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Design and Development of a Novel Physiologically-Based Combination Approach to Oral Colon Delivery

CHIM/09

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Abstract

The design and manufacturing of oral colon drug delivery systems with improved targeting ability were undertaken after a preliminary literature survey highlighting the main issues of currently marketed drug products, intended for inflammatory bowel disease (IBD) treatment. First, a more consolidated time-based approach was applied, as technology transfer was faced for a 5-aminosalicylic acid prototype formulation evaluated in a human pharmacoscintigraphy study. Subsequently, an original combination strategy leveraging multiple physiological characteristics of the intestine was developed for enhanced site selectivity of drug release into the large bowel. Particularly, a double-coated delivery system was devised, wherein an enteric soluble outer film, containing a microbially-degradable polysaccharide as a channeling agent, and a water-swellable/erodible inner layer, composed of hydrophilic cellulose derivatives of broad use and availability, would act synergistically to prevent early release to the small intestine on the one hand, and release failure on the other. The experimental activities were mainly focused on the formulation and manufacturing of the enteric outer film, and particularly on the selection of a proper polysaccharide pore former out of naturally-occurring ones already described for colon delivery purposes. The screening encompassed high-amylose starch, high-methoxylated pectin, as such or as a polyelectrolyte complex with chitosan, and chondroitin sulfate sodium as a polyelectrolyte complex with chitosan. Fluid bed spray-coating technique was used, aqueous- or hydro-alcoholic-based when the former mode was proved unfeasible. Coating formulas were set by establishing the weight ratio between the enteric-soluble methacrylic acid copolymer (Eudragit® S) and the polysaccharide, as well as the nature and percentage amount of the excipients, *i.e.* plasticizer and anti-tacking agent. Minitablets, either as such or provided with the inner swellable/erodible layer, composed of low-viscosity hydroxypropyl methylcellulose, and hydroxypropyl cellulose capsular devices for modified-release, fabricated by injection-molding, were prepared and used as multiple- and single-unit cores, respectively. Process parameters were identified and adjusted on a case-by-case basis. The resulting coated systems underwent physico-technological characterization and *in vitro* release testing using compendial media. Based on release results, high-amylose starch, pectin and its polyelectrolyte complex with chitosan were

shown potentially suitable polysaccharide candidates. On the other hand, chondroitin sulfate sodium was discarded on account of fast leaching from the coating layer, thus impairing the relevant barrier properties. The role of colonic bacterial strains on the release performance of the formulations developed so far was evaluated. To this end, a previously described method for preparation of simulated colonic fluid (SCF), consisting in culture medium of proper composition inoculated with fecal samples from IBD patients, was adapted and improved. Furthermore, a testing procedure was devised. Clear differences were thereby highlighted in the case of amylose- and pectin/chitosan-containing films depending on whether SCF or culture medium not inoculated with fecal samples, used as a control, was employed for the test.

Preface

In the pharmaceutical field, advanced therapeutic carrier systems are increasingly studied to meet more and more sophisticated medical needs and deliver innovative drugs often involving bioavailability constraints.

Over the last few decades, colon delivery has been representing a research topic of considerable interest in the oral delivery area. This would primarily be due to the spread of inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, which have ceased to be confined to the industrialized Western countries to become more of a global health problem [1,2].

In addition, much effort has been put into the search for more convenient modes of administration for biological drug, such as peptide ones, also encompassing oral release strategies. Overall, these have frequently leveraged the less threatening environment of the large bowel, where digestive and brush-border peptidases are known to be less concentrated than in the small intestine and the mucosa is more sensitive to absorption enhancers [3].

For colon delivery purposes, drug release has to be hindered during gastric residence and small intestinal transit of the dosage form and, subsequently, be prompted to take place following passage through the ileocecal valve [4]. In order to meet these complex formulation challenges, different approaches have been explored and described in the literature, all having inherent pros and cons. Physiological patterns of gastrointestinal pH, microbial population, transit time and intraluminal pressure have commonly been exploited. It is indeed well-known that the microbiota is by far more abundant and diversified in the colon than in the small intestine. Bacterial species resident in the large bowel are capable of catalyzing a variety of enzymatic reactions that are not occurring in the upper

intestine [5-7]. Accordingly, various synthetic or natural polymers that are selectively degraded by colonic micro-organisms have been used as carrier moieties in prodrugs and as coating or bulking excipients in drug delivery systems. Such polymers generally bear glycoside or azo bonds that are liable to cleavage by the colon microbiota. Synthetic azo polymers have enjoyed modest success mainly because of toxicological concerns and possible regulatory constraints [8-13]. On the other hand, natural polysaccharides, e.g. pectin [14-16], chitosan[17,18], galactomannan [19] and starch [20-22], are generally endowed with hydrophilicity properties, which may impair protection of the conveyed drug during transit of the dosage form within the proximal gastrointestinal tract.

In pH-dependent colon delivery formulations, typical gastroresistant polymers with pH-dependent solubility, soluble in a 5.5 to 7.0 pH range, have been applied as coating agents aimed at shielding the drug core from gastrointestinal contents during stomach residence and transit throughout the proximal small bowel [23-26]. Although pH values in the above-mentioned range may physiologically be reached in the jejunum and ileum, such coatings would be expected to completely dissolve in the distal small bowel or even in the large intestine based on their relatively high thickness. This, however, may hinder release when exposure of the coating to pH above threshold is not sufficiently long-lasting to prompt dissolution of the enteric-soluble polymer throughout the entire layer.

The transit time throughout the small intestine was reported to be fairly consistent (3 h ± 1 SE) and poorly dependent on the size and density of the dosage forms as well as on the feeding state of the subjects [27,28]. Once in the colon, solid substrates generally reside for longer periods than in the small bowel

because of less frequent propulsive peristaltic waves [29]. Therefore, systems intended to release the active ingredient after a lag phase of suitable duration, i.e. lasting throughout the whole small intestine transit, and provided with an enteric film, aimed at preserving integrity of the formulation until gastric emptying, have been exploited as time-dependent colon delivery platforms [30-35]. Recently, acceleration of small intestinal transit was observed under pre-feed administration regimens, and criticism has accordingly been addressed to the time-dependent approach [36].

Finally, hydrostatic pressure established in the colon lumen has also been exploited for targeting purposes, because of more intense smooth muscle contractions as compared with the small intestine and of the higher viscosity of the luminal fluid that would lead to more effective mechanical impact on solid contents [37-39]. Relatively brittle hydrophobic films, capable of withstanding the pressure conditions undergone in the proximal gut though not those built up more distally, have accordingly been explored to attain selective disintegration of single-unit dosage forms in the large bowel. However, this kind of formulations has not been far-reaching, being undertaken by a limited number of researchers only.

It is thus evident that, although the above-described formulation strategies are all based on valid concepts, none of them is free from major limitations. Among such strategies, those relying on microbiota and pH are especially popular, the largest amount of research work being focused on delivery systems derived from each or both of them. Combinations of the two approaches have indeed be propounded to overcome respective limitations that are mainly due to variability

in the parameters that they exploit. However, the sole commercially-available drug products with colon delivery claims, indicated for IBD therapy, imply pH-dependent release technologies.

Based on these premises, it was hypothesized that the design of an alternative formulation strategy, carried out taking account of the limitations involved in the previously investigated ones, could have added value to the field of oral colon delivery, thus ultimately helping achieve progress in the outcome of IBD therapy through enhanced exposure of the inflamed intestinal region to the administered drugs. The identification of a more reliable strategy, in accordance with the above-stated hypothesis, required that the well-established delivery strategies be critically evaluated for their pros and cons, to build up a starting background for subsequent activities. Furthermore, more in-depth insight was needed on the time-dependent approach, which was previously proposed although the inherent potential has not been extensively assessed so far. Finally, with the aim of overcoming the limitations of the prior-art strategies while taking advantage of related steps forward in the area of colon delivery, a novel formulation approach was deemed worth setting up based on exploitation of combined physiological parameters, which would be aimed to overcome issues related to the variability of each of them.

Therefore, the goals of the research work overall performed have been to review pH-sensitive colon delivery systems and gain in-depth understanding of the inherent limitations and advantages (Chapter 1), to explore the potential of a different, and so far less investigated, physiologically-based approach, such as the time-dependent one developed in the research group within which this research

work has been carried out (Chapter 2), and to finally set up a novel combined formulation strategy that may offer better chances of effective colon targeting (Chapter 3).

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Chapter 1

Enteric Coatings for Colonic Drug Delivery: State of the Art

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ABSTRACT

Oral colonic drug delivery has been pursued through differing formulation strategies. Among these, the pH-dependent approach relies on gastrointestinal pH profile and uses enteric soluble polymers, mostly having dissolution threshold above pH 7, as coating agents for drug-containing cores. A considerable number of mesalazine products in the form of enteric-coated formulations are currently available on the market for the treatment of inflammatory bowel disease. Such a design strategy, however, has been shown not to consistently provide the site selectivity of release needed for a better therapeutic outcome. Several attempts to overcome the drawbacks associated with enteric-coated colon delivery systems have recently been reported, generally aimed to improve reliability of the relevant targeting performance. In the present article, these research efforts are thoroughly reviewed and discussed.

ENTERIC COATINGS FOR COLONIC DRUG DELIVERY

Colon delivery systems for oral administration have grown in popularity since the 90s, primarily because of the increasing incidence of inflammatory bowel disease (IBD) that has broadly been demonstrated to benefit from topical pharmacological treatment [1]. Moreover, selective release of biotechnological drugs to the large bowel has been proposed as a viable strategy to have their oral bioavailability enhanced with respect to gastric and/or small intestinal delivery as yielded by conventional peroral dosage forms [2,3].

So far, a wide range of targeting formulation approaches have been explored, which are generally based on physiological parameters typically differing between the large bowel and more proximal regions of the digestive tract [4]. Enteric-coated systems, in particular, are intended to pursue colon delivery by exploiting differences in the pH of gastrointestinal fluids. Polymethacrylates with a pH-dependent dissolution threshold ranging from pH 6.0 to 7.0 are mainly used as coating agents aimed at protecting the drug core from gastric and small intestinal contents, Eudragit® S (EuS), Eudragit® L (EuL) and Eudragit® FS (EuFS) (Evonik Industries) being popular brands thereof [5]. This is notably the formulation strategy behind most of anti-inflammatory drug products that are commercially available worldwide for the therapy of ulcerative colitis and Crohn's disease (IBD).

It is well known, however, that the dissolution pH thresholds of the employed enteric-soluble polymers may physiologically be exceeded within the small bowel, particularly in its distal portions.

In addition, acidic pH values were disclosed in the right colon of healthy subjects by a radiotelemetry study performed almost thirty years ago [6]. The fall in pH would be due to accumulation of short-chain fatty acids in the caecum and proximal large intestine resulting from bacterial fermentation activities. It ensues that exposure of the coating polymers to fluids having pH above their dissolution threshold may not be long enough to enable drug release within the target site, particularly in the presence of high coating levels that are generally resorted to in order to prevent drug release into the upper gut. Such issues may become especially challenging in the case of ulcerative colitis sufferers, who reportedly undergo pathology-related alterations in the pH of colonic contents [7].

Indeed, the reliability of pH-dependent formulations has recurrently been questioned over the last decades.

In the early 90s, the time and site of disintegration for EuS-coated tablets dosed to fasted healthy volunteers were found to be highly variable [8]. It was thereby concluded that enteric-coated formulations would be unsuitable for consistent colonic release.

In a more recent ɣ-scintigraphy investigation, it was demonstrated that EuS films, irrespective of whether they were applied as an organic solution or an aqueous dispersion of the polymer, were unfit to provide selective drug release into the colon [9]. Indeed, when organic coating systems were used, the dosage forms were subject to disintegration failure, whereas aqueous EuS-coated units always disintegrated before the target site had been reached. In agreement with this evidence of early release, a newly identified goal of ileo-colonic targeting was proposed.

The risk of a lack of timely disintegration for organic EuS-coated tablets was confirmed under fasted, fed and pre-feed administration regimens, the latter consisting in a standard breakfast taken 30 min post-dose [10]. By concomitant radiotelemetric measurements, remarkable variability was highlighted in intestinal pH values. This finding, along with possibly insufficient residence time within regions having pH above the dissolution threshold of the coating polymer and paucity of water available, was deemed to hinder attainment of predictable performance from such formulations. Importantly, the duration of exposure to the acidic gastric fluid, which may penetrate the enteric layer and then delay neutralization of carboxyl groups upon pH rise, was also shown to impact on the release profile.

Lately, by administering differing marketed mesalazine formulations for modified release to fasted healthy volunteers and measuring drug concentrations in gastrointestinal fluid and faeces samples, it was hypothesized that a tableted system coated with EuS may in some cases fail to completely release its drug load [11].

Issues of poorly site-selective disintegration would concern not only single- but also multiple-unit EuS-coated formulations. Indeed, either early drug absorption or disintegration failure were observed from pellets coated with an organic solution of the polymer [12].

Coatings based on EuFS were more specifically proposed for colonic release. In spite of a slightly higher dissolution pH threshold, which helped overcome the risk of premature release into the small intestine, a tendency to early disintegration was still demonstrated for dosage forms coated with such a polymer

[9,13]. However, an intact unit was also seen in the descending colon at the end of imaging [9].

In view of the limitations encountered, much effort has been devoted to improving the colon targeting effectiveness of the pH-based approach. Particularly, the risk of delivery failure has been faced by exploiting further physiological characteristics of the large bowel, such as the enzymatic activity of the resident bacteria, as a synergistic trigger for *in situ* release, or promoting dissolution of the enteric-soluble coating polymer when its pH threshold has been reached.

High-amylose maize starch (resistant starch) was blended with EuS because of its susceptibility to selective colonic microbial degradation [14]. Tablets coated with this mixture were consistently shown to disintegrate at the ileo-caecal junction or in the colon irrespective of the feeding regimen.

In order to promote a rapid disintegration of EuS layers, swelling agents were added to the organic coating solution as solid particles [15]. The time elapsed between 5% and 70% *in vitro* release (pulse time) at pH 7.5 from hard-gelatin capsules coated according to this technology was reduced with respect to a EuS-coated reference formulation. Through the use of $^{13}C_6$-glucose and ^{13}C-urea as markers of the time and site of release, it was besides demonstrated that disintegration in fasted healthy volunteers would occur in the caecum and colon and not be slowed down as compared with an uncoated capsule [15,16].

A dual EuS coating was also designed aiming to face the problem of incomplete release. The dual coating included an outer layer obtained from an organic solution of the polymer and an inner one resulting from an alkaline

aqueous solution thereof that also contained a buffering agent [17]. The internal layer was expected to accelerate the dissolution of the overlaid one in pH>7 intestinal fluid by creating an additional dissolution front at its inner surface. This coating technology proved advantageous to expedite release from EuS-coated tablets intended for ileo-colonic release [17,18].

EXPERT OPINION

The earliest attempts to pursue oral colon targeting were based on a pH-dependent approach exploiting luminal pH differences that occur throughout the gastrointestinal tract. Such a strategy is still in use and has so far yielded numerous anti-inflammatory drug products approved for various IBD treatment options. Most of them are mesalazine dosage forms provided with a polymer coating soluble at pH above 7.

It is by now clearly evident, however, that while neutral to slightly alkaline pH values are reached in the small intestine, an acidic environment is found in the caecum and proximal colon. Such circumstances imply major risks of premature drug release into the small bowel on the one hand, and of release failure on the other, which need to be taken into proper account especially in the case of IBD sufferers and may impact on the successful outcome of the anti-inflammatory therapy. Nevertheless, it is generally believed that, in spite of diverse release patterns, there would not be any clinical differences among the various enteric-coated oral formulations for topical treatment of IBD [19,20].

Irrespective of the clinical outcome of mesalazine products based on gastrointestinal pH, it is undeniable that the targeting effectiveness of the

approach concerned still requires to be improved. In this respect, notable benefits could arise from exploitation of further regional colonic characteristics that, by a combination of release-triggering mechanisms, may help overcome patho-physiological variability issues related to each single parameter.

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Chapter 2

In Vitro and Human Pharmacoscintigraphic Evaluation of an Oral 5-ASA Delivery System for Colonic Release

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ABSTRACT

5-aminosalicylic acid (5-ASA) is the most widely used drug for the treatment of ulcerative colitis. The benefits of targeted delivery of 5-ASA to the large intestine are well known, resulting in reduced systemic absorption and increased local concentrations at the disease site. In the present study, a 5-ASA colon delivery system based on the time-dependent strategy, exploiting the relatively consistent small intestinal transit time (SITT), was manufactured and evaluated *in vitro* as well as *in vivo*. The system was obtained by successive spray-coating of an immediate-release tablet core with low-viscosity HPMC and Eudragit® L. The enteric film was effective in preventing release during the acidic stage of the *in vitro* test, while the HPMC coating brought about reproducible lag phases prior to release in phosphate buffer medium. A γ-scintigraphy investigation pointed out that, following administration to fasted and fed volunteers, disintegration of the units never occurred prior to colon arrival. In all cases, a lag time preceded the appearance of the drug and its N-acetyl metabolite in the bloodstream, which was found to correlate with the time of disintegration in a linear mode. The plasma levels of the drug and metabolite as well as their cumulative urinary recovery were relatively low with respect to those reported when 5-ASA is delivered to the small bowel.

KEYWORDS

Oral colon delivery, time-dependent release, 5-aminosalicylic acid, hydroxyl-propyl methylcellulose, spray-coating, pharmacoscintigraphy.

INTRODUCTION

5-aminosalicylic acid (5-ASA), also known as mesalazine or mesalamine, is the first-line and most widely used treatment for remission and maintenance of remission of mild-to-moderate ulcerative colitis (UC) [1-3]. UC is a potentially debilitating chronic inflammatory pathology that, together with Crohn's disease, affects over three million people in Europe and North America, showing a currently increasing trend in developing countries of non-Western areas [4,5].

Because it has been proved to act locally, selective delivery of 5-ASA to the disease site, via oral or rectal administration, is generally pursued [6,7]. This enhances the efficacy and tolerability associated with the therapy, by increasing the dose fraction reaching the inflamed mucosa and reducing that entering the systemic circulation due to poor colonic absorption.

In order to obtain effective levels of 5-ASA in the large bowel following oral intake, proper delivery strategies need to be used [8-10]. The vast majority of commercially-available 5-ASA products relate to a pH-dependent strategy [11,12]. The basic concept behind that is the aboral increase in the gastrointestinal pH, which ranges from acidic values of the gastric fluid to neutral or slightly alkaline ones of the distal intestine contents. Accordingly, coated dosage forms provided with an enteric film are used, and polymers soluble at pH > 6.0 or 7.0, primarily polymethacrylates such as Eudragit® S and Eudragit® L, are generally employed as the coating agents [13,14]. Nevertheless, it has broadly been highlighted that these coatings may not be suitable for site-selective release to the colon [15-17]. Indeed, while the dissolution pH threshold of such polymers can physiologically be exceeded in the small intestine, it may fail to be reached in

the proximal colon, where acidic values are often encountered particularly in active phases of UC [18,19]. Moreover, there is large intra- and inter-patient variability in the intestinal pH profile. Therefore, the likelihood of both premature release and of release failure is to be accounted for. In the lack of effective and reliable targeting, mucosal levels of 5-ASA may vary, thus affecting the clinical response rate [20].

Although no related drug products are found on the marketplace, colon delivery approaches that leverage physiological parameters other than the luminal pH have been proposed and extensively studied [21-24]. Among these, the time-dependent strategy exploits the small intestinal transit time (SITT) [25,26]. It has been shown that SITT has duration of 3±1 h (mean±sd) and is relatively little influenced by variables such as the fasted/fed state of subjects, the kind of dosage forms and, in the case of solids, the size and density of the latter [27]. In addition, because it is no inherent physiological characteristic of the colonic region, SITT would to a lesser extent undergo pathological alterations associated with UC as compared with the local pH and the composition of the microbiota [28].

Time-dependent delivery systems are generally coated dosage forms, wherein a pH-independent coating material is responsible for a programmable lag phase prior to release [13,26]. In order to turn time-programmed into site-selective release, the impact of unpredictable gastric residence time is to be overcome, which is commonly accomplished through the use of a gastroresistant outer coating. Furthermore, the lag phase needs to be sufficiently extended to cover the whole small intestinal transit. Taking account of the generally long-lasting transit of dosage forms in the large intestine, lag phases of 6 to 7 h may reasonably

prevent early drug release into the proximal gastrointestinal tract while still allowing for targeted delivery to the ascending colon [29]. According to the nature of the inner coating polymer and the formulation of the core, the drug release may be deferred through a range of mechanisms [30]. When swellable hydrophilic polymers, typically soluble cellulose derivatives, are used as coating agents, release is delayed due to their slow interaction with the aqueous gastrointestinal fluids and consequent dissolution and/or erosion in the hydrated state [31].

A previously described delivery system, provided with a functional low-viscosity HPMC layer applied by spray-coating, has been proved to fulfill the desired release behavior *in vitro* and *in vivo*, with lag phases increasing in duration as a function of the thickness of such a layer [32,33]. Moreover, colonic breakup was highlighted by γ-scintigraphy following administration of placebo units to healthy volunteers. Various configurations of this delivery platform were proposed by using different cores [34-37] and alternative manufacturing techniques [38-42]. Particularly, in the form of coated insulin minitablets, the system brought about more than two-fold bioavailability and ten-fold pharmacological availability vs a reference uncoated formulation for immediate release when administered to diabetic rats [43].

Based on the current need for effectively targeted pharmacological therapy of IBD, the aim of the present study was to manufacture, according to the above-mentioned delivery technology, an oral system for colonic release of 5-ASA, and to evaluate the relevant *in vitro* as well as *in vivo* performance. The manufacturing step involved (i) preparation of immediate-release tablet cores

containing 400 mg of the drug, (ii) application of a low-viscosity HPMC layer of approximately 750 µm in thickness, by virtue of previous *in vivo* studies of analogous systems [33,38,43], and (iii) enteric coating of the HPMC-coated units. After physico-technological and *in vitro* release characterization, combined pharmacokinetic and scintigraphic investigation into the performance of the final delivery system was carried out in healthy volunteers.

MATERIALS AND METHODS

MATERIALS

5-aminosalcylic acid (5-ASA, Chemi, I), povidone (Kollidon®25 BASF, D) and crospovidone Kollidon® CL, BASF, D), glyceryl dibehenate (Compritol®888 ATO, Gattefossé, F), lactose (Pharmatose DCL11 DMW, NL), samarium oxide (Sigma-Aldrich, US), hydroxypropyl methylcellulose (HPMC, Methocel® E50, Colorcon, US; MW 65-80 kDa), polyethylene glycol (PEG 6000, Hoechst, UK), poly(methacrylic acid, methyl methacrylate) 1:1 copolymer (Eudragit® L30D, Evonik, D), triacetin and talc (Tradeco, I).

METHODS

Manufacturing and physico-technological characterization of the 5-ASA delivery system

In order to reduce the apparent volume of the active pharmaceutical ingredient powder and improve its flow properties through wet granulation, 1.5 l of povidone aqueous solution (10% w/v) was slowly added to 1.6 kg of 5-ASA powder and kneaded in a high shear mixer at 100 rpm for 5 min (Roto Junior 10L

Zanchetta-Romaco, I). The wet mass was forced through a 250 µm net sieve of an oscillating granulator (AR400 Erweka, D). The resulting granules were oven-dried at 40 °C for 20 h and sieve-calibrated (500 µm). 0.396 kg of lactose as a filler, 0.072 kg of crospovidone as a superdisintegrant, 0.032 kg of talc as an anti-tacking agent, 0.032 kg of glyceryl behenate as a lubricant, and 0.040 kg of samarium dioxide for radiolabeling were added and mixed in a V-blender (Erweka, D). The mixture was tableted by a rotary machine (AR 13 Ronchi, I; concave punches diameter 11 mm, curvature radius 9 mm) set to provide adequate mechanical characteristics of the cores, so that these could withstand the subsequent coating steps while maintaining prompt disintegration properties upon exposure to aqueous media.

Tablets were checked for weight (n=20), height and diameter (digital micrometer Mitutoyo, J; n=20), crushing strength (crushing tester TBH30 Erweka, D; n=10), friability (friabilometer TA3R Erweka, D) and disintegration time (three-position disintegration apparatus Advanced Products, I; n=6). The weight, height, diameter, crushing strength, friability and disintegration time were 555.2±2.8 mg, 6.453±0.037 mm, 11.072±0.004 mm, 81±19 N, <1 % and <1 min, respectively.

The 5-ASA tablet cores were coated in fluid bed equipment (Uniglatt, Glatt, D) using an aqueous solution of Methocel® E50 (7.5% w/v) and PEG 6000 (0.5% w/v), used as a plasticizer,under the following process conditions: batch size 1 kg, inlet air temperature 60-64 °C, outlet air temperature 39-44 °C, nebulizing air pressure 3.5 bar, nozzle port size 1.2 mm. The coating level was monitored by measuring the weight and height as well as diameter (digital micrometer) of

samples (n=10) collected at successive time points. The coating thickness was calculated as half of the mean difference between the height and diameter of coated units and cores, respectively.

Coated systems having selected weight gains, in a 10-50% range (M10, M20, M30, M40 and M50), were cross-sectioned and the thickness of the applied layer was measured on images acquired by digital microscope (Dyno-Lite Pro AM-413T, AnMo Electronics Co., TW). Thickness data were the mean of measurements performed in 8 different regions of the HPMC layer of each of 3 coated units.

Enteric coating dispersions (Eudragit® L30D 35% w/v; Triacetin 3% w/v, Talc 7% w/v, deionized water 55 w/v) were applied onto HPMC-coated cores having nominal 50% weight gain to give final gastroresistant systems (GM50). The coating process was run up to approximately 3% weight gain using the same fluid bed apparatus under the following conditions: batch size 1 kg, inlet air temperature 60-62 °C, outlet air temperature 39-41 °C, nebulizing air pressure 1.5 bar, nozzle port size 1.2 mm. Mass uniformity of the final double-coated system was assessed (CV=0.58).

SEM photomicrographs of cross-sectioned final double-coated units were taken and used to assess the thickness of the enteric coating and confirm that of the HPMC layer. Samples were gold sputtered using a plasma evaporator under vacuum, and photomicrographs were acquired at an accelerated voltage of 10 kV at different magnifications (Leo 1430, Carl Zeiss, CH).

In vitro evaluation

For release testing, a three-position disintegration apparatus was used in place of a dissolution equipment to avoid adhesion of swollen HPMC layer of the coated system to the vessels, which would impact on data reliability [32]. Each unit was inserted into a basket-rack assembly so that only one of the 6 available tubes was occupied. The basket-rack assemblies moved in separate vessels at a constant 29 to 32 cycles/min frequency through a 55±2 mm distance, immersed in 800 ml of fluid at 37±1 °C, i.e. hydrochloric acid solution pH 1.2 for 2 h (gastroresistant systems only) and/or phosphate buffer pH 6.8.

Fluid samples were withdrawn continually for spectrophotometrical assay (λ=244 nm) at fixed time points and subsequently reintroduced into the vessel. Release tests (n=3) were carried out under sink conditions, here meant as those at which the concentration of the drug was <20% of its solubility in phosphate buffer pH 6.8 at 37 °C (~3.5 g/l) [44].

In vitro lag time was expressed as the time required for 10% drug release in phosphate buffer pH 6.8 ($t_{10\%}$) and calculated by linear interpolation of the experimental data immediately before and after this release percentage.

Pharmacoscintigraphic evaluation

The *in vivo* evaluation was carried out as an open-label, single-dose study in 6 healthy male Caucasian volunteers aged 18 to 45 years, who received the test formulation (GM50) in fasted and fed conditions according to a randomized two-period crossover design (washout of 7 days). The investigation was undertaken at the U.L.B., Faculté de Médecine, Hôpital Erasme, Service des Radioisotopes

(Brussels, BE). The protocol was granted approval by a registered ethics committee, in compliance with local regulations. The study was performed in accordance with the Declaration of Helsinki and its subsequent revisions. The enrolled subjects gave written informed consent and, prior to beginning, underwent a thorough medical examination. These controls were repeated at discharge. No medications were allowed during the study period.

Formulation GM50 was irradiated in a neutron flux of $2 \cdot 10^{12}$ cm^{-2}/s for 4 min, so that the stable isotope ^{152}Sm could be converted into the γ-emitting radionuclide ^{153}Sm [45]. Release testing after neutron irradiation confirmed that no alterations were brought about by such a procedure. At the time of dosing, the formulation was radiolabelled with 3.0 MBq. For each study session, 1.85 MBq ^{99m}Tc-colloid in 100 ml of water was also administered to outline the gastrointestinal tract.

On each administration day, at 8.00 a.m., all volunteers took orally a single dose of the radiolabeled formulation with 100 ml of tap water at room temperature. In the fed administration regimen, a standard breakfast of same composition was served 30 min before intake. Standard lunch, snack and dinner were served to all volunteers at 4, 8 and 12 h post-dose, respectively. At 24 h post-dose, breakfast was allowed as prescribed in the balanced standard diet the volunteers had to follow in the 3 days preceding administration. Neither food nor drink apart from these meals were permitted (except for water).

The subjects were placed in supine position under the collimator of the γ-camera, and images of 2 min in duration were acquired just before intake (t=0) and at the scheduled time points of 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12

and 24 h post-dose. Actual imaging times are reported in the Results and Discussion section. Gastric emptying time (GE), colon arrival time (CA), small intestinal transit time (SITT) and time of disintegration (t_d) as well as site of disintegration (s_d), i.e. when and where dispersed fragments from disintegrated units were first observed, respectively, were assessed. Time of disintegration after gastric emptying ($t_{d\text{-}GE}$) was calculated by subtracting GE from t_d.

Blood sampling was scheduled just before intake of the dosage form (t=0), every hour up to 12 h and at 24 h post-dose. Actual sampling times are reported in the Results and Discussion section. Urine was also collected prior to administration, from 0 to 12 h and from 12 to 24 h. 5-ASA and its metabolite N-acetyl 5-ASA (Ac5-ASA) were assayed in the plasma and urine by a HPLC validated method with fluorescence detection adapted from Fischer et al., 1981 [46]. The limit of quantitation, defined as the analyte concentration that provides a relative response with a coefficient of variation not exceeding 20, was 5 ng/ml for both 5-ASA and Ac5-ASA in the plasma, and, in urine, 0.25 µg/ml for 5-ASA and 5 µg/ml for Ac5-ASA.

Lag time prior to appearance of the drug ($t_{lag5\text{-}ASA}$) and of the metabolite ($t_{lagAc5\text{-}ASA}$) in the bloodstream was expressed as the time at which 5-ASA and Ac5-ASA were first assayed in the plasma.

RESULTS AND DISCUSSION

In vitro evaluation

Tablet cores containing 5-ASA underwent aqueous spray-coating with low-viscosity HPMC as previously described, in order to obtain a lag phase prior to

release, according to the time-dependent approach to colon delivery [33]. The operating parameters were preliminarily set up, and slight adjustments were progressively required due to the growing mass of the substrate. Coated formulations having nominal 10, 20, 30, 40 and 50 % weight gain (codes M10, M20, M30, M40 and M50) were subjected to physico-technological and release characterization. Consistent weight and coating thickness as well as a smooth surface were observed (Table I, Figures 1 and 2).

Table I. Weight, weight gain and coating thickness data relevant to uncoated cores and HPMC-coated systems

Formulation	Weight *(mg)* Mean±sd	Weight gain *(%)*	Coating thickness* *(µm)* Mean±sd
Uncoated core	555.2±2.8	-	-
M10	615.2±5.4	10.8	159.5±17.6
M20	669.0±5.8	20.5	306.8±33.1
M30	720.1±5.3	29.7	453.4±35.6
M40	782.8±5.0	41.0	633.9±31.5
M50	838.5±4.7	51.0	764.8±33.4

*measured by digital microscope

Figure 1. Photographs of uncoated cores and HPMC-coated systems having increasing coating level (ruler scale in cm).

A linear correlation (R^2=0.9973) was found between the weight gain of the units and the thickness of the HPMC layer (Figure 3). The pursued nominal thickness of 750 μm corresponded to a weight gain of approximately 50%. The coated systems, subjected to crushing test, were deformed without breaking at applied forces up to 300 N. Only in the case of M10, signs of rupture were noticed.

The release profiles from uncoated cores and HPMC-coated units, obtained in phosphate buffer pH 6.8 under sink conditions, are reported in Figure 4. A lag phase preceded the onset of 5-ASA release in all cases. By increasing the coating level, the duration of the lag phase was extended. A slow release phase due to outward drug diffusion was especially evident in the case of formulations having the greater weight gain (M40 and M50). Afterwards, release was prompt and quantitative, associated with the observed breakup of the swollen polymer layer. The relationship between the coating thickness and the duration of the lag phase, expressed as $t_{10\%}$, was found linear (Figure 5). This finding was consistent with previous ones relevant to coated systems having tableted cores of smaller size, cores of different nature (hard and soft gelatin capsules) or functional polymeric layers fabricated by different techniques (injection-molding, fused deposition modelling) [38,47,48].

Formulation M50, having coating thickness of approximately 765 μm (~68 mg/cm^2), was thus provided with an enteric film intended to prevent interaction of the HPMC layer with the aqueous fluid in the stomach, thus overcoming the influence of highly variable gastric residence time on the intestinal site of release.

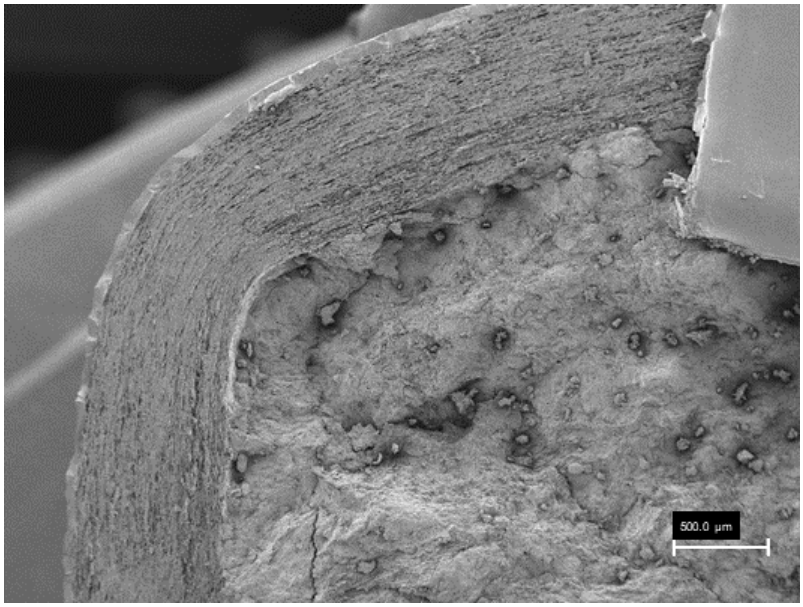

Figure 2. SEM photomicrograph of the cross-section of a final double-coated system (GM50).

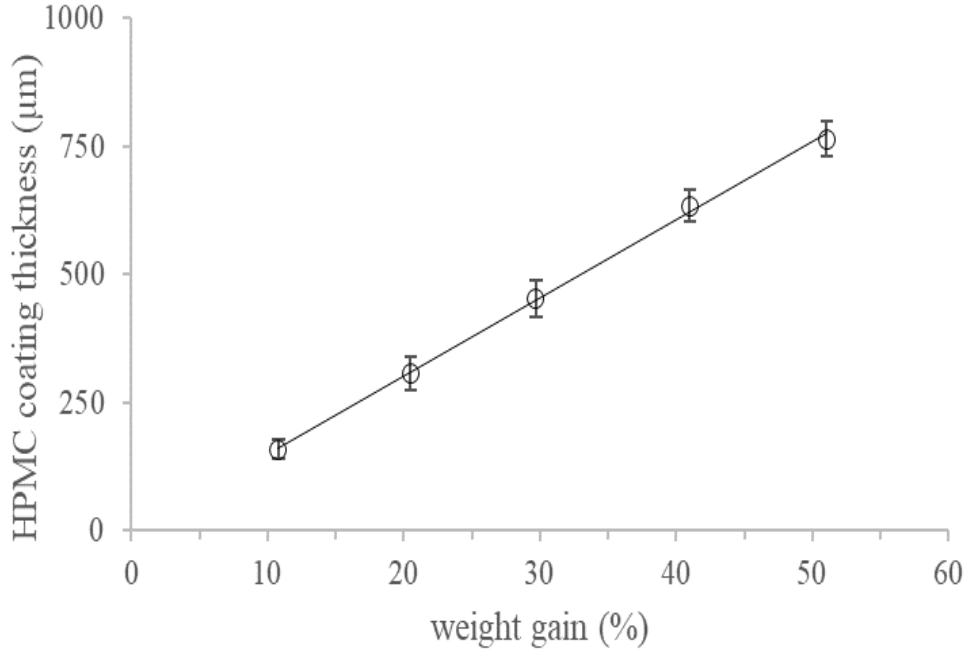

Figure 3. Relationship between weight gain and HPMC coating thickness.

Application of the enteric soluble polymer led to an increase in thickness of approximately 60 µm (~4.5 mg/cm^2) in the final double-coated system (GM50). Evaluated for release according to the compendial two-stage testing procedure, the gastroresistant units were proved to withstand 2 h in HCl solution. The release pattern observed after switching to phosphate buffer pH 6.8 was consistent with that shown by the corresponding non-gastroresistant formulation, with non-significantly different $t_{10\%}$ (p=0.79) although included in a broader range (Figure 6). Enteric coating was thus demonstrated not to impact on the performance of the HPMC layer.

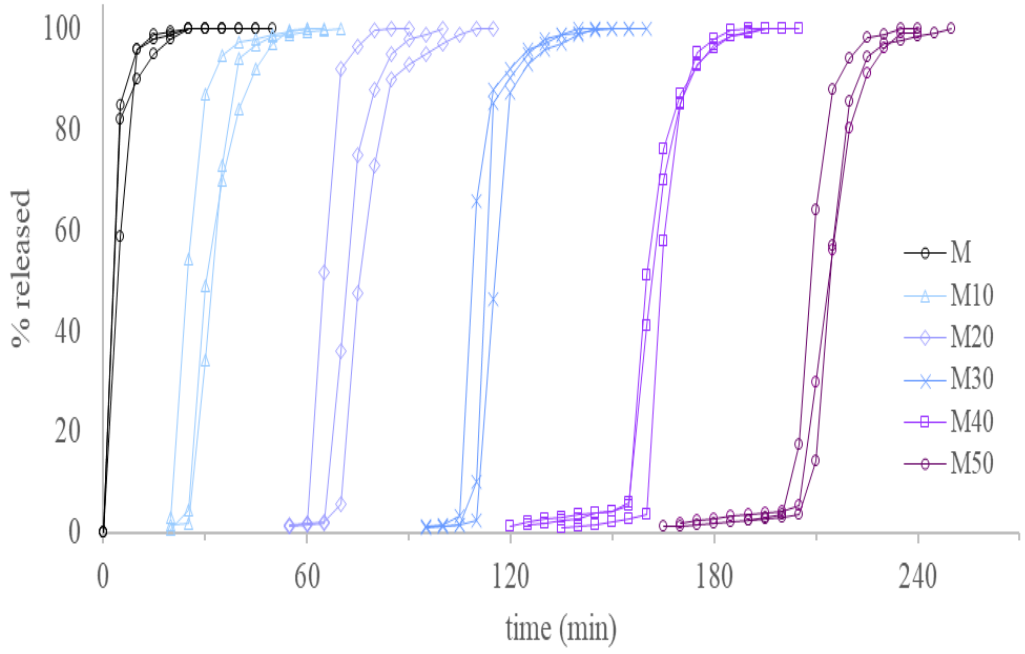

Figure 4. Release profiles of 5-ASA from uncoated cores and HPMC-coated systems having increasing coating level (phosphate buffer pH 6.8).

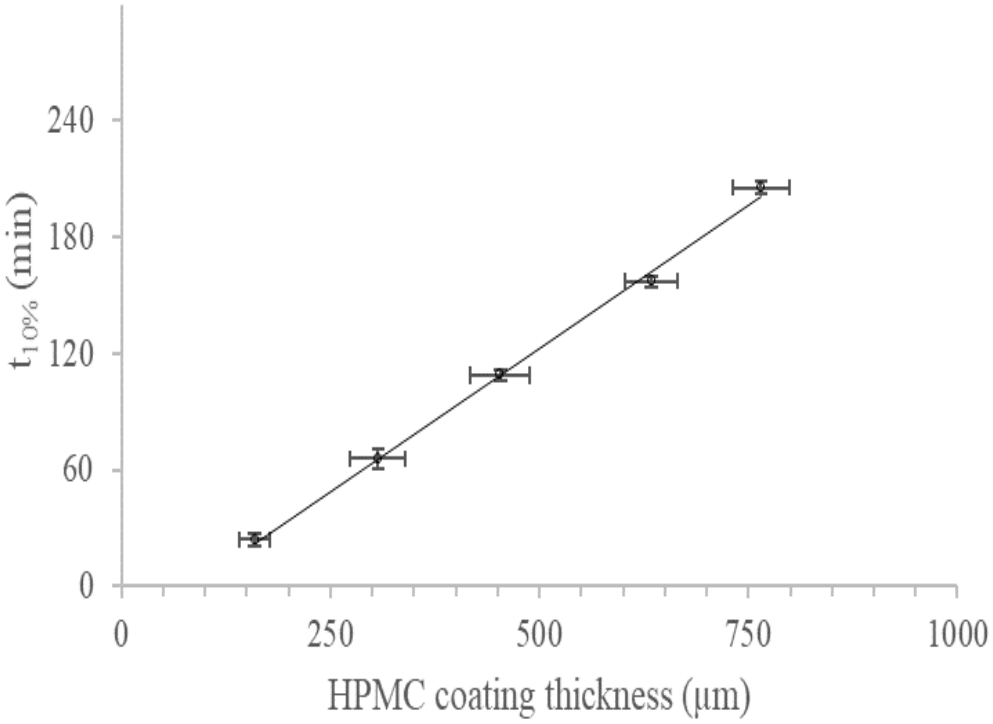

Figure 5. Relationship between HPMC coating thickness and 5-ASA in vitro lag time ($t_{10\%}$).

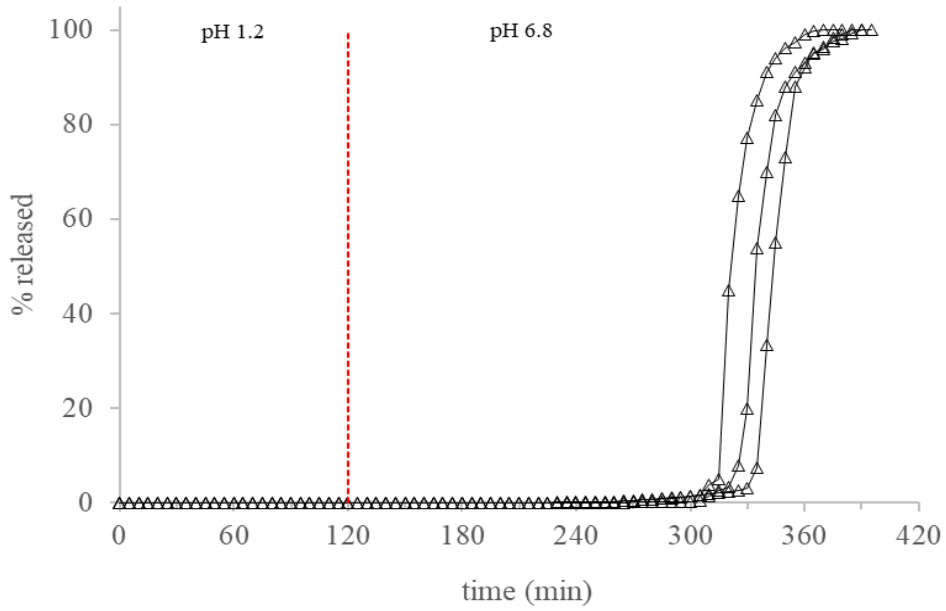

Figure 6. Release profile of 5-ASA from the final double-coated system (GM50).

In vivo evaluation

A pharmacoscintigraphic investigation was undertaken in 6 healthy male volunteers, under fasted and fed conditions. γ-scintigraphy was used to study the transit of the administered dosage forms (GM50) throughout the gastrointestinal tract and the site as well as time of relevant breakup. Moreover, 5-ASA and its N-acetyl derivative, resulting from intestinal and hepatic metabolism, were assayed in plasma samples collected within the imaging time frame in order to concurrently monitor their concentration in the systemic circulation [20,49,50]. Cumulative urinary recovery of both compounds was also assessed.

All volunteers concluded the trial without any remarkable side effect or alteration in the main clinical parameters that could be related to the treatment. Pharmacoscintigraphic results are presented in Tables II and III as well as in Figures 7 and 8. Horizontal bars are superimposed to concentration vs time curves of 5-ASA and Ac5-ASA to highlight concomitant position and disintegration of the units within the gastrointestinal tract.

A lag phase followed by appearance of the drug and its metabolite in the plasma was in all cases observed. As expected, 5-ASA levels turned out far lower than Ac5-ASA ones [49,51]. Overall, the concentrations of the drug and metabolite pointed out poor absorption, possibly consistent with distal intestinal release [50,52]. This was in line with cumulative urinary recovery of 5-ASA and Ac5-ASA, which never exceeded 20% of the administered dose.

Gastric residence was variable in duration, being generally shorter in the fasted regimen ($p < 0.05$).

Table II. Scintigraphic data under fasted and fed conditions

FASTED CONDITION						
Subject	Gastric emptying time GE (h)	Small intestinal transit time SITT (h)	Colon arrival time CA (h)	Time of disintegration t_d (h)	Time of disintegration after gastric emptying $t_{d\text{-}GE}$ (h)	Site of disintegration s_d
1	1.75	3.00	4.75	12.00	10.25	TC
2	0.33	4.50	4.83	4.83	4.50	C
3	0.42	1.50	1.92	11.00	10.58	AC
4	0.42	4.58	5.00	10.00	9.58	AC
5	0.58	4.50	5.08	#	#	#
6	0.58	1.59	2.17	# (12.55)	# (11.97)	#
Mean	0.68	3.28	3.96	9.46	8.73	
sd	0.53	1.47	1.49	3.19	2.85	

FED CONDITION						
Subject	Gastric emptying time GE (h)	Small intestinal transit time SITT (h)	Colon arrival time CA (h)	Time of disintegration t_d (h)	Time of disintegration after gastric emptying $t_{d\text{-}GE}$ (h)	Site of disintegration s_d
1	1.83	1.92	3.75	#	#	#
2	2.33	2.50	4.83	5.83	3.50	AC
3	1.41	3.51	4.92	8.92	7.51	AC
4	2.50	2.50	5.00	12.90	10.40	AC
5	1.92	3.00	4.92	# (13.87)	# (11.95)	#
6	0.58	3.59	4.17	10.25	9.67	TC
Mean	1.76	2.84	4.60	9.48	7.77	
sd	0.70	0.65	0.51	2.94	3.10	

Caecum (C), ascending colon (AC), transverse colon (TC).
Time (t_d) and site (s_d) of disintegration refer to when and where dispersed fragments of units were first observed
Definite time and anatomical position of disintegration could not experimentally be assessed since dispersed fragments were seen at 24 h only. Disintegration occurred between 12 and 24 h in the colon. Times of disintegration that could be estimated on the basis of the correlation in Figure 9 are in brackets.

Table III. Pharmacokinetic data under fasted and fed conditions

FASTED CONDITION						
Subject	5-ASA lag time $t_{lag5\text{-}ASA}$ (h)	Ac5-ASA lag time $t_{lagAc5\text{-}ASA}$ (h)	5-ASA cumulative urinary recovery (mg)		Ac5-ASA cumulative urinary recovery (mg)	
			0-12 h	0-24 h	0-12 h	0-24 h
1	11.70	10.70	0.00	0.64	0.00	29.36
2	5.83	4.83	0.87	2.58	24.75	83.63
3	11.00	10.00	0.00	0.69	0.00	17.77
4	11.00	9.07	0.00	0.68	0.00	19.04
5	§	§	0.00	1.62	0.00	34.02
6	11.20	11.20	0.00	1.12	0.00	25.18
Mean	10.15	9.16	0.15	1.22	4.13	34.83
sd	2.43	2.55	0.36	0.77	10.10	24.68

FED CONDITION						
Subject	5-ASA lag time $t_{lag5\text{-}ASA}$ (h)	Ac5-ASA lag time $t_{lagAc5\text{-}ASA}$ (h)	5-ASA cumulative urinary recovery (mg)		Ac5-ASA cumulative urinary recovery (mg)	
			0-12 h	0-24 h	0-12 h	0-24 h
1	§	§	0.00	0.00	0.00	0.00
2	5.20	5.20	0.00	0.59	25.99	47.99
3	8.03	7.30	0.35	0.65	6.57	14.72
4	12.10	11.00	0.00	1.19	0.00	27.45
5	12.00	12.00	0.00	1.15	0.00	26.56
6	9.32	9.32	0.00	1.67	0.00	68.5
Mean	9.33	8.96	0.06	0.88	5.43	30.87
sd	2.90	2.76	0.14	0.58	10.41	24.30

§ Definite time of appearance of 5-ASA and Ac5-ASA in plasma could not experimentally be assessed since these were assayed at 24 h only. The appearance of drug and metabolite occurred between 12 and 24 h.

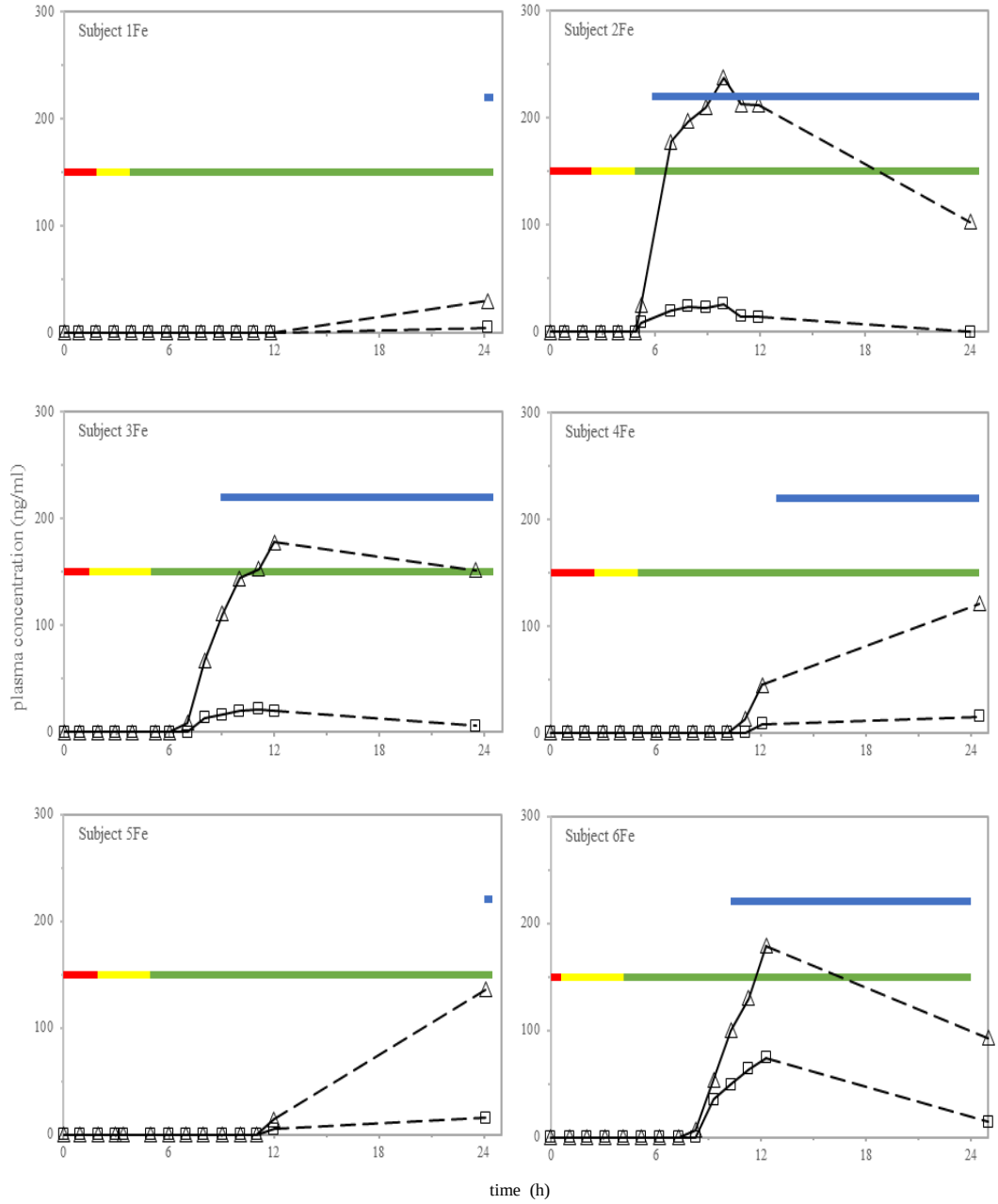

Figure 7. 5-ASA (□) and Ac5-ASA (Δ) plasma concentrations vs time profiles following administration of the final double-coated system (GM50) to fasted volunteers (the dashed portion of the curves indicates the 12-24 h time frame during which no experimental data were collected and does not reflect the actual time course of concentration). Red, yellow and green bars (bottom) indicate gastric, small intestinal and colonic residence, respectively; the blue bar (top) indicates disintegration.

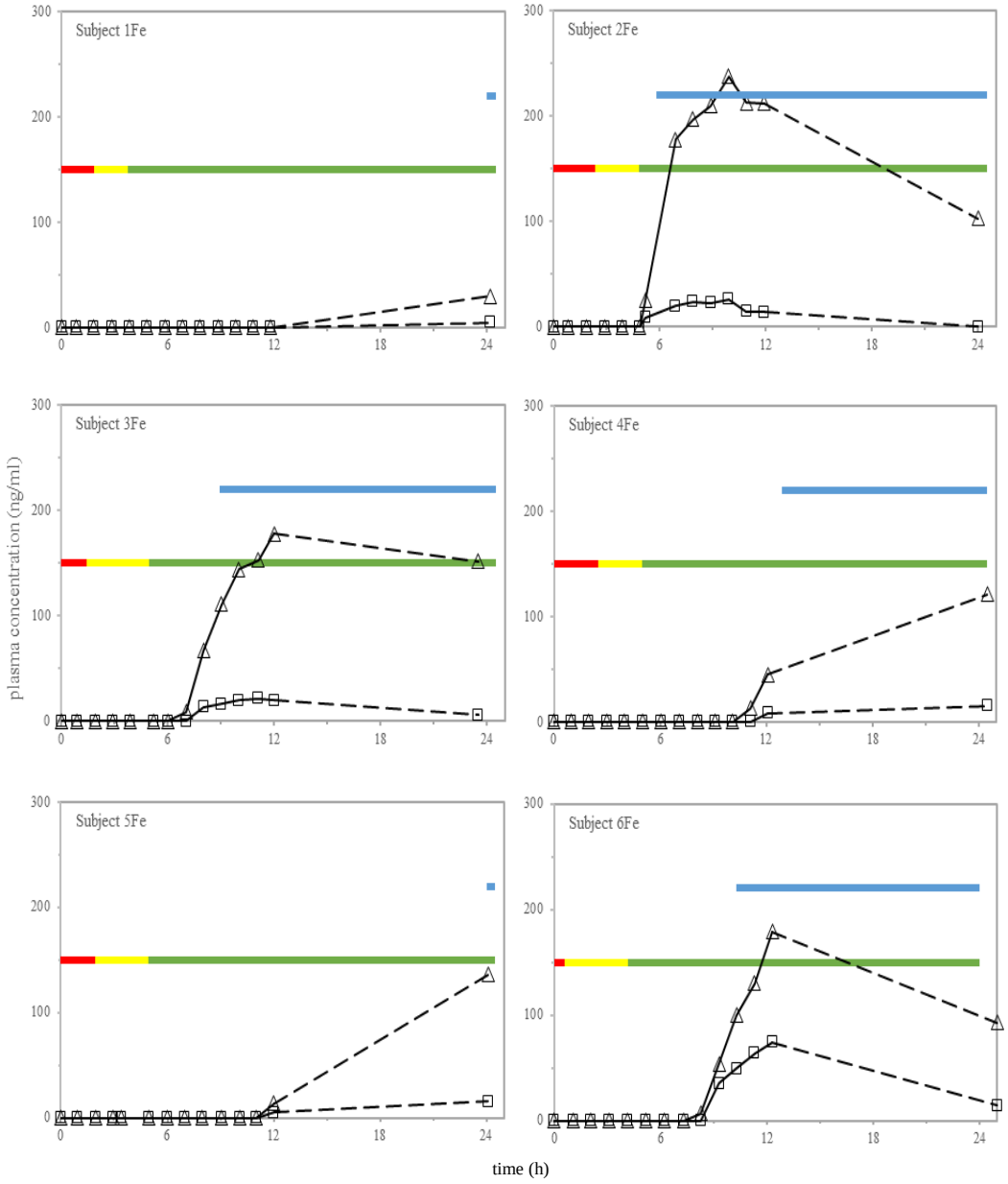

Figure 8. 5-ASA (□) and Ac5-ASA (Δ) plasma concentrations vs time profiles following administration of the final double-coated system (GM50) to fed volunteers (the dashed portion of the curves indicates the 12-24 h time frame during which no experimental data were collected and does not reflect the actual time course of concentration). Red, yellow and green lines (bottom) indicate gastric, small intestinal and colonic residence, respectively; the blue line (top) indicates disintegration.

Both in fasted and fed subjects, average SITT was in good agreement with previous findings, which the time-dependent colon delivery approach relies on. Colon arrival was always within 5 h post-dose. Notably, in no cases disintegration occurred before the units had entered the large bowel, and residual radioactivity, ranging from 10 to 100%, was still observed in the colon at 24 h after intake.

In 8 cases out of 12, disintegration of the units and appearance of 5-ASA and/or Ac5-ASA took place within 12 h after administration, the metabolite being detected either earlier than the parent drug or at the same time point. In those 8 cases, the time at which disintegration was first observed after gastric emptying ($t_{d\text{-}GE}$) turned out to be 8.73±2.85 h and 7.77±3.10 h for fasted and fed subjects, respectively, indicating no major influence of food intake (p=0.66). The site of disintegration (s_d), i.e. where dispersed fragments from disintegrated units were evident for the first time, was the caecum in one case (2Fa), the ascending colon in 5 cases (3Fa, 4Fa, 2Fe, 3Fe, 4Fe) and the transverse colon in the remaining 2 cases (1Fa, 6Fe). The time of disintegration (t_d), i.e. when the dispersed fragments were first seen, followed (1Fa, 3Fa, 4Fa, 2Fe, 3Fe, 4Fe, 6Fe) or coincided with (2Fa) the time at which Ac5-ASA was first assayed in the plasma ($t_{lagAc5\text{-}ASA}$), the metabolite being detected generally earlier than the parent drug because of its higher concentration. Diffusion of 5-ASA through the swollen hydrophilic polymer coating may thus have taken place before breakup of the units. The time elapsing from the detection of Ac5-ASA in the plasma to that of dispersed fragments in the intestine, i.e. the difference between $t_{lagAc5\text{-}ASA}$ and t_d, was 0.81±0.56 h in fasted subjects and 1.27±0.59 h in fed ones. A good linear

correlation was found between $t_{lagAc5\text{-}ASA}$ and t_d in both fasted and fed regimens, with the slope of regression lines reflecting the occurrence of metabolite absorption prior to breakup of the units (Figure 9).

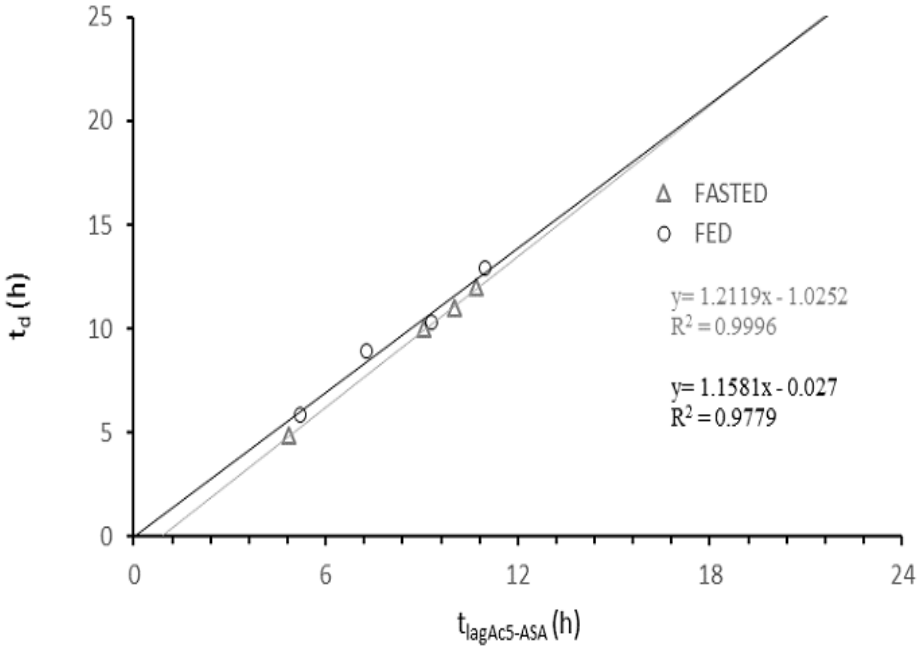

Figure 9. Relationship between Ac5-ASA in vivo lag time ($t_{lagAc5\text{-}ASA}$) and time of disintegration (t_d).

In those 4 cases where disintegration was not observed within 12 h after intake (5Fa, 6Fa, 1Fe, 5Fe), a comprehensive evaluation of the performance of the dosage form was hindered by the lack of information between 12 h and 24 h, time interval during which neither imaging nor blood sampling were scheduled. These specific cases are discussed into detail as follows.

In subject 6Fa, 5-ASA and Ac5-ASA appeared in the plasma 11.2 h after intake, while disintegration was not seen in the images taken up to 12 h. Fragments from the disintegrated unit were observed at 24 h post-dose in the

transverse and descending colon, and residual radioactivity was 83%. The plasma concentrations of drug and metabolite, along with their overall cumulative urinary recovery, were of such an extent as to indicate that breakup would have occurred within the “blind” time lapse well before the 24th hour post-dose. Through the linear relationship presented in Figure 9, breakup was estimated at 12.55 h. Based on colon arrival time, this would reasonably be consistent with the unit being positioned in the ascending and/or transverse colon.

Analogous considerations can be drawn from scintigraphic and pharmacokinetic data relevant to subject 5Fe. In this case, t_d was estimated at 13.87 h. At 24 h post-dose, radioactivity in the transverse and descending colon was still 100%.

In the same subject under fasted condition (5Fa), plasma levels of drug and metabolite as well as the disintegrated unit were detected at 24th hour only, fragments of the dosage form being positioned in the transverse and descending colon. Therefore, estimation of t_d based on the linear regression model was not possible in this instance, and no hypothesis could accordingly be made about the site of disintegration. However, plasma concentrations and urinary recovery of 5-ASA and Ac5-ASA, relatively high and comparable with data from the same subject in the fed state, suggest breakup of the unit should be traced back to several hours before the last sampling time at 24 h after intake.

In subject 1Fe, only very low 5-ASA and Ac5-ASA plasma levels, and as little as 10% of residual radioactivity in the distal part of the descending colon, were found at 24 h. By the same time, neither the drug nor the metabolite was

recovered in the urine. These results would indicate that breakup occurred just before the last time point and the unit was voided soon after disintegration.

Overall, the administered dosage forms disintegrated and released their drug load in the large intestine, under both fasted and fed conditions of the subjects participating in the study.

CONCLUSIONS

In the present work, an oral 5-ASA delivery system aimed at time-dependent colonic release was manufactured and evaluated *in vitro* as well as in healthy volunteers, by γ-scintigraphy and pharmacokinetics techniques. The system encompassed an immediate-release drug-containing tablet core, a low-viscosity HPMC layer deferring the onset of release and an enteric outer coating protecting the inner formulation from contact with gastric fluid during poorly predictable stomach residence.

The expected *in vitro* performance was confirmed, with lag time extended as a function of the HPMC coating level. A phase of diffusive release of a relatively small percentage of the drug appeared with increasing coating thickness.

The imaging study showed that disintegration of the administered units was in no cases observed prior to colon arrival. Breakup mainly occurred in the caecum, ascending or transverse colon. Notably, the small intestinal transit time was proved fairly reproducible and consistent with the tenets of the time-dependent formulation strategy. These findings were independent of the fasted or fed dosing regimen.

A lag phase following administration always preceded the appearance of the drug and its main metabolite in the bloodstream. An agreement was found between this lag time and the onset of disintegration of the units. 5-ASA and Ac5-ASA were generally detected approximately 1 h before breakup could be observed, which may reflect the diffusive release phase noticed in the *in vitro* study. After the lag phase, the concentrations of drug and metabolite reached in the plasma were relatively low as compared with data obtained following delivery of the drug to more proximal regions of the gut. This was consistent with the mean cumulative urinary recovery of 5-ASA and Ac5-ASA over 24 h that was below 10% of the drug dosed.

On average, the time taken for the dosage form to disintegrate after emptying from the stomach, where swelling of the functional HPMC coating was prevented by the gastroresistant film, turned out slightly longer than should in principle be pursued according to the time-dependent colon delivery approach. However, the results obtained were mostly satisfactory in terms of large intestinal targeting. Therefore, though with due caution in view of the need for avoiding early release into the small bowel, formulation changes could be implemented to possibly shorten the *in vivo* lag time, thus offering better chances of drug delivery into the proximal colon and reducing the risk of release failure.

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Chapter 3

Oral Colon Delivery Platform Based on a Novel Combination Approach: Design Concept and Screening of Naturally-Occurring Polysaccharides as Pore Formers

to be submitted for publication

The contribution given by Saliha Moutaharrik to the work associated with this manuscript was review of the literature, formulation and manufacturing of the delivery systems, relevant *in vitro* characterization, setup and preparation of simulated colonic fluid as well as release testing using such a fluid.

ABSTRACT

An oral colon delivery platform based on an original combination strategy, leveraging multiple physiological characteristics of the intestine, was proposed. The system comprised a drug-containing core, a water-swellable/erodible inner layer based on a hydrophilic cellulose derivative and an enteric soluble (Eudragit® S) outer coating, containing a microbially-degradable polysaccharide as a channeling agent, acting synergistically to prevent early release to the small intestine on the one hand, and release failure on the other. Formulation and manufacturing of the enteric outer coating were mainly addressed, with special reference to selection of a proper naturally-occurring polysaccharide pore former. In this respect, high-amylose starch, high-methoxylated pectin, as such or as a polyelectrolyte complex with chitosan, and chondroitin sulfate sodium as a polyelectrolyte complex were evaluated. Spray-coating technique was used, either aqueous or hydro-alcoholic when the former mode was proved unfeasible. Coating formulas were set by establishing the weight ratio between the enteric-soluble methacrylic acid copolymer and the polysaccharide, as well as the nature and percentage amount of the excipients, i.e. plasticizer and anti-tacking agent. Minitablets provided with the inner swellable/erodible layer, composed of low-viscosity hydroxypropyl methylcellulose, and hydroxypropyl cellulose capsular devices for modified-release, fabricated by injection-molding, were prepared and used as coating cores. Process parameters were identified and adjusted on a case-by-case basis. The resulting coated systems underwent physico-technological characterization and *in vitro* release testing using compendial media. The role of the microbiota

on the release performance of the formulations developed so far was explored. To this end, simulated colonic fluid (SCF), consisting in culture medium of proper composition inoculated with fecal samples from IBD patients, was used. Clear differences were thereby highlighted in the performance of amylose- and pectin/chitosan-containing coatings depending on whether SCF or culture medium not inoculated with fecal samples, used as a control, was employed for the test.

KEYWORDS

Oral colon delivery, hydroxylpropyl methylcellulose, Eudragit® S, high-amylose starch, pectin, chitosan, spray-coating.

INTRODUCTION

Targeted drug delivery to the colonic region of the gastrointestinal (GI) tract has received considerable attention in the last decades. The scientific interest in this area has been driven by the need to better treat local disorders of the colon such as inflammatory bowel disease (IBD) including ulcerative colitis and Crohn's disease, and irritable bowel syndrome. The colon is also known as a possible gateway to the systemic circulation.

Over the past years, several strategies have been used for achieving colon-targeted drug delivery systems based on physiological features of the intestine. As a result, the design of time-based, enzymatically-degradable and pH-sensitive drug delivery systems has mainly been carried out [1].

The vast majority of commercially available products intended for colon delivery, indicated for the therapy of IBD, are based on the pH-dependent formulation strategy. This approach is based on exploitation of pH changes along the GI tract. The enteric polymers used for pH-dependent systems dissolve above pH values in the 5.5-7 range, thus preventing release of drug in the stomach and providing a delayed release profile. Eudragit® S (pH>7), Eudragit® L (pH>5.5), and Eudragit® FS (pH>7) (Evonik Industries) are mostly employed. However, the dissolution pH threshold of such a polymers is often exceeded in the small intestine while pH values below it are typical of the proximal colon. For this reason, Eudragit®-coated systems may undergo premature release or release failure. In the former case, the drug would be released before the colonic region is reached, while in the latter, no release would occur and the delivery platform may be voided intact. γ-scintigraphy

studies of pH-sensitive delivery systems have shown erratic behavior *in vivo* [2-5]. To overcome limitations associated with such an approach, various strategies have been proposed. Schellekens and coworkers have developed a new delivery platform, which is based on a combination of Eudragit® S and superdisintegrants intended to ameliorate the pulsatile release kinetics and site targeting [6-8]. In particular, croscarmellose sodium (Ac-di-sol®)-containing systems have shown the most satisfactory results among the investigated swelling agents. Another approach was based on the concept of double coating, involving the application of two separate enteric layers: an inner one of partially neutralized polymer along with a buffer salt, and a standard outer one. Using Eudragit® L30D-55, this dual coating was demonstrated to accelerate drug release *in vitro* and within the human proximal small intestine as compared with a conventional single coating [9]. Thus, Eudragit® S-coated systems intended for colon targeting were also prepared, providing faster drug release for both single- and multiple-unit dosage forms *in vitro* [10,11]. Finally, Ibekwe et al. introduced a new design concept based on a single coating layer composed of Eudragit® S in admixture with bacterially-degradable polysaccharide (resistant starch). In this case, the hydrophilic component should undergo selective degradation by the colonic microbiota, that would aid rupture of the enteric film in case it is not exposed to pH above the dissolution threshold for a sufficient time lapse at the targeted site [12]. All the proposed delivery systems were tested in healthy volunteers to assess the site of disintegration, which was consistently seen at the ileocecal junction, or in the proximal large intestine. Therefore, in spite of the increased chances to avoid

failure of drug release in the large bowel, the premature release issue is still open, and may even be worsened by the presence of a hydrophilic component within the coating layer.

Based on these premises, the present work is focused on the design, manufacturing and evaluation of a novel oral colon delivery platform based on a combined strategy, which would be aimed to overcome the limitations associated with the above-mentioned approach, addressing not only the issue of release failure but also that of premature release.

MATERIALS AND METHODS

MATERIALS

Paracetamol for direct compression (Rhodapap™ DC 90, Novacyl, CN), microcrystalline cellulose (Avicel® PH-101, FMC, BE), sodium starch glycolate (Explotab® CLV, JRS, D), vinylpyrrolidone-vinyl acetate copolymer (Kollidon® VA 64, BASF, D), hydrophilic fumed silica (Aerosil® 200, Evonik, D), magnesium stearate (Recordati, I), hydroxypropyl cellulose (HPC; Klucel™ LF, Eigenmann & Veronelli, I), polyethylene glycol (PEG 400 and 1500, Clariant, D), Hydroxypropyl methylcellulose (HPMC; Methocel® E50, Colorcon, UK), methacrylic acid-methyl methacrylate copolymer (1:2) (EuS; Eudragit® S, Evonik, D), high-amylose corn starch (Amylo N460, Roquette, FR), high-methoxylated pectin (pectin HM, Aglupectin HS-RP, Silvateam Food Ingredients, I), chondroitin sulfate sodium (ScanDroitin, ZPD, DK; Cr), chitosan (MADAR Corporation, UK), triethyl citrate (TEC; Fluka, CH), glyceryl monostearate (GMS; Gattefossé, FR), polysorbate 80 (Tween 80;

A.C.E.F., I), ammonium acetate (Carlo Erba, I), sodium hydroxide (VWR chemicals, BE), ammonia solution 25 % v/v (Carlo Erba, I), glacial acetic acid (Merk KGaA, D) and hydrochloric acid 37 % v/v (ACROS, D), 1-butanol (J.T. Baker, NL), ethanol 96 % (VWR, FR).

METHODS

Manufacturing of delivery systems

Minitablet cores: a paracetamol DC (80 %), Avicel® PH 101 (12.5 %), Explotab® CLV (4.5 %), Kollidon® VA 64 (2.0 %), Aerosil® 200 (0.5 %) and magnesium stearate (0.5 %) powder mixture was tableted by rotary press (AM-8S, Officine Ronchi, I) equipped with concave punches (4 mm diameter, 4 mm curvature radius), resulting in tablets with a nominal weight of 40 mg.

Molded capsular devices: HPC was oven-dried at 40 °C for 24 h and blended with PEG 1500 (9:1 w/w) before molding by a bench-top micromolding machine (BabyPlast 6/10P, Cronoplast S.L., ES) equipped with a capsular mold having two interchangeable inserts [13]. Matching caps and bodies were obtained, giving final capsules with a nominal thickness of 600 μm that were manually filled with paracetamol DC (~130 mg).

Coating: the obtained minitablets were coated with an aqueous solution of HPMC (8% w/w + 10% PEG 400 on the dry polymer by tangential-spray fluid bed (Glatt GPCG 1.1, Glatt, DE) [14]. HPMC-coated minitablets and molded HPC capsules were then coated with Eudragit® S in admixture with high-amylose starch, pectin HM as such or as a polyelectrolyte complex with chitosan and chondroitin sulfate sodium as a polyelectrolyte complex with chitosan, by bottom-spray fluid bed (Mini-Glatt, Glatt, DE) or pan-coater

equipped with a pan of 1 L capacity (GS Coating, Morandi, IT), respectively. The coating formulas and process parameters set up through the experimental work are reported in the Results and Discussion section. In all cases, a 7:3 solid weight ratio was maintained between Eudragit® S and the polysaccharides as such or as a polyelectrolyte complex, and TEC and GMS were added as plasticizing and anti-tacking agents, respectively. Fine water dispersion of GMS was prepared under heating (75 °C for 15 min) before use. All coated systems were oven-cured at 40 °C for 24 o 48 h depending on the coating formula. Curing conditions were experimentally established.

Characterization of delivery systems

Physico-technological characterization: the obtained systems were checked for coating level, i.e. percentage weight gain (%), amount of polymer applied per unit area (mg/cm^2) and coat thickness (µm) by digital micrometer (Mitutoyo, JP). All these parameters were obtained by performing weight and thickness measurements before and after coating, and the final values resulted from subtraction. Core surface was calculated by equation (1), which incorporates curvature radius (R), radius (r) and height (h) of the unit:

(1) $$Surface = 4\pi(R-a)\left(R-\sqrt[2]{R^2-a^2}\right)+2\pi a h_{cpr}$$

SEM analysis: the cross-sectional morphology of coated systems was analyzed by means of a scanning electron microscope (SEM, LEO 1430, Zeiss, DE) after gold-sputtering in a plasma evaporator under Argon flow (Auto sputter coater, Agar, UK; voltage 10 mA; time 3 min). Photomicrographs were acquired at an accelerated voltage of 7 kV at differing magnifications.

Release testing: coated systems (n=3) were tested for release by USP 42 paddle apparatus (Dissolution System 2100B, Distek, US), paddle speed 100 rpm, in the case of minitablets, and by adapted USP 42 disintegration apparatus (Sotax DT3, Sotax, CH), dipping speed 31 cycles/min, in the case of capsules, using 800 mL of 0.1 N HCl for 2 h and then phosphate buffer (PB) pH 7.4 at 37 °C as the immersion fluids [13]. Fluid samples were automatically withdrawn at successive time points. The drug released over time was assayed by spectrophotometer at 248 nm.

Release testing in simulated colonic fluid (SCF): in order to evaluate enzymatic digestion of the microbially-degradable component, the coated systems were pretreated in USP 42 dissolution paddle apparatus (100 rpm, 800 mL) in the case of minitablets and in BioDis (10 dpm, 200 mL) in the case of capsules, first immersed in HCl 0.1 N for 2 h and afterwards in PB pH 4.5 for another 2 h before being transferred into 120 mL flasks containing culture medium, either inoculated with fecal samples collected from IBD patients to give SCF, or not inoculated (CM) for comparison [15]. 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 solubilized in 1 L distilled water (pH 6.5 ± 0.2) were used for the preparation of CM, which was sterilized in autoclave before use [15,16]. SCF consisted of 100 mL of culture medium inoculated with 1 g of fecal residues, then incubated under anaerobic conditions at 37 °C and horizontally shaken (50 rpm) for at least 20 h before adding the pre-treated formulations. During the test, 2 mL fluid samples were manually withdrawn, centrifuged (13,000 rpm, 5 min) and then filtered (0.22 µm) before being analyzed by HPLC (Thermo

Fisher Scientic Ultimate 3000 Series, Waltham, USA) for the amount of drug released. A Gemini® 5 μm C18 110 Å, 150 × 4.6 mm column (Phenomenex, UK) was employed. The mobile phases consisted of (A) water adjusted to pH 2 with orthophosphoric acid and (B) acetonitrile, and 10 μL samples were injected for HPLC analysis. The flow rate was set at 1 mL/min and a gradient program: 0-10 min, 5-20% B; 10-11 min, 20-5% B, was followed. Paracetamol was detected spectrophotometrically at 248 nm [17].

RESULTS AND DISCUSSION

The novel drug delivery platform here presented was devised in the form of a drug-containing core, a swellable/erodible inner layer and a Eudragit® S based outer film wherein a microbially-degradable naturally-occuring polysaccharide was dispersed. The external film is intended to protect the system during transit throughout the proximal GI tract. The incorporated degradable polysaccharide should act as a site-selective channeling agent following digestion by the colonic microbiota, thus speeding up the enteric film breakdown in case it is not exposed for a sufficient time lapse to pH above the dissolution threshold of the gastroresistant polymer. Finally, the internal polymer layer would protect the drug core for an additional pH-independent lag time in case the outer coat fails to reach the colonic region intact.

Various polysaccharides were investigated as pore formers. In particular, high-amylose resistant starch, pectin and chondroitin sulfate sodium, which are extensively reviewed in scientific literature for colon targeting purposes, were chosen.

Disintegrating minitablets provided with low-viscosity HPMC coating and molded HPC capsular devices were used as multiple- and single-unit substrate cores for application of the Eudragit® S layers. Both types of dosage form were conceived for time-controlled release and verified to impart the desired initial lag phase. The molded capsules were considered of particular interest in view of the ability to convey a variety of drug formulations that could even be filled in extemporaneously, and the possibility of having the relevant release governed by the swellable/erodible polymer shell [18]. Moreover, the use of injection-molding in the pharmaceutical field would offer considerable advantages in terms of solvent-free processing, versatility, patentability and suitability for continuous manufacturing [19].

Coating formulations based on Eudragit® S in admixture with high-amylose starch (Amylo) were first set up and evaluated for process and performance upon application to minitablet cores. Hydro-alcoholic and aqueous spray-coating were employed. In the former, two different percentages of TEC, i.e. 10 and 20 % based on dry polymers, were used to evaluate a possible influence of plasticization extent, whereas a higher amount of TEC was required (35 % on dry polymers) in the latter (Table I).

Both coating processes were successfully carried out. The resulting operating parameters and coating levels reached are reported in Table II and III, respectively. The coating level of the outer film was set at approximately 7 mg/cm^2 of Eudragit® S based on literature finding [20].

Table I: % composition of the coating systems

Components	EuS/Amylo coating Hydro-alcoholic TEC 10*	EuS/Amylo coating Hydro-alcoholic TEC 20*	EuS/Amylo coating Aqueous TEC 35*	EuS/pectin coating Aqueous	EuS/pectin-chitosan coating Aqueous	EuS/chondroitin SS-chitosan coating Aqueous
Eudragit® S	3.45	3.44	5.46	2.88	3.23	3.68
Amylo N 460	1.48	1.47	2.34	-	-	-
Pectin HM	-	-	-	1.24	1.15	-
Chondroitin SS	-	-	-	-	-	0.95
Chitosan	-	-	-	-	0.23	0.63
Triethyl citrate	0.49	0.98	2.73	2.02	2.25	1.84
Glyceryl monostearate	0.25	0.25	0.27	0.29	0.32	0.18
Tween® 80	0.10	0.10	0.11	0.11	0.13	0.07
NaOH 1 N	-	-	-	-	5.42	-
Acetic acid 3% v/v	-	-	-	-	-	31.53
Ammonium acetate 5 M	-	-	-	-	-	16.82
Water	36.97	36.97	86.36	92.01	17.88	42.46
NH_3 1 N	-	-	2.73	1.44	1.61	1.84
HCl 0.1 N	-	-	-	-	66.00	-
1-Butanol	2.96	2.95	-	-	-	-
96° Ethanol	54.11	53.85	-	-	-	-

*on dry coating polymers

Table II: coating process parameters

	HPMC Coating	EuS/Amylo Coating Hydro-alcoholic		EuS/Amylo Coating Aqueous	EuS/pectin coating Aqueous		EuS/pectin-chitosan coating Aqueous		EuS/chondroitin SS-chitosan coating Aqueous
Equipment	Glatt GPCG 1.1	Mini-Glatt	Pan coater	Mini-Glatt	Mini-Glatt	Pan coater	Mini-Glatt	Pan coater	Mini-Glatt
Core	Tablets	Tablets	HPC capsules	Tablets	Tablets	HPC capsules	Tablets	HPC capsules	Tablets
Nozzle pore size (mm)	1.2	0.5	0.8	0.5	0.5	0.8	0.5	0.8	0.5
Atomizing air pressure (bar)	2	0.2	0.2	0.2	1.0	0.2	1.0	0.2	1.0
Pattern air pressure (bar)	-	-	0.2	-	-	0.3	-	0.3	-
Drying air volume (m^3/h)	100	40	32	40	35-47	32	37-47	32	38-47
Inlet air temperature (°C)	59	40	40	40	40-43	46-50	40	45-51	40
Outlet air temperature (°C)	53	-	-	-	-	-	-	-	-
Product temperature (°C)	52	30-32	21-24	29-33	32-35	28.30	30-34	29.31	31-34
Spray rate (g/min/kg)	3-5	12-18	22-26	20-27	14-15	14-17	12-13	7-9	15-16
Post drying time (min - °C)	30 -59	10 - 40	5 – 40	10 - 40	5 - 40	5 – 45	10 - 40	5 - 50	10 - 40
Curing	-	*24 h-40 °C*	*24 h-40 °C*	*24 h-40 °C*	48 h-40 °C	48 h-40 °C	48 h-40 °C	48 h-40 °C	*48 h-40 °C*

Table III: coating levels

	Weight gain (%)	Applied amount (mg EuS/cm^2)	Thickness (µm)
Inner layer (minitablet cores**)**			
Batch code		-	
HPMC 70	10.9	-	79.0
HPMC 100	17.0	-	104.2
HPMC 200	35.2	-	201.4
HPMC 270	48.5	-	273.2
Outer layer (minitablet cores)			
Batch code			
HPMC 0+EuS/Amylo W/Et 10*	15.3	7.1	98.0
HPMC 100+EuS/Amylo W/Et 10*	14.7	7.2	107.1
HPMC 200+EuS/Amylo W/Et 10*	15.0	7.5	105.7
HPMC 270+EuS/Amylo W/Et 10*	13.8	7.1	99.2
HPMC 0+EuS/Amylo W/Et 20*	17.0	7.2	106.1
HPMC 100+EuS/Amylo W/Et 20*	15.0	6.7	94.0
HPMC 200+EuS/Amylo W/Et 20*	13.7	6.4	114.6
HPMC 270+EuS/Amylo W/Et 20*	13.8	6.4	105.7
HPMC 0+EuS/Amylo W 35*	19.0	7.3	105.8
HPMC 100+EuS/Amylo W 35*	11.6	4.55	101.4
HPMC 200+EuS/Amylo W 35*	11.4	6.7	106.6
HPMC 270+EuS/Amylo W 35*	14.8	6.4	104.5
HPMC 0 + EuS/pectin	39.4	13.5	231.1
HPMC 70+ EuS/pectin	39.2	13.5	233.4
HPMC 100 + EuS/pectin	36.2	13.1	252.7
HPMC 200 + EuS/pectin	42.5	15.6	281.3
HPMC 0+ EuS/pectin-chitosan	41.8	14.7	228.7
HPMC 70+ EuS/pectin-chitosan	40.2	13.8	235.9
HPMC 100+ EuS/pectin-chitosan	39.5	14.3	265.0
HPMC 200+ EuS/pectin-chitosan	38.0	14.0	244.6
HPMC 0 + EuS/chondroitin SS-chitosan	20.5 / 37.1	7.1 / 12.5	142.9 / 237.5
Outer layer (HPC capsule cores)			
Batch code			
HPC cps+ EuS/Amylo W/Et 20*	12.3	7.3	105.9
HPC cps + EuS/pectin	34.7	14.2	204.7
HPC cps + EuS/pectin-chitosan	32.7	14.6	199.4

*on dry coating polymers

The resulting systems exhibited satisfactory physico-technological characteristics and smooth outer surface. The cross-section morphology of two-layer units was analyzed by SEM, which confirmed the quality of the HPMC coat and highlighted, as expected, structural differences between EuS/Amylo films attained via hydro-alcoholic and aqueous spray-coating technique (Figure 1). Indeed, while the former appeared continuous, the latter displayed discontinuity signs that were ascribed to a lack of extensive coalescence.

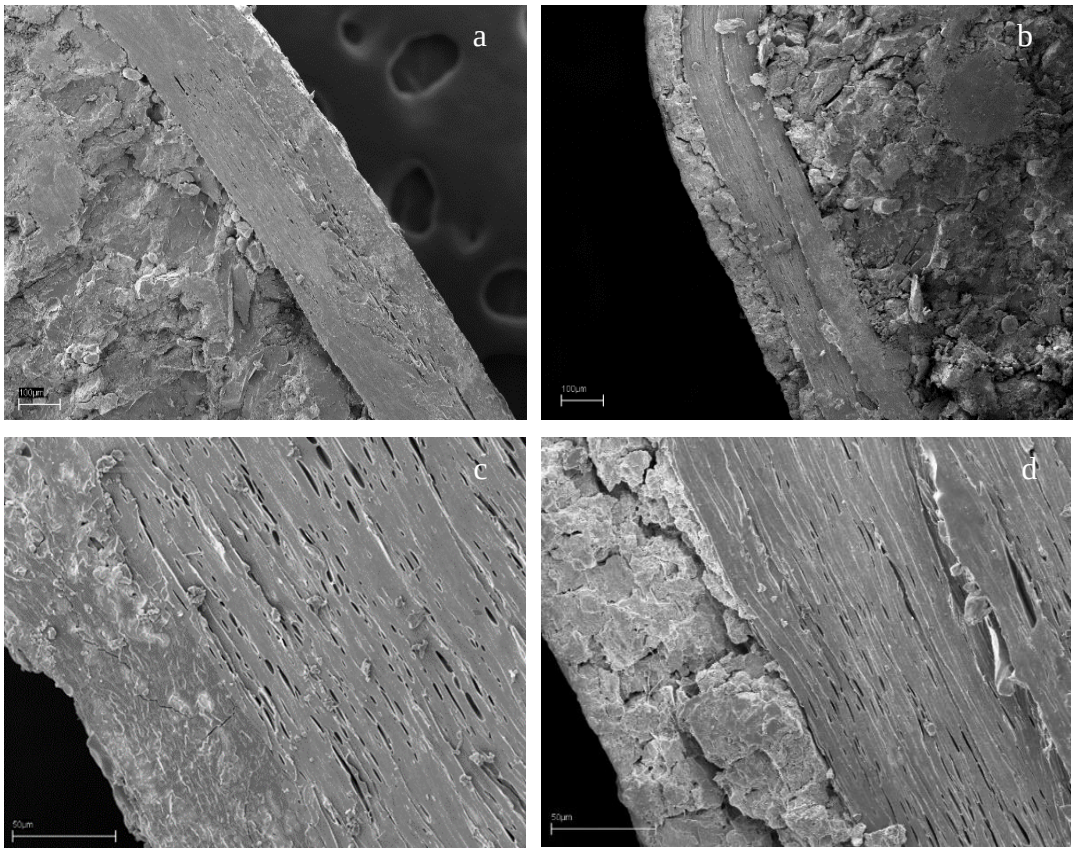

Figure 1: SEM photomicrographs of cross-sectioned two-layer systems having minitablet core, HPMC coating of 200 µm in nominal thickness and outer EuS/Amylo layer containing TEC at 20%, applied by hydro-alcoholic (a,c) or aqueous (b,d) spray-coating.

Release testing

The various coated formulations obtained were subjected to *in vitro* evaluation. All systems proved to withstand 2 h of testing in HCl 0.1 N, thus confirming gastroresistance according to compendial requirements. After switching to PB pH 7.4, pulsatile release profiles were obtained (Figure 2-4). The average lag time increased as a function of the HPMC coat thickness, as desired. With 270 μm HPMC layers, the lag phase was markedly extended as compared with thinner coatings. However, a relatively long phase of slow diffusive release occurred. An increase in the percentage of TEC shortened the lag time, especially in the case of systems devoid of HPMC coating. This might be ascribed to a faster dissolution of the EuS film that, in the absence of an underlying HPMC coat, would not be offset by the delay imparted by the latter. Longer lag times were mostly observed from hydro-alcoholic coated units as compared with aqueous coated ones, most likely because of poorer quality of the coating besides partial neutralization of the polymethacrylate in the water dispersion, which has been proved to shorten its dissolution time [21]. Generally, the release performance was demonstrated reproducible. Furthermore, early stability issues were noticed in the case of EuS/Amylo aqueous spray-coated systems tested for release after 3 months of storage under ambient conditions (data not shown). This would confirm the insufficient quality of the outer coat that was hypothesized to be due to a lack of extensive coalescence. Formulation changes in this respect would therefore be needed, especially envisaging increased percentage amounts of plasticizer.

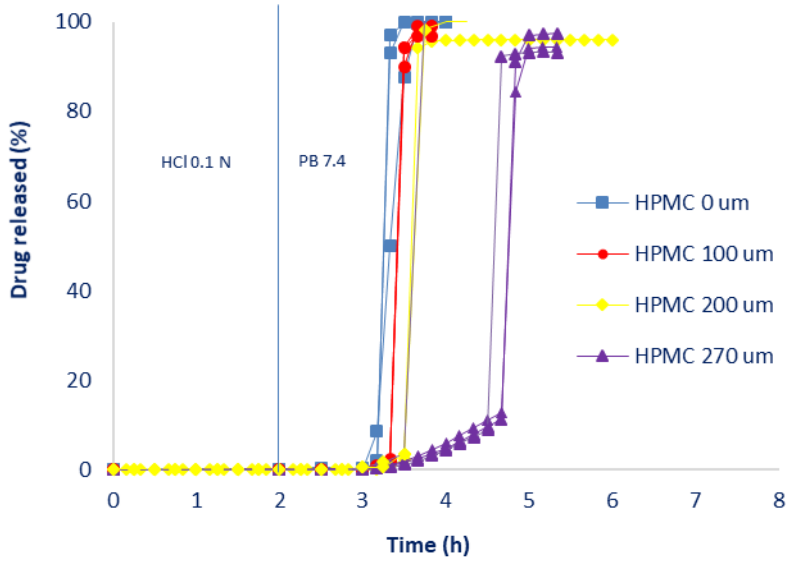

Figure 2: release profiles of paracetamol from two-layer systems having minitablet core, increasing HPMC coating level and outer EuS/Amylo layer containing TEC at 10%, applied by hydro-alcoholic spray-coating.

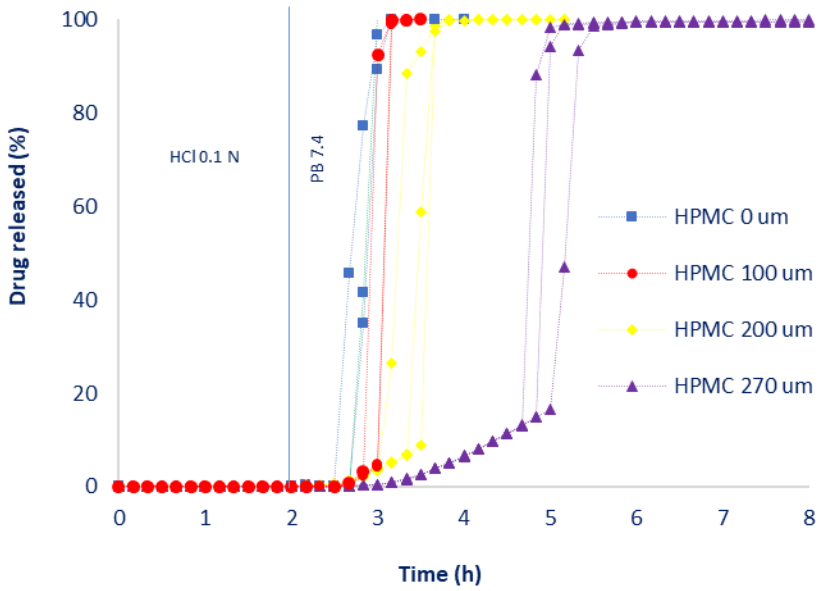

Figure 3: release profiles of paracetamol from two-layer systems having minitablet core, increasing HPMC coating level and outer EuS/Amylo layer containing TEC at 20%, applied by hydro-alcoholic spray-coating.

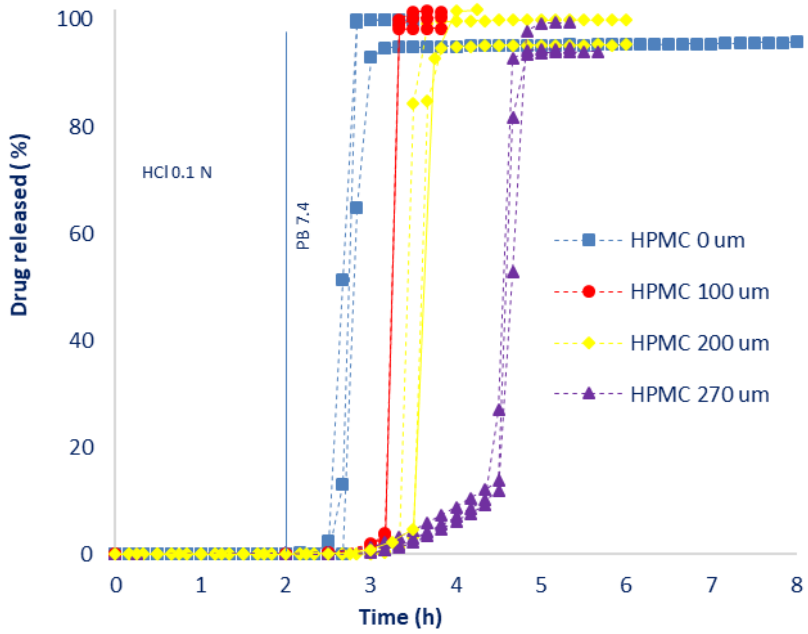

Figure 4: release profiles of paracetamol from two-layer systems having minitablet core, increasing HPMC coating level and outer EuS/Amylo layer applied by aqueous spray-coating.

Based on these results, the hydro-alcoholic coating formulas were selected for application onto molded HPC capsular devices for pulsatile release. Given the marked swelling of the polymer shell, the formulation containing the higher amount of TEC was preferred. Preliminary coating trials were carried out using commercial hard-gelatin capsules in order to restrain the number of HPC capsular devices to be used, as these required to be individually manufactured and manually filled. This step eased subsequent adjustment of the operating conditions with the molded shells. A feasible coating process was finally set up in spite of the critical characteristics of the core, possibly involving sticking and shrinking phenomena as well as nebulization issues due to the inherent mass and shape (Table II).

The coated HPC capsules showed rough surface that was highlighted by SEM analysis (Figure 5). However, they exhibited prompt and quantitative release in

PB pH 7.4 after reproducible lag times that, as expected, were not only due to the applied coating but also to the swellable/erodible core shell (Figure 6). This pointed out the good outcome of the coating process and the possibility of preserving the original release behavior of the capsule cores.

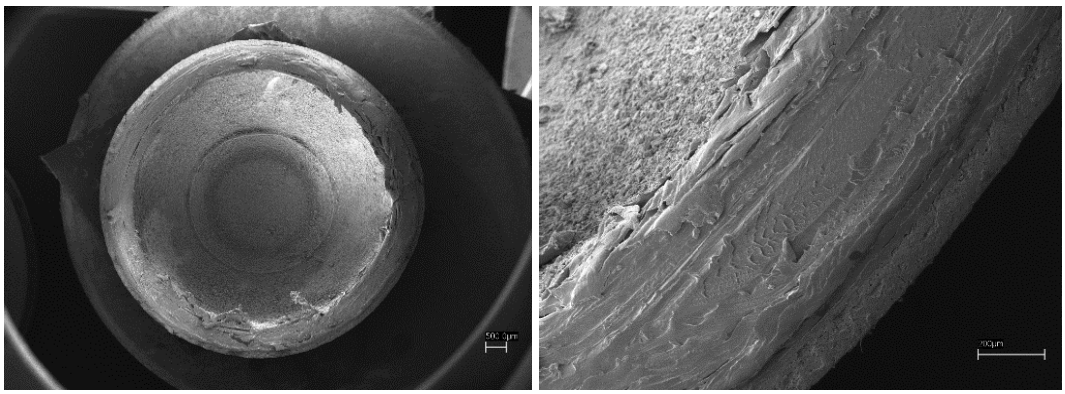

Figure 5: SEM photomicrographs of cross-sectioned HPC capsules provided with EuS/Amylo layer containing TEC al 20%, applied by hydro-alcoholic spray-coating.

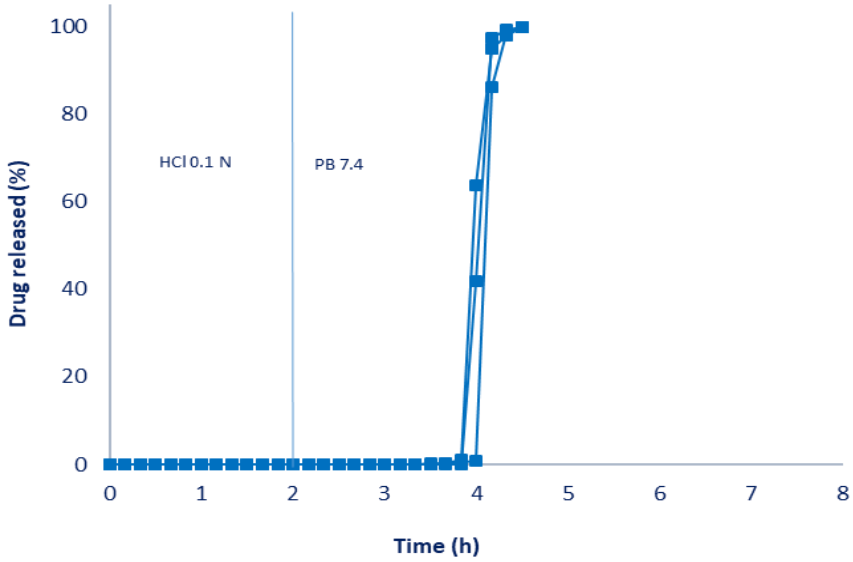

Figure 6: release profiles of paracetamol from HPC capsules provided with EuS/Amylo layer containing TEC at 20%, applied by hydro-alcoholic spray-coating.

Pectin-based formulations were subsequently studied. In order to limit the impact of its water solubility, possibly impairing effective protection of the core by the external coating, a high-methoxylation grade of pectin, having reduced solubility, was chosen. The aqueous film-coating formula developed, and the process parameters used when coating minitablets as the starting core are shown in Table I and II, while the coating levels reached are reported in Table III, respectively. The resulting coated systems showed satisfactory physico-technological characteristics. However, the minitablets coated with Eudragit® S in admixture with pectin HM up to nominal 7 mg of polymethacrylate per unit area did not generally withstand 2 h of testing in HCl 0.1 N (data not shown). Therefore, the EuS/pectin coat thickness was progressively increased in an attempt to strengthen its barrier properties. With doubled coating level, gastroresistance was obtained along with pulsatile release (Figure 7). Although the lag phases were thereby extended, undesired diffusive release issues were observed. Analyzed by SEM, two-layer systems having 14 mg of Eudragit® S/cm^2 were shown to possess uniform coat thickness all over the surface of the tablet, even at the rounded edges that are known to represent challenging areas as compared with flat surfaces (Figure 8).

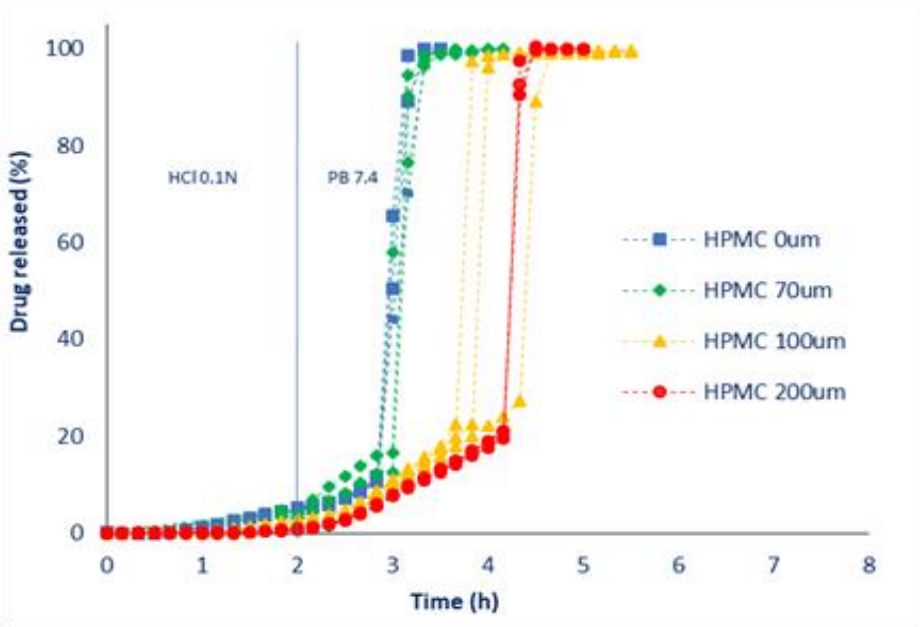

Figure 7: release profiles of paracetamol from two-layer systems having minitablet core, increasing HPMC coating level and outer EuS/pectin layer (14 mg/cm^2 of EuS).

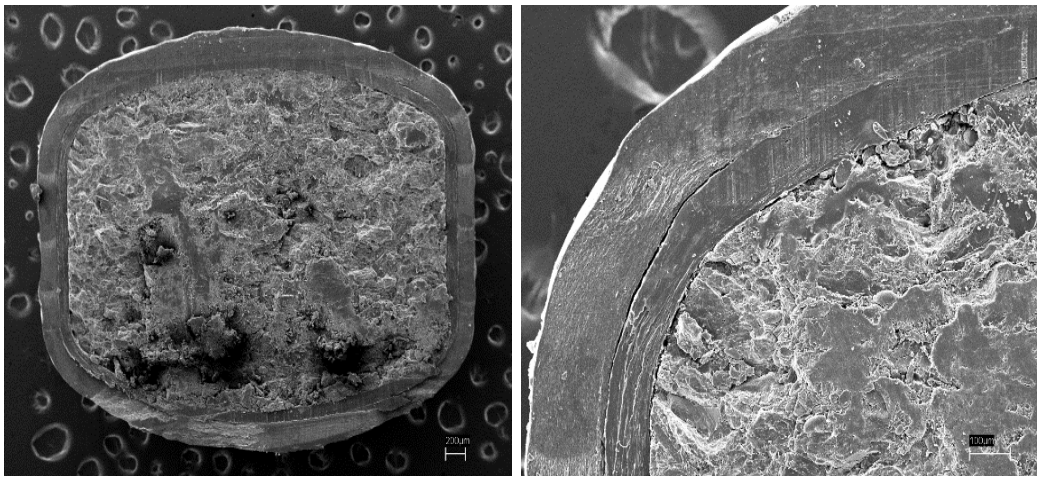

Figure 8: SEM photomicrographs of cross-sectioned two-layer systems having minitablet core, HPMC coating of 100 µm in nominal thickness and outer EuS/pectin layer (14 mg/cm^2 of EuS).

In order to further improve the performance of the Eudragit® S layer, pectin HM was used as a polyelectrolyte complex with chitosan, which was expected to limit the problem of its early leaching out of the coat [22]. Chitosan is also known to be microbially degradable in the large intestine [23]. An aqueous coating dispersion of the pectin-chitosan polyelectrolyte complex was prepared (Table I). In the case of minitablet cores, the process parameters used for the formulation including pectin as such proved generally appropriate for application of the polyelectrolyte complex-based one, except for a slight decrease in the spray rate (Table II). SEM images displayed the good quality of the EuS/pectin-chitosan layer (Figure 9). Also with the new film-forming formulation, a doubled coating level was needed in order to achieve gastroresistance (Figure 10). The release test demonstrated that effective protection of the drug-containing core along with longer lag phases were generally obtained with respect to pectin HM used as such.

On the other hand, by increasing the coating level of HPMC, lag time was extended though a diffusion phase was observed before quantitative release of the drug.

Subsequently, the developed film-coating formulas based on pectin HM as such or as a polyelectrolyte complex were applied onto molded HPC capsules under the operating conditions reported in Table II, up to 14 mg/cm^2 of Eudragit® S. The EuS/pectin-chitosan coated HPC capsules exhibited uniform thickness of the film all around the shell and good relevant quality.

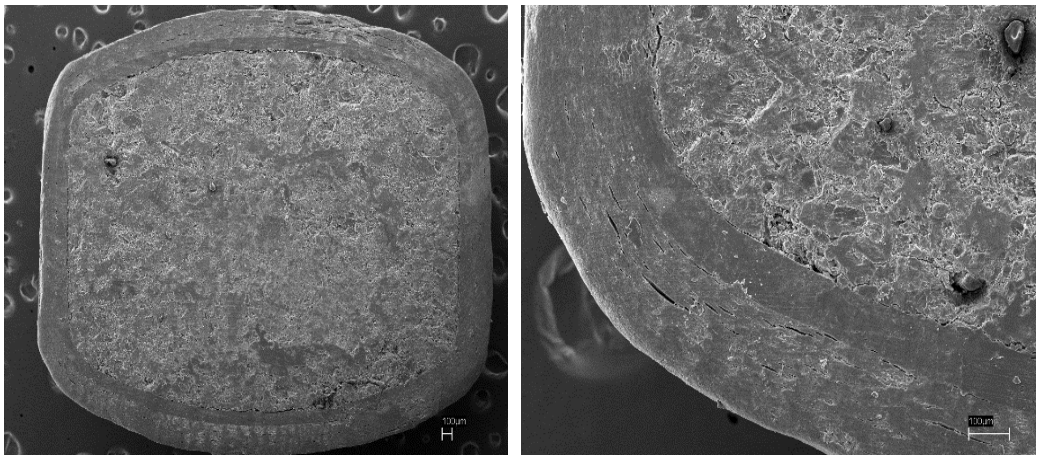

Figure 9: SEM photomicrographs of cross-sectioned two-layer systems having minitablet core, HPMC coating of 100 µm in nominal thickness and outer EuS/pectin-chitosan layer (14 mg/cm^2 of EuS).

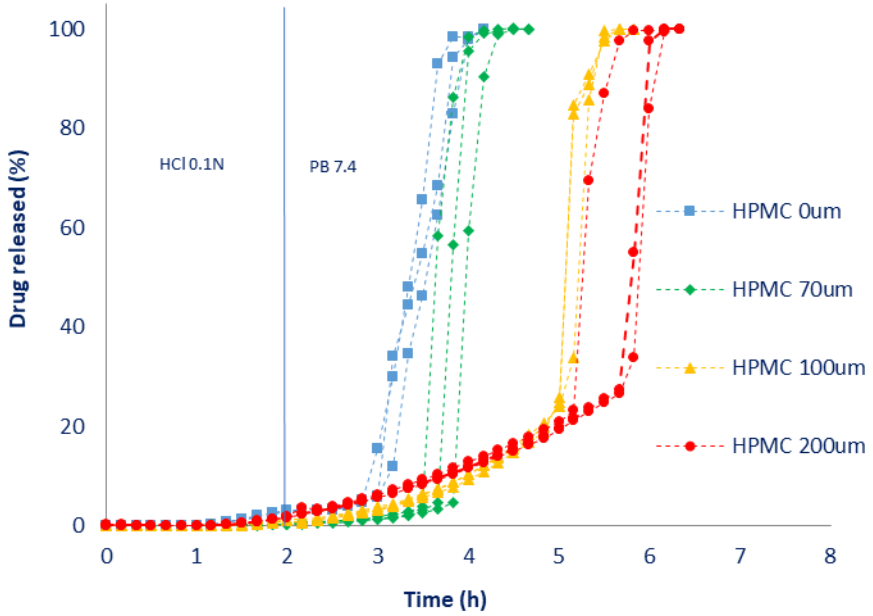

Figure 10: release profiles of paracetamol from two-layer systems having minitablet core, increasing HPMC coating level and outer EuS/pectin-chitosan layer (14 mg/cm^2 of EuS).

Photomicrographs of shells having pectin- or polyelectrolyte complex-based coatings are shown in Figure 11. Good quality of the applied layers also in this case was highlighted. Satisfactory *in vitro* release results were attained using pectin HM as such or as a polyelectrolyte complex with chitosan (Figure 12). Longer lag phases were observed when the polyelectrolyte complex was dealt with, as pursued. In agreement with the release profiles from EuS/Amylo hydro-alcoholic coated capsules, reproducible performance with no diffusion phase was obtained.

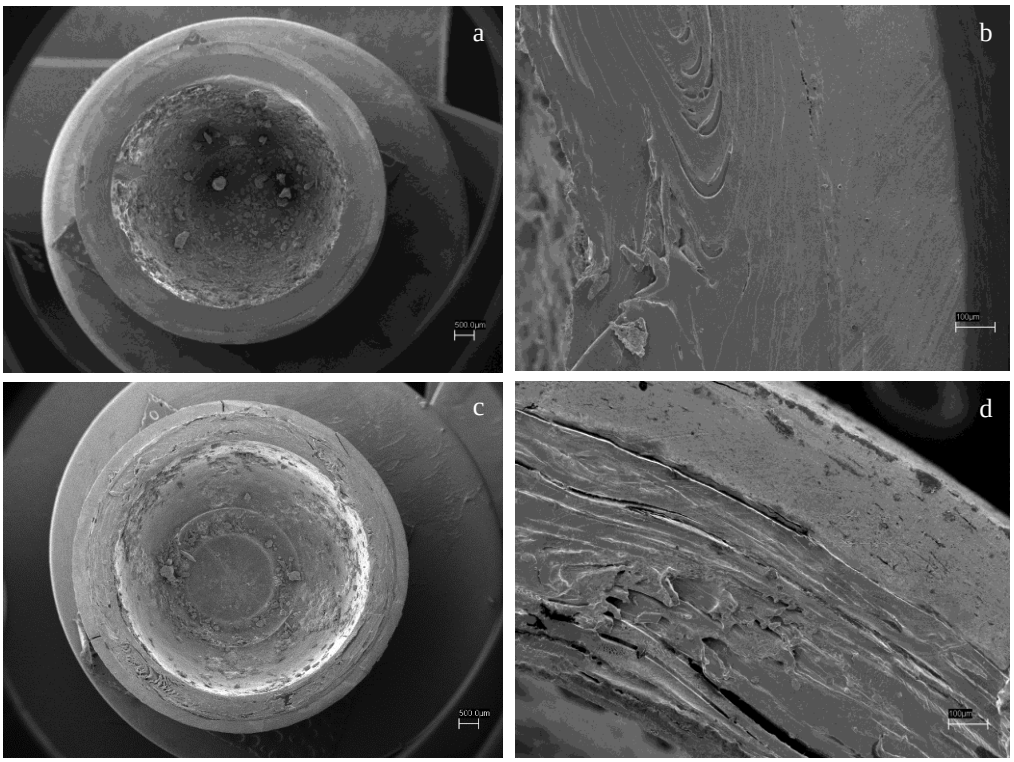

Figure 11: SEM photomicrographs of cross-sectioned HPC capsules provided with EuS/pectin (a,b) and EuS/pectin-chitosan (c,d) coatings.

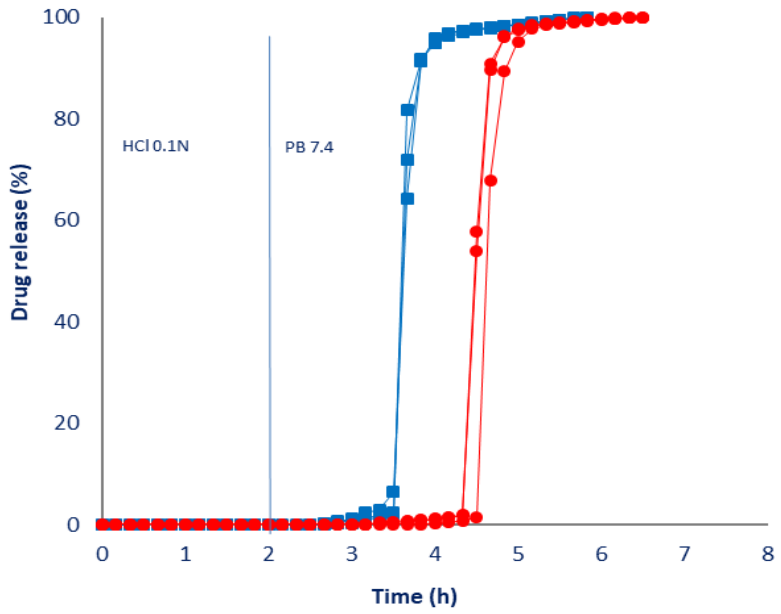

Figure 12: release profiles of paracetamol from HPC capsules provided with EuS/pectin (square) and EuS/pectin-chitosan (circles) coating (14 mg/cm^2 of EuS).

Based on relevant literature reports and the previous experience with pectin, chondroitin sulfate sodium was used as a polyelectrolyte complex with chitosan only [24-27]. An aqueous dispersion was developed and used under the processing conditions set up (Table I and II). Although coating layers having acceptable physico-technological characteristics were achieved, gastroresistance was not even obtained with the nominal coating level of 14 mg/cm^2 of Eudragit® S (Figure 13). For this reason, chondroitin sulfate sodium was discarded, and coatings containing such an excipient were deemed unsuitable for use in the proposed delivery platform, as they would most likely fail to serve as a barrier throughout the proximal gastrointestinal tract.

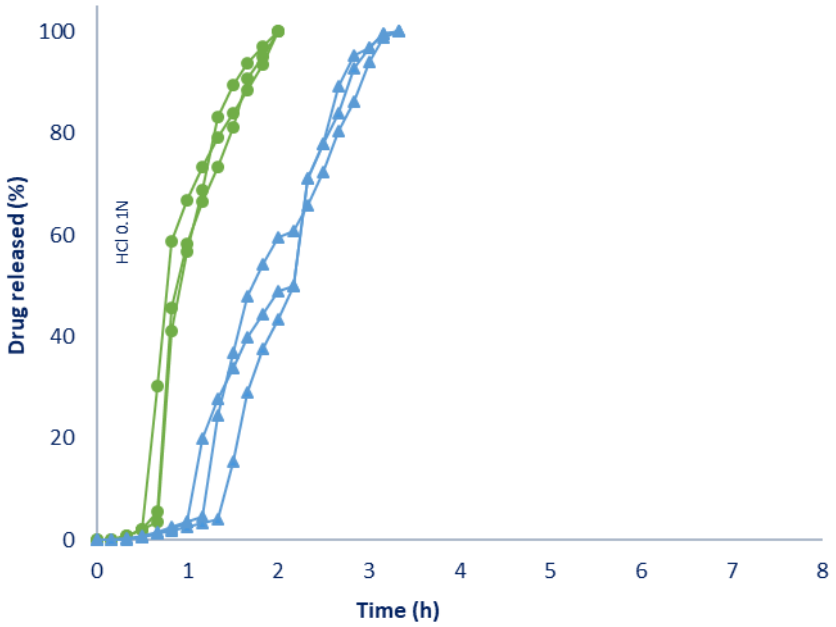

Figure 13: release profiles of paracetamol from minitablets provided with EuS/chondroitin SS coating corresponding to 7 (circles) and 14 (triangles) mg/cm^2 of EuS.

Release testing in simulated colonic fluid (SCF)

After verifying the release performance of the formulations developed in conventional hydrochloric and phosphate buffer media, release testing was repeated by employing simulated colonic fluid (SCF), *i.e.* culture medium inoculated with fecal samples collected from IBD patients, in order to assess the role of the microbiota on the overall performance. To this end, a test method needed to be devised, involving pretreatment of the systems in HCl for 2 h and afterwards in PB pH 4.5 for another 2 h before being transferred into 120 mL flasks containing either SCF or culture medium as such (CM) for control, both at pH 6.5. These conditions were chosen to preserve integrity of the enteric film at pH below dissolution threshold and allow evaluation of the impact of the polysaccharide degradation on drug release. When high-amylose starch-

containing systems obtained from minitablet cores by hydro-alcoholic spray-coating were evaluated, the use of SCF highlighted clear differences *vs*. control in the case of the coating formula with the higher amount of plasticizer (Figure 14 and 15). This was probably due to the better quality of the coat that would be able to withstand the testing conditions in the absence of fecal bacterial strains. The impact of microbial digestion was also well evident with coated HPC capsules, release being observed only in simulated colonic fluid (Figure 16).

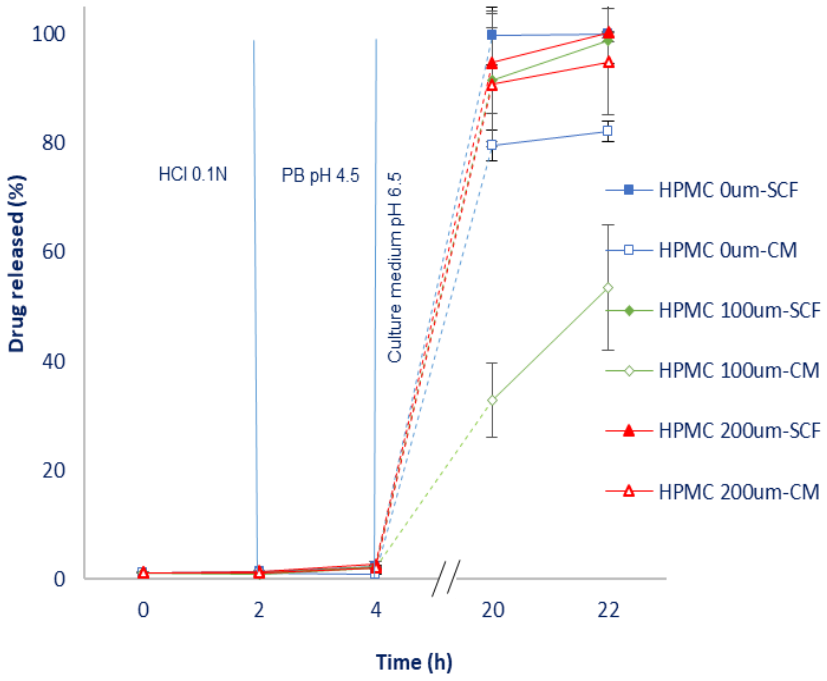

Figure 14: release profiles of paracetamol from two-layer systems having minitablet core, increasing HPMC coating level and outer EuS/Amylo layer (TEC 10 %) applied by hydro-alcoholic spray-coating, tested in culture medium either as such (CM) or inoculated with fecal samples (SCF).

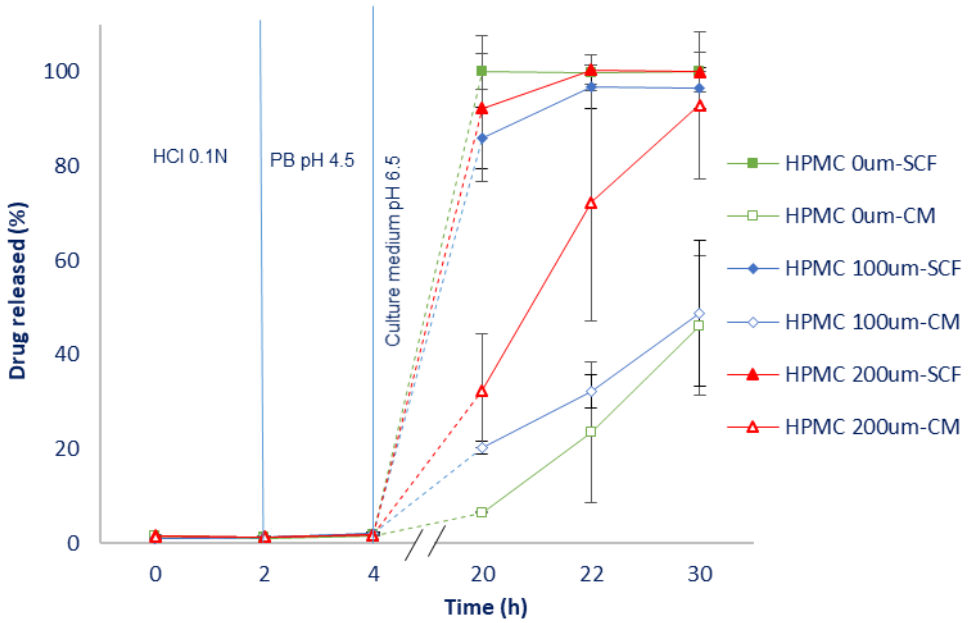

Figure 15: release profiles of paracetamol from two-layer systems having minitablet core, increasing HPMC coating level and outer EuS/Amylo layer (TEC 20 %) applied by hydro-alcoholic spray-coating, tested in culture medium either as such (CM) or inoculated with fecal samples (SCF).

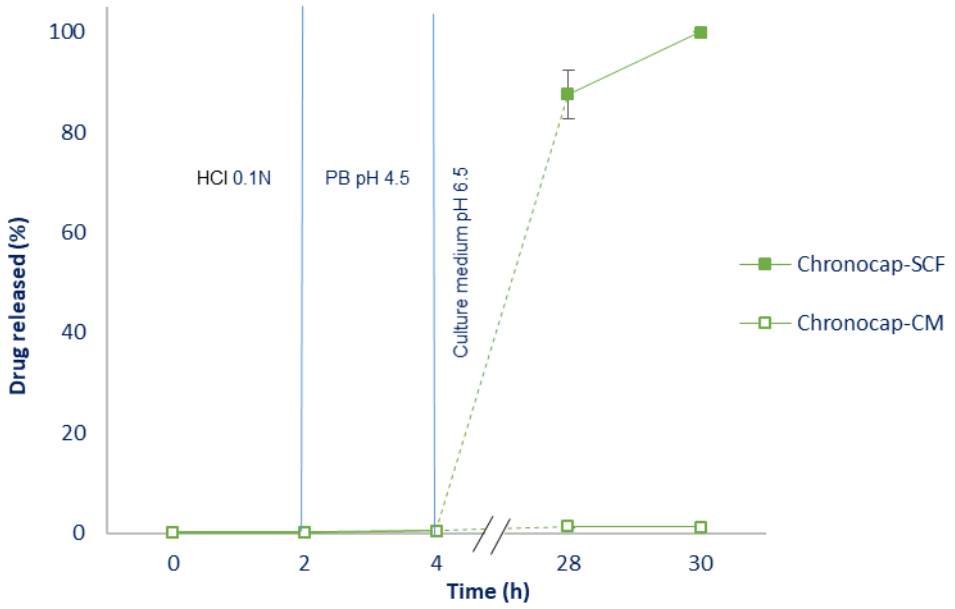

Figure 16: release profiles of paracetamol from HPC capsules provided with EuS/Amylo coating (TEC 20%) applied by hydro-alcoholic spray-coating, tested in culture medium either as such (CM) or inoculated with fecal samples (SCF).

With pectin HM-containing systems, clear dependence of release on the presence of microbial strains was observed when testing the formulations containing the polyelectrolyte complex only (Figure 17). Indeed, no differences were noted with pectin HM alone, probably due to its early leaching out of the coat as a result of dissolution rather than of enzymatic digestion (Figure 18). HPC capsules provided either with pectin- or pectin-chitosan polyelectrolyte complex-containing coating showed initial diffusion phase, which was far more evident in the case of the former system (Figure 19).

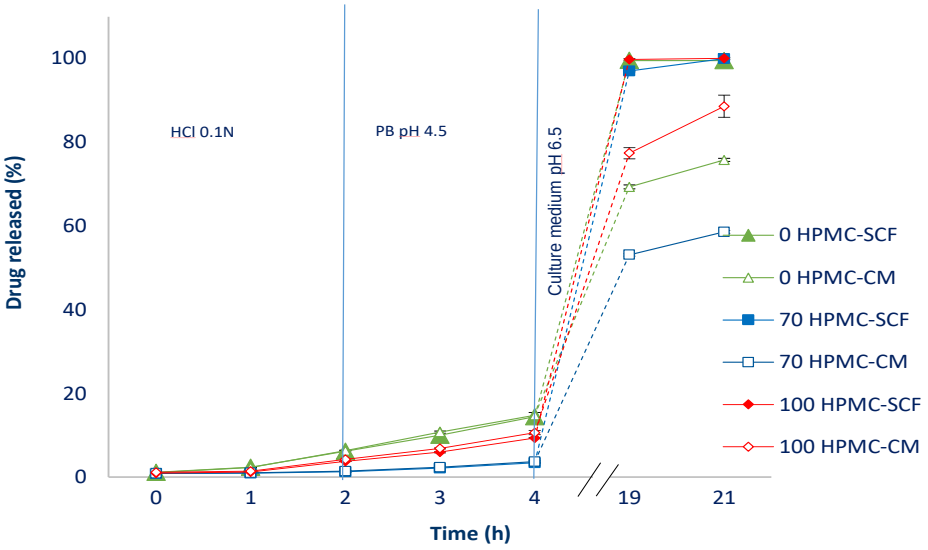

Figure 17: release profiles of paracetamol from two-layer systems having minitablet core, increasing HPMC coating level and outer EuS/pectin-chitosan layer (14 mg/cm^2 of EuS), tested in culture medium either as such (CM) or inoculated with fecal samples (SCF).

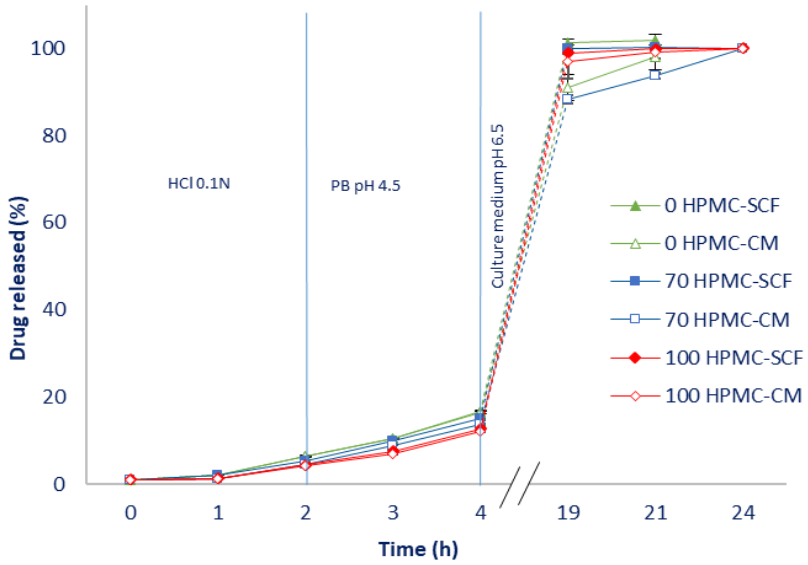

Figure 18: release profiles of paracetamol from two-layer systems having minitablet core, increasing HPMC coating level and outer EuS/pectin layer (14 mg/cm^2 of EuS), tested in culture medium either as such (CM) or inoculated with fecal samples (SCF).

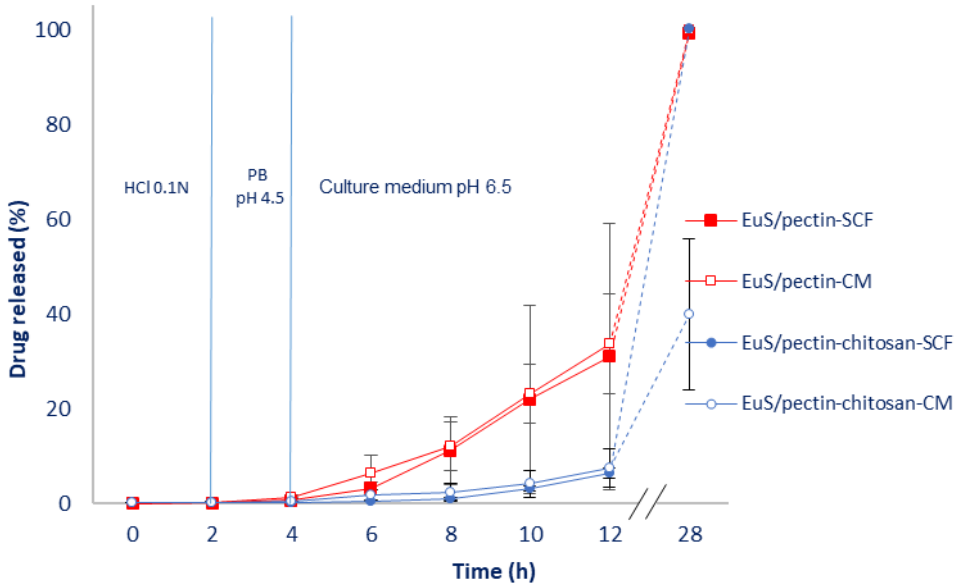

Figure 19: release profiles of paracetamol from HPC capsules provided with EuS/pectin or EuS/pectin-chitosan coating (14 mg/cm^2 of EuS), tested in culture medium either as such (CM) or inoculated with fecal samples (SCF).

CONCLUSIONS

High-amylose starch and pectin HM as such or as a polyelectrolyte complex with chitosan were proved suitable for incorporation as pore formers into Eudragit® S films, which were applied by spray coating to different drug-containing cores, such as HPMC-coated minitablets and molded HPC capsules. Adequate physico-technological characteristics and potentially interesting release patterns were generally attained from all coated systems tested in HCl 0.1 N and PB pH 7.4. The release performance was also evaluated by the use of simulated colonic fluid of proper composition in order to evaluate the role of microbiota. EuS/Amylo hydro-alcoholic coated systems, containing 20 % of TEC on the dry polymer mass, and EuS/pectin-chitosan coated ones, having both minitablet and molded HPC capsules cores, showed clear dependence of release profiles on microbiota, thus supporting the relevant design concept based on site-selective enzymatic degradation of the polysaccharide component in the colonic region.

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Concluding Remarks

The research work carried out within the present doctoral project has been focused on oral colonic drug delivery, encompassing existing formulation strategies, mostly based on exploitation of single physiological parameter inherent to the gastrointestinal tract, and a newly-devised approach, leveraging a combination of such variables in pursuit of improved targeting reliability.

Overall, the knowledge gained in this field through the obtained results can be summarized as follows.

The vast majority of anti-inflammatory drug products that are commercially available, indicated for IBD therapy, are based on the pH-dependent formulation strategy, and particularly on Eudragit® S coatings having dissolution pH threshold of 7.0. This is often physiologically exceeded in the small intestine, while pH values below such a threshold are typical of the proximal colon.

The site of disintegration for Eudragit® S-coated systems, investigated by γ-scintigraphy, was found to be highly variable and poorly reliable irrespective of whether the acrylic polymer was applied by organic or aqueous spray-coating i.e., as a solution or a dispersion.

When organic spray-coating was dealt with, the dosage forms were subject to disintegration failure, which was ascribed to possibly insufficient residence time within regions having pH above the dissolution threshold of the coating polymer and paucity of water available at the distal intestine. When aqueous spray-coating was carried out, the Eudragit® S-coated units always disintegrated before the colon was reached.

Issues of poorly site-selective disintegration were not only encountered with single- but also with multiple-unit Eudragit® S-coated formulations.

On the other hand, the potential of the time-dependent formulation strategy was in-depth explored by contributing, through technology transfer activities, the test formulation for a proof-of-concept investigation. Such a formulation consisted in a swellable/erodible system, as previously developed by the research unit wherein this project has been carried out, and comprised a disintegrating 5-aminosalicylic (5-ASA) tableted core, a functional low-viscosity HPMC layer and an enteric outer film.

The human study pointed out fairly reproducible small intestinal transit time irrespective of the fasted or fed state of the subjects, consistent with the basic principles of the time-dependent delivery approach.

By imaging, breakup of the administered units was always seen after their passage through the ileocecal valve, in an anatomical region extending from the caecum to the transverse branch.

An *in vivo* lag phase was always observed before detection of the drug and its acetyl metabolite in the plasma, aligned with the time span preceding the onset of disintegration of the dosage forms, thus confirming the ability of the delivery system to defer release throughout the gastrointestinal tract.

Relatively low plasma levels of the drug and metabolite, as well as urinary recovery thereof, were found as compared with average values resulting from 5-ASA delivery to upper regions of the gut, in apparent agreement with poor absorptive properties of the distal bowel.

However, the *in vivo* lag phase net of gastric emptying time, *i.e.* the delay time provided by swelling/erosion of the functional polymer layer after relevant contact with the aqueous intestinal fluid, was more extended than pursued relying on the

time-dependent colonic delivery targeting approach. This strengthened the perceived need for design and implementation of a combined formulation strategy preventing release failure and thereby offering greater chance of proximal colon targeting.

Consistent with previous findings, an original delivery platform exploiting multiple physiological variables was proposed. Particularly, a time-dependent HPMC layer was associated with an enteric-soluble outer coating containing a microbially degradable polysaccharide channeling agent. All components were generally regarded as safe (GRAS) excipients. While the HPMC coating would prevent early release in case of failure of the enteric one, the polysaccharide dispersed within the latter would rule out the risk of release failure.

In this respect, identification and screening of naturally-occurring polysaccharide candidates to be used as microbiota-sensitive pore formers were the core research steps. High-amylose starch and high-methoxylated pectin, as such or as a polyelectrolyte complex with chitosan, were proved suitable for incorporation into Eudragit® S films applied by spray-coating onto different drug-containing cores having inherent time-controlled release performance, such as HPMC coated minitablets and molded HPC capsule shells.

Satisfactory physico-technological and *in vitro* release characteristics were obtained with all the polysaccharides investigated.

Release testing using simulated colonic fluid with proper microbially-relevant composition indicated a role of enzyme digestion on breakup of the enteric outer film, thus enabling potentially site-selective drug release performance.

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