Guar Gum as a Microbially Degradable Component for an Oral Colon Delivery System Based on a Combination Strategy: Formulation and *In Vitro* Evaluation

Saliha Moutaharrik[1], Gabriele Meroni[2], Alessio Soggiu[2], Anastasia Foppoli[1], Matteo Cerea[1], Luca Palugan[1], Francesca Caloni[3], Piera Anna Martino[2*], Andrea Gazzaniga[1], Alessandra Maroni[1]

[1]*Università degli Studi di Milano, Department of Pharmaceutical Sciences, Sezione di Tecnologia e Legislazione Farmaceutiche "M.E. Sangalli", via G. Colombo 71, 20133 Milan, Italy; saliha.moutaharrik@unimi.it, anastasia.foppoli@unimi.it, matteo.cerea@unimi.it, luca.palugan@unimi.it, andrea.gazzaniga@unimi.it*

[2]*Università degli Studi di Milano, Department of Biomedical, Surgical and Dental Sciences, One Health Unit, via Pascal 36, 20133 Milan, Italy; gabriele.meroni@unimi.it, alessio.soggiu@unimi.it, piera.martino@unimi.it*

[3]*Università degli Studi di Milano, Department of Environmental Science and Policy, via Celoria 10, 20133 Milan, Italy; francesca.caloni@unimi.it*

*corresponding author: Tel +390250318083, E-mail piera.martino@unimi.it, via Pascal 36, 20133 Milan, Italy

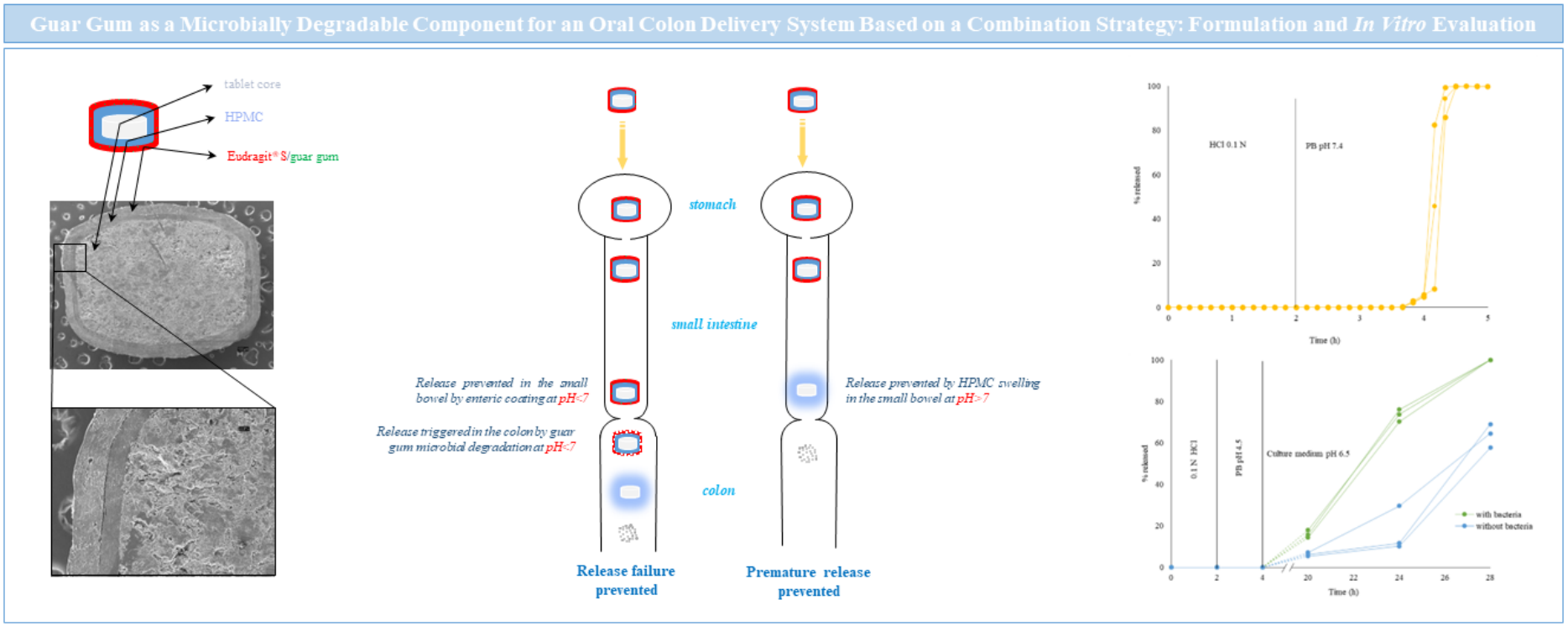
ABSTRACT

Oral colonic delivery has widely been pursued exploiting naturally occurring polysaccharides that are degraded by the resident microbiota. However, the hydrophilicity of these polymers may impair their targeting performance. In the present study, a double-coated delivery system leveraging intestinal microbiota, pH and transit time, was proposed in search of more reliable colonic release. This system comprised a tablet core, an inner swellable hydroxypropyl methylcellulose (HPMC) layer and an outer coating based on Eudragit® S and guar gum. Both layers were applied by spray-coating. In 0.1 N HCl followed by phosphate buffer pH 7.4, guar gum was demonstrated not to impair the barrier properties of the enteric film when incorporated in dispersed form. Lag phases of consistent duration were imparted by the HPMC layer and synergistically extended by the overlaid Eudragit® S/guar gum coating. The delivery systems were also evaluated in simulated colonic fluid (SCF) containing fecal bacteria from an IBD patient, showing faster release than in the presence of β-mannanase and in control culture medium. SCF was obtained by an experimental procedure purposely adopted to enable multiple tests from a single sampling and processing run, thus reducing the time, costs and complexity involved and enhancing replicability.

KEYWORDS

Oral colon delivery, guar gum, hydroxypropyl methylcellulose, Eudragit® S, spray-coating, *in vitro* release test.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Naturally occurring polysaccharides selectively degraded by the resident microbiota have widely been used in the field of colon delivery [1–3]. This is mainly due to their well-known safety profiles resulting from natural origin and wide usage as food components, which makes them more attractive from a regulatory perspective. However, their colon targeting performance could be impaired by the inherent hydrophilicity and, in some cases, water solubility that would question the protection they are expected to afford before the delivery system reaches the colon. In order to overcome such drawbacks, a variety of approaches have been explored. These primarily encompass the chemical modification of polysaccharides and their use in admixture with insoluble polymers, such as ethyl cellulose, polymethacrylates or cellulose acetate [4].

Also, natural polysaccharides were combined with enteric soluble polymers to improve the targeting effectiveness of the pH-dependent colon delivery strategy [5]. Coatings based on high-amylose starch and Eudragit® S blends were proposed to avoid the issue of release failure that has been observed with merely pH-dependent formulations [6–9]. In the proximal gastrointestinal (GI) tract, the enteric polymer would shield amylose from the aqueous fluids, while selective microbial degradation of the polysaccharide in the colonic region would lead to the formation of pores within the film and its consequent rupture.

As pH values higher than 7 are encountered in the upper GI tract, a hydrophilic polymer layer based on low-viscosity hydroxypropyl methylcellulose (HPMC) was applied beneath the Eudragit® S/high-amylose starch coating to provide an extra delay prior to release in case the enteric film loses integrity before colon arrival [9]. Indeed, erodible coating layers based on swellable hydrophilic cellulose derivatives have broadly been used to defer the onset of drug release for chronopharmaceutical or time-dependent colon delivery purposes [10–14] .

As an alternative to starch, high-methoxyl pectin was used as a site-selectively degradable polysaccharide [15]. Whether with starch or pectin, drug release from these delivery systems

was accelerated in simulated colonic fluid (SCF) *vs.* the reference culture medium. SCF was inoculated with fecal bacteria from inflammatory bowel disease (IBD) patients, thereby more closely reflecting the human microbial population in the diseased colon as compared with media prepared with selected enzymes, bacterial species or rat cecal/colonic contents/homogenates that have mostly been employed so far [16]. Evaluated in an HLA-B27 transgenic rat colitis model versus Pentasa®, pectin-containing systems were proved to reduce the overgrowth of *Escherichia coli,* the alteration of the healthy gut microbiota and the progression of inflammation [15].

However, using starch required a preliminary heat treatment so that it could resist degradation by pancreatic amylases in the small bowel and acquire selective degradability by the microbiota [7,8]. On the other hand, because the inherent water solubility negatively affected the barrier properties of the enteric coating, pectin was coupled with positively charged chitosan to limit early leaching from the film [15,17].

Guar gum is a linear galactomannan extracted from guar seeds that consists in a (1-4)-linked β-D-mannose backbone with single (1-6)-bonded α-D-galactose side groups, characterized by a 2:1 mannose/galactose ratio [18,19]. It is mainly produced in India for a wide range of industrial applications, including food, prebiotic fiber supplements, cosmetics and pharmaceuticals.

In the drug formulation field, it is mainly used as a thickener. Moreover, it has been investigated as an ethanol-insoluble excipient for incorporation into modified-release ethyl cellulose coatings to prevent the relevant dissolution in alcohol-rich media [20,21]. Given its proven susceptibility to selective microbial degradation in the large intestine, it has also been exploited for colon delivery purposes [22–26]. Although it has not extensively been investigated in this respect, matrix, compression-coated and film-coated systems based on guar gum have been described to date [27–30].

In the present work, guar gum was evaluated as a different natural polysaccharide pore former for the above-described delivery systems in pursuit of improved performance and more straightforward manufacturing. Particularly, the coating formulation based on Eudragit® S and guar gum as well as the relevant spray-coating conditions were set up. Double-coated delivery systems were thus obtained and fully characterized. In order to evaluate the relevant *in vitro* behavior, different media were employed. The role played by microbial degradation was assessed using media containing either guar gum-hydrolyzing enzymes (*β*-mannanase) or fecal bacteria from an IBD patient diagnosed with Crohn's disease, in search of a reliable and replicable testing procedure.

2. MATERIALS AND METHODS

2.1. MATERIALS

Acetaminophen for direct compression (Rhodapap™ DC 90, Novacyl, Lyon, France), microcrystalline cellulose (Avicel® PH-101, FMC Co., San Colombano al Lambro, Italy), sodium starch glycolate (Explotab® CLV, JRS Rettenmaier Italia, Castenedolo, Italy), vinylpyrrolidone-vinyl acetate copolymer (Kollidon® VA 64, BASF Italia Spa, Cesano Maderno, Italy), hydrophilic fumed silica (Aerosil® 200, Evonik Degussa Italia Spa, Pandino, Italy), magnesium stearate (Carlo Erba Reagents, Srl, Cornaredo, Italy), hydroxypropyl methylcellulose (HPMC, Methocel™ E50, Colorcon Ltd, Dartford, United Kingdom), polyethylene glycol (PEG 400, Clariant SE, Sulzbach am Taunus, Germany)methacrylic acid-methyl methacrylate copolymer (1:2) (EuS, Eudragit® S, Evonik Degussa Italia Spa), guar gum (GG, Gasid, Volvera, Italy), viscosity of 1% aqueous solution at 25 °C is 3620 cps, d_{10}=24.6, d_{50}= 56.1, d_{90}=125.0), triethyl citrate (TEC, Honeywell International Inc, Charlotte, North Carolina, United States), glyceryl monostearate (GMS; Gattefossé SA, Saint-Priest, France),

polysorbate 80 (Tween® 80; ACEF Spa, Fiorenzuola d'Arda, Italy), ammonia solution 25 % v/v (Carlo Erba Reagents Srl), ethanol 96 % (VWR International Srl, Milan, Italy), bovine serum albumin (BSA, Merck KgaA, Darmstadt, Germany), endo-1,4-β-mannanase from *Cellvibrio japonicus* 5,000 U/mL (E-BMACJ, Megazyme Ltd. Bray, Ireland), Beef extract (ThermoFisher Scientific, Milan, Italy), yeast extract (ThermoFisher Scientific), tryptone (ThermoFisher Scientific), sodium chloride (NaCl, Carlo Erba Reagents Srl), L-cysteine hydrochloride (Fisher Scientific) and glycerol (Carlo Erba Reagents Srl).

2.2. METHODS

2.2.1. Manufacturing of delivery systems

Immediate-release tablets (40 mg nominal weight) were prepared from a mixture of acetaminophen 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 %) (Turbula mixer; Willy A. Bachofen AG, Muttenz, Switzerland; 12 + 3 min, 200 rpm). Tableting was performed by a rotary press (AM-8S, Officine Meccaniche F.lli Ronchi, Cinisello Balsamo, Italy) equipped with concave punches (4 mm diameter, 4 mm curvature radius). The resulting tablets were characterized for their weight (analytical balance BP211D Sartorius Italy Srl, Varedo, Italy; n = 20), friability (friabilometer TA3R Erweka GmbH, Langen, Germany), crushing strength (crushing tester TBH30 Erweka GmbH; n = 10), height and diameter (digital micrometer, Mitutoyo Italiana Srl, Lainate, Italy; n = 20) as well as disintegration time (three-position disintegration apparatus DT3 Sotax Srl, Milan, Italy; n = 6). The weight, height, diameter, crushing strength (mean ± s.d.), friability and disintegration time were 40.16 ± 1.13 mg, 3.13 ± 0.03 mm, 4.03 ± 0.02 mm, 42.9 ± 4.7 N, < 1% and <5 min, respectively.

The tablets were coated with an aqueous solution of HPMC (8% w/w) and PEG 400 (10% w/w on dry polymer) by tangential-spray rotary fluid bed (Glatt GPCG 1.1, Glatt GmbH, Binzen, Germany) up to a nominal thickness of 100 μm [9,12,15]. The operating conditions were as follows: 1.2 mm nozzle port size, 2 bar atomizing air pressure, 100 m^3/h drying air

volume, 60 °C inlet air temperature, 50-53 °C product temperature, 49-52 °C outlet air temperature and 3-5 g/min/kg spray rate. HPMC-coated tablets were then coated with an aqueous or a hydro-alcoholic dispersion of Eudragit® S and guar gum by bottom-spray fluid bed (Mini-Glatt, Glatt GmbH). The coating formulas and process parameters set up through the experimental work are reported in the Results and Discussion section. Eudragit® S was suspended in deionized water at a concentration of 17.6 or 21.4 % w/w according to whether guar gum was added or not, respectively, and the resulting dispersion was partially neutralized (15 % of theoretical amount of polymethacrylate) with 1 N ammonia added dropwise under magnetic stirring [9,15]. TEC (70 or 50 % w/w based on dry Eudragit® S, with or without guar gum, respectively) and a fine 5 % w/w water dispersion of GMS (10 or 5 % based on dry Eudragit® S with or without guar gum, respectively) obtained under vigorous stirring at 75 °C, also containing Tween® 80 (40 % w/w based on dry GMS), were then incorporated. Guar gum was used either as an aqueous solution or a hydro-alcoholic (80:20 v/v water:ethanol) dispersion [29,30]. The previously prepared Eudragit® S dispersion was slowly added to the guar gum solution or dispersion under magnetic stirring, up to a 7:3 solid weight ratio between the polymethacrylate and the polysaccharide. The coating processes were carried out until 7 and 14 mg/cm^2 of Eudragit® S was applied. Finally, the coated systems were oven-cured at 40 °C for 24 h.

2.2.2. Characterization of delivery systems

Physico-technological characterization

The obtained systems were checked for coating level, *i.e.* weight gain (%; *n*=100), amount of polymer applied per unit area (mg/cm^2; *n*=100) and coat thickness (µm; *n*=20). The surface of uncoated or HPMC-coated tablets was calculated by the following equation, which incorporates the curvature radius (R), radius (r) and height (h) of the unit:

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

The coating thickness was measured in 10 different regions of each of 3 cross-sectioned coated tablets by a digital microscope (Dyno-Lite Pro AM-413T, AnMo Electronics Co., Hsinchu, Taiwan) and expressed as the mean of such measurements.

Cross-sectioned coated systems were also analyzed using a scanning electron microscope (SEM, LEO 1430, Carl Zeiss S.p.A., Milan, Italy) after gold-sputtering in a plasma evaporator under Argon flow (Auto sputter coater, Agar Scientific Ltd, Stansted, United Kingdom; voltage 10 mA; time 3 min). Photomicrographs were acquired at an accelerated voltage of 9 mV at 25x and 100x magnifications.

Release testing

Release tests (*n*=3) were carried out by USP 43 paddle dissolution apparatus (Dissolution System 2100B, Distek Strumenti & Misure Srl, Napoli, Italy; paddle speed 100 rpm) in 800 mL of 0.1 N HCl for 2 h and then phosphate buffer (PB) pH 7.4 at 37 °C. Fluid samples were automatically withdrawn at successive time points. The drug released was assayed by spectrophotometer (Lambda 35, PerkinElmer® Italia, Milan, Italy; λ 248 nm). Testing was repeated after 6 months of storage at 25±2 °C and 60±5 % relative humidity. Lag time was calculated as the time taken for 10 % release ($t_{10\%}$) in phosphate buffer pH 7.4 by linear interpolation of the closest data before and after this release percentage.

Drug release was also studied in fluids enriched with guar gum-hydrolyzing enzymes or fecal bacteria from an IBD patient. In this case, the double-coated systems were pretreated in 0.1 N HCl for 2 h and then phosphate buffer pH 4.5 for further 2 h using the USP 43 paddle apparatus (100 rpm, 800 mL). After pretreatment, a different testing procedure was followed according to whether the enzyme- or the bacteria-containing fluid was used. In the former case, sodium phosphate buffer (100 mM) pH 6.5 or pH 7.0, containing bovine serum albumin (BSA, 0.5 mg/mL) as recommended by the enzyme product supplier, was used as such or after addition of endo-1,4-*β*-mannanase from *C. japonicus* (64 µL), to give simulated colonic fluid. The pretreated double-coated systems were tested with (simulated colonic fluid) or without (phosphate buffer with BSA as a control) enzymes employing the same apparatus (50 rpm, 250 mL). In the latter case, after obtaining informed consent, fecal samples from a young (25 years old) female donor diagnosed with Chron's disease were aseptically collected and, within 6 h, diluted to a final concentration of 100 mg/mL with sterile saline solution supplemented with 10 % v/v glycerol, using glass beads to help dispersion. The final suspension was vacuum filtrated using sterile gauze swabs (Rays Spa, Osimo, Italy). Subsequently, 10 mL aliquots were obtained and frozen in sealed tubes at -20°C. The day of the experiment, 3 frozen aliquots were allowed to thaw at room temperature before being added each to a flask containing 90 mL of culture medium (CM) to give simulated colonic fluid. CM was prepared by dissolving 1.5 g of beef extract, 3 g of yeast extract, 5 g of tryptone, 2.5 g of NaCl, 0.3 g L-cysteine hydrochloride in 1 L of deionized water and sterilized by autoclaving at 121 °C for 15 min [9,15,31]. The 3 flasks were placed in a sealed plastic bag with an anaerobic gas generator sachet (AnaeroGen^{TM}, ThermoFisher Scientific, Massachusetts, United States) and incubated for 24 h at 37 °C under horizontal shaking (50 rpm). Three further flasks containing culture medium as such (control) were incubated under the same conditions. Afterwards, the pretreated double-coated systems were transferred each into one of the 6 flasks. During the test, the above-described temperature,

hydrodynamics and anaerobiosis conditions were maintained. At programmed time points, 1 mL fluid samples were withdrawn, centrifuged (13,000 rpm, 5 min), filtered (0.22 μm, VWR International Srl), and the drug released was assayed through validated HPLC (Waters, Milford, Massachusetts, United States) method [9,15,32]. An Acclaim™ 5 μm C18 120 Å, 150 × 4.6 mm column (ThermoFisher Scientific, Massachusetts, United States) was used, and the mobile phase consisted of (A) water adjusted to pH 2 with orthophosphoric acid and (B) acetonitrile. A gradient program was applied as follows: 0–10 min 5–20 % B; 10–11 min 20–5 % B. Flow rate and injection volume were set to 1 mL/min and 10 μL, respectively. Acetaminophen was detected spectrophotometrically at 248 nm.

3. RESULTS AND DISCUSSION

The drug delivery platform here proposed was based on a combination approach leveraging on multiple variables of the gastrointestinal tract for reliable colon targeting performance. Particularly, a dispersion of Eudragit® S and guar gum was applied to tablets of 4 mm in diameter, containing acetaminophen as a drug tracer, already coated with low-viscosity HPMC. While the enteric soluble polymer was intended to protect the inner formulation at pH values typical of the proximal gastrointestinal tract, the naturally occurring polysaccharide would impart microbial degradability in the large bowel, and the swellable/erodible layer would shield the drug core for an additional time lapse in the event the outer coating ceased to perform its barrier function before colon arrival of the delivery system.

The Eudragit® S/guar gum coating formulation needed to be set up, and uncoated tablets were employed as the substrate cores. An aqueous polymethacrylate dispersion was used. High percentages of plasticizer TEC (70 % on dry polymer) and of anti-tacking agent GMS (10 % on dry polymethacrylate) were required to promote the formation of a homogeneous film while counteracting the relevant stickiness [15]. This Eudragit® S dispersion was mixed with an aqueous solution of guar gum to a 7:3 solid weight ratio between the two polymers or, alternatively, applied as such for comparison purposes. Solutions of guar gum having different concentrations (1.5, 3 or 4.5 % w/w) were evaluated to assess the relevant impact on viscosity and, consequently, sprayability of the final blend. Only the guar gum solution with the lowest concentration allowed feasible nebulization through the nozzle in use. The composition of the coating formulations is shown in Table I. The aqueous Eudragit® S/guar gum dispersion was successfully applied onto the tablet cores. The coating conditions used for Eudragit® S alone were only slightly adjusted except for the spray rate, which was reduced to approximately 35 % due to the increased viscosity after adding guar gum (Table II). The coating level was set at nominal 7 and 14 mg/cm^2 of Eudragit® S. At the end of the process, the coated units underwent

a curing phase of 24 h at 40 °C, which was longer than usually required for the polymethacrylate. This was indeed shown to be the minimum necessary to provide better physical stability characteristics in the case of previously described Eudragit® S coatings containing different polysaccharides, *i.e.* high amylose starch, pectin and chitosan, at the same nominal weight ratio [9,15]. As shown in Table III, the resulting systems exhibited reproducible thickness of the coating and amount of polymethacrylate applied per unit surface area, close to the nominal values.

Table I: % composition of Eudragit® S-based coating dispersions

Components	Eudragit® S aqueous	Eudragit® S/guar gum Aqueous	Eudragit® S/guar gum hydro-alcoholic
Eudragit® S	13.14	2.74	5.30
Guar gum	-	1.17	2.30
Triethyl citrate	6.57	1.95	3.84
Glyceryl monostearate	0.66	0.19	0.38
Tween® 80	0.26	0.08	0.15
Deionized water	72.80	92.5	74.2
1 N NH_3	6.57	1.37	2.69
Ethanol (96°)	-	-	11.06

Table II: process parameters used with Eudragit® S-based coating dispersions

	Eudragit® S aqueous	Eudragit® S/guar gum aqueous	Eudragit® S/guar gum hydro-alcoholic
Nozzle pore size (mm)	0.5	0.5	0.5
Atomizing air pressure (bar)	1.0	1.0	1.0
Drying air volume (m^3/h)	34 – 38	36 – 40	35 – 38
Inlet air temperature (°C)	38-40	40	40
Product temperature (°C)	30 – 32	33 – 35	34 – 35
Spray rate (g/min/kg)	37 – 41	13 – 15	15 – 16

Table III: HPMC and Eudragit® S/guar gum coating levels

Batch code	HPMC inner coating		Eudragit® S/guar gum outer coating			
	weight gain (%)	thickness (µm)	weight gain (%)	Eudragit® S amount applied (mg/cm^2)	guar gum amount applied (mg/cm^2)	thickness (µm)
$EuS_{aqueous}$	-	-	14.8	7.3	-	116.2 ± 4.8
			29.6	14.7	-	238.9 ± 7.4
$EuS/GG_{aqueous}$	-	-	19.2	6.6	2.8	119.4 ± 2.3
			38.4	14.0	5.6	232.3 ± 3.9
$EuS/GG_{hydro\text{-}alcoholic}$	-	-	20.5	7.0	3.0	139.2 ± 4.4
			41.1	14.2	6.1	260.1 ± 6.5
$HPMC_{100}$ - $EuS/GG_{hydro\text{-}alcoholic}$	15.0	98.2 ± 2.3	20.3	8.2	3.2	167.9 ± 3.6
			33.7	12.4	5.3	264.4 ± 6.2

The coated formulations were evaluated for release in 0.1 N HCl and then phosphate buffer pH 7.4. There was no release in the acid stage of the test was observed from Eudragit® S-coated units irrespective of the coating level, with highly reproducible release profiles following pH change (Figure 1a). In the case of Eudragit® S/guar gum-coated systems, gastroresistance was only obtained at the higher coating level (Figure 1b). However, a diffusional release phase was observed in 0.1 N HCl, which was ascribed to the hydrophilic nature and water solubility of the polysaccharide pore former, weakening the barrier properties of the enteric film.

The use of guar gum as a hydro-alcoholic (water:ethanol, 80:20 v/v %) dispersion was thus explored [29,30]. This also allowed a higher concentration of polysaccharide to be employed because of the lower viscosity imparted when present in the form of dispersed particles rather than a solution. The solid weight ratio between Eudragit® S and guar gum was maintained at 7:3 (Table I). The coating parameters used for the aqueous Eudragit® S/guar gum formulation proved suitable for the hydro-alcoholic one. Despite the increased percentage of guar gum in the coating formula, it was possible to raise the spray rate slightly. The *in vitro* evaluation of the resulting systems showed that all units withstood 2 h of testing in HCl 0.1 N. In phosphate buffer pH 7.4, the time taken for the polymethacrylate/guar gum to dissolve was dependent on the coating level (Figure 1c). As desired, no diffusional release was observed, indicating that a properly performing enteric layer could be obtained by incorporating guar gum as a hydro-alcoholic suspension. With both nominal amounts of polymethacrylate, the time preceding the onset of release from tablets coated with Eudragit® S and guar gum was even extended *vs.* Eudragit® S as such. To further challenge the formulation, the acid stage of the *in vitro* test was prolonged over 5 h, and drug release was found to be prevented throughout the entire duration of exposure of the Eudragit® S/guar gum-coated systems to 0.1 N HCl (data not shown). After 6 months of storage of Eudragit® S/guar gum-coated systems under ambient conditions, release

profiles were consistent with those achieved immediately after coating, thus indicating that the quality of the applied layer was maintained over time.

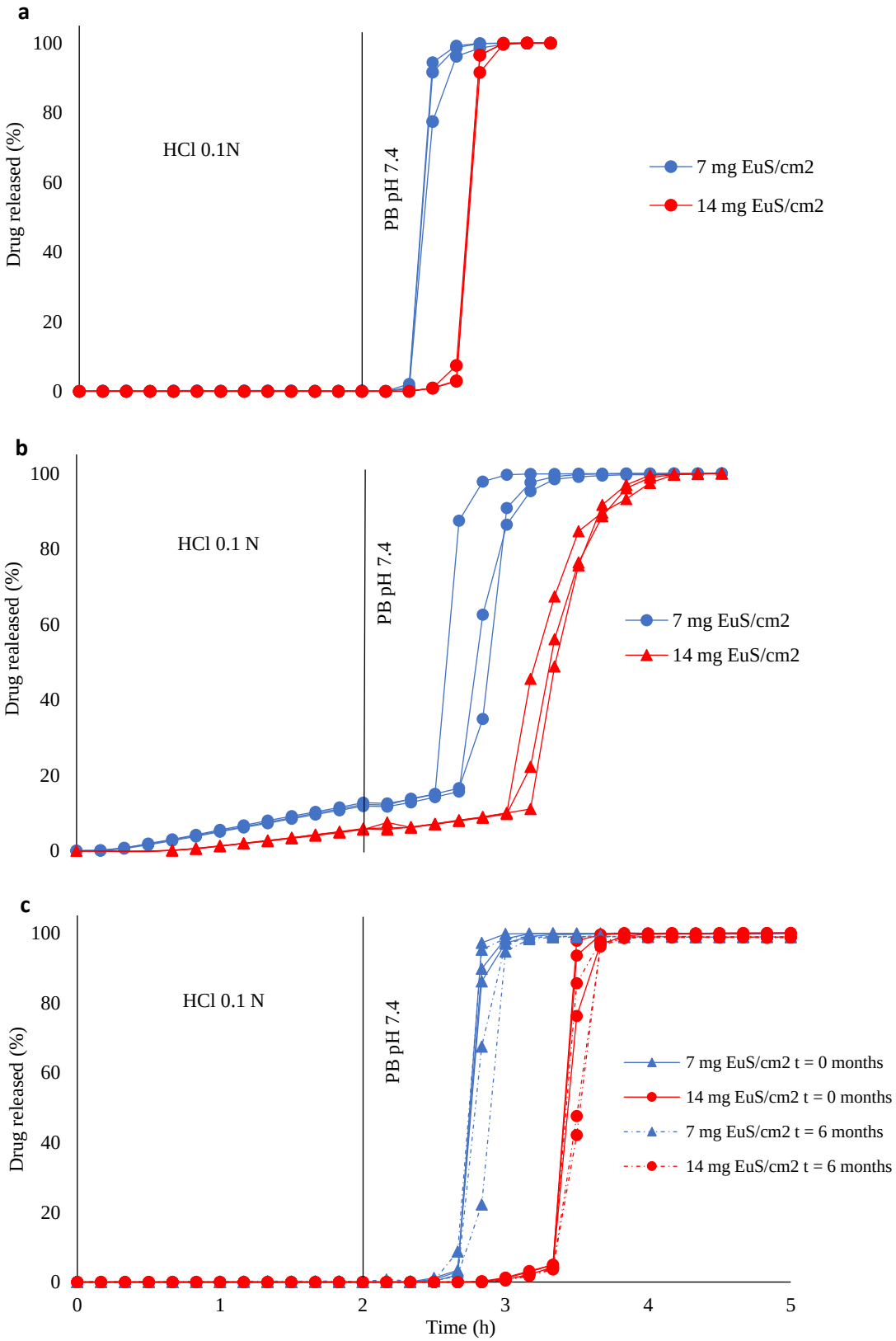


Figure 1: release profiles of acetaminophen from systems coated with (a) aqueous Eudragit® S, (b) aqueous Eudragit® S/guar gum and (c) hydro-alcoholic Eudragit® S/guar gum formulations up to 7 and 14 mg/cm² of polymethacrylate. The solid and dotted plots indicate release profiles obtained immediately after coating and following 6 months of storage under 25±2 °C/60±5 % conditions, respectively.

Based on these results, the hydro-alcoholic formulation of Eudragit® S/guar gum was selected for application onto HPMC-coated tablets to manufacture the proposed colon delivery system. Indeed, the above-mentioned coating dispersion was applied onto disintegrating tablets provided with a 100 µm HPMC layer. The process was not affected by the different characteristics of the substrate and, despite the inherent stickiness of the hydrophilic polymer coating already applied, the operating parameters did not require any changes (Table II). The resulting double-coated systems exhibited highly consistent thickness of the polymethacrylate-based layer, as shown by the low data variability in Table III. The cross-section morphology of the coated units was analyzed by SEM. Continuous HPMC and Eudragit® S/guar gum layers were highlighted. The dispersed coating formula used for the application of the outer layer did not impact on its structure, although a less smooth surface was observed than with the HPMC coating derived from an aqueous polymer solution (Figure 2).

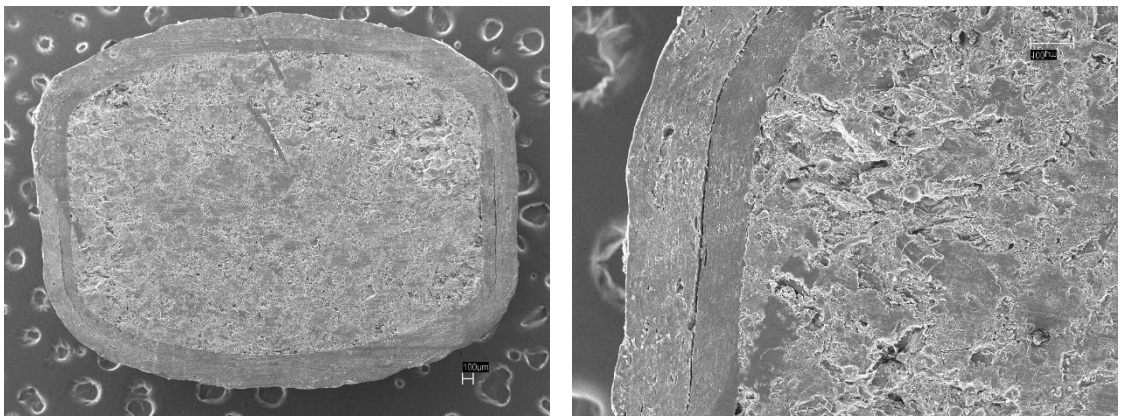
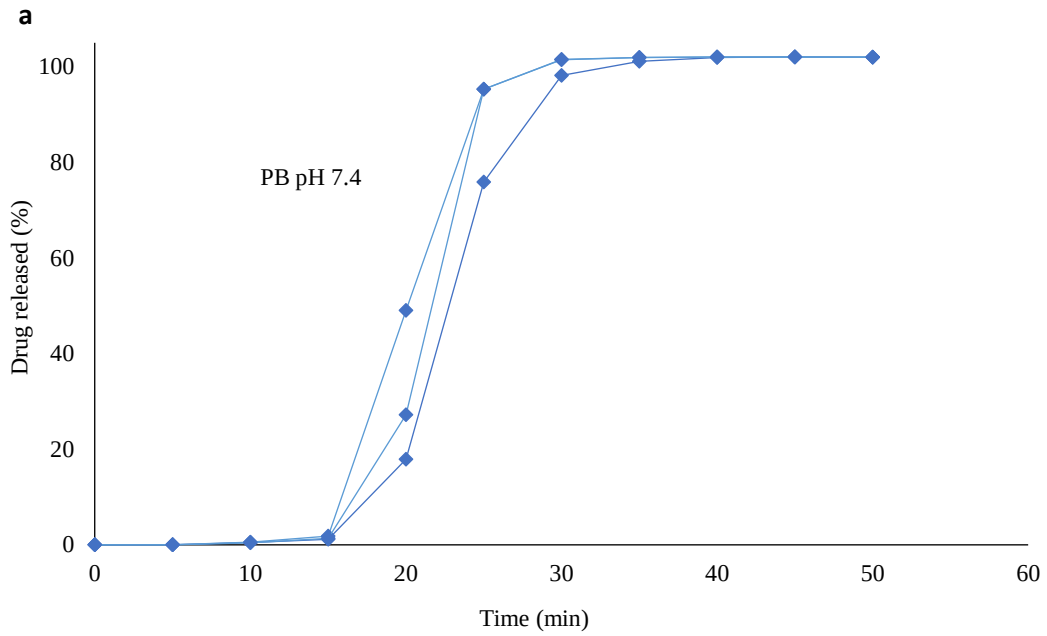


Figure 2: SEM photomicrographs of cross-sectioned double-coated systems having tablet core, HPMC (100 µm) inner layer and Eudragit® S/guar gum (14 mg EuS/cm^2) outer layer. Magnification 25x (left) and 100x (right), scale bars correspond to 100 µm.

The *in vitro* release performance of double-coated systems was first evaluated using compendial fluids (Figure 3). After the medium was changed to phosphate buffer pH 7.4, the time needed for drug release was considerably increased as compared with systems provided with the sole Eudragit® S/guar gum layer. Indeed, as desired, HPMC deferred the onset of release through swelling and dissolution upon hydration. The duration of such a lag phase was consistent irrespective of whether the outer coating level was 7 or 14 mg/cm^2 of polymethacrylate. Notably, the lag time, net of the time taken for dissolution of the Eudragit® S/guar gum layer (39.8 ± 2.4 min and 74.9 ± 5.1 min with 7 and 14 mg/cm^2, respectively), was much longer when the HPMC layer was coupled with the latter (53.7 ± 5.9 min and 49.2 ± 5.3 min with 7 and 14 mg/cm^2 of Eudragit® S, respectively) rather than when applied alone (16.7 ± 0.9 min). A synergistic effect could thus be hypothesized. The external layer would shield HPMC from extensive exposure to the aqueous medium and, until complete dissolution of the polymethacrylate, water would penetrate through the hydrophilic guar gum domains. Also, the outer coating may mechanically hinder swelling of the polymer, which might account for the limited diffusional phase observed prior to quantitative release [33,34].



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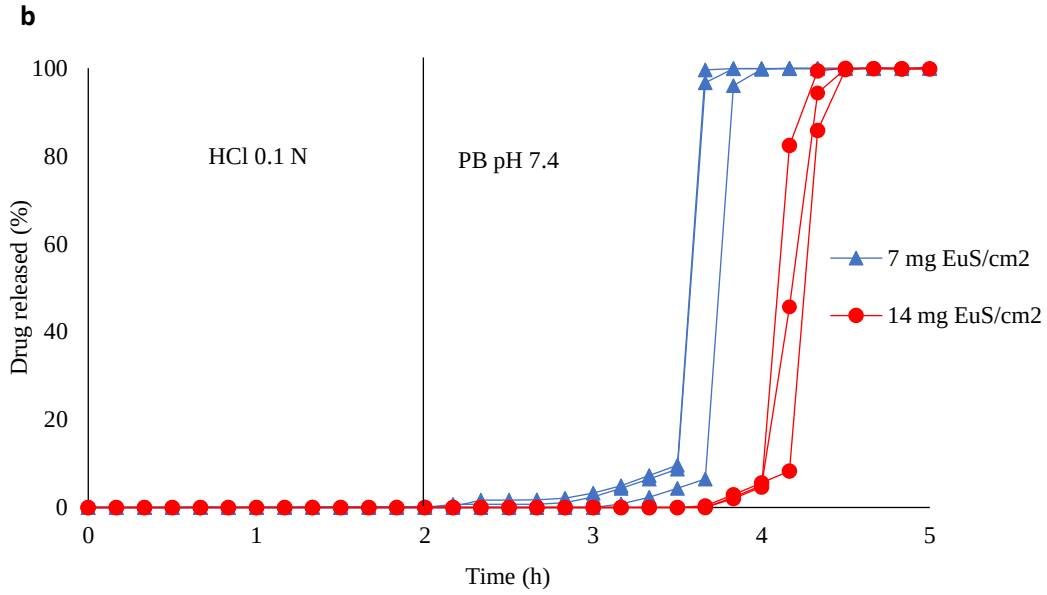


Figure 3: release profiles of acetaminophen from systems coated with (a) HPMC (100 µm), and (b) HPMC (100 µm) and hydro-alcoholic Eudragit® S/guar gum layer formulations (7 and 14 mg/cm² of Eudragit® S).

315 In order to study the potential effect of guar gum on the release performance of the double-coated tablets in the colon environment, two different testing methods were implemented. The former exploited selected hydrolase enzymes, *β*-mannanase, that degrade the polysaccharide [35,36]. A commercially available *β*-mannanase-containing product in the form of an aqueous

dispersion was diluted in phosphate buffer pH 6.5 to obtain the simulated colonic fluid. To simulate exposure to upper gastrointestinal fluids, the double-coated systems were pretreated in 0.1 N HCl for 2 h and then in phosphate buffer pH 4.5 for another 2 h before being transferred into such a medium. The pH of the sequential media employed did not exceed the dissolution threshold of the enteric soluble polymer Eudragit® S, so that any role of microbial degradation of guar gum, expected to allow for drug release in case a pH value of 7 is not reached or maintained in the proximal colon, could be highlighted.

As shown in Figure 4a, no drug was detected in the first 4 h of the test, while a slow release was observed in the phosphate buffer media with or without *β*-mannanases. The release profiles were overlapping, thus indicating no major influence of enzyme degradation. To rule out that this result might have been affected by the pH of simulated colonic fluid being lower than the optimum value for *β*-mannanase activity, the test was repeated using phosphate buffer pH 7.0 to dilute the enzyme product. Also in this case, the drug was slowly released over a long period of time (Figure 4b). As compared with the pH 6.5 fluid previously used, the release started earlier irrespective of the presence of the enzyme, reasonably due to the test being performed at a pH value corresponding to the dissolution pH threshold of Eudragit® S. Only a slightly increased release rate was shown when *β*-mannanases were present. However, this difference was not statistically significant.

The latter testing approach utilized fecal bacterial strains from an IBD patient. In this case, simulated colonic fluid was obtained by a purposely applied procedure adapted from those currently employed for handling and processing biological specimens for fecal microbiota transplantation (FMT) [37]. An analogous method was also used in investigations aimed at assessing/predicting the stability of drugs in the distal intestine [38–40]. According to FMT guidelines, stool samples collected from a Crohn's disease patient were processed within 6 h by

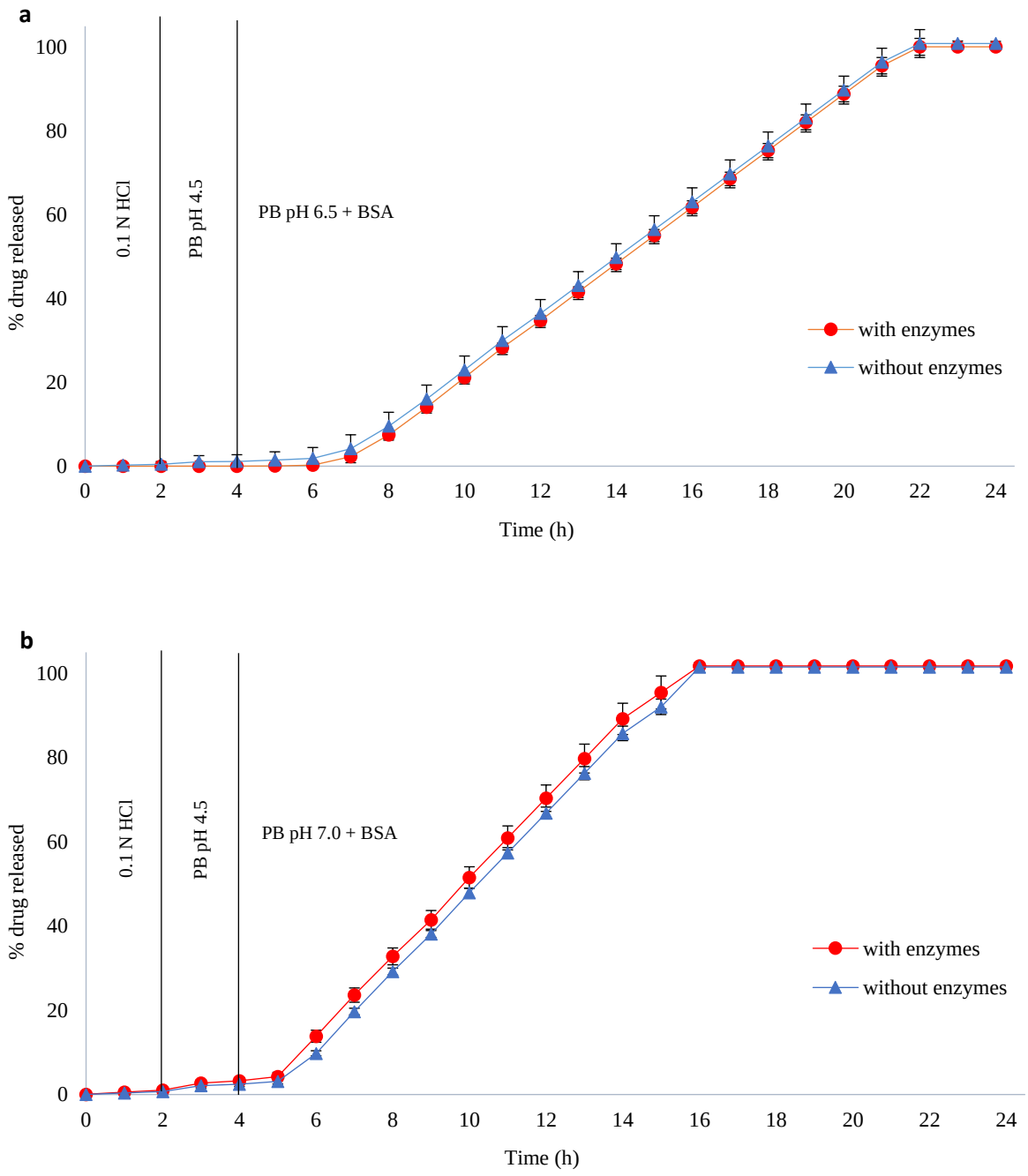
dilution in saline solution supplemented with glycerol (10 % v/v), filtration and partitioning into 10 mL aliquots for storage at -20 °C [41].

Before release testing, the frozen specimens were thawed, and the bacteria were allowed to grow in sterile culture medium under anaerobic conditions for 24 h at 37° C for full recovery of metabolic activities. After pretreatment in 0.1 N HCl and phosphate buffer pH 4.5, as described above, the dosage forms were immersed either in the simulated colonic fluid or in control culture medium without bacteria.

Release of the tracer drug was prevented under the acidic conditions of the first 4 h of the test and started after pH change to 6.5. A slow release was also observed in the control culture medium, which could be ascribed to water penetration into the core and consequent diffusion of the dissolved tracer drug via the hydrophilic domains of the external coating. However, the release rate was notably higher in simulated colonic fluid containing fecal bacteria. Despite a c blind time lapse, involved by the long testing procedure and need for manual sampling, at 20 h the amount of drug released was still low (15.9±1.8 % and 6.1±0.8 % in the presence and absence of bacteria, respectively). The differences between the release profiles in the two fluids were well evident at 24 h, when 73.4±3.0 % and 17.2±10.8 % of the drug was released in the test and the control medium, respectively. At 28 h, 100 % release was reached in simulated colonic fluid.

These results would point out an impact of microbial degradation on the release performance of the double-coated systems, supporting the combination of Eudragit® S with guar gum to reduce the risk of release failure reported in regard to the pH-dependent colon delivery approach. Interestingly, microbiota alterations possibly induced by the pathological conditions did not appear to hamper polysaccharide breakdown by the bacteria.

The testing method based on fecal bacteria, unlike *β*-mannanases, was found effective in highlighting the performance of the guar gum-containing formulation. Moreover, because a set of processed fecal samples was ready for use simply by thawing, the inherent complexity of dealing with simulated colonic fluid enriched with bacteria was partially overcome. Indeed, the procedure set up helped streamline the *in vitro* release study and, importantly, solved the problem of inter-test variability in the composition of the bacterial population.



375 *Figure 4: release profiles of acetaminophen from double-coated systems (14 mg/cm^2 of Eudragit® S) upon exposure to 0.1 N HCl for 2 h, phosphate buffer pH 4.5 for 2 h and then BSA-containing phosphate buffer enriched with β-mannanase having (a) pH 6.5 or (b) pH 7.0.*

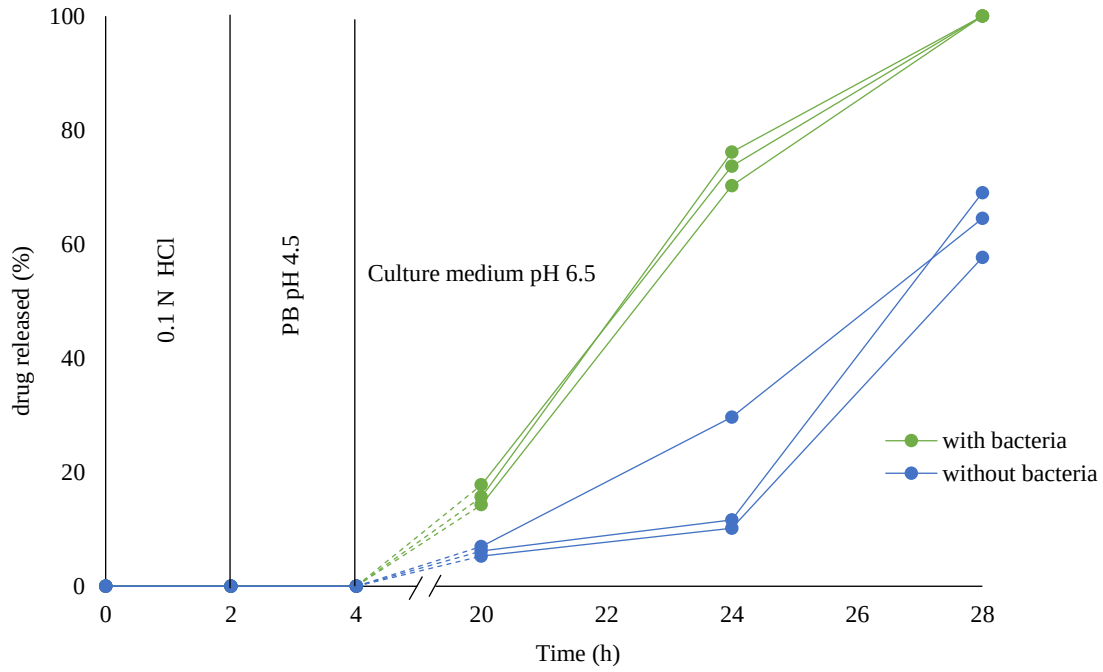


Figure 5: release profiles of acetaminophen from double-coated systems (14 mg/cm^2 of Eudragit® S) upon exposure to 0.1 N HCl for 2 h, phosphate buffer pH 4.5 for 2 h and then culture medium inoculated with fecal bacteria or culture medium as such.

4. CONCLUSIONS

Oral colon delivery is of interest for a range of therapeutic goals, leveraging small molecules, biologics, prebiotics and/or probiotics. Recently, inherent limitations of formulation strategies based on a single physiological parameter and advantages arising from combined approaches have been highlighted. In this respect, the incorporation of naturally occurring polysaccharides prone to microbial degradation into a Eudragit® S coating and the addition of an HPMC layer underneath, delaying the onset of release through polymer swelling and erosion, were proposed to reduce the risks of premature release in the small intestine and release failure reported with pH-dependent colon delivery platforms.

Guar gum, a galactomannan well known for its prebiotic properties, was here investigated as a colonic degradable pore former for the enteric layer of a double-coated delivery system according to the novel design concept mentioned above. The application of Eudragit® S/guar gum films onto HPMC-coated tablets via spray-coating technique proved feasible after setup of the formulation and processing conditions. The *in vitro* release study indicated that gastric resistance of the resulting dosage forms was not impaired by the presence of the polysaccharide in the outer coating. Coupled with the HPMC inner layer, such a coating was hypothesized to yield a synergistic effect on the release performance. Indeed, the duration of lag phases at intestinal pH was longer than when HPMC was applied alone. The capability of guar gum to trigger the release of the loaded drug in the colon environment was evaluated by *in vitro* release testing in simulated colonic fluid either containing *β*-mannanase or fecal bacterial strains derived from an IBD patient. While no effects of the naturally occurring polysaccharide were highlighted by the use of enzymes, a significantly faster release was found in the presence of bacteria. In this case, simulated colonic fluid was obtained through an experimental procedure that was profitably applied for the first time to the study of colon delivery systems intended for

actuation by the resident microbiota. Because it allowed multiple tests to be carried out from a single fecal collection and processing run, not only such a procedure reduced the time, costs and complexity of these investigations, but also ensured improved replicability, thus addressing the main shortcomings of microbially relevant release testing.

5. ETHICAL STATEMENT:

CONSENT TO PARTICIPATE:

Informed consent was obtained from the donor of the fecal sample that was employed in the study for *in vitro* purposes.

CONSENT FOR PUBLICATION:

The authors state that the donor of the fecal sample that was employed in the study provided informed consent for publication of personal information.

COMPETING INTERESTS:

The authors have no relevant financial or non-financial interests to disclose.

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AUTHORS' CONTRIBUTIONS:

Saliha Moutaharrik: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing

Gabriele Meroni: Methodology, Formal analysis, Investigation, Writing - Review & Editing

Alessio Soggiu: Validation, Data curation, Visualization

Anastasia Foppoli: Methodology, Formal analysis, Writing - Review & Editing

Matteo Cerea: Validation, Data Curation, Visualization

Luca Palugan: Methodology, Formal analysis, Resources

Francesca Caloni: Data curation, Validation, Writing - Review & Editing

Piera Anna Martino: Validation, Formal analysis, Resources, Writing - Review & Editing, Supervision

Andrea Gazzaniga: Conceptualization, Resources, Writing - Review & Editing, Supervision

Alessandra Maroni: Conceptualization, Writing - Original Draft, Writing - Review & Editing, Project administration, Supervision

DATA AVAILABILITY STATEMENT:

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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