Research article

Film coatings based on "Swanlac® ASL 10" an aqueous shellac ammonium salt and inulin for oral controlled delivery in the distal part of GIT

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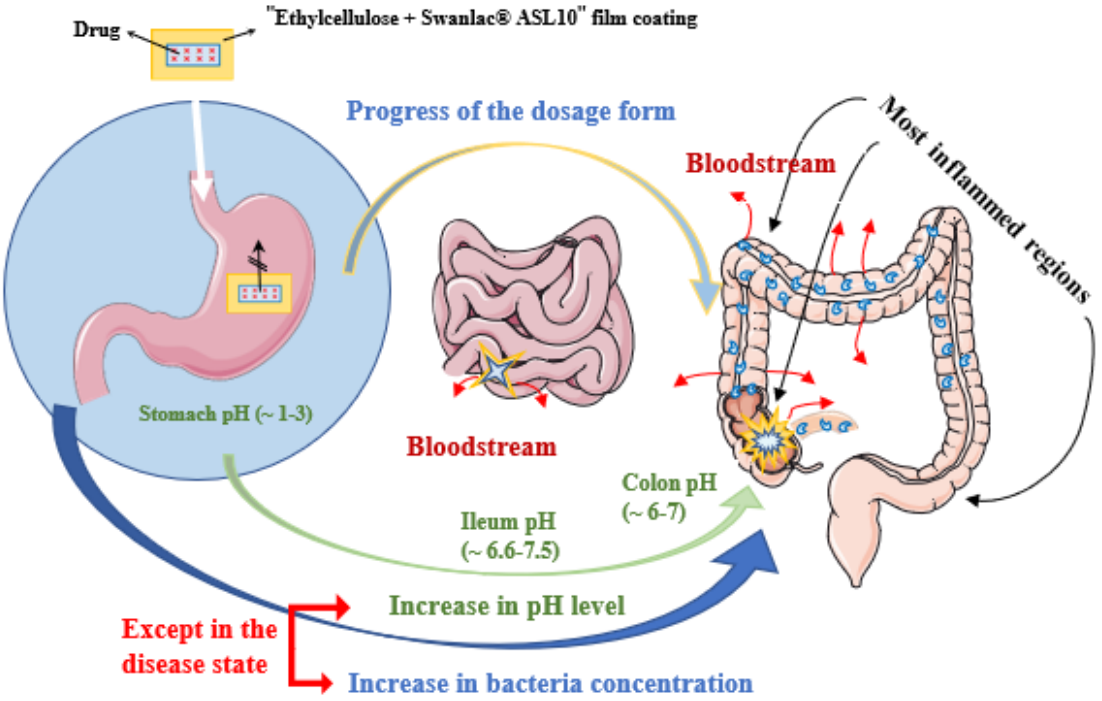
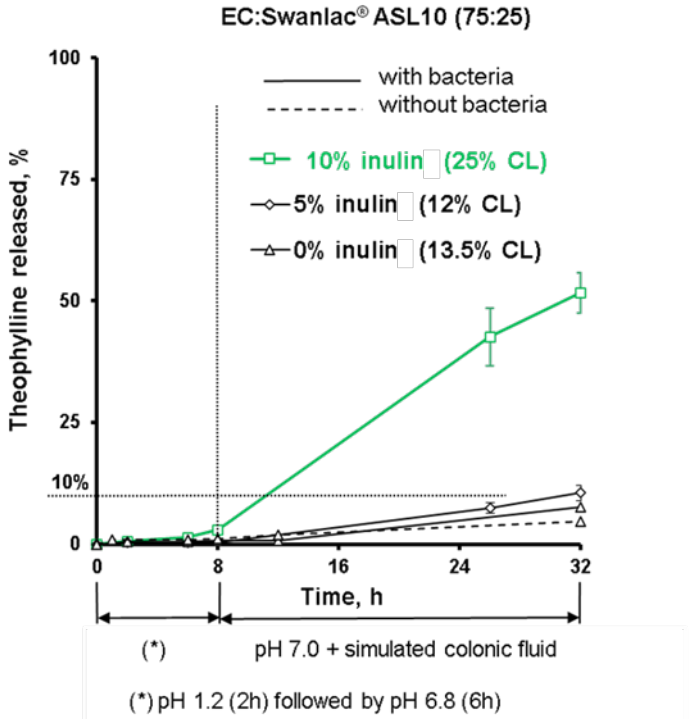
Abstract

Over the past decades, increasing interests took place in the realm of drug delivery systems. Beyond treating intestinal diseases such as inflammatory bowel disease, colon targeting can provide possible applications for oral administration of proteins as well as vaccines due to the lower enzymatic activity in the distal part of GIT. To date, many strategies are employed to reach the colon. This article encompasses different biomaterials tested as film coatings and highlights appropriate formulations for colonic drug delivery. A comparison of different films was made to display the most interesting drug release profiles. These films contained ethylcellulose, as a thermoplastic polymer, blended with an aqueous shellac ammonium salt solution. Different blend ratios were selected as well for thin films as for coated mini-tablets, mainly varying as follows: (80:20); (75:25); (60:40). The impact of blend ratio and coating level was examined as well as the addition of natural polysaccharide "inulin" to target the colon. *In vitro* drug release was measured in 0.1 M HCl for 2 h followed by phosphate buffer saline pH 6.8 to simulate gastric and intestinal fluids, respectively. Coated mini-tablets were exposed to fresh fecal samples of humans in order to simulate roughly colonic content. Several formulations were able to fully protect theophylline as a model drug up to 8 h in the upper GIT, but allowing for prolonged release kinetics in the colon. These very interesting colonic release profiles were related to the amount of the natural polysaccharide added into the system.

Keywords: film coatings; oral controlled release; aqueous shellac salt solution; inulin; colon targeting; reservoir systems.

Graphical Abstract:


Drug
"Ethylcellulose + Swanlac® ASL10" film coating
Progress of the dosage form
Bloodstream
Most inflammed regions
Stomach pH (~ 1-3)
Bloodstream
Colon pH
(~ 6-7)
Ileum pH
(~ 6.6-7.5)
Increase in pH level
Except in the
disease state
Increase in bacteria concentration


EC:Swanlac® ASL10 (75:25)
with bacteria
without bacteria
10% inulin (25% CL)
5% inulin (12% CL)
0% inulin (13.5% CL)
Theophylline released, %
100
75
50
25
10%
0
0
8
16
24
32
Time, h
(*) pH 7.0 + simulated colonic fluid
(*) pH 1.2 (2h) followed by pH 6.8 (6h)

Introduction

Colon-targeted delivery is a suitable alternative approach for the oral administration of drugs that may be degraded or cause side-effects in the upper gastrointestinal tract (*e.g.* irritations or premature release and drug absorption into blood stream) [1–4]. For instance, protein- and peptide-based drugs, which are formulated in colon-targeted delivery systems, may be protected from hydrolysis due to the low activity of proteolytic enzymes in the distal part of the GIT (colon) [5, 6]. With 10 million people affected worldwide, inflammatory bowel diseases (IBD), including Crohn's disease (CD) and Ulcerative Colitis (UC), are a concrete example of the limits encountered with conventional dosage forms. IBD are known to show moderate to severe symptoms, and only benefit from symptomatic oral or parenteral treatments. UC and CD share many clinical features, both are characterized by relapsing-remitting cycles of mucosal inflammation [7]. To date, there is no cure for IBD. Only therapeutic strategies aimed at maintaining remission episodes can be commonly used. Glucocorticoids are commonly prescribed for acute exacerbations of UC and CD, but their frequently use can lead to serious systemic side-effects, which often cause a non-acceptance of the treatment. Other therapies, including oral aminosalicylates (5-ASA), antibiotics (metronidazole) and parenteral immunosuppressive agents (azathioprine) can temporarily maintain remission for certain types of IBD. According to the literature, the effect of 5-ASA as a standard treatment in Crohn's disease is controversial and may lead commonly to the failure of the treatment [8]. Unfortunately, 70 % of IBD patients will require at least one surgical intervention in their lifetime [9]. However, conventional dosage forms may also cause certain adverse effects once they have been distributed throughout the body. Thus, prolonged local drug delivery would be the appropriate tool to increase the treatment efficacy and decrease serious systemic side effects. Oral dosage forms must be completely protected in the upper part of the GIT, and thus drug release must begin in the distal part of the GIT (most inflamed area) in IBD patients [10]. It should be emphasized that oral route is considered as the preferred route of administration due to its well-established acceptability, cost-effectiveness, manufacturing advantages and drug stability. In addition, these dosage forms offer advantages such as better compliance, greater

convenience, reduced risk of both cross-infections and needle stick injuries [11]. Because of these advantages, oral administered systems continue to dominate the majority of the market. Indeed, around 40 % of therapies available in pharmacies are formulated into oral solid dosage forms, such as tablets, capsules or granules. The main current strategies are based on *) the integration of prodrugs into formulations, **) pH-sensitive drug delivery, ***) time-dependent systems and ****) microbiota sensitive systems. Given that the transit time of IBD patients is affected (often accelerated), time-dependent delivery as a single approach is not appropriate and could lead to the failure of the treatment. The pH-responsive colonic delivery approach has been adopted clinically, with several products established on the market, and others introduced for the treatment of IBD (*e.g.* Asacol®, Salofalk®, Lialda® containing mesalamine and Budenofalk® containing budesonide). For colon targeting, the microbiota-sensitive approach exploits the fact that there are around one hundred billion CFU (Colony Forming Unit)/mL of bacteria in the colon, and with hundreds of different species. The colonic microbiota is known to have a metabolic potential equal to or greater than that of the liver, which can be exploited to deliver drug in the distal part of GIT. Importantly, many complex carbohydrates are selectively degraded and fermented by the microbiota located in the colon.

Because of many intra- and inter-individual variations in pH levels along the GIT, the bacteria-sensitive strategy become increasingly very attractive for colon targeting. Moreover, due to the inflammation, the mucosa becomes more acidic in IBD patients than in healthy subjects.

The pH drops to 6.4 (+/- 0.6) in the right colon and to 7.0 (+/- 0.7) in the left colon in healthy subjects [12]. Colonic pH is characterized by values fluctuating mainly between pH 5 and pH 8, with the mean pH value of 6.5±0.3 [13]. Nugent et al. reported that the right colonic luminal pH can be as low as 4.7 by patients in both active and inactive ulcerative colitis [14]. It was also found that by patients suffering from Crohn's disease a low colonic luminal pH, which was similar to that reported by patients suffering from active ulcerative colitis [15]**.**

However, these impacts in pH fluctuations are not systematic. Consequently, the use of pH-sensitive polymers alone would have limitations in these conditions. The aim of this study is to determine a reliable system capable to prevent premature drug release in the upper GIT, but

also able to deliver drug in time-controlled manner at the site of action, in both diseased and healthy subjects. Importantly, this system would have the features of a dual-stimuli-triggered form, which is sensitive to both pH and colonic microbiota. *In vitro* drug release was measured under conditions simulating the contents of the upper GIT (0.1 M HCl and phosphate buffer pH 6.8) and the colon (fresh fecal samples from IBD patients) to simulate pathophysiological conditions. Ethylcellulose (EC) was chosen as a thermoplastic polymer associated with ammonium-based aqueous shellac solution (Swanlac® ASL10) in film coatings for mini-tablets. Shellac is known for its good film-forming properties and is recognized as safe by the Food and Drug Administration (FDA) [16]. It is often used as an enteric coating material for oral dosage forms, and its use in sustained-release, colon-targeting formulations has been also reported [17]. Interestingly, only low coating levels (= CL) are required to ensure negligible drug release in the upper part of the GIT. However, dosage forms based on aqueous ammonium-based shellac solution (Swanlac® ASL10) show better mechanical properties than alcoholic-based shellac solution. In addition, shellac (water insoluble under pH 7.0) is described in the US Pharmacopeia (USP) and the European Pharmacopeia (Ph. Eur.) as a natural resinous oligomer (Mw ≈ 1000 Da) secreted by the insect Kerria lacca (a parasite of certain trees particularly found in India, Burma, Thailand, and southern China) [18–22]. This is an important feature of nature to protect insects and their larvae from tropical rains.

This natural compound is an anionic polymer composed of aleuritic acid and cyclic terpene acids, which constitute the hydrophobic and hydrophilic parts respectively and linked by ester bonds (Figure 1) [23, 24].

Shellac, called LAC after refining, becomes a water-soluble salt after dissolution in alkaline water via an acid-base neutralization reaction. The carboxyl groups of shellac, when dissolved in ammonia water, react with the ammonia to form a water-soluble ammonium shellac salt solution. As ammonia is not a very strong base, an excess of ammonia is required in the solution. The ions are solvated and, as they dry, solvation is lost, forming a dense contact between the ions.

However, the use of aqueous shellac ammonium salts and EC provides an overall hydrophobic system, which allows for controlled and prolonged release. Colonic drug delivery will be optimized in this work by the addition of polysaccharides, which are degraded by the microbiota in the colon.

Herein, the use of ammonium-based shellac solution (Swanlac® ASL10) ensured product stability for at least a year, and offered a number of perspectives such as weak viscosity, ready and easy use for aqueous coating systems. This ammoniated shellac solution has already demonstrated its enteric coating release capacities on theophylline tablets, and provided aesthetically attractive, robust and stable coatings [25]. To prepare an aqueous solution, an acid-base neutralization reaction is required, in which shellac becomes a shellac salt [26]. Different alkalis result different shellac salts with different film properties. Aqueous shellac ammonium salt solutions are prepared commercially with ammonium hydrogen carbonate or ammonium hydroxide. The ammonium chloride is released, and then carboxylic acid moieties generate dimers by hydrogen bonding interactions, which contribute further to stable and rugged film coating.

Materials and Methods

Materials

Mini-tablets cores containing theophylline: Microcrystalline cellulose (Avicel PH 102, FMC Biopolymer); magnesium stearate (Merck, Saint-Quentin-Fallavier, France); theophylline anhydrous powder (BASF, Ludwigshafen, Germany).

Mini-tablets cores without theophylline: Placebo mini-tablets (Chemische Fabrik Budenheim, Germany).

Film and tablet coatings: Ethylcellulose dispersion (Aquacoat ECD 30, DuPont Nutrition, Wilmington, DE, USA); triethyl citrate (TEC, Alfa Aesar, Karlsruhe, Germany); maltodextrin (Glucidex Maltodextrin 19, Roquette, Lestrem, France); aqueous shellac ammonium salt solution (Swanlac® ASL 10, A.F. Suter, Witham, United-Kingdom); Inulin (Orafti® HP, Beneo, Mannheim, Germany); dicalcium phosphate (dicalcium phosphate anhydrous, Rhône-Poulenc,

Antony, France).

Simulated gastric and intestinal fluids: Hydrochloric acid pH 1.2 (0.1 M HCl); phosphate buffer pH 6.8 (PBS 6.8).

Simulated colonic fluid: Culture medium inoculated with fresh fecal samples from patients.

Culture medium: Extracts from beef and tryptone (Pancreatic digest of casein, Becton Dickinson, Sparks, USA); yeast extract (Oxoid, Hants, UK); sodium chloride (J. T. Baker, Deventer, Netherlands); L-cysteine hydrochloride hydrate (Acros Organics, Geel, Belgium); Ringer solution (Merck, Darmstadt, Germany).

HPLC-UV analysis:

Mobile phase: Acetonitrile (VWR, Fontenay-sous-Bois, France); phosphate buffer pH 6.8 (PBS 6.8).

Column: C18 column (Gemini® 5 µm C18 110 Å, 100 mm x 4.6 mm; Phenomenex, Le Pecq, France).

Preparation of polymeric films

Polymeric films were prepared by mixing ethylcellulose with aqueous shellac ammonium salt solution (with or without maltodextrin) at different blend ratios: (90:10); (80:20); (70:30); (60:40) respectively. The blend ratios expressed the proportion of dry mass of ethylcellulose compared to the dry mass of the second fraction (shellac with or without maltodextrin).

Ethylcellulose was first plasticized with 25 % TEC (referring to the dry mass of ethylcellulose) for 24 h with a magnetic stirrer. Then, aqueous shellac ammonium salt solution (with or without maltodextrin) was added for 3 h to get a homogeneous dispersion. 1 % of theophylline anhydrous powder (w/w, referring to the total dry mass of the film) was added to the dispersion and mixed for 2 h. Please note that the drug was dissolved molecularly into the dispersion (solubility in H_2O: 7.360 g/L at 25 °C [27]). Films containing dicalcium phosphate were made by adding 10 % of the mineral (w/w, referring to dry shellac mass). After mixing, the films were poured into teflon molds and dried in an oven at 60 °C for 24 h. They were cut into pieces and their thicknesses were measured using a thickness gauge (MiniTest 600, ElektroPhysik,

Köln, Germany). The mean thickness of films was 350 µm. Samples were measured in triplicate.

Preparation of non-coated and coated mini-tablets

Non-coated mini-tablets were prepared as follows: microcrystalline cellulose was mixed with 10 % theophylline anhydrous for 10 minutes, then lubricated with 1 % magnesium stearate for further 5 minutes using a 3D powder blender mixer at 20 rpm (Turbula T2C, Willy A. Bachofen, Basel, Switzerland). The powder was then compressed into mini-tablets using an automatic single-punch tablet machine (Korsch, EKO/DMS, Berlin; Germany). These mini-tablets were mixed with placebo mini-tablets with the same dimensions from Chemische Fabrik (BudenHeim, Germany) to get the appropriate batch mass for coating process (400 g). Tablets diameter was 5 mm.

Afterwards, the batch was coated using a drum coater module (Solidlab 1 Hüttlin, Syntegon Technology, Waiblingen, Germany) with a 0.5 mm nozzle. Mini-tablets coating was carried out using following parameters: 4 g/min spray rate, 60 °C inlet air temperature, 40 °C outlet air temperature, 1 bar atomizing air pressure, 0.5 bar shaping air pressure, rotation speed was set at 25 rpm (rotations per minute).

The sprayed dispersion was a blend of ethylcellulose and aqueous shellac ammonium salt solution, with and without the addition of 5 % and 10 % inulin. Blend ratios were varied as follows: 80:20; 75:25; 60:40. Different coating levels were used, ranging from 10 % to 35 %, depending on the blend ratio. Coated mini-tablets with various polymer:polymer ratios at different coating levels were cured in an oven at 60 °C for 24 h, which has been shown in previous studies to be enough for polymeric coalescence and complete film formation of systems based on ethylcellulose. Please note that coating levels represent the weight gain after coating, calculated with the initial tablet's weight.

Control of quality of non-coated mini-tablets

The quality control of mini-tablets before their coating was carried out according to Ph. Eur. 11.1 (section 2.9: "Pharmaceutical technical procedures"). The criteria controlled were as follows: uniformity of tablet mass, uniformity of drug content, hardness, friability, disintegration of tablet, *in vitro* dissolution test, tablet height and diameter.

The mean mass of 20 mini-tablets should not exceed a 10 % standard deviation value. The drug content was determined UV-spectrophotometrically (UV-1650 PC; Shimadzu, Champs-sur-Marne, France) at a wavelength of 275 nm. The mean drug content should be in the range of 85 to 115 %.

The friability of the mini-tablets was determined gravimetrically, and should be less than 1 %.

Theophylline release from the mini-tablets was measured using the USP 32 dissolution apparatus (paddle method, 80 rpm, 37 °C) in 300 mL phosphate buffer pH 6.8 (USP 32) (Sotax; Basel, Switzerland). At predetermined time points, 3 mL samples were withdrawn and analyzed spectrophotometrically (UV-1650 PC; Shimadzu, Champs-sur-Marne, France) for their drug content (λ=275 nm). All experiments were conducted in sextuplicate.

Optical microscopy

Macroscopic pictures of coated mini-tablets were taken with a trinocular stereomicroscope (Optika microscopes, Italy). Pictures were taken at t=0 h, t=8 h and t=24 h after incubation of mini-tablets into simulated gastrointestinal (gastric, intestinal and colonic) fluids.

In-vitro drug release from polymeric films and coated mini-tablets

Upon exposure to simulated gastric and intestinal fluids:

Polymeric films were incubated in flasks (1 sample per flask) containing 100 mL 0.1 M HCl and stirred at 80 rotations per minute (using a horizontal shaker, at 37 °C, GFL 3033, Gesellschaft Für Labortechnik, Burgwedel, Germany). After 2 h, the medium was completely replaced with phosphate buffer pH 6.8 to simulate intestinal fluids.

Coated mini-tablets were incubated under the same conditions as free films (2 h in 0.1 M HCl pH 1.2 followed by 6 h in PBS pH 6.8) using a USP III dissolution apparatus (Bio-Dis, Varian, Paris, France) at 20 dips per minute "dpm" and 37 °C. 20 dips per minute are considered as harsh conditions in this work [28].

At pre-determined times, 3 mL samples were withdrawn and measured using UV-spectrophotometry (UV-1650 PC; Shimadzu, Champs-sur-Marne, France) at λ = 275 nm for theophylline concentration. The range of concentrations of the calibration curve was [1-10 mg/L] and r^2 was 0.99998 and 0.9997 for HCl and PBS, respectively.

Upon exposure to simulated colonic fluid:

After incubation in simulated gastric and intestinal fluids, mini-tablets were transferred into 100 mL flasks containing: (1) 100 mL culture medium inoculated with fresh human feces (obtained from IBD patients giving written informal consent), and (2) culture medium without feces for reasons of comparison. Mini-tablets were incubated in triplicates for each aspect tested (ratio or coating level). The samples were gently agitated (50 rpm; Stuart, Cole-Parmer; Villepinte, France) at 37 °C in anaerobic atmosphere (AnaeroGen 2.5 L; Thermo Scientific; Illkirch, France). Culture medium was prepared as follows, according to previous publications [28–31]: 1.5 g beef extract, 3 g yeast extract, 5 g tryptone, 2.5 g NaCl and 0.3 g L-cysteine hydrochloride hydrate were dissolved in 1 L distilled water (pH 7.0 ± 0.2). Afterwards, the solution was sterilized in an autoclave. Culture medium inoculated with patients' feces was prepared by diluting fecal samples (approximately 1 g) 1:200 with cysteinated Ringer solution. At pre-determined times, 2 mL of culture media were withdrawn, centrifuged for 10 min at 15000 rpm (Centrifuge Universal 320; Hettich, Tuttlingen, Germany), filtered (0.22 µm, Millex-HU; Merck Millipore, Tullagreen, Ireland) and quantified by HPLC (Waters E2695 ALLIANCE HPLC) for their drug content using an equipment with a pump, an auto sampler and coupled to UV-Vis detector. For the detection of theophylline as a model drug, the mobile phase was prepared by mixing 90 % phosphate buffer saline 6.8 with 10 % acetonitrile (v/v). Samples were injected into a C18 column (Gemini® 5 µm C18 110 Å, 100 mm x 4.6 mm;

Phenomenex, Le Pecq, France) at a flow rate of 0.6 mL/min. The drug was detected at λ = 275 nm [32]. The range of concentrations of the calibration curve was [5-150 mg/L] and r^2 was 0.9998.

Results and discussion

In-vitro drug release from polymeric films

An ideal microbially-triggered system for prolonged release should protect the drug in the upper GIT and release it in the distal part of the GIT (e.g., ileocecal and colonic region) due to the presence of the microbiota. Numerous release kinetic profiles were obtained in this study for controlled delivery. Some of them were able to suppress drug release for up to 8 h in the upper GIT. Others showed the ability to deliver the drug in a prolonged manner, irrespective of the presence of microbiota. This phenomenon could be interesting and appealing for therapies with prolonged drug delivery, unsusceptible to the microbiota. However, these systems should release drug in zero-order kinetics in order to control the delivered drug doses. Figure 1 stands for the molecular structure and related physicochemical properties of shellac.

The in vitro drug release profile from a polymeric film should enable the estimation of drug release from coated solid dosage forms. Since these polymeric films containing drug are matricial systems, coated mini-tablets (reservoir systems) will deliver the drug more slowly due to the run-through of the polymeric barrier surrounding the mini-tablets. It was relevant to investigate and compare different polymeric films to determine the most suitable for controlled and prolonged drug delivery in the distal part of the GIT. Figure S1 (supplementary data) illustrates the *in vitro* drug release profile from polymeric films based on Ethylcellulose:Shellac (EC:Swanlac® ASL10) composition at different blend ratios (90:10; 80:20; 70:30; 60:40), in the presence or absence of dicalcium phosphate.These polymeric films were incubated in 0.1 M HCl for the first 2 h, then in 6.8 phosphate buffer for the next 6 h simulating the upper GIT. Films were loaded with 1 % theophylline anhydrous (w/w, referring to the total dry mass of the film). As shown in Figure S1, theophylline release from films with dicalcium phosphate (dotted

lines) was slower than without dicalcium phosphate (full lines) with respect to the polymer:polymer ratio. Dicalcium phosphate was chosen because several sources mentioned the fact that minerals can retain drug into the delivery systems, which can be very beneficial for the controlled release [33]. Dotted lines showed very interesting trend: all kinetic profiles have been impacted and modified. In vitro drug release became slower and less pronounced with dicalcium phosphate.

Except for the ratio (90:10), which showed no significant difference between with and without dicalcium phosphate), all other ratios (80:20, 70:30, 60:40) showed improved controlled release profiles. This can be attributed to the fact that the drug release rate from polymeric films with the ratio (90:10) was initially low. Thus, the impact of such mineral excipients can be a very useful tool for controlled drug delivery.

Importantly, the ratio (80:20) exhibited 26 % release from films with dicalcium phosphate versus 65 % without dicalcium phosphate. Moreover, 70:30 blend ratio displayed 55 % release from films with dicalcium phosphate, versus 80 % without dicalcium phosphate.

Finally, the ratio (60:40) has only released 44 % of drug from films with dicalcium phosphate, whereas a completely release of drug content can be seen been from polymeric films without dicalcium phosphate.

It is clear that the addition of this mineral can impact the profile of drug release by slowing down the drug diffusion in the outer bulk fluid. This behavior could be attributed to the drug being captured or adsorbed into the porous scaffold of crystals [33]. As this mechanism was not investigated in our studies, we have not conducted any further experiments with dicalcium phosphate at present, but these results could be considered promising for colon targeting.

The ethylcellulose fraction impacted drug diffusion due to its hydrophobic nature, which can decrease drug release in simulated GIT fluids, irrespective of the pH level. Indeed, ethylcellulose is known for its water barrier functions, and is introduced into the coating material of solid dosage forms in order to decrease the hydrophilicity of polymers (*e.g.* polysaccharide) and reduce their premature dissolution. This allows controlled management on

the release profile of drug substances. The ethyl-ether groups which substitute hydroxyl end groups of cellulose make it water insoluble polymer in the GIT.

Although EC is insoluble at any pH in the gastro-intestinal tract, it swells slowly upon exposure to gastric fluid [34], making it permeable to water [35]. This amount of water uptake is controlled with respect to film coating thicknesses.

In vitro drug release from film coatings based on EC relies on the coating level, drug solubility, and the way the polymer is carried out during the coating process (*e.g.*, aqueous dispersion or organic solution). The influence of EC over other polymers on the control of drug delivery was demonstrated by Shah et al. and Karrout et al. [28, 36, 37]. Ethylcellulose forms a rigid coat that decreases polymer leaching and, consequently, drug release, due to the close packing and shorter interchange chain distance [38].

As the swelling of dry shellac is known at pH 6.8, an ‘‘open’’ network with a larger average distance between polymeric chains is expected, whereas a fairly ‘‘tight’’ network is formed at pH 1.2. The latter can be beneficial for the protection in the upper GIT, particularly when mixed with thermoplastic hydrophobic polymers.

As EC:Swanlac® ASL10 blends showed good controlled-release properties, these polymers were selected for further studies involving an additional polysaccharide, which should be degraded by the colonic microbiota for colon targeting.

Due to the presence of the microbiota in high concentrations in the distal part of the GIT (ileocecal and colonic regions), we could imagine prolonged and controlled drug delivery once these regions are reached. As in the conditions depicted in Figure S1, we investigated thin polymeric films based on EC:Swanlac® ASL10 with the addition of maltodextrin, a hydrophilic polysaccharide that can be degraded by the microbiota enzymes secreted in the distal part of the GIT (Figure 2).

Thin polymeric films were characterized at different thicknesses: 70 µm, 250 µm and 400 µm, using different blend ratios: [70:(30)] and [50:(50)] [EC:(Swanlac® ASL10 50 % + maltodextrin 50 %)] as indicated in the diagram. The goal was to better understand the behaviour and properties of these blended polymeric film coatings, considering: (*) polymer:polymer blend ratios, (**) film thickness, and (***) the influence of maltodextrin on drug release from these matricial systems. All experiments were carried out in triplicates. Overall, we can easily see the negative evolution as the mean thickness increases due to the increase in diffusion pathway length. This phenomenon is well studied in the literature and is explained by the increase in polymeric network density [39], which increases the diffusion pathway. Obviously, thicknesses of 400 µm corresponded to the slowest kinetic profiles obtained.

The 70 µm thin film (Figure 2) exhibited an immediate release for both [70:(30)] and [50:(50)] polymeric blend ratios due to the decreased diffusion pathway and polymeric network density.

Based on this understanding, we can approximately select the best candidate that could be used to coat the mini-tablets for prolonged and controlled drug delivery in the distal part of the GIT.

This partial release evidenced that the composition, as a single film coating, was sufficiently impermeable to protect the drug under these conditions. However, it should be emphasized that such matrix polymeric films based on EC can present a balanced concentration gradient between the inner and outer site (“bulk release medium”) of the polymeric networking.

The obtained results from the release kinetic profiles showed prolonged drug release, unaffected by the increased addition of hydrophilic polysaccharide.

Interestingly, reducing the ethylcellulose fraction in favor of the second fraction (shellac+maltodextrin) from 70:(30) to 50:(50) only had little impact on the drug release profiles. This can be probably attributed to the fact that also the hydrophobic backbone of shellac will interact and increase significantly the hydrophobic nature of these new developed systems.

400 This formulation seems to be an appropriate tool to target the colon with such dual-triggered
401 stimuli system (pH and microbiota sensitive system).

402 Importantly, shellac provides in this case a pH-dependent property, while maltodextrin or other
403 natural polysaccharides could be obviously substrates of the microbiota.

404 The formulation of shellac mixed with EC in the investigated blend ratios (70:(30) and 50:(50))
405 could be used as good candidates for colon targeting.

406 Promising free polymeric films have been identified with low drug diffusion upon exposure to
407 release media simulating the stomach and small intestine fluids (Figure 2).

408 These physicochemical characteristics are interesting in terms of film coatings for oral dosage
409 forms. Certainly, from a coated tablet core, the drug has a longer pathway to cross: Firstly, the
410 drug has to be dissolved within the tablet core. Secondly, it must be diffused through the
411 polymeric barrier to the outer side of the tablets. In the reservoir system, drug should pass over
412 through the coating layer or pass through hydrophilic pores in the film coating to reach the bulk
413 release medium.

414 *In-vitro drug release from coated mini-tablets*

415 Quality control of uncoated mini-tablets was carried out in accordance with Ph. Eur. 11.1.
416 Figures S2, S3, S4, S5 (supplementary data) represent the various quality tests carried out and
417 also highlight the conformity of the mini-tablets. Importantly, the uncoated mini-tablets used
418 complied with Ph. Eur. 11.1 and could therefore be used for film coatings.

419 Figures 3, 4 and 5 show the *in vitro* release kinetics of theophylline from coated mini-tablets
420 with EC:Swanlac® ASL10 blends at different ratios (80:20; 75:25; 60:40, respectively) and
421 coating levels. Coating levels correspond to the weight gain after the coating of mini-tablet
422 cores. The increase of the coating level leads to a thicker and denser coating material on the
423 tablet, which increases the diffusion pathway.

424 40g of mini-tablets were withdrawn at pre-determined time points during the process after each
425 coating level indicated in the diagram. The following EC:Swanlac® ASL10 ratios were chosen:

(80:20); (75:25); (60:40) and the coating levels were: 10, 12, 13.5, 15, 18, 20, 25, 30, 35 %, depending on the polymer:polymer ratio.

The mini-tablets were then incubated in 0.1 M HCl for 2 h, followed by phosphate buffer pH 6.8 for 6 h to simulate the upper GIT (Figures 3a, 4a and 5a). Moreover, to simulate the entire GIT, the same oral dosage forms were incubated in 0.1 M HCl for 2 h, then followed by PBS pH 6.8 for 6 h, before being transferred into simulated colonic fluid with and without fresh feces samples (Figures 3b, 4b and 5b).

A comparison between ratios and coating levels was performed to determine the influence of each one upon theophylline release under these conditions as well as to select the best candidate for colon targeting.

As shown in Figure 3, EC:Swanlac® ASL10 (80:20, using 13.5 to 27 % coating level) exhibited none or little diffusion in the simulated gastric and intestinal fluids up to 8 h (Figure 3a), which protects the drug release in these media. However, the same results were obtained when followed by 24 h incubation in colonic medium. There was no significant difference in the release profile in the presence or absence of fecal samples up to 32 h (Figure 3b).

A comparison of the release kinetics of coated mini-tablets and films shows a good estimate of protection against water uptake and subsequent drug diffusion (polymeric films vs. real coated mini-tablets).

Figure 4 shows no release up to 8 h, which indicates that polymer:polymer ratio (75:25) protects completely this dosage form in the upper GIT similarly to ratio (80:20).

After 24 h incubation in simulated colonic medium, a slight onset of drug release was observed. Both (80:20) and (75:25) ratios are (at least at the 15 % coating level) very useful film coatings for colonic drug delivery under these conditions.

Please note that 24 h incubation time in the colon was chosen in order (i) to better exploit the microbiota *in vitro*, which is a long process for proliferation (sufficient time for bacteria to be

multiplicated), and (ii) to better simulate the colonic transit time, which can be ranged from 18 to 34 as well varied considerably from one subject to the other (sex, age, disease etc.) [40].

Drug protection is possible in the upper GIT using these dosage forms (Figure S6), but unfortunately also in the lower GIT without the microbiota, these systems start to release drug in a slow time-controlled manner. It is worth pointing out that these coating materials could offer the following advantages: (1) suitable for lower-dose therapies “chronotherapy”, (2) easy to be administered.

460 This is consistent with the literature: indeed, shellac-based coating layers remain intact in the
461 stomach until they reach the pH-threshold, which is greater than 7.0. Shellac enables drugs to
462 be transported to the colonic compartment for a topical alleviation of local affections [41–44].

463 For reasons of comparison, Karrout et al. [28] demonstrated *in vitro* 5-ASA release from
464 Pentasa® pellets and Asacol® capsules. They showed the premature 5-ASA release from these
465 commercially available dosage forms in the upper GIT. These outcomes were consistent with
466 previous reports in the literature [45, 46].

467 Figure 5 illustrates *in vitro* theophylline release profiles from coated mini-tablets at (60:40)
468 blend ratio. Upon exposure to the upper GIT (Figure 5a), the evolution of drug release can be
469 seen as function of coating level. Surely, we selected mini-tablets releasing very low amount in
470 the upper GIT for each exploited formulation. For instance, (75:25) and (60:40) blend ratios
471 were tested at different coating levels throughout the whole GIT in order to adjust the
472 appropriate coating level required for a prolonged drug delivery, which can protect drug in the
473 upper GIT and be fermented/degraded in the distal GIT.

474 Shellac does not dissolve at pH 1.2 and maintains its barrier functions, allowing for gastric
475 resistance. Mini-tablets coated with 30 % level were the most robust and stable, which released
476 only 14 % of drug concentration during 8 h of incubation.

477 Interestingly, all other coating levels (15 %, 20 % and 25 %) reached the same release profile
478 of drug delivery as the coating level 30%. This again, highlighted the impact of the
479 ethylcellulose amount on the hydrophobicity of these advanced dud delivery systems. Surely,
480 this can confirm the above obtained results with polymeric films.

481 In Figure 5b, the same experience was performed with subsequent incubation in simulated
482 colonic fluid for a further 24 hours. As in Figure 5a, the 20 % coating level (full line) reached
483 around 25 % drug release after 8 h (in the upper GIT), which was twice as important as the
484 other coating levels. Almost the entire amount of drug was delivered using this coating level
485 after 32 h, as compared to the others, reaching about 72 % drug release (ratios and coating levels
486 are indicated in the diagram). For all the tests, we did not notice any significant difference in

drug release with or without bacteria. Shellac is a non-fermentable polymer, like ethylcellulose, and is not degraded by bacterial enzymes. There is only a passive diffusion of drug through the film coating, which increases in the upper GIT as shellac fraction in the blend ratio increases. Moreover, a change in the color of the mini-tablets can be seen in Figure 6 and explained as follows: the raw material of aqueous shellac ammonium salt has a dark orange tint. The overall system contained less ethylcellulose and was less hydrophobic. Shellac is known to have good film-forming properties and is mainly used as enteric coating, which can be considered a best candidate for colon targeting [16, 47].

As for its molecular structure, shellac contains non-polymerized carboxyl groups on cyclic terpene acids, making it a weak acid with a pKa between 5.6 and 7.0, depending on the type and grade [24, 48].

At pH < pKa, the carboxyl groups are protonated and produced strong intermolecular hydrogen bonding. The latter renders the shellac polymer tightly arranged and, thus, lead to a film coating with high modulus of rigidity [16, 49]. As the pH of the surrounding medium increases, the carboxylic groups dissociate and shellac swells. The swelling of shellac at pH 6.8 is known. This swelling precedes its dissolution, which is located around pH 7.3 due to its ionization above this pH value [16, 49, 50].

<u>The different shellac mechanisms reactions are indicated below:</u>

$R - COOH + NH_4^+ + OH^- \rightarrow R - COO^- + NH_4^+ + H_2O$

(Shellac ammonium salt solution)

After coating and drying

$R - COO^- NH_4+$ (Ammonium shellac salt film)

After digestion and contact to stomach with 0.1 M HCl (pH 1.2)

$R - COOH + NH_4Cl$

The swelling reduces the barrier functions of the coating and allows water to penetrate into the tablet core. Other parameters to be considered are mechanical properties and brittleness, which are shown to be much better using ammonium aqueous shellac solution than the alcoholic

514 solution. Luangtana et al. [24] reported that the high amount of shellac in thin composite
515 films based on alcoholic solution leads to weak mechanical properties and high brittleness.
516 Thus, the higher the shellac content in the dosage form, the lower the strength and strain are
517 generated.

518 If we combine these characteristics with the lower overall hydrophobicity of the system, we can
519 explain the kinetic profiles of this composition. The pH in which this enteric polymer dissolves
520 is controlled by the pKa and the amount of carboxyl groups [51]. As the amount of shellac
521 increases, its pH properties have an impact on the overall behaviour of the system and early
522 release occurs (Figure 5).

523 We have an interesting avenue, which can be explored in this study, related to the high
524 dissolution pH-threshold of shellac. This feature permits prolonged and controlled release in
525 the upper, or even in the entire GIT, for up to 32 h when combined with ethylcellulose.
526 Moreover, the pH in the distal part of the GIT is lower in IBD patients, giving us the opportunity
527 to use this natural resin polymer well below its dissolution pH threshold (pH 7.0). In this case,
528 adding polysaccharides degraded by the intestinal microbiota will be required to trigger the
529 degradation of such new developed systems.

530 From another point of view, Figure S6 (supplementary data) displays theophylline release as a
531 function of ratios at various coating levels (15 %; 20 %; 25 %; 30 %) in the upper GIT. This
532 compilation of release kinetic profiles allows us to evaluate the release kinetic profile evolution
533 when increasing EC fraction and select the best candidate for colon targeting. We can also
534 assess the impact of coating level, irrespective of blend ratios. The increase of the coating level
535 will lead to thicker and denser film coating, impeding the easy and quickly formation of pores
536 and subsequently diffusion of the drug at the site of action [36]. It has to be pointed out that this
537 phenomenon can be considered as advantageous in the protection of drug in the upper GIT, but
538 unfortunately disadvantageous in drug triggering into the colon.

539 For mini-tablets tested up to pH 6.8, drug release was linked to the swelling of both shellac and
540 ethylcellulose, followed by drug diffusion through the coating [43]. Although the shellac

coating is resistant at acidic pH, small amounts of water can penetrate the shellac-based material in the case of aqueous products containing ammonium salts, which were used for this study [49].

Even if shellac is highly hydrophobic, its proportion must be considered when developing and optimizing these drug delivery systems. Importantly, the (60:40) ratio seems to fit the required profile for colon targeting, which have shown a zero order kinetic profile irrespective of bacterial triggering action (Figure 5b). Given these considerations, optimization is possible by exploiting the toughest ratios (namely (80:20) and (75:25) ratios) and adding a natural polysaccharide for inducing a microbial-triggered-release, instead of a diffusion or pH dependent release triggering. The diffusion of drug into the surrounding media depends on water uptake and dry mass loss of the dosage form, which is controlled by the nature and the quantity of the added polysaccharide. The phenomenon of drug release from coated mini-tablets and pellets with bacteria and pH-sensitive polymeric film coatings include swelling of the polymer, erosion, and the dissolution of the polysaccharide creating pores/channels into the coating layer, thus facilitating diffusion of water and drug release.

It is necessary to find an appropriate balance between an overly resistant system and a suitable bacteria sensitive dosage form. Interestingly, natural polymers can also show comparable or superior performance to synthetic polymers in such applications. Shellac possesses a combination of characteristics such as good film formability, accurate pH-responsiveness, and amphiphilicity, making it a promising processing material [52].

The use of natural polysaccharides is of utmost importance due to their prebiotic and other multiple physiological activities, which can have in some cases beneficial effects by IBD patients [53].

As a polysaccharide, inulin was chosen and incorporated into polymeric films based on EC:Swanlac® ASL10 at different percentages. The goal was to determine the impact of this hydrophilic and biodegradable polysaccharide, as well as the appropriate amount used to target the distal part of the GIT. Figure 7 displays theophylline release as a function of the

568 concentration of additional inulin in polymeric films. The amount of the added
569 polysaccharide was ranged from 0 % to 30 % (w/w, referring to the total dry mass of the film
570 (EC+Shellac+TEC)). Please note that inulin was chosen due to its excellent mechanical
571 properties (flexibility) [54], as well to its resistance to digestive enzymes in the upper GIT, but
572 fortunately degraded by inulinases of Bifidobacterium genus located in the colon [55, 56].
573 The blend ratio of EC:Swanlac® ASL10 were the same as for the coated mini-tablets (described
574 above), namely 80:20 and 75:25. For comparison purposes, all films were standardized with an
575 average of 600 µm thickness. The increase of film thicknesses (600 µm) were chosen in order
576 to study its impact on drug release from polymeric films including the bacteria fermented
577 polysaccharide inulin.
578 As it can be seen in figure 7 a, films containing high amount of inulin showed premature drug
579 release due to the increased amount of the hydrophilic polysaccharide (inulin). As expected,
580 drug release increased with increasing inulin amount. As it can be seen in Figure 7a, the addition
581 of 30 % inulin (w/w, referring to the total dry mass of the film (EC+Shellac+TEC)) leads to 60
582 % of drug release. Considering that polymeric films (matrix system) containing high amount of
583 inulin showed more or less fast drug release when placed into an aqueous medium, drug release
584 from mini-tablets (reservoir system) will take more time to be released.

585 In a same way, Figure 7b clearly shows slowed drug release from films based on (EC:Swanlac®
586 ASL10; 75:25). Less than 50 % release was achieved with the two highest amounts of inulin,
587 while lower concentrations led to 20 to 30 % release up to 8 h. Data from Fig 3a and 4a should
588 not be compared directly to data from figure 7 because the dosage form is not the same. In this
589 case, the added inulin to the system can extremely impact the drug release behavior of such
590 advanced drug delivery systems.

591 Please note that inulin has little hydrophilic properties compared to other polysaccharides. This
592 may explain the retention capacity of this compound, which was further supported by Benzine
593 et *al* [32]. They investigated *in vitro* theophylline release from hot melt extrudates using several
594 polysaccharides over 24 h in the same media as for the upper GIT (HCl 0.1 M followed by PBS

595 6.8) and showed that inulin was one of the most resistive materials, with only 13 % release
596 up to 24 h. These formulations give a good estimation of their use on mini-tablets, as the drug
597 has a longer pathway from the inner core to the outer side at the site of action. These polymeric
598 films could be used to protect drug within the upper GIT, with the possibility of *in situ*
599 degradation caused by the metabolic activity of the microbiota. Following these observations,
600 the same compositions with the same blend ratios were tested to coat mini-tablets. The
601 percentages of inulin were selected between 5 and 20 %. The aim of this study was to find a
602 colon-targeted formulation capable of showing an immediate or prolonged drug release within
603 the lower GIT only, due to a stimulus provoked by microbiota degradation in this region.
604 According to Figure 7, which shows drug release from polymeric films, we could easily
605 incorporate inulin into the coating layer, as data in the upper GIT showed little influence on
606 drug release. Shellac brings here a pH dependency, which is interesting for a dual-triggered
607 stimuli system. Its presence promotes the dissociation of formulation as pH evolves. Inulin
608 serves as a substrate for the microbiota, but also increases the hydrophilicity and thus the entry
609 of water into the coating. Figure 8a stands for theophylline drug release of mini-tablets in the
610 upper GIT, coated with EC:Swanlac® ASL10 (80:20) and 5 % inulin,using different coating
611 levels.

612 In accordance with the results observed with polymeric films (Figure 7), the addition of inulin
613 did not lead to any significant difference in drug release in the upper GIT. The formulations
614 were clearly resistant, showing less than 5 % theophylline release up to 8 h. The same
615 formulations were then subsequently incubated in simulated colonic medium with and without
616 fresh fecal samples to further evaluate this formulation for colon targeting. Figure 8b shows
617 negligible differences for 5 % and 10 % inulin. For a blend ratio of (80:20), the influence was
618 found to be very low. Therefore, more polysaccharide would be required in this case to trigger
619 drug release in the distal part of GIT. Polymeric films did not show differences either for this
620 ratio (figure 7).

621 On the other hand, Figure 9 was conducted in the same conditions with EC:Swanlac® ASL10
622 (75:25) blend ratio.

623 Importantly, the same behavior can be observed in the upper GIT. These robust systems can
624 withstand their incubation in the upper GIT, even with the adjunction of a hydrophilic
625 component. Nonetheless, Figure 9b highlights some interesting data for colon targeting.
626 Different drug release profiles can be depicted upon exposure to the entire gastrointestinal tract,
627 including 24 h in colonic medium. Interestingly, the addition of 10 % inulin has showed 55 %
628 release during 24 h upon exposure to culture medium inoculated with fecal samples. However,
629 the addition of 5 % inulin, which displayed only 10 % drug release, was not sufficient to trigger
630 drug release in the colon. For reasons of comparison, *in vitro* drug release of the same
631 formulation (10 % inulin) has been carried out in the entire GIT without fecal samples.
632 Importantly, *in vitro* drug release was very low and not impacted by culture medium without
633 bacteria. Obviously, inulin serves as a substrate for the microbiota. We believe that release
634 will be faster and higher by increasing the amount of the polysaccharide fraction (15, 20, 30 %)
635 under these conditions. Please note that coating level was increased as the amount of inulin
636 increased for more protection in the upper GIT, since the system becomes more hydrophilic:
637 12 % CL was exploited with 5 % inulin and 25 % CL with 10 % inulin. Therefore, the need to
638 protect the drug is superior when the hydrophilicity of such systems rises. These new developed
639 systems based on polysaccharides need to be more protected by thicker coating. It is important
640 to note that there can be a saturation of the enzymatic activity as well as secretion in the utilized
641 closed test dissolution set up. This phenomenon will not be limited in vivo due to the motility
642 and peristalsis, ensuring continuous degradation of the formulation (open ecosystem). Figure
643 10 represents macroscopic pictures of these mini-tablets coated with Ethylcellulose:Swanlac®
644 ASL10 (75:25) and containing 5 or 10 % inulin.

From another point of view, Figure S7 indicates drug release as a function of the blend ratio (80:20 and 75:25) and the amount of inulin added (5 % and 10 %) in the coating layer.

Interestingly, (80:20) blend ratio containing 5 % inulin displays a double percentage of theophylline release compared to the one of (75:25) ratio (21 % vs 10.5 % respectively, Figure S7a). However, the opposite trend could be observed in case of 10 % inulin (Figure S7b).

10 % inulin in such new developed systems provided relevant clue for colonic drug delivery. It is to emphasize that 10 % inulin will be the minimum amount at which a colon targeted profile is conceivable under these conditions, protecting the drug in the upper GIT but triggering drug release in the lower GIT. Here, inulin as an excipient showed interesting and significant variations on theophylline drug release over time, providing a potential dual (pH and bacteria sensitive)-triggered stimuli system. This new developed formulation containing 10 % inulin as a biodegradable polysaccharide confirmed *in vitro* efficacy, which offers very promising perspectives and deserves to be further exploited in preclinical studies.

Conclusion

Novel polymeric film coatings for prolonged and controlled drug delivery in the distal part of GIT, based on Ethylcellulose:Swanlac® ASL10 blends containing inulin, have been identified. Some of them have demonstrated the ability to completely protect the drug in the upper GIT, which may be a useful tool for protein-based therapies via oral route. Clearly, the contribution of EC to protect drug and hinder premature film dissolution is of overriding importance. Surprisingly, Shellac ASL 10 plays an operational key role in the efficiency of these film coatings, which must be adjusted in the polymer blending to avoid potential failure of the concept/technology. It is of utmost importance to optimize these novel technologies in order to achieve appropriate triggered release kinetics at the site of action, as well in physiological as in pathological conditions. One thing is clear: this new pharmaceutical technology based on Ethylcellulose:Shellac which may or may not include a polysaccharide, can be used for prolonged release in the lower GIT of fragile drugs such as protein and polypeptide-based drugs that are destroyed in the upper GIT and preferentially released in the colon.

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M. Penning	Methodology; Validation; Investigation; Writing - Review & Editing

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Figure Captions

Fig. 1 Chemical structure and properties of shellac with permission of Thombare et al. [57]

Fig. 2 Impact of the thickness on *in vitro* theophylline release from polymeric films based on Ethylcellulose:(Swanlac® ASL10 + maltodextrin) and plasticized with 25 % TEC. Thicknesses and the blend ratio are indicated in the diagram. Polymeric films containing 1 % theophylline were incubated in 0.1 M HCl pH 1.2 (2 h) followed by phosphate buffer pH 6.8 (6h).

Fig. 3 Impact of the coating level on *in vitro* theophylline release from coated mini-tablets with Ethylcellulose:Swanlac® ASL10 blend (80:20) upon exposure to (a) the upper GIT: 0.1 M HCl (2 h) followed by phosphate buffer pH 6.8 (6h), and (b) the entire GIT: simulated gastric and intestinal fluids followed by culture medium inoculated with or without fresh fecal samples (24h). For reasons of comparison mini-tablets were incubated in culture medium without fresh fecal samples (24h).

Fig. 4 Impact of the coating level on *in vitro* theophylline release from coated mini-tablets with Ethylcellulose:Swanlac® ASL10 blend (75:25) upon exposure to (a) the upper GIT: 0.1 M HCl (2 h) followed by phosphate buffer pH 6.8 (6h), and (b) the entire GIT: simulated gastric and intestinal fluids followed by culture medium inoculated with or without fresh fecal samples (24h). For reasons of comparison mini-tablets were incubated in culture medium without fresh fecal samples (24h).

Fig. 5 Impact of the coating level on *in vitro* theophylline release from coated mini-tablets with (60:40) Ethylcellulose:Swanlac® ASL10 blending upon exposure to (a) the upper GIT: 0.1 M HCl (2 h) followed by phosphate buffer pH 6.8 (6h), and (b) the entire GIT: simulated gastric and intestinal fluids followed by culture medium inoculated with or without fresh fecal samples (24h). For reasons of comparison mini-tablets were incubated in culture medium without fresh fecal samples (24h).

Fig. 6 Macroscopic pictures of coated mini-tablets with Ethylcellulose:Swanlac® ASL10 (60:40) blends. The coating levels are indicated on the left hand side. The potential exposure to the release media is indicated at the top.

Fig. 7 Impact of inulin amount (0 to 30 %; w/w referring to the total mass of the film) on *in vitro* theophylline release from polymeric films based on Ethylcellulose:Swanlac® ASL10 blends ratio: (a) 80:20 and (b) 75:25. Polymeric films are incubated in 0.1 M HCl (2 h) followed by phosphate buffer pH 6.8 (6h).

Fig. 8 Impact of (a) the coating level on *in vitro* theophylline release from coated mini-tablets with Ethylcellulose:Swanlac® ASL10 blend (80:20) with 5 % inulin added, and (b) the amount of inulin (0, 5 and 10 %) into the film coatings. Coated mini-tablets are exposed to (a) 0.1 M HCl (2 h) followed by phosphate buffer pH 6.8 (6h), and (b) simulated gastric and intestinal fluids as well as simulated colonic fluid with and without fresh fecal samples.

Fig. 9 Impact of (a) the coating level on *in vitro* theophylline release from coated mini-tablets with Ethylcellulose:Swanlac® ASL10 blend (75:25) with 5 % inulin added, and (b) the amount of inulin (0, 5 and 10 %) into the film coatings. Coated mini-tablets are exposed to (a) 0.1 M HCl (2 h) followed by phosphate buffer pH 6.8 (6h), and (b) simulated gastric and intestinal fluids as well as simulated colonic fluid with and without fresh fecal samples.

Fig. 10 Macroscopic pictures of coated mini-tablets with Ethylcellulose:Swanlac® ASL10 (75:25) blends. The percentages of inulin are indicated on the left hand side. The potential exposure to the release media is indicated at the top.

References

[1] Tran PHL, Tran TTD. Current Film Coating Designs for Colon-Targeted Oral Delivery. Curr Med Chem. 2021; 28: 1957–1969

[2] Jones RGA, Martino A. Targeted localized use of therapeutic antibodies: a review of non-systemic, topical and oral applications. Crit Rev Biotechnol. 2016; 36: 506–520

[3] Vass P, Démuth B, Hirsch E, Nagy B, Andersen SK, Vigh T, et al. Drying technology strategies for colon-targeted oral delivery of biopharmaceuticals. J Control Release. 2019; 296: 162-178

[4] Truong-Le V, Lovalenti PM, Abdul-Fattah AM. Stabilization challenges and formulation strategies associated with oral biologic drug delivery systems. Adv Drug Deliv Rev. 2015; 93: 95–108

[5] Philip AK, Philip B. Colon targeted drug delivery systems: a review on primary and novel approaches. Oman Med J. 2010; 25: 79-87 [6] Awad A, Madla CM, McCoubrey LE, Ferraro F, Francesca KHG, Buanz A, et al. Clinical translation of advanced colonic drug delivery technologies. Adv Drug Deliv Rev. 2022; 181: 114076

[7] Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet Lond Engl. 2017; 389: 1756–1770

[8] Barberio B, Segal JP, Quraishi MN, Black CJ, Savarino EV, Ford AC. Efficacy of Oral, Topical, or Combined Oral and Topical 5-Aminosalicylates, in Ulcerative Colitis: Systematic Review and Network Meta-analysis. J Crohns Colitis. 2021; 15: 1184–1196

[9] Gallo G, Kotze PG, Spinelli A. Surgery in ulcerative colitis: When? How? Best Pract Res Clin Gastroenterol. 2018; 32–33: 71–78

[10] Sousa T, Paterson R, Moore V, Carlsson A, Abrahamsson B, Basit AW. The gastrointestinal microbiota as a site for the biotransformation of drugs. Int J Pharm. 2008;

363: 1–25 [11] Alqahtani MS, Kazi M, Alsenaidy MA, Ahmad MZ. Advances in Oral Drug Delivery. Front Pharmacol. 2021; 12: 618411

[12] Evans DF, Pye G, Bramley R, Clark AG, Dyson TJ, Hardcastle JD. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut 1988; 29: 1035–1041

[13] Koziolek M, Grimm M, Becker D, Iordanov V, Zou H, Shimizu J, et al. Investigation of pH and Temperature Profiles in the GI Tract of Fasted Human Subjects Using the Intellicap® System. J Pharm Sci 2015; 104: 2855–2863

[14] Nugent SG, Kumar D, Rampton DS, Evans DF. Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosalicylates and other drugs. Gut 2001; 48: 571–577

[15] Sasaki Y, Hada R, Nakajima H, Fukuda S, Munakata A. Improved localizing method of radiopill in measurement of entire gastrointestinal pH profiles: colonic luminal pH in normal subjects and patients with Crohn's disease. Am J Gastroenterol 1997; 92: 114–118

[16] Yuan Y, He N, Dong L, Guo Q, Zhang X, Li B, et al. Multiscale Shellac-Based Delivery Systems: From Macro- to Nanoscale. ACS Nano. 2021; 15: 18794–18821

[17] Zhang C, Chen Z, He Y, Xian J, Luo R, Zheng C, et al. Oral colon-targeting core-shell microparticles loading curcumin for enhanced ulcerative colitis alleviating efficacy. Chin Med. 2021; 16: 92

[18] Panchapornpon D, Limmatvapirat C, Nuntanid J, Luangtana-Anan M, Sriamornsak P, Puttipipatkhachorn S, et al. Development of Shellac from Source available in Thailand as an alternative Polymer for Postharvest Treatment. Thai J Agric Sc. 2011; 224-229

[19] Chen Q, Zhang H, Zheng H, Sun Y. Solubility of Lac Resin in Aqueous Ammonia and Physico-chemical Properties. Food Sci. 2013; 77-82

[20] Al-Gousous J, Penning M, Langguth P. Molecular insights into shellac film coats from different aqueous shellac salt solutions and effect on disintegration of enteric-coated soft gelatin capsules. Int J Pharm. 2015; 484: 283–291

[21] Specht F. PhD Thesis. Kiel University. 1995; p.22

[22] Specht F, Saugestad M, Waaler T, Müller B. The application of shellac as an acidic polymer for enteric coating. Pharm Tech Europe. 1998; 9 [23] Farag Y, Leopold CS. Physicochemical Properties of Various Shellac Types. Dissolution Technol. 2009; 16: 33–39

[24] Limmatvapirat S, Limmatvapirat C, Puttipipatkhachorn S, Nuntanid J, Luangtana-Anan M, et al. Enhanced enteric properties and stability of shellac films through composite salts formation. Eur J Pharmaceutics and Biopharmaceutics. 2007; 67: 690-698

[25] Habashy R, Khoder M, Zhang S, Pereira B, Bohus M, Tzu-Wenig Wang J, et al. An innovative wax-based enteric coating for pharmaceutical and nutraceutical oral products. Int J Pharm 2020; 591: 119935

[26] Data Sheet SWANLAC ASL 10. www.afsuter.com. https://www.afsuter.com/product/aqueous-shellac-solution (accessed 13th march 2023)

[27] Theophylline water solubility at 25°C. www.pubchem.com. Available via Theophylline water solubility (accessed 13 October 2022)

[28] Karrout Y, Neut C, Wils D, Siepmann F, Deremaux L, Flament MP, et al. Novel polymeric film coatings for colon targeting: Drug release from coated pellets. Eur J Pharm Sci. 2009; 37: 427–433

[29] Karrout Y, Neut C, Wils D, Siepmann F, Deremaux L, Flament MP, et al. Peas starch-based film coatings for site-specific drug delivery to the colon. J Appl Polym Sci. 2011; 119: 1176–1184

[30] Karrout Y, Neut C, Siepmann F, Wils D, Ravaux P, Deremaux L, et al. Enzymatically degraded Eurylon 6 HP-PG: ethylcellulose film coatings for colon targeting in inflammatory bowel disease patients. J Pharm Pharmacol. 2010; 62: 1676–1684

[31] Karrout Y, Neut C, Wils D, Siepmann F, Deremaux L, Dubreuil L, et al. Colon targeting with bacteria-sensitive films adapted to the disease state. Eur J Pharm Biopharm. 2009; 73: 74–81

[32] Benzine Y, Siepmann F, Neut C, Danede F, Willart JF, Siepmann J, et al. Hot melt extruded polysaccharide blends for controlled drug delivery. J Drug Deliv Sci Technol. 2019; 54: 101317

[33] Levingstone TJ, Herbaj S, Dunne NJ. Calcium Phosphate Nanoparticles for Therapeutic Applications in Bone Regeneration. Nanomater Basel Switz 2019; 9: E1570

[34] Rathbone MJ, Hadgraft J, Roberts MS, Lane ME. Modified-Release Drug Delivery Technology. 2nd Edition. CRC Press. Epub ahead of print 28 May 2008, 2013; Volume 2. Available from https://doi.org/10.3109/9781420045260

[35] Hamedelniel EI, Bajdik J, Pintye-Hódi K. Optimization of preparation of matrix pellets containing ethylcellulose. Chem Eng Process Process Intensif. 2010; 49: 120–124

[36] Shah N, Sharma OP, Mehta T, Amin A. Design of experiment approach for formulating multi-unit colon-targeted drug delivery system: in vitro and in vivo studies. Drug Dev Ind Pharm. 2016; 42: 825–835

[37] Benzine Y, Siepmann F, Neut C, Danede F, Willart JF, Siepmann J, et al. Injection-molded capsule bodies and caps based on polymer blends for controlled drug delivery. Eur J Pharm Biopharm. 2021; 168: 1–14

[38] Esseku F, Adeyeye MC. Bacteria and pH-sensitive polysaccharide-polymer films for colon targeted delivery. Crit Rev Ther Drug Carrier Syst. 2011; 28: 395–445

[39] Haaser M, Karrout Y, Velghe C, Cuppok Y, Gordon KC, Pepper M, et al. Application of terahertz pulsed imaging to analyse film coating characteristics of sustained-release coated pellets. Int J Pharm. 2013; 457: 521–526

[40] Stillhart C, Vučićević K, Augustijns P, Basit AW, Batchelor H, Flanagan TR, et al. Impact of gastrointestinal physiology on drug absorption in special populations-An UNGAP review. Eur J Pharm Sci. 2020; 147: 105280

[41] Ravi V., Siddaramaiah, Pramod Kumar TM. Influence of natural polymer coating on novel colon targeting drug delivery system. J Mater Sci Mater Med. 2008; 19: 2131–2136

876 [42] Roda A, Simoni P, Magliulo M, Nanni P, Baraldini M, Roda G, et al. A new oral
877 formulation for the release of sodium butyrate in the ileocecal region and colon. World J
878 Gastroenterol. 2007; 13: 1079–1084

879 [43] Farag Y, Leopold CS. Investigation of drug release from pellets coated with different
880 shellac types. Drug Dev Ind Pharm. 2011; 37: 193–200 [44] Maghrabia AE,
881 Boughdady MF, Meshali MM. New Perspective Enteric-Coated Tablet Dosage Form for
882 Oral Administration of Ceftriaxone: In Vitro and In Vivo Assessments. AAPS
883 PharmSciTech. 2019; 20: 306

884 [45] Wilding IR, Kenyon CJ, Hooper G. Gastrointestinal spread of oral prolonged-release
885 mesalazine microgranules (Pentasa) dosed as either tablets or sachet. Aliment Pharmacol
886 Ther. 2000; 14: 163–169

887 [46] Schellekens RCA, Stuurman FE, van der Weert FHJ, Kosterink JGW, Frijlinket HW. A
888 novel dissolution method relevant to intestinal release behaviour and its application in
889 the evaluation of modified release mesalazine products. Eur J Pharm Sci. 2007; 30: 15–
890 20

891 [47] SSB Pharma - Shellac for pharmaceutical applications USP / Ph. Eur. Available from
892 SSB Pharma

893 [48] Buch K, Penning M, Wächtersbach E, Maskos M, Langguth P. Investigation of various
894 shellac grades: additional analysis for identity. Drug Dev Ind Pharm. 2009; 35: 694–703

895 [49] Theismann E-M, Keppler JK, Knipp J-R, Fangmann D, Appel E, Gorb SN, et al.
896 Adjustment of triple shellac coating for precise release of bioactive substances with
897 different physico-chemical properties in the ileocolonic region. Int J Pharm. 2019; 564:
898 472–484

899 [50] Patel A, Heussen P, Hazekamp J, Velikov KP. Stabilisation and controlled release of
900 silibinin from pH responsive shellac-colloidal particles. Soft Matter. 2011; 7: 8549

901 [51] Limmatvapirat S, Limmatvapirat C, Luangtana-Anan M, Nunthanid J, Oguchi T, Tozuka
902 Y, et al. Modification of physicochemical and mechanical properties of shellac by partial
903 hydrolysis. Int J Pharm. 2004; 278: 41–49

904 [52] Yuan Y, He N, Xue Q, Guo Q, Dong L, Haruna MH, et al. Shellac: A promising natural
905 polymer in the food industry. Trends Food Sci Technol 2021; 109: 139–153

906 [53] Kanwal S, Aliya S, Xin Y. Anti-Obesity Effect of Dictyophora indusiata Mushroom
907 Polysaccharide (DIP) in High Fat Diet-Induced Obesity via Regulating Inflammatory
908 Cascades and Intestinal Microbiome. Front Endocrinol. 2020; 11: 558874

909 [54] Mensink MA, Frijlink HW, Van Der Voort Maarschalk K, Hinrichs WLJ. Inulin, a
910 flexible oligosaccharide. II: Review of its pharmaceutical applications. Carbohydr Polym
911 2015; 134: 418–428

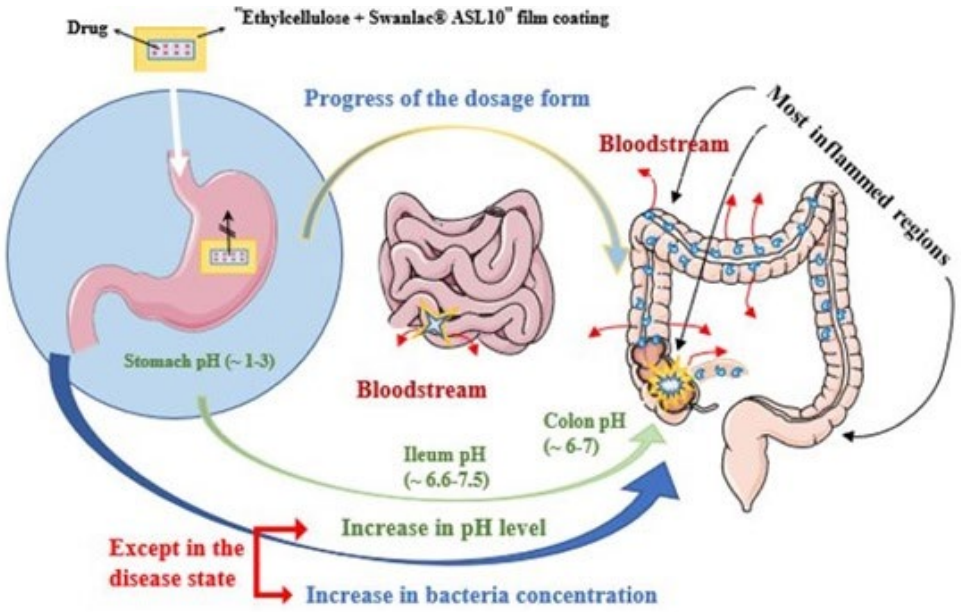
912 [55] Afinjuomo F, Abdella S, Youssef SH, Song Y, Garg S. Inulin and Its Application in Drug
913 Delivery. Pharm Basel Switz 2021; 14: 855

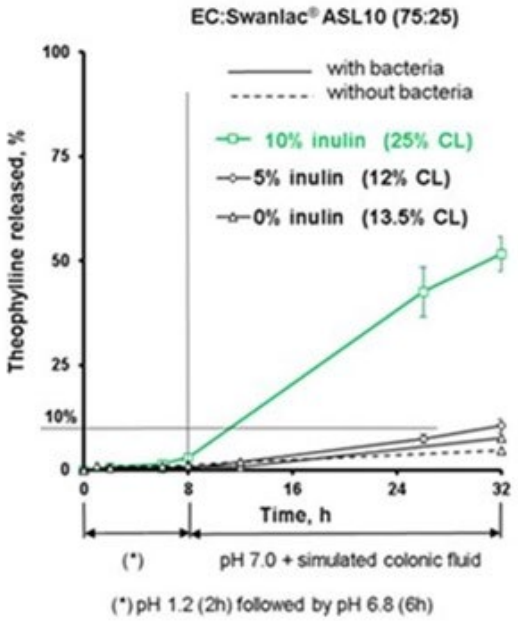
914 [56] López-Molina D, Navarro-Martínez MD, Rojas-Melgarejo F, Hiner ANP, Chazarra S,
915 Rodriguez-Lopez J. Molecular properties and prebiotic effect of inulin obtained from
916 artichoke (Cynara scolymus L.). Phytochemistry 2005; 66: 1476–1484

[57] Thombare N, Kumar S, Kumari U, Sakare P, Yogi RK, Prasad N, et al. Shellac as a multifunctional biopolymer: A review on properties, applications and future potential. Int J Biol Macromol. 2022; 215: 203–223

[58] Farag Y, Leopold CS. Development of shellac-coated sustained release pellet formulations. Eur J Pharm Sci Off J Eur Fed Pharm Sci 2011; 42: 400–405

Graphical Abstract





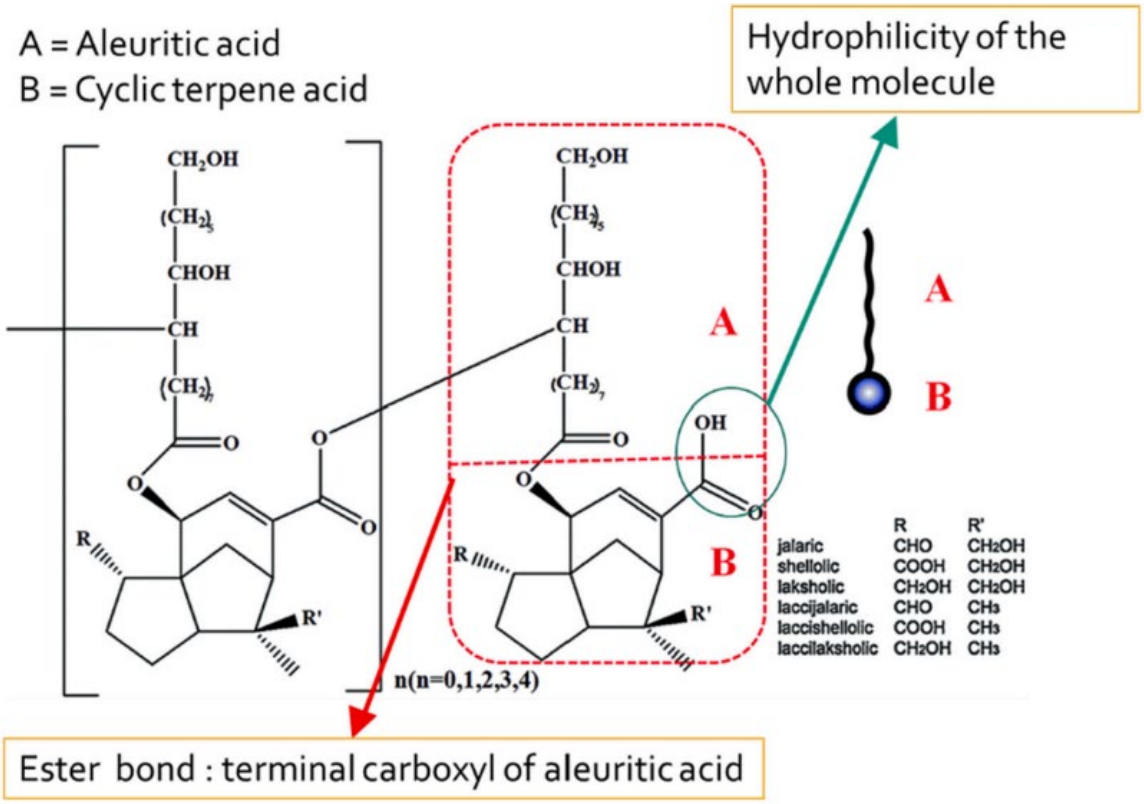
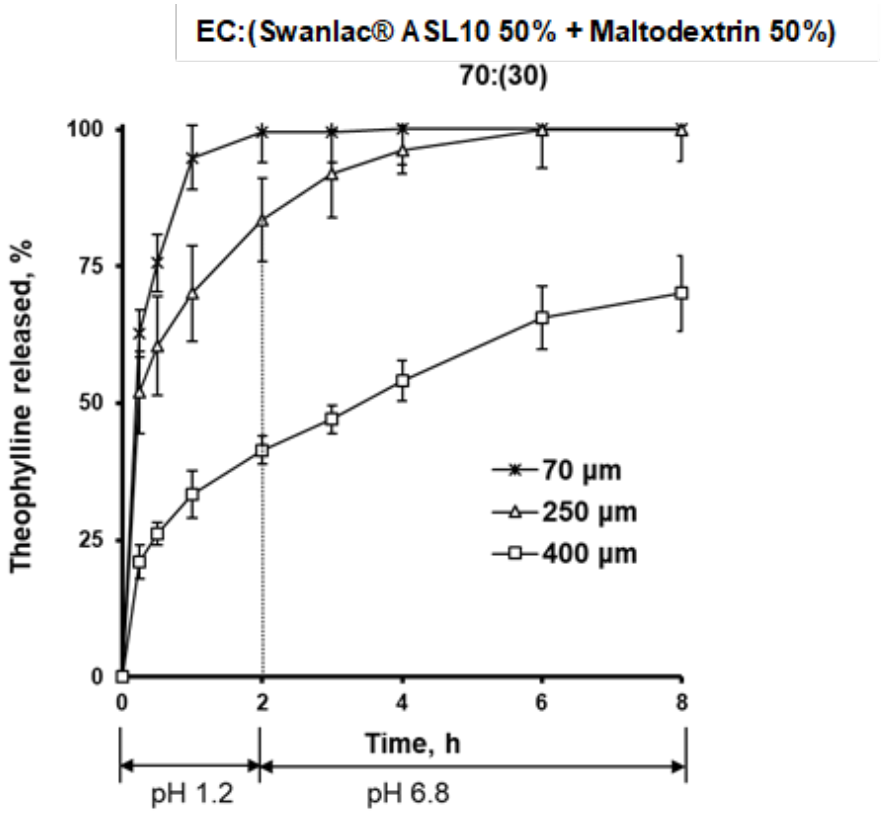

A = Aleuritic acid
B = Cyclic terpene acid
Hydrophilicity of the whole molecule
A
B
n(n=0,1,2,3,4)
Ester bond : terminal carboxyl of aleuritic acid
R R'
jalaric CHO CH2OH
shellolic COOH CH2OH
laksholic CH2OH CH2OH
laccijalaric CHO CH3
laccishellolic COOH CH3
laccilaksholic CH2OH CH3

Figure 1

a)


EC:(Swanlac® ASL10 50% + Maltodextrin 50%)
70:(30)
Theophylline released, %
100
75
50
25
0
0
2
4
6
8
Time, h
pH 1.2
pH 6.8
70 µm
250 µm
400 µm

b)

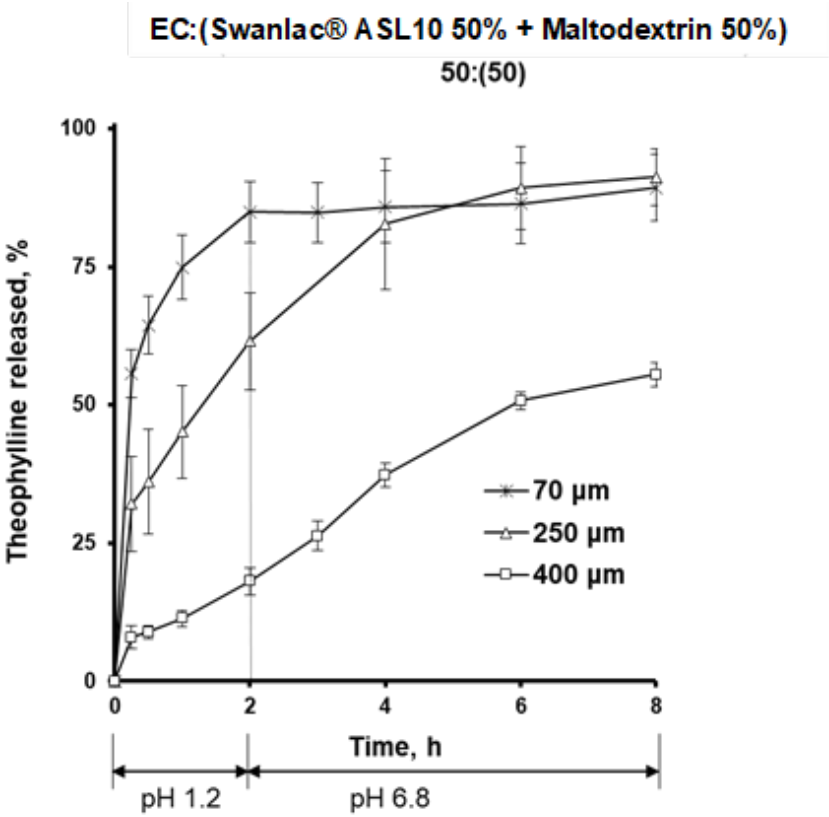
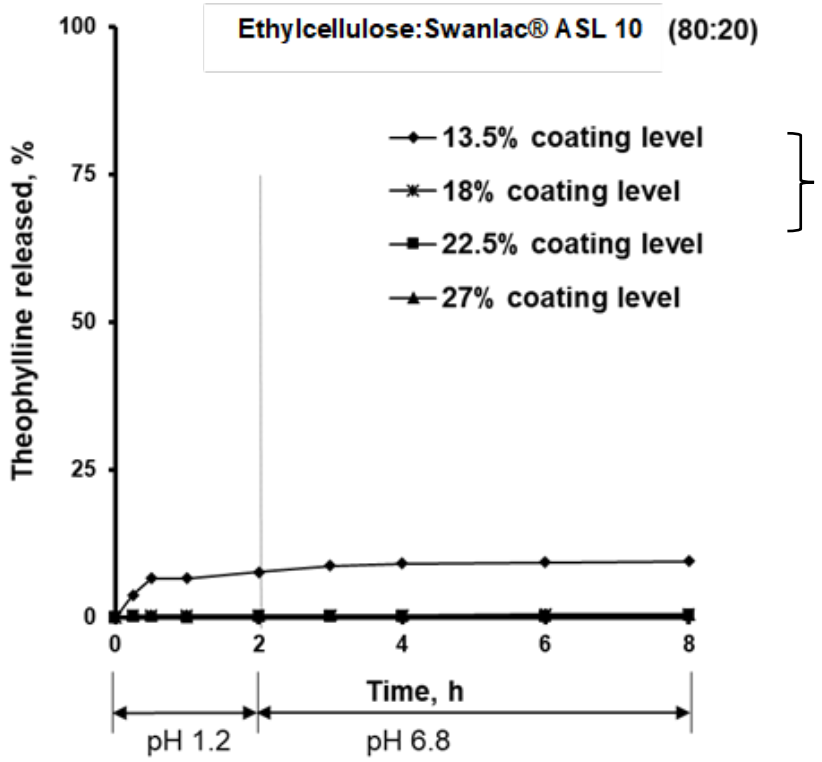

EC:(Swanlac® ASL10 50% + Maltodextrin 50%)
50:(50)
Theophylline released, %
100
75
50
25
0
0
2
4
6
8
Time, h
pH 1.2
pH 6.8
70 µm
250 µm
400 µm

Figure 2

a) **Upper GIT**



Human fresh fecal samples

b) **Entire GIT**

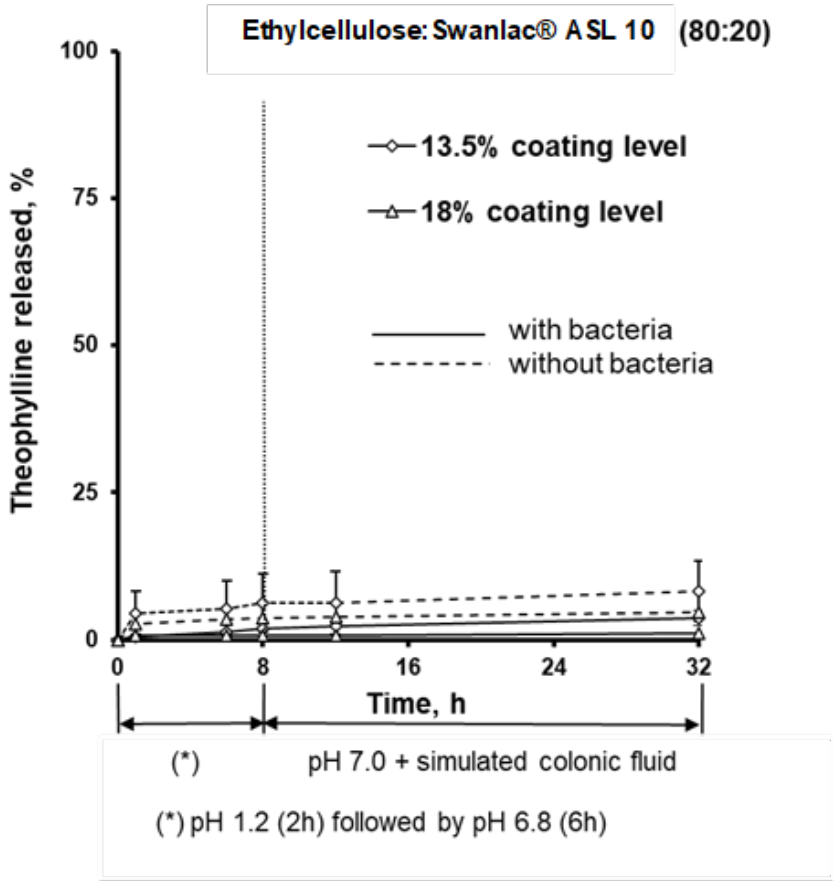
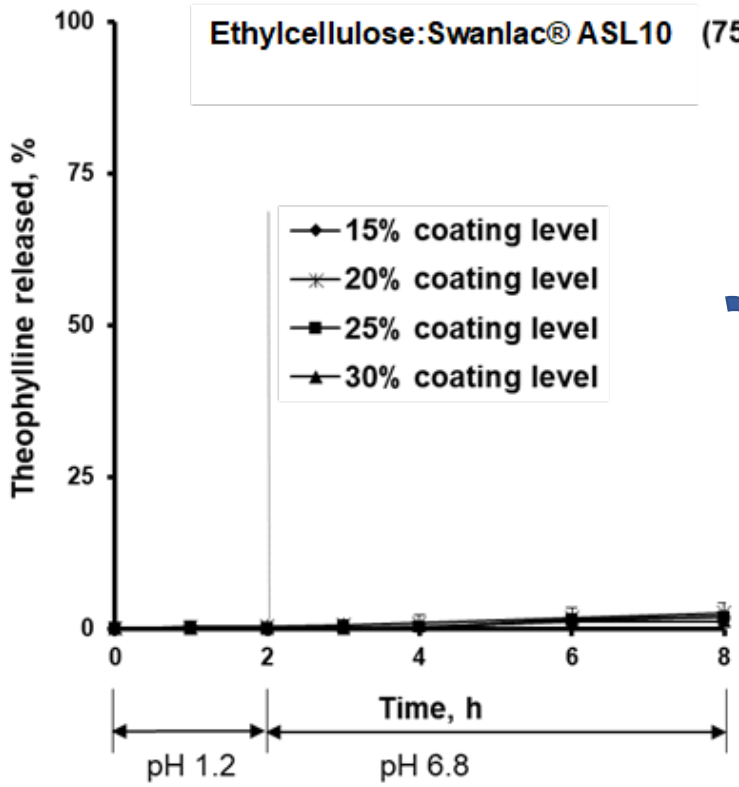


Figure 3

a) **Upper GIT**



Human fresh fecal samples

b) **Entire GIT**

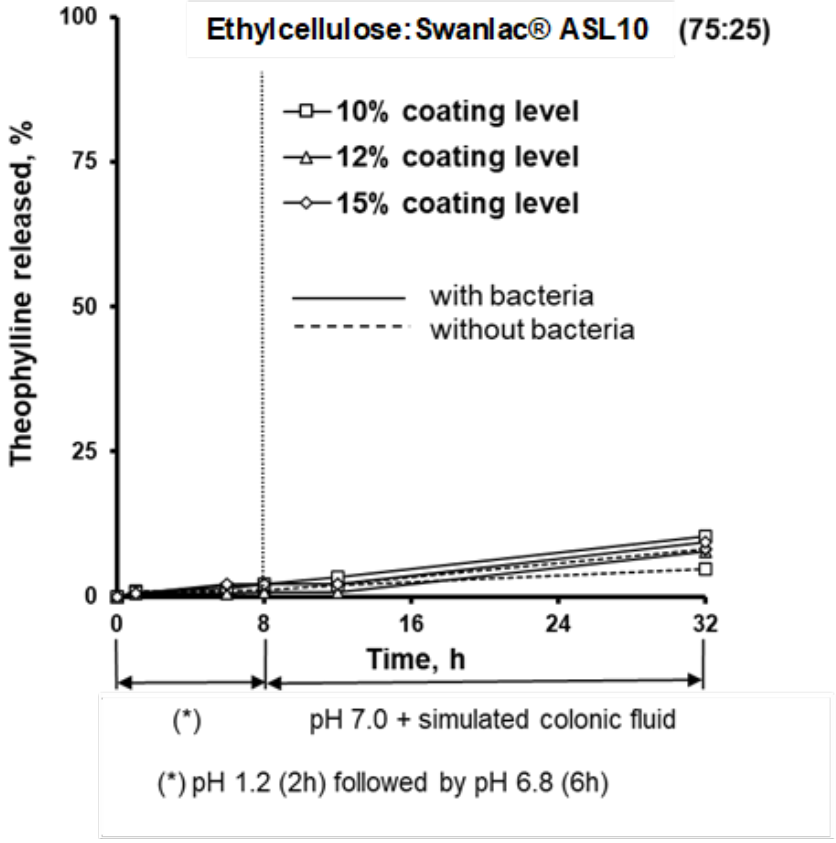
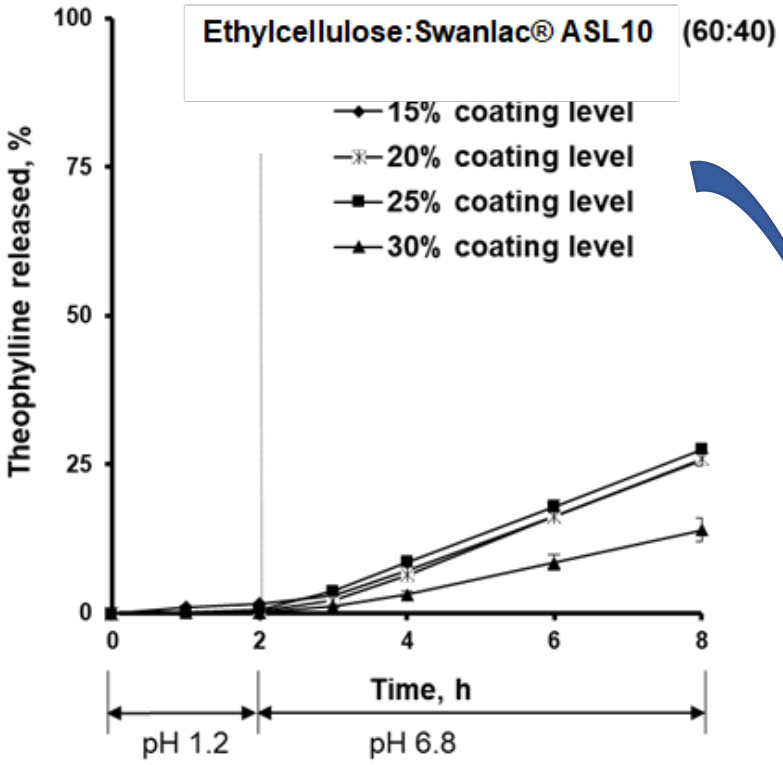


Figure 4

a) **Upper GIT**



Human fresh fecal samples

b) **Entire GIT**

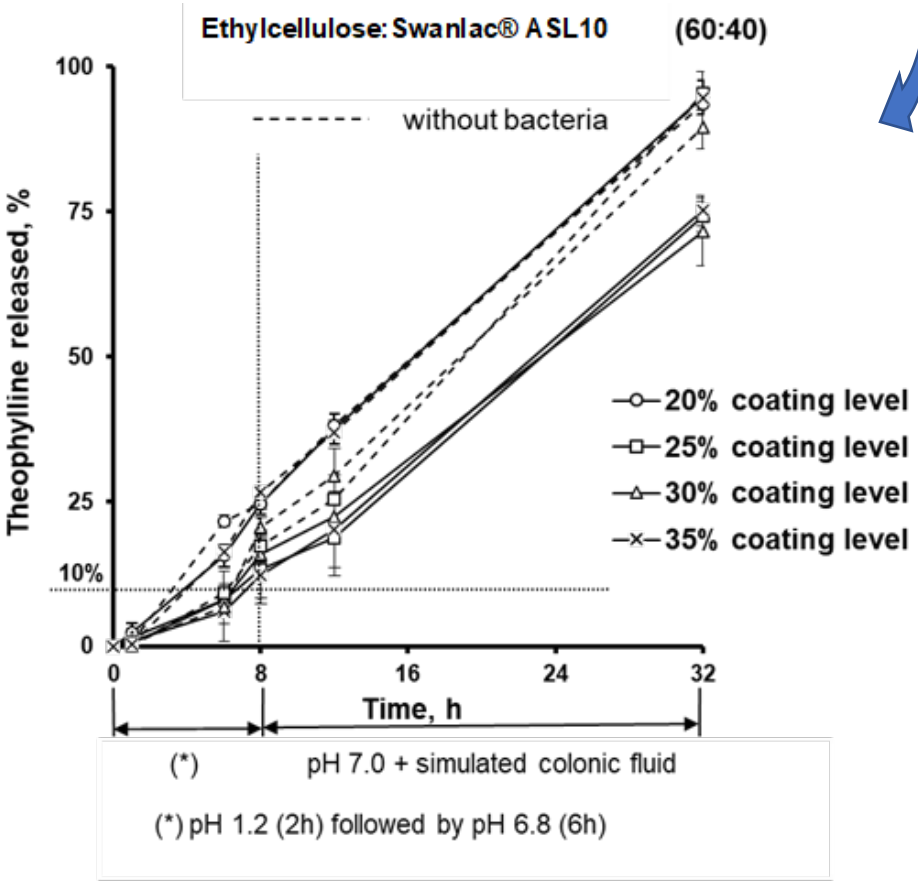


Figure 5

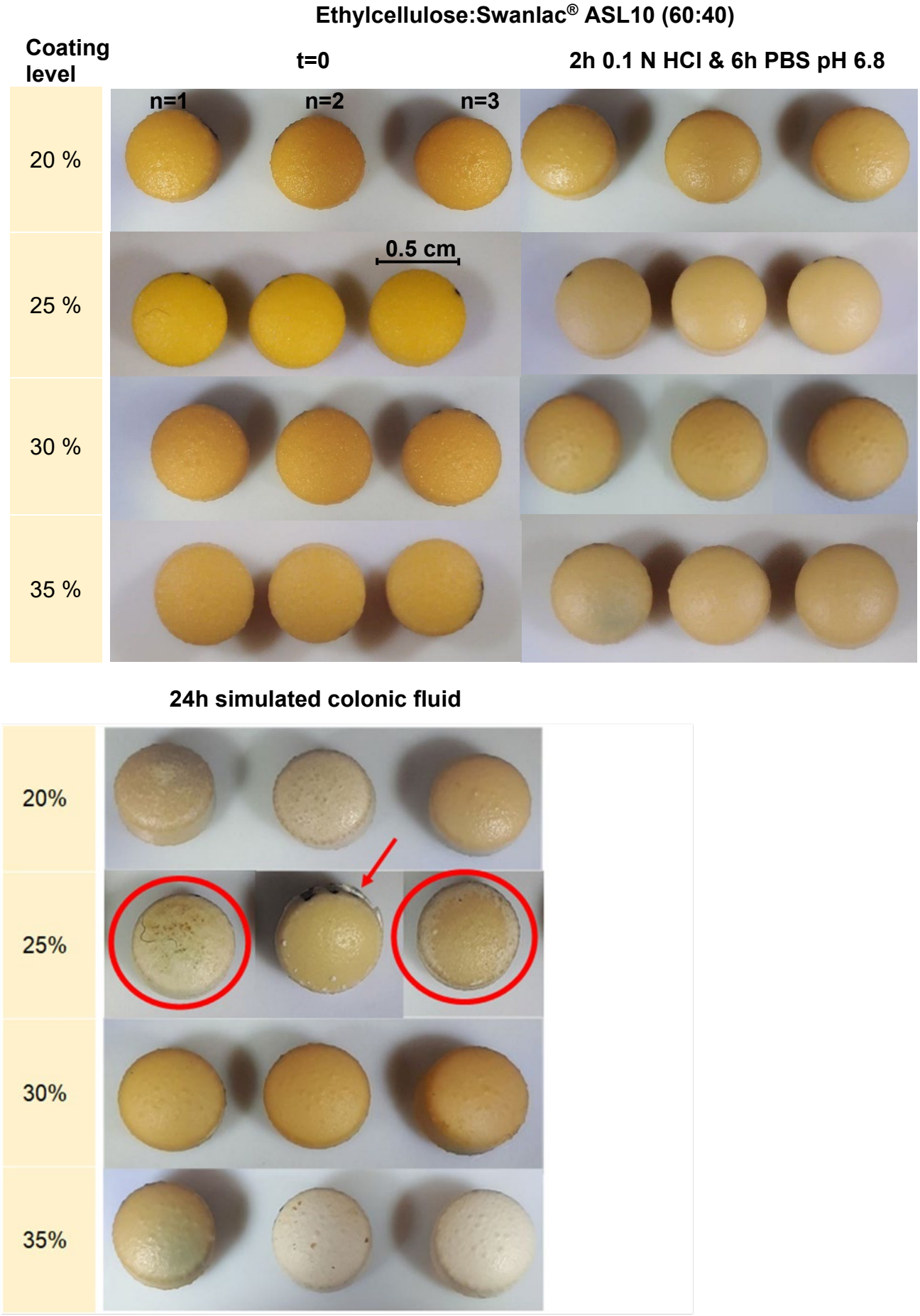
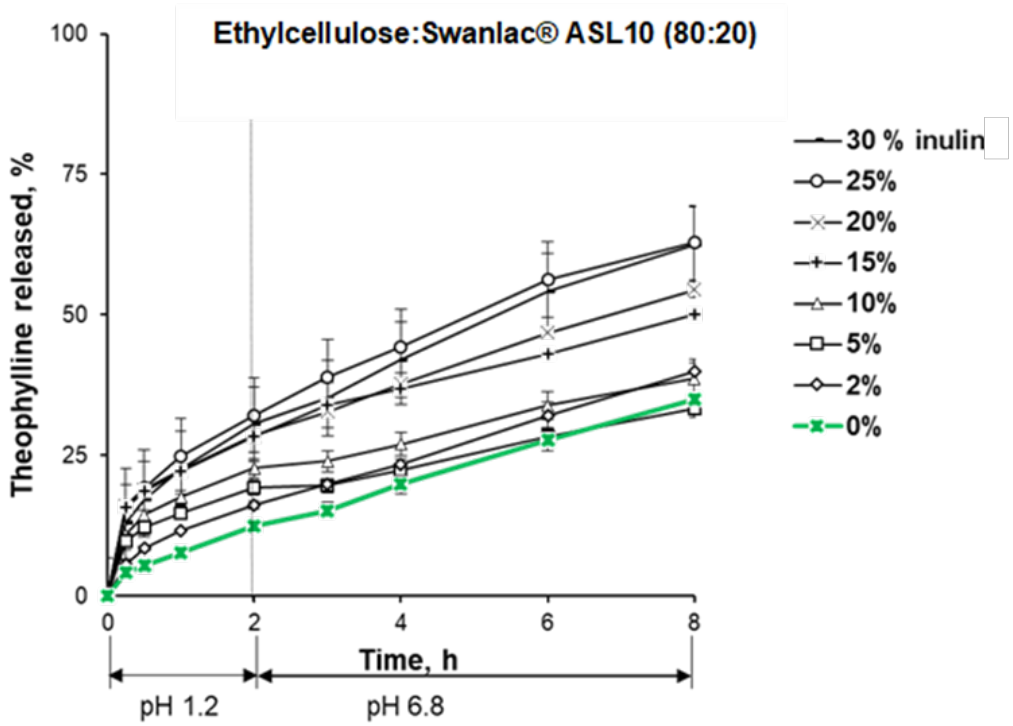

Ethylcellulose:Swanlac® ASL10 (60:40)
Coating level
t=0
2h 0.1 N HCl & 6h PBS pH 6.8
n=1
n=2
n=3
20 %
25 %
30 %
35 %
0.5 cm
24h simulated colonic fluid
20%
25%
30%
35%

Figure 6

a) Upper GIT



b) Upper GIT

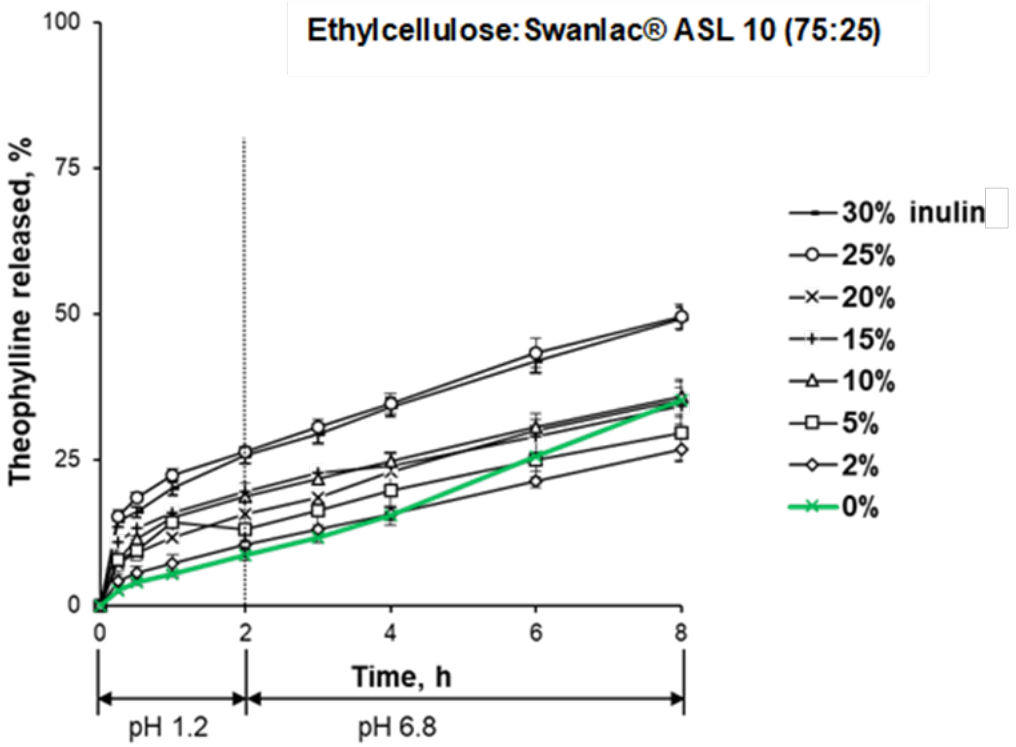
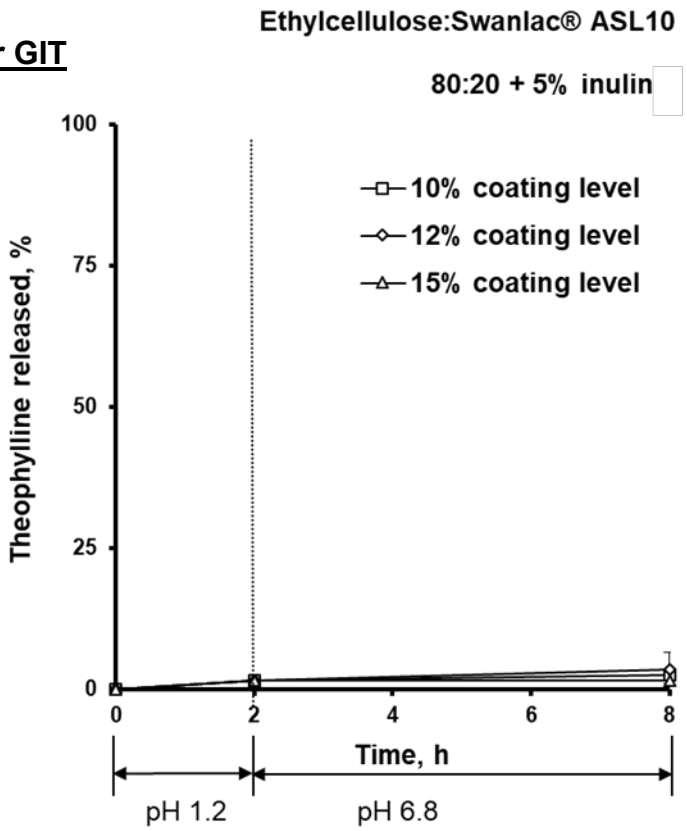


Figure 7

a) **Upper GIT**



Human fresh fecal samples

b) **Entire GIT**

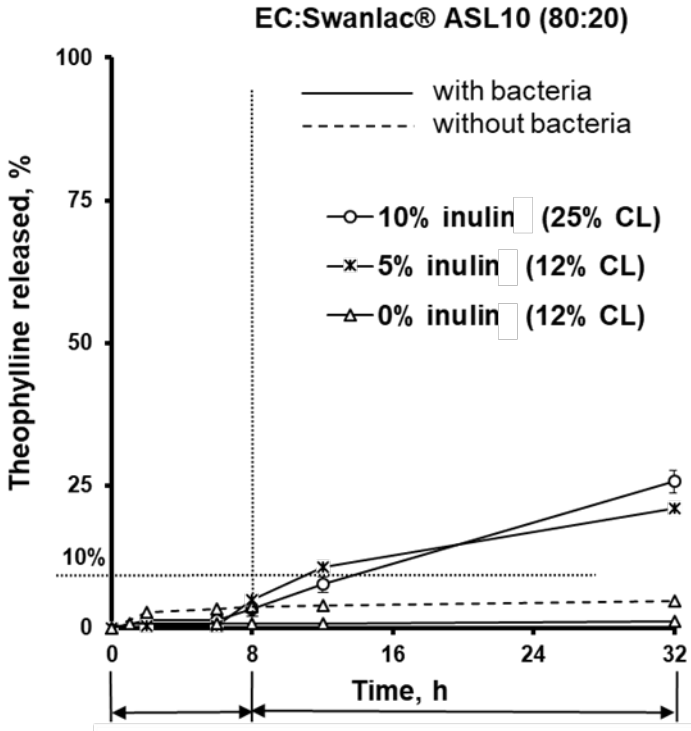
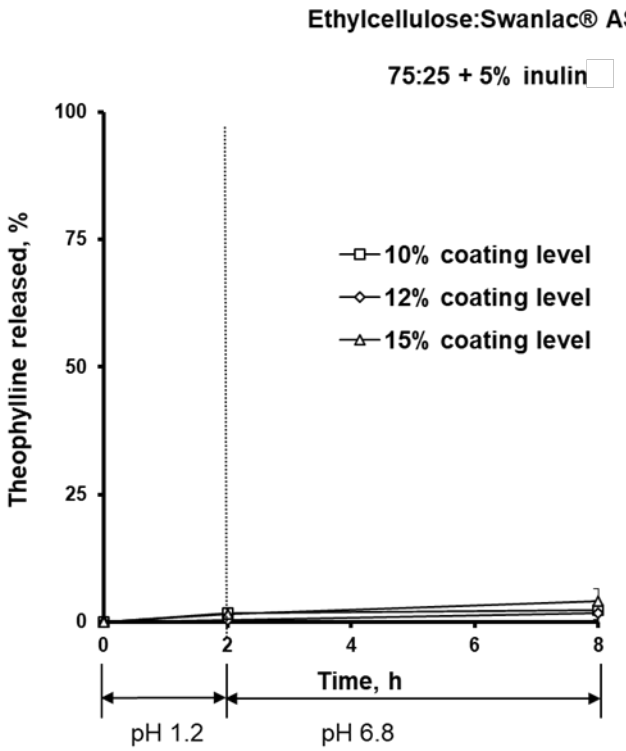
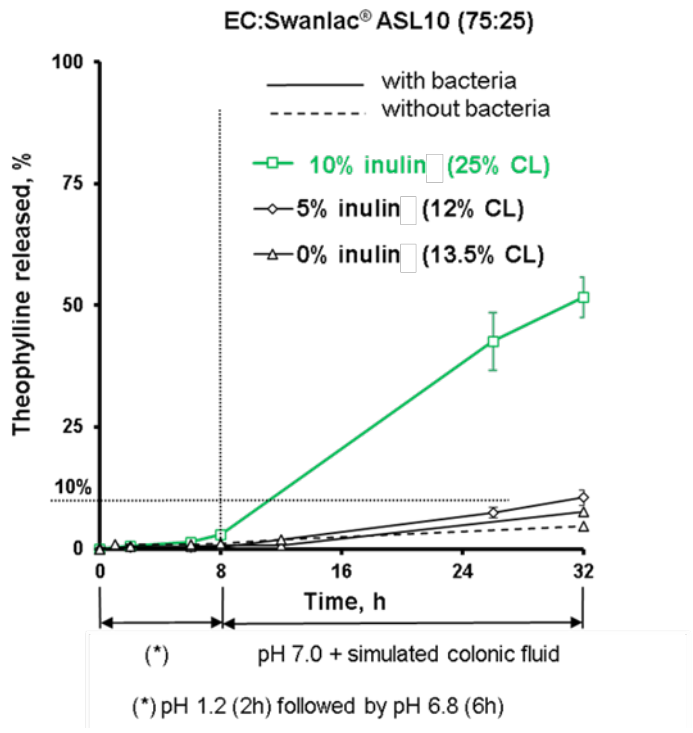


Figure 8

a) **Upper GIT**



b) **Entire GIT**



Human fresh fecal samples

Figure 9

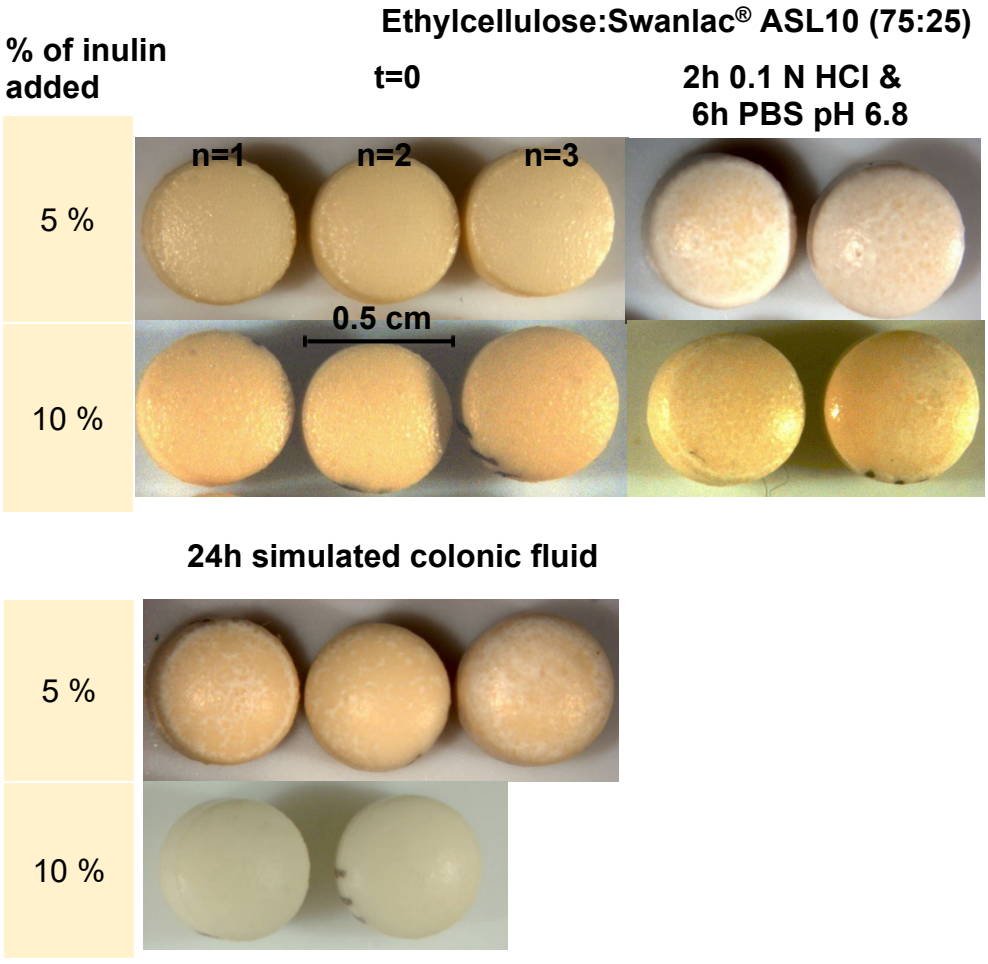

Ethylcellulose:Swanlac® ASL10 (75:25)
% of inulin added
t=0
2h 0.1 N HCl &
6h PBS pH 6.8
n=1
n=2
n=3
5 %
0.5 cm
10 %
24h simulated colonic fluid
5 %
10 %

Figure 10