could compromise the enteric properties of the coated tablets. This was mixed for 2 hours and 20% TEC (based on total polymer weight) and 5% GMS (based on total polymer weight) were added and mixed for further 2 hours. The GMS dispersion was prepared at a concentration of 5% w/w. Polysorbate 80 (40% based on GMS weight) was dissolved in distilled water followed by the dispersion of GMS. This preparation was then heated to 75 °C for 15 minutes while strongly stirring magnetically in order to form an emulsion. The emulsion was cooled at room temperature while stirring. The final preparation was coated onto 5-ASA tablet cores in a fluid bed bottom-spray coating machine (Strea-1 Aeromatic AG, Bubendorf, Switzerland) until 5% total weight gain (TWG) was obtained. The spray coating parameters were as follows: spraying rate 14 ml/min/kg tablets, atomising pressure 0.2 bar and inlet air temperature 40 °C.

As a comparator, tablets were also coated with Eudragit® S, applied as an organic solution. Briefly, 20% TEC (based on polymer weight) was dissolved in 96% ethanol followed by

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Eudragit® S while stirring mechanically and mixing continued for 1 hour. 10% GMS emulsion, prepared as described above, was added and mixed for 30 minutes prior to spraying onto 400 mg 5-ASA tablets using a bottom-spray coating machine (Strea-1 Aeromatic AG, Bubendorf, Switzerland) until 5% TWG was reached. The coating parameters were as follows: spraying rate 16 ml/min/kg tablets, atomising pressure 0.2 bar and inlet air temperature 40 °C.

2.3.3 Scanning electron microscopy (SEM)

The morphology of the surface and cross-sectioned coated tablets was evaluated by SEM. Samples were placed on SEM stubs and fixed using carbon discs before being gold coated using an EMITEC K 550 sputter coater for three minutes at 40 mA. The samples were then transferred to a Phillips XL20 Scanning Electron Microscope for imaging.

2.3.4 Robustness in upper GI tract - Dissolution in Hanks buffer pH 6.8

Drug release from coated tablets was assessed in pH 6.8 Hanks buffer (simulating the proximal small intestine) after pre-exposure to 0.1N HCl (fasted stomach simulation). The drug release profiles of 5-ASA were carried out using a USP II apparatus (Model PTWS, Pharmatest, Hainburg, Germany). The tests were conducted in triplicate, in 900 ml dissolution medium maintained at 37 ± 0.5°C. A paddle speed of 50 rpm was employed. The amount of 5-ASA released from the coated tablets was determined at 5-min intervals by an in-line UV spectrophotometer at a wavelength of 330 nm. The pH was kept constant at pH 6.8 by means of constant CO_2 purging into the medium throughout the dissolution run as described in the literature (Liu *et al.* 2011).

2.3.5 Release in the ileo-colonic region - Dissolution in Krebs buffer pH 7.4

Drug release in pH 7.4 Krebs buffer (simulating the ileo-colonic fluid) was assayed after pre-exposure of coated tablets to 0.1N HCl. The assay was performed as described above in a USP II dissolution apparatus at 37 ± 0.5°C and using 900 ml of pH 7.4 Krebs buffer. The tests were conducted under sink conditions (< 30% of 5-ASA saturation solubility in this buffer) (Fadda *et al.* 2009). The pH was kept constant at pH 7.4 by means of constant CO_2 purging into the medium throughout the dissolution run (Varum *et al.* 2011). The amount of 5-ASA released from the tablets was determined at 5 min intervals by an in-line UV spectrophotometer at a wavelength of 301 nm (acid stage) and 330 nm (buffer stage). Data were processed using Icalis software (Icalis Data Systems Ltd., Berkshire, UK).

2.3.6 Drug release in human faecal slurry pH 6.8

The *in vitro* assay using human faecal material used to test the formulations was based on the method described by Hughes and collaborators (Hughes *et al.* 2008) and optimised by Basit and co-workers (Yadav *et al.* 2013, Sousa *et al.* 2014). The basal medium used to allow bacterial growth was prepared and mixed to a ratio of 1:1 with a faecal slurry, which was prepared by homogenising fresh human faeces (pooled from three different healthy donors) in phosphate buffered saline (pH 6.8) at a concentration of 40% w/w. The final concentration of the prepared faecal slurry (diluted with basal medium) was 20% w/w. The donors had not received antibiotic treatment for at least three months before carrying out the studies using the faecal slurry. Tablets were tested in 210 ml of faecal slurry adjusted to the required pH under continuously stirring. The tests were carried out in an anaerobic chamber (at 37°C and 70% RH). Tablets were placed in small baskets composed of a flexible mesh (SEFAR NITEX^{TM} mesh size of 2000 μm) and were added to plastic transparent containers each containing 210 mL of feacal slurry adjusted

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to pH 6.8. Further pH adjustment were made each 30 minutes in the event of pH drop. Faecal slurry samples (1.5 mL) were collected hourly up to 9 hours and the last sample was colleted at 24 hours. Samples were then centrifuged at 13,000 rpm for 10 minutes. The supernatant was collected from the eppendorfs using 1mL syringes and filtered through 0.22 µm filters (Millex GP syringe-driven filter units, Millipore, Ireland). Thereafter, 100 µl of the filtered supernatant was transferred using a micropipette (Gilson, Inc., USA) into labeled 2 mL amber glass HPLC vials and diluted with 900 µl of mobile phase (95% Water, 5% Methanol and 0.05 % TFA). 5-ASA content was determined using High Pressure Liquid Chromatography (Agilent technologies 1200 Series) using a LiChroCART 250-4 colum, RP-18 (5 µm) (Merck Chemicals) and with a UV detection set at 228 nm. A mobile phase flow rate of 1 mL /min was utilized and column temperature was set at 40°C.

3 Results and Discussion

3.1 Starch suspension development and characterisation

The properties of raw starch are critical quality attributes for the functionality of starch and the overall quality of the final coated drug product. Resistant starch is starch that resists digestion in the upper GI tract by amylolytic enzymes and becomes available to colonic bacteria as an energy substrate. Resistant starch needs to be processed in order for it to be digested. It requires a high temperature and moisture in order to allow for gelatinisation, granule rupture and leakage of amylose into solution to occur. The morphological and physical changes of starch during heat treatment were investigated. Gelatinisation of starches is an important property that varies significantly amongst starch species. During gelatinisation, the starch granules swell to many times their original size, starch solubilisation increases, amylose leaches out, granule optical birefringence is lost, native crystallites melt and eventually the entire starch granule collapses (Lund and Lorenz 1984, Liu *et al.* 1991). The effect of gelatinisation on the morphology of

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starch granules and birefringence can be assessed qualitatively by PLM. Raw starch granules show a characteristic maltese cross-pattern and refract polarised light through their intact crystalline regions giving rise to one of the most unique characteristics of starches i.e. its birefringence (Buleon *et al.* 1998). Upon heating an aqueous starch suspension, starch granules start to swell and the cross patterns disappear. As this takes place in the early stages of the gelatinisation process, the loss of birefringence is a good indicator of the onset of the gelatinisation process (Liu *et al.* 1991). It can be clearly seen that the loss of birefringence occurs significantly earlier as the processing temperature increases as described in Figure 1 and Figures S1 and S2 (Supplementary information). Upon boiling, loss of birefringence is already seen at 5 minutes, indicating that the internal crystalline structure of the starch granule has been modified. Therefore, for a manufacturing process, a shorter gelatinisation procedure is an advantage.

During the gelatinisation process, amylose chains can form complexes with hydrophobic molecules such as fatty acids and butanol, resulting in the so-called V-complexes (Schoch 1942). Furthermore, the presence of butanol during the gelatinization process contributes to lower the gelatinization temperature, perhaps due to a surface tension phenomenon (Schoch 1942). The longer the alkyl chains in the amylose complex, the slower the transformation from single helix (V-complex) to double-helices, which is associated with retrogradation (Hopkins and Jelinek 1948). These V-complex structures are more resistant than free amylose chains however, during storage, these complexes compete with the retrogradation process of amylose leading to a higher susceptibility to enzymatic digestion by alpha amylases. In addition to temperature and water content, butanol also appears to have an effect on gelatinization (Figure 2).

In order to confirm that the crystalline structure of the starch has been disrupted upon heat treatment, samples of raw material and suspension that had undergone heat treatment were

analysed by XRPD. Raw resistant starch presents a type B polymorphic form. The very high intensity found at 2θ of 18° (Figure 3A) is consistent with the highly ordered crystalline structure of the amylose-lipid complexes in the starch granules (Shi *et al.* 1998). The crystallinity of resistant starch is maintained after suspending it in water and butanol for 24 hours (Figure 3B), without applying heat. Crystallinity gradually decreased with increasing temperature, and at boiling temperatures, all diffraction peaks disappeared (Figure 3C). In the absence of butanol, the peak at 2θ of 18° was maintained, thus it appears likely that the butanol decreases the gelatinisation temperature of starch by dissociating the highly ordered amylose-lipid complexes within the starch granule. The effect of heating duration was evaluated by heating the starch suspensions at temperatures just below the boiling point of the suspension (i.e. 80 and 85°C) up to 2 hours (Figure 3D-E). These conditions were insufficient to make the resistant starch fully amorphous.

From a process and product development perspective it is important that the properties of intermediates (i.e. starch suspension) can be maintained over sufficient time to allow more flexibility during large-scale manufacturing. In this regard, the particle size of the starch suspension was evaluated prior and after boiling and over the period of 1 month. The particle size of the starch granules clearly increased upon boiling, also indicating that the starch granules are gelatinized. However, particle size distribution was retained upon storage of the suspension for at least 1 month at room temperature (Figure 4).

3.2 Characterisation of Phloral™ coated tablets

In Figure 5 the morphology of coatings prepared with resistant starch processed at increasing temperatures can be seen. Heating up to 75°C resulted in rougher coatings with clearly visible particles on the surface, most likely starch particles. Upon boiling the starch, coated tablets prepared with such a suspension show a smoother coating and with large particles on the surface

as a result of the gelatinised starch as shown by the PLM images in Figure 2 and particle size distribution in Figure 4. As seen in Figure S3, the mixture of starch and Eudragit® S resulted in a compact film with homogeneous distribution of starch across the film, as revealed by SEM and iodine staining. In order to evaluate the performance of Phloral™ coated tablets in an environment resembling that of the human colon, the tablets were exposed to human faecal slurry, prepared from human faeces and stored under anaerobic conditions. The microbiota in the human colon largely outnumber the total human cells (Eckburg *et al.* 2005), therefore the catabolic potential is not represented by a restricted selection of enzymes and specific concentrations. Furthermore, over 50% of colonic bacteria species produce starch degrading enzymes (Macfarlane and Englyst 1986). Using this model of the human colon, it is demonstrated that resistant starch processing conditions have a significant impact on the digestibility of the coated tablets (Figure 6). When starch is not processed, coated tablets do not disintegrate within 24 hours in pH 6.8 human faecal slurry, demonstrating that the gelatinisation process is key to allowing improved digestibility of resistant starch. Only when starch is processed at 75°C and preferably when it is boiled, the coated tablets can be effectively digested by bacterial enzymes resulting in drug release (Figure 6).

In comparison, Eudragit® S coated tablets did not disintegrate over 24 hours in faecal slurry, only diffusion of drug occurred due to swelling (Figure 6). In contrast, 5-ASA release from Phloral™ coated tablets started significantly earlier following two hours exposure to simulated colonic fluids and was complete within five hours; suggesting that drug release can indeed be effectively triggered by colonic bacteria when coated tablets are not exposed to fluid with pH above 7.

Both Phloral™ and Eudragit® S coated tablets were fully robust (release $\leq$ 5%) in simulated gastric and upper small intestinal conditions (2 hours in 0.1N HCl followed by pH 6.8 Hanks buffer > 8 hours), indicating that premature release prior to arrival in the ileo-colonic region is

not expected *in vivo* (data not shown). This is further supported by several *in vivo* gamma scintigraphy studies with Eudragit® S (Schroeder *et al.* 1987, Ibekwe *et al.* 2006), providing the enteric properties and Phloral™ (Ibekwe *et al.* 2008b) coated tablets. In the case of Phloral™ coated tablets, Eudragit® S has an additional function; it controls the swelling of starch during transit through the upper gut. Due to its high amylose content, the starch component of the coating is resistant to digestion by pancreatic amylases secreted by the pancreas.

When tested in pH 7.4 Krebs buffer, which simulates the luminal composition of the ileo-colonic region (Fadda and Basit 2005), drug release from Phloral™ coated tablets matches drug release from standard Eudragit® S coated tablets (Figure 7). This demonstrates that the presence of starch in the coating does not have any impact on the enteric properties of Eudragit® S. Therefore, *in vivo*, if both formulations are exposed for a sufficient time to luminal fluid above pH 7, they are expected to perform similarly. However, GI pH and transit times are highly variabile (Varum *et al.* 2010, Fischer and Fadda 2016, Nandhra *et al.* 2020), which contributes to the overall variability in the performance of modified release systems. Furthermore, in UC patients, the pH in the colon is often lower than in healthy subjects (Fallingborg *et al.* 1993, Press *et al.* 1998, Nugent *et al.* 2001), which in combination with often accelerated transit (Davis *et al.* 1991, Hebden *et al.* 2000), can contribute to reported cases of intact tablets (Ibekwe *et al.* 2006, McConnell *et al.* 2008c).

The presence of starch in the coating can act as a second drug release trigger, due to its digestion by colonic bacteria (Macfarlane and Englyst 1986). This shows that despite the starch being embedded into the Eudragit® S matrix, it is still reachable by starch degrading enzymes. The degradation of starch by the enzymes weakens the coating structure, leading to drug release, even if the pH of the medium is below the pH trigger of Eudragit® S (pH = 7). There are four groups of starch-converting enzymes: (i) endoamylases, (ii) exoamylases, (iii) debranching enzymes and (iv) transferases. Endoamylases such as α-amylase hydrolyses α,1-4 glycosidic on

amylose and amylopectin while exoamylase such as β-amylases hydrolyse both α,1-4 glycosidic and α,1-6 glycosidic bonds like amyloglucosidase (van der Maarel *et al.* 2002). Debranching enzymes such as isoamylase can only hydrolyse α,1-6 glycosidic bonds. *In vivo* digestion of starch is a complex process in which several enzymes are involved and is extremely difficult to accurately simulate such a process using *in vitro* methods. A synergistic effect of an endoamylase (α-amylase) and an exoenzyme (amyloglucosidase) were used in an *in vitro* dissolution system to evaluate the enzymatic digestion of starch and consequent drug release from coated tablets.

These results demonstrate the superiority of a two-trigger mechanism to induce drug release, particularly in conditions of low colonic pH and/or fast transit times, as often seen in some groups of patients with UC (Davis *et al.* 1991, Fallingborg *et al.* 1993, Nugent *et al.* 2001). Due to the inherent challenges encountered in the colon, the induction of drug release mediated by these two triggers would offer a significant added benefit to allow a timely and complete drug release. This is particularly important in the colon of UC patients, where the limited fluid volume available and fast transit times through inflamed regions may limit full exposure of the mucosa to the delivered active pharmaceutical ingredient.

It has been demonstrated by a gamma-scintigraphy study, in healthy human subjects, that the combination of a pH and bacteria trigger mechanism (Phloral™) resulted in more accurate ileo-colonic targeting (Ibekwe *et al.* 2008b, Allegretti *et al.* 2019), acting as a fail-safe system in case one of the triggers is not activated. A novel 1600 mg 5-ASA colonic targeted formulation comprising the Phloral™ technology, embedded into the OPTICORE™ technology,(Varum *et al.* 2020) has successfully achieved accurate colonic targeting as proven by means of gamma-scintigraphy (unpublished data). In addition, a 1600 mg 5-ASA product (Asacol 1600, Octasa 1600, Yaldigo 1600), has been shown to be safe and efficacious in a Phase III clinical trial

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(D'Haens *et al.* 2017), and has now been launched and marketed in multiple territories worldwide.

4 Conclusions

An ideal colonic release system should be able to cope with the inherent variability in GI physiology. Systems relying on more than a single trigger (such as pH) can offer an alternative option to initiate drug release in a large, diverse patient population. The inclusion of a human microbiota-trigger to an enteric coated system can provide guaranteed drug release, even if the drug product is not exposed to the desired pH or for a sufficient time in the ileo-colonic region. The incorporation of resistant starch into the Eudragit S coating does not affect product robustness nor the dissolution properties mediated by pH. For resistant starch to be more susceptible to digestion by colonic bacteria, however, a short processing step involving the presence of moisture and increased temperature is required. The dual trigger mechamism offered by the coating is designed to allow for accurate and fail-safe drug release in the colon. In sum, the Phloral™ technology ultimately provides a superior drug delivery approach for the treatment of IBD and related conditions.

Declaration of interest

OPTICORE™ coating technology is patented and owned by Tillotts Pharma AG. Felipe Varum and Roberto Bravo are co-inventors of the technology but do not hold any commercial rights on the use of the technology. Phloral™ technology has been developed and is proprietary technology of UCL School of Pharmacy.

Acknowledgments

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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.

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Figure Captions

Fig. 1. Polarised light microscopy (PLM) images of resistant starch (Amylo N-400) suspensions containing butanol heated at 50°C for different periods of time. Magnification of x10.

Fig. 2. Light microscopy images of (Amylo N-400) aqueous suspensions boiled for 3 minutes in absence (A) or presence of butanol (B). Magnification of x10.

Fig. 3. X-ray difractograms of Amylo N-400 raw material (A), Amylo N-400 dispersed in water and butanol for 24 hours (B) Amylo N-400 boiled with water and butanol (C), Amylo N-400 heated at 80°C for 120 min (D) and Amylo N-400 heated at 85°C for 120 min (E).

Fig. 4. Particle size distribution expressed as (A) Xcmin (minimum length) above and (B) Femax (maximum length) below from starch suspensions prior to cooking (water and butanol),after cooking and after suspension storage up to 30 days (T1 = 1 day, T7 = 7 days and T30 = 30 days). Results expressed as average of three measurements and respective standard deviation.

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Fig. 5 Scanning electron pictures of the surface of tablets coated with Phloral™ coating prepared with starch suspension processed at different temperatures.

Fig. 6. Drug release from Phloral™ coated tablets, prepared with starch suspensions processed at increasing temperature: room temperature (F1), 50°C (F2), 60°C (F3), 75°C (F4) and boiling (F5), in faecal slurry pH 6.8. For comparison reasons drug release from Eudragit® S coated tablets is represented (F6). Data represented as average of three independent measurements and standard deviation.

Fig. 7. Drug release from coated tablets in 0.1N HCl (2 hours) followed by pH 7.4 Krebs buffer. Data represented as average of three independent measurements and standard deviation.

Table Captions

Table 1. Formulation summary

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Table 1. Formulation summary

Coating type	**Phloral coating**					**Eudragit® S coating**
Formulation n°	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Starch suspension processing temperature (°C)	Room temp.	50	60	75	92 (boiling)	Not applicable

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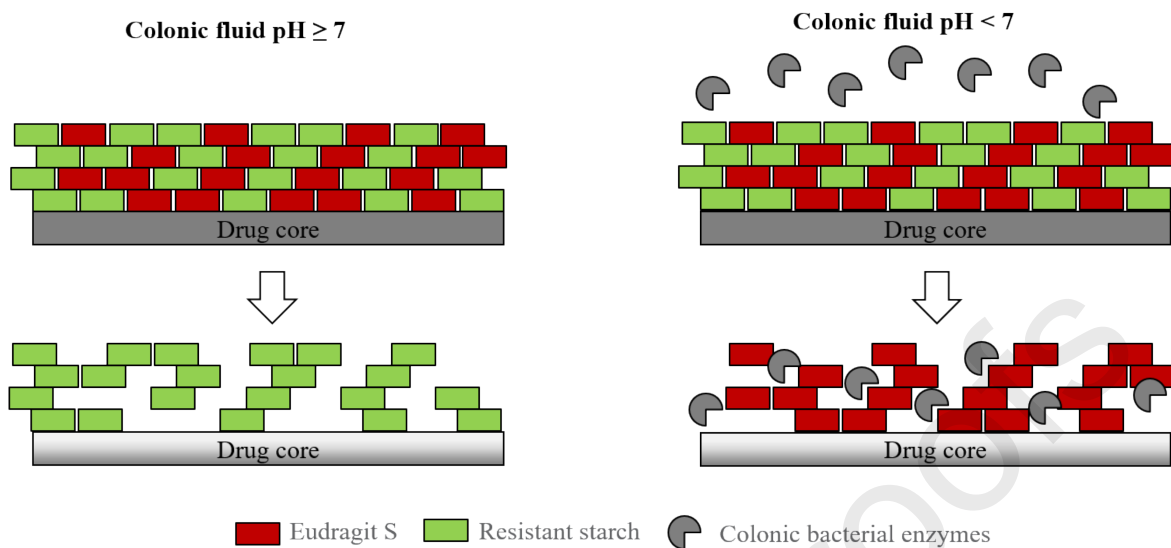

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Declaration of interests

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

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Declaration of interest

OPTICORE™ coating technology is patented and owned by Tillotts Pharma AG. Felipe Varum and Roberto Bravo are co-inventors of the technology but do not hold any commercial rights on the use of the technology. Phloral™ technology has been developed and is proprietary technology of UCL School of Pharmacy.

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