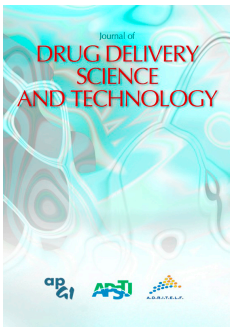
Journal Pre-proof



Development and evaluation of budesonide-based modified-release liquid oral dosage forms

Federica Ronchi, Antonio Sereno, Maxime Paide, Ismaël Hennia, Pierre Sacré, George Guillaume, Vincent Stéphenne, Jonathan Goole, Karim Amighi

PII: S1773-2247(19)30732-4

DOI: https://doi.org/10.1016/j.jddst.2019.101273

Reference: JDDST 101273

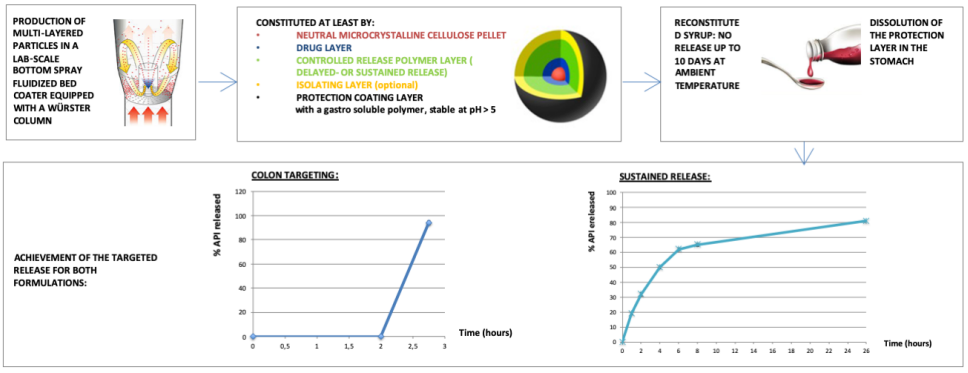
To appear in: *Journal of Drug Delivery Science and Technology*

Received Date: 22 May 2019

Revised Date: 31 August 2019

Accepted Date: 9 September 2019

Please cite this article as: F. Ronchi, A. Sereno, M. Paide, Ismaë. Hennia, P. Sacré, G. Guillaume, V. Stéphenne, J. Goole, K. Amighi, Development and evaluation of budesonide-based modified-release liquid oral dosage forms, *Journal of Drug Delivery Science and Technology* (2019), doi: https://doi.org/10.1016/j.jddst.2019.101273.



Development and evaluation of budesonide-based modified-release liquid oral dosage forms

Federica Ronchi[1], Antonio Sereno[1†], Maxime Paide[1], Ismaël Hennia[1], Pierre Sacré[2], George Guillaume[2], Vincent Stéphenne[2], Jonathan Goole[1], Karim Amighi[1]

[1]Laboratory of Pharmaceutics and Biopharmaceutics, Université libre de Bruxelles, Campus de la Plaine, CP 207, Boulevard du Triomphe, Brussels, 1050, Belgium

[2]BePharBel Manufacturing, Courcelles, Belgium

Corresponding author: Karim.Amighi@ulb.ac.be, boulevard du Triomphe, CP 207, Access 2, Campus de la Plaine, Building BC- 1050 Belgium

Keywords: Budesonide, multi-layered particles, liquid syrup, oral dosage form, colon targeting, sustained release.

Table of abbreviations and acronyms

API: Active pharmaceutical ingredient

BUD: Budesonide

CV: Coefficient of variation

GIT: Gastrointestinal tract

GMS: Glyceryl monostearate

HCL: Hydrochloric acid

HPLC: High performance liquid chromatography

K30: Kollidon® 30

LOQ: Limit of quantification

MUDF: Multiple-unit dosage form

MUPS: Multi-unit pellets system

PBS: Phosphate-buffered saline

PVP: Polyvinylpyrrolidone (Povidone)

RH: Relative humidity

rpm: Rotation per minute

SEM: Scanning electron microscopy

SOP: Standard operating procedure

SUDF: Single-unit dosage form

T°: Temperature (°C)

TEC: Triethyl citrate

TGA: Thermogravimetric analysis

UK: United Kingdom

USA: United States of America

Abstract

Modified-release oral drug delivery dosage forms are widely used in the pharmaceutical field to overcome all the potential issues imposed by the physiological variabilities of the gastrointestinal tract as well as to maintain drug concentrations within the therapeutic window. In the market, they are available only as solid dosage forms such as capsules or tablets. The development of a liquid oral dosage form with modified-release properties has been keenly awaited. This form could increase the compliance of patients with a swallowing impairment (i.e. paediatric, older or critically ill patients) and, consequently, the efficacy of the therapeutic treatment. In this study, budesonide was used as a model drug to develop a modified-release liquid oral dosage form (i.e. colonic-release, sustained-release). For this purpose, multi-layered particles were obtained, starting from small microcrystalline cellulose neutral cores (Cellets® with a mean diameter lower than 500 µm), in a lab-scale fluid-bed coater. Poly(meth)acrylate polymers commonly available under the trade name of Eudragit®, such as S100, RS PO, RL100 and E100, were used to get defined drug release profiles. They were also used to guarantee the stability of the reconstituted liquid syrup during 2 weeks of storage at room temperature.

1. Introduction

Among the different routes of administration, the oral route is still the most commonly used due to its ease of administration [1]. Oral dosage forms can be classified into immediate- and modified-release systems. Although immediate-release oral dosage forms may provide a rapid onset of therapeutic response, they are not able to control the release of a drug [2]. Such dosage forms have to be administered several times per day to maintain the drug concentration within the therapeutically effective range for the whole duration of the treatment. This can result in fluctuating drug levels in the bloodstream and in a decrease in compliance by patients [3, 4]. Currently, modified-release formulations, such as sustained-release systems, are being used to treat chronic illness [4, 5]. Advantages of modified-release dosage forms include their ability to provide temporal or spatial control of the release thanks to the use of suitable polymeric excipients [6, 7]. For bioavailability, efficacy or safety reasons, the selective delivery of drugs to specific gastrointestinal sites is pursued. This is done to protect the loaded drugs from biological fluids or potential issues imposed by the physiologic variabilities of gastrointestinal tract (GIT) (e.g. pH, the commensal flora, enzymatic activity, surface area and gastrointestinal transit time). It also prevents delivery of the drug outside the so-called absorption window, ensuring its release in the target site as extensively as possible [8]. An example of this latter case is colon delivery, for which it is necessary to prevent the release of active ingredients in the entire gastric residence and the small intestinal transit. Site-selective release is to be sought based on environmental differences between the small and the large intestine, such as the quali-quantitative composition of the microbiota, the pH of fluids, the intraluminal pressure and the transit time [6, 9, 10].

Solid dosage forms are the most frequently used drug delivery systems for oral administration [7]. They differ in both size and the number of units administered as a single dose, including single-unit dosage forms (SUDF) and multiple-unit dosage forms (MUDF) [11]. The most important characteristic of MUDFs in comparison to SUDFs is their lower susceptibility to dose dumping and to a faster gastric emptying. This is because the subunits of a MUDF may be distributed more

evenly throughout the gastro intestinal tract [12, 13]. Such advantages result in fewer adverse effects, better bioavailability, lower variability in the drug release and, consequently, better compliance by patients [14].

However, the ability to take such solid dosage forms may be compromised in patients with swallowing impairments (dysphagia), especially paediatric, elderly or critically ill patients [15-18]. Swallowing issues have been described as dosage forms getting stuck in the throat, an uncomfortable feeling, the need for repeated swallowing attempts, gagging, choking, coughing while swallowing or vomiting [19]. It may result in alteration of the dosage form, omission of doses or discontinuation of medications [20, 21, 16]. Therefore, a modified-release liquid oral dosage form, has been highly awaited for patients with swallowing impairment.

In a previous study, a new technology based on gastro-resistant small omeprazole-loaded multi-layered pellets dispersed in syrup was developed [22]. In this study, budesonide (BUD), a potent corticosteroid used in the management of asthma and allergic rhinitis, the treatment of various skin disorders and the treatment of inflammatory bowel diseases (e.g. ulcerative colitis, Crohn's disease), was used as a model drug [23-26]. BUD is commercially available in the form of pH-dependent enteric-coated preparations, mainly for the local treatment of Crohn's disease, a chronic inflammatory bowel disorder of unknown aetiology that may affect any part of the gastrointestinal tract in both children and adults. However, the most common sites of inflammation are the distal ileum and/or the ascending colon [27]. BUD was employed to verify the feasibility of this new technology. The technology was constituted of multi-layered particles dispersed in a liquid vehicle, with diverse kinds of release (i.e. colon-targeting and sustained-release).

The aim of this work was to demonstrate the feasibility of the new technology by developing budesonide-based modified-release liquid oral dosage forms that present a delayed release of the drug in the colon or a sustained release, and to verify the short-term stability of drug release after dispersion of the multicoated particles in a syrup.

2. Materials and methods

2.1 Materials

Microcrystalline cellulose pellets (Cellets® 263, Process Center GMBH & Co, Germany) with a mean diameter, D(50), ranged between 200 and 300 µm were used as neutral core. Budesonide (Sterling Spa, Corciano, Italy) was used as a model drug. Eudragit® S100 (an anionic copolymer based on methacrylic acid and methyl methacrylate (1:2)), in the form of powder (Evonik® Industries, Darmstadt, Germany), was used as a colon-targeting polymer. Eudragits RS PO and RL 100 (insoluble copolymers of ethyl acrylate, methyl methacrylate and a low content of a methacrylic acid ester with quaternary ammonium groups (1:2:0.1 for RS PO and 1:2:0.2 for RL 100, respectively)) in the form of powder and granules (Evonik® Industries, Darmstadt, Germany) were used to provide sustained-release formulations. Eudragit® E100 (cationic copolymer based on dimethylaminoethyl methacrylate, butyl methacrylate and methyl methacrylate in the ratio 1:2:1) in the form of granules (Evonik® Industries, Darmstadt, Germany) was used as a gastro-soluble polymer. Povidone (Kollidon® K30, D-BASF, Germany) and talc (micronized 10 µm talc, Sigma Aldrich, USA) were used as a binder and a bulk agent, respectively. Microfine lactose (Lactochem® Microfine 201, Borculo Domo, Netherlands) was used as hydrophilic agent. Triethyl citrate (TEC, Alfa Aeser, USA) was used as plasticizer. Glyceryl monostearate (GMS, D-BASF, Germany) and aluminium oxide (Sigma Aldrich, United States) were employed as bulk agents because of their anti-electrostatic properties.

Neosorb sorbitol (Roquette, France), Avicel® RC-591 (microcrystalline cellulose and sodium carboxymethylcellulose, FMC, USA), Kollidon® K30 (polyvinylpyrrolidone, PVP, D-BASF, Germany), anhydrous sodium carbonate (Sigma Aldrich, United States) and dihydrate disodium hydrogen phosphate (Merck, Germany) were used for the preparation of the dry syrup.

2.2 Production of multicoated particles

Neutral microcrystalline cellulose pellets (1-1.5 Kg) were transferred into a lab-scale bottom-spray fluidized bed coater (SLFLL_3, Lleal s.a., Spain) fitted with a Würster insert.

To provide a colonic release, the pellets were coated with four successive coating layers: a drug layer; a colon-targeting polymer layer to prevent the early release of budesonide in the upper part of the GIT; an isolating layer to avoid interaction between layers 2 and 4; and a gastro-soluble polymer layer to avoid drug release in the syrup. To provide a sustained release, the pellets were coated with three successive coating layers: a drug layer; a sustained-release layer including two insoluble polymers in a ratio to obtain a progressive release of budesonide over a prolonged period of time; and a gastro-soluble polymer layer to avoid drug release into the syrup. The compositions of the coating dispersions are presented in the section "Results and discussions".

For both formulations, all the coating dispersions were filtered through a 200 µm sieve before starting the coating processes. During all the coating processes, the dispersions were continuously stirred to prevent sedimentation of insoluble particles. The coated pellets obtained after each coating steps (1^{st}, 2^{nd}, 4^{th} for the colon-targeted formulation and 1^{st}, 2^{nd}, 3^{rd} for the sustained-release formulation) were sieved to discard potential agglomerates before continuing the coating process with the next coating layer. After the sieving (30 Hz for 5 min, 500 µm sieve, Rhewum vibrating apparatus, Germany) only 1 Kg of the coated pellets obtained from the previous step was used for the next coating step.

2.2.1 Preparation of coating dispersions for colon targeting

For the preparation of the first coating dispersion, budesonide and PVP were dissolved in ethanol using a gentle stirring system (blade stirrer, Janke & Kunkel, model RW20, Ika Labortechnik, Germany). Then, talc was dispersed at 300 rpm. The second coating dispersion contained Eudragit® S100, which was solubilized in an isopropanol-water mixture (87:13) at 500 rpm for 2 hours using the blade stirrer. TEC and talc were then added under the same conditions.

The third coating suspension was an ethanolic solution composed of PVP, in which talc was suspended at 300 rpm using the blade stirrer.

The outermost layer dispersion was prepared by solubilizing the Eudragit® E100 granules in an ethanol-water mixture (6:94) at 500 rpm. After its complete solubilization, GMS and aluminium oxide were dispersed using a T25 Ultra-Turrax (IKA®, Staufen, Germany) at 13 500 rpm. Then, talc was added to the suspension at 500 rpm using the blade stirrer.

The coating parameters used for the application of each coating layer in the fluid-bed coater are listed in Table 1.

Table 1. Coating parameters used to produce the budesonide colon-targeted formulation

Coating parameters	First coating: drug layer	Second coating: delayed-release layer	Third coating: isolating layer	Fourth coating: protective coating layer
Inlet air temperature (°C)	35-37	40-42	33-35	33-35
Outlet air temperature (°C)	28-30	32-34	26-28	26-28
Product temperature (°C)	30-32	35-37	28-30	28-30
Air flow (m^3/h)	15-25	90-95	90-95	70-85
Spraying rate (g/min)	10-12	10-12	10-12	10-12
Air pressure (Bar)	1.5-1.7	1.7-1.9	1.6-1.8	1.6-1.8

2.2.2 Preparation of coating dispersions for sustained release

As usual, for the preparation of the first coating dispersion, the soluble compounds were previously dissolved in ethanol-budesonide and PVP with a blade stirrer (Janke & Kunkel, model RW20, Ika

Labortechnik, Germany). Microfine lactose was dispersed using a T25 Ultra-Turrax (IKA®, Staufen, Germany) at 13.500 rpm. Then, talc was added using the blade stirrer at 300 rpm.

The second coating dispersion contained Eudragit® RS PO and RL100, which were dissolved in an isopropanol-water mixture (with the ratio 87:13) at 500 rpm for 2 hours. Then, TEC and talc were dissolved and dispersed, respectively. Considering the nature of polymers employed in the second coating layer, this approach prevents the potential risk of interaction with Eudragit® E100 as all the polymers used are positively charged. In this case, the use of isolating layers was not necessary. The last coating dispersion was equivalent to that used for the colon-targeting formulation. For this reason, the preparation was made following the same procedure.

The coating parameters used for the application of each coating layer are listed in Table 2.

Table 2. Coating parameters used to produce the budesonide sustained-release formulation

Coating parameters	First coating: drug layer	Second coating: sustained-release layer	Third coating: protective coating layer
Inlet air temperature (°C)	33-35	28-30	33-35
Outlet air temperature (°C)	25-27	25-27	26-28
Product temperature (°C)	26-28	26-28	28-30
Air flow (m^3/h)	15-25	70-85	70-85
Spraying rate (g/min)	10-12	10-12	10-12
Air pressure (Bar)	1.5-1.7	1.7-1.9	1.6-1.8

2.3 Characterization of multicoated pellets

2.3.1 Coated-pellet agglomerates

To determine the amount of coated-pellet agglomerates and to discard any potential agglomerates, the coated pellets obtained after each step were sieved (i.e. after the 1st, 2nd and 4th coating for the colon-targeted formulation and the 1st, 2nd, and 3rd coating for the sustained-release formulation). This step avoids multiple coating or the presence of broken agglomerates during the next steps in the coating process. The sieving was done at 30 Hz for 5 min, using a 500 µm sieve (Rhewum vibrating apparatus, Germany). From the sieved pellets obtained from the previous step, 1 Kg was then used as a starting point for the next coating step.

2.3.2 Particle-size distribution

For both formulations, the particle-size distribution of the multicoated pellets was evaluated after each coating step, after withdrawing the agglomerates by sieving. This evaluation was made by laser diffraction (Mastersizer® 3000, Malvern Instruments, UK), using a dry sample dispersion accessory (Aero S). The SOP (Standard operating procedure) used for the analysis was: Fraunhofer scattering; dispersive air pressure, 0.5 bar; vibrating rate, 50%; measurement time, 10s.

2.3.3 *Quantification of budesonide*

For both formulations, the drug content was determined by grinding the coated pellets in a mortar and weighing an amount of powder containing a theoretical content equivalent to 3 mg of budesonide. The drug was extracted using an ethanol-water-methanol mixture in the ratio 7:37.2:55.8 under sonication.

The high performance liquid chromatography (HPLC) system used was a series 1200 Agilent Technologies system (USA), equipped with a single pump, an autosampler and a diode-array UV detector. The column used was an end-capped RP-18 (Purospher® STAR, Germany). The chromatographic conditions were set as follows: wavelength, 225 nm; flow rate, 1.5 mL/min; temperature, 30°C; injection volume, 100 µL; run time, 8 min. The LOQ (limit of quantification) of

this method coincided with 0.2 μg/mL and the LOD (limit of detection) coincided with 0.5 μg/mL, which corresponded to 0.01% and 0.02% w/w budesonide content in the coated dry pellets, respectively.

All the data are the means of five determinations. The coating process efficiency was determined by expressing the mean drug content as a percentage of the theoretical drug loading.

2.3.4 *Thermogravimetric analysis*

To evaluate the residual amount of solvent after each coating layer and in the final batch, thermogravimetric analysis (TGA) (Q500, TA Instruments, USA) was performed. Approximately 10 mg of intact coated sample was loaded in platinum pans and heated from 30°C to 170°C at a heating rate set at 10°C/min.

2.3.5 Characterization of the film structure

The morphology of both the external surfaces and inner structure of the multicoated pellets was evaluated using scanning electron microscopy (SEM). For the analysis of the external surface, a sample of several pellets were fixed and “sprinkled” onto a conductive, adhesive tape placed on a sample holder. All preparations were sputtered with gold/palladium to obtain an electrically conductive surface. The samples were immediately analysed to avoid any change in characteristics. The analysis was performed using a high-resolution field emission scanning electron microscope (7 kV, Jeol JSM using an oil diffusion pump 6610LA, Tokyo, Japan). For the cross-section analysis, pellets were embedded in a light curing resin (Heraeus Technovit 2200 light cure). After hardening (≈ 2 minutes), the embedding was cooled in liquid nitrogen. Then, the embedding was fractured using a plier and dried before sputtering with gold/ palladium to obtain an electrically conductive surface (10 kV, Jeol JSM using a turbo molecular pump IT 300, Tokyo, Japan). The coated pellets

were observed at different magnifications between 250x, 500x and 1000x.

2.3.6 *Dissolution test*

A Distek 2100C USP 29 dissolution apparatus (Distek Inc., North Brunswick, NJ, USA), Type II (paddle method), with a rotational speed of 100 rpm and a temperature set at 37°C, was used for the dissolution tests.

For the colonic-release formulation, the dissolution test was carried out for 2 hours in 750 mL of acid medium pH 1.2 (HCL 0.1N) and for 45 minutes in 1 000 mL of phosphate buffer medium (PBS) [0.05M] at pH 7.5. Indeed, to mimic distal intestine pH value (pH 7.5), 250 mL of tri-sodium phosphate dodecahydrate was added to the acidic medium. The dissolution tests on the sustained-release budesonide multi-layered pellets were performed in one of two different manners, depending on the coating step at which the test was done. When the dissolution test was performed on the pellets obtained after the modified-release coating (2^{nd} coating), 1 000 mL of PBS 0.05 M at pH 7.5 was employed as a buffer medium and 6 mL of sample was withdrawn after 1, 2, 4, 6, 8 and 24 hours. The withdrawn volumes were replaced with equal volumes of blank medium to maintain the volume of the dissolution medium constant during the whole test. When the dissolution test was performed after the protective-coating layer (3^{rd} coating), the test involved 750 mL of acid medium pH 1.2 (HCL 0.1N) for 2 hours followed by 22 hours in PBS 0.05 M at pH 7.5. As before, 6 mL of sample was withdrawn after 1 and 2 hours in the acidic medium, and 1, 2, 4, 6, 8 and 22 hours in the buffer medium and replaced with an equal volume of blank medium. A maximum dissolution test duration of 24 hours was selected because when using a liquid dosage form based on a suspension of small particles, GI transit times are shorter than for solid dosage forms.

The amount of drug released was detected by HPLC (Agilent, USA) in both acid and phosphate buffer medium (see 2.3.3. Quantification of budesonide) after filtration and propriate dilution of the withdrawn samples. The dissolution tests were performed on dry multi-layered pellets and on multi-layered pellets dispersed extemporaneously in the reconstituted syrup; in both cases, the syrup

contained 3 mg of budesonide. The percentages of drug released were quantified at the predetermined times and averaged (n=6).

2.4 Preparation of the syrup and stability of the dispersed pellets

To evaluate the stability of the budesonide-loaded multicoated pellets in a liquid dosage form, a "conventional" syrup was prepared. Kollidon® 30 (10% w/w), sorbitol (60% w/w) and the buffering agents (disodium hydrogen phosphate dihydrate) were dissolved in water at 60°C ± 5°C at 500 rpm using a blade stirrer (Janke & Kunkel, model RW20, Ika Labortechnik). Then, Avicel® RC-591 (2% w/w) was dispersed at 400 rpm. The suspension was cooled at room temperature and the final volume was adjusted with water to 100 mL. The pH was set at 7.5 ± 0.2 with sodium carbonate (0.24% w/w). For both formulations, multi-coated pellets were added to reach a concentration of budesonide of 3 mg/10 mL of dispensed dose and the syrups were poured into closed amber glass bottles. The final preparations were stored at 25°C/50% ± 5 RH.

Both the drug content and dissolution profiles of budesonide were evaluated at time zero, and after 1 and 2 weeks of storage using the methods described before. To quantify the amount of budesonide in the syrup (i.e. inside and outside the pellets), 10 mL of sample was dispersed in a mixture made of 20 mL purified water and 40 mL ethanol before being sonicated for 20 minutes. Then, another 30 mL of ethanol was transferred into a 100 mL amber volumetric flask to be sonicated for other 30 minutes. Samples obtained from an appropriate dilution (1:10) of the filtered extracts with the dilution phase (methanol-water 60:40) were injected into the HPLC to be quantified.

The amount of budesonide that was diffused outside the pellets in the syrup during the storage was also investigated. A sample of 5 mL of the syrup was dispersed in 10 mL of ethanol before being centrifugated for 5 minutes, at 2 000 rpm and at 20°C (Haerus Multifuge X1R centrifuge, Thermo Scientific, USA). Samples obtained from an appropriate dilution (1:150) of the filtered extracts with the dilution phase (methanol-water 60:40) were injected into the HPLC to be quantified.

311

312 *2.4.1 Statistical evaluation*

313 The Student's t-test was used to determine whether the means of two sets of data were significantly
314 different from each other. In this case, the test was done on the amount of BUD released at time
315 zero from the colon-targeted formulation, compared with the amount obtained after 1 and 2 weeks
316 of storage. If the *p* value is higher than 0.05, no significant differences are present between the two
317 sets of data [28].

318 In addition, the similarity factor (f_2) was used to determine the similarity of dissolution profiles for
319 the sustained-release formulation. This test is recommended in the FDA's Guidance for Industry for
320 profiles that include different time points, such as this one. To compare the dissolution profiles
321 obtained, the same test conditions were set and the same dissolution time points were examined. If
322 the f_2 value is included in the range between 50 and 100, the two dissolution profiles are considered
323 similar [29].

324

325
326
327 **3. Results and discussion**
328
329 *3.1 Characterization of multicoated particles*

330 3.1.1 *Optimization of the coating procedure*

331 The lab-scale bottom-spray fluid-bed coater used to produce both budesonide formulations is a
332 prototype adapted for coating small particles with polymeric films [22]. It has the following
333 structural components: a hole distribution plate composed of eight concentric circumferences with
334 holes of different diameters, 4.0 mm, 0.8 mm and 1.8 mm, in sequence respectively; a main
335 chamber 96 cm high; and a metal filter structure with a size aperture of 250 µm. Also, the position
336 of the cylinder (placed 3 cm away from the perforated plate) was adjusted to improve the particle
337 flow and the distribution of coating on the particle surfaces.

338

339 *Budesonide-loaded colonic-release multicoated pellets*

340 During the first coating, the main issue was shown to be the spray drying of the ethanolic dispersion
341 when the inlet temperature was too high. It was demonstrated that the yield of the process was
342 improved from 80% to 95% when the inlet temperature was set and limited to a 35-37°C range,
343 with a corresponding product temperature of 30-32°C.
344 The pH in the caecum and in the ascending colon drops to slightly acidic values because of an
345 anaerobic bacterial metabolism that results in a local accumulation of short-chain fatty acids.
346 However, the traverse and descending branches are restored to a neutral to slightly alkaline
347 environment due to the absorption of fermentation products. In vivo pH variabilities in the intestine
348 do not permit a specific drug release to be predicted at the entry to the colon. However, such
349 changes in the pH have been studied to achieve colon-targeting of drugs through the application of
350 pH-sensitive coatings [30, 31]. To avoid an early release of drug in the upper part of the
351 gastrointestinal tract and to attempt to release it at the correct site of the colon, Eudragit® S100 was
352 used in the second coating as the pH-sensitive colonic polymer. Different percentages of coating
353 were evaluated from 17% to 25% w/w of total solids, which corresponded to 12.4% to 18.1% w/w
354 of Eudragit S®100. The aim was to evaluate the appropriate amount of coating to allow a good
355 protection in acidic gastric medium as well as a fast drug release in intestinal buffer medium at
356 around pH 7.5, which corresponds to that of the ileum (i.e. the last section of the small intestine
357 before entering to the ascending colon). The results obtained in this paper demonstrate that the
358 formulation selected for the second coating was able to provide gastro-resistance and a high release
359 of budesonide in the buffer medium at pH 7.5 during the dissolution test (see 3.4).
360 The third coating was an ethanolic dispersion of PVP and talc. This coating aimed to avoid potential
361 interaction between the anionic and the cationic polymers present in the second and in the fourth
362 coating, respectively.

For the fourth coating, the main issue was the difficulty in reaching sufficient integrity of the protective film based on Eudragit E®100, a cationic polymer. The addition of hydrophobic substances to the polymer was able to increase the strength of the protective film as a barrier against the liquid vehicle in the reconstituted syrup. This barrier prevented, or at least decreased, the liquid's swelling and gel-forming properties, which are normally activated after prolonged contact with neutral pH media [32]. The composition of the selected formulation is shown in Table 3.

Table 3: Qualitative and quantitative compositions (%w/w) of each layer present in the selected budesonide-loaded colonic-release multi-layered pellets

Substance	Core	First coating: drug layer	Second coating: delayed-release layer	Third coating: isolating layer	Fourth coating: protective coating layer
Neutral core	48.56				
Budesonide		0.73			
PVP		0.24		2.01	
Talc		0.24	3.04	2.01	6.57
Eudragit S100			12.44		
TEC			1.69		
Eudragit E100					20.45
GMS					1.01
Aluminium oxide					1.01
Ethanol		√		√	√
Isopropanol			√		
Water			√*		√*
% coating		**1.21**	**17.17**	**4.02**	**29.04**

* The percentages of water in the coating dispersions were 15% and 6% for the second and fourth coating layers, respectively.

Budesonide-loaded sustained-release multicoated pellets

In addition to the spray-drying issue already encountered with the colonic-release multi-layered pellets, the poor solubility of budesonide (BUD) in water (10.7 mg/ L at 25°C) was another issue to solve when developing a sustained-release dosage form. Therefore, it was decided to add lactose as a hydrophilic and hydrosoluble compound to increase the hydrophilic environment around the drug upon contact with aqueous fluids. As ethanol was used as a solvent to dissolve budesonide and PVP

for the first coating, micronized lactose and talc were used to obtain a homogeneous coating suspension.

The second coating was made of water-insoluble polymers to obtain a sustained release of BUD. Eudragit® RS PO and RL 100 were selected as they were compatible with each other, allowing the use of different ratios to modulate the release profiles of BUD from the developed sustained-release multilayered pellets. Two batches produced with 100% w/w of each polymer were used as references for high (RL) and low (RS) permeability barrier films to control the drug release. Three polymer ratios were also evaluated, namely RS-RL 50:50, RS-RL 70:30, and RS-RL 30:70 to obtain coatings with intermediate permeabilities between the low- and high- permeability barrier films. The results obtained in this paper demonstrate that the formulation selected for the second coating was able to provide a sustained release of BUD during the 24-hour dissolution test done in a PBS 0.05 M buffer medium pH 7.5.

After the second coating, the outermost coating was based on Eudragit® E100. This coating was similar to that used for the colon-targeted coated pellets and was applied to avoid the early release of the drug in the reconstituted syrup during storage. The composition of the selected formulation is shown in Table 4.

Table 4: Qualitative and quantitative compositions (%w/w) of the selected formulation for budesonide sustained-release pellets

Substance	Core	First coating: drug layer	Second Coating: sustained-release layer	Third coating: protective coating layer
Neutral core	57.83			
Budesonide		0.87		
Lactose		1.74		
PVP		0.29		
Talc		0.29	2.14	7.22
Eudragit RS PO			1.28	
Eudragit RL100			2.99	
TEC			0.64	
Eudragit E100				22.49
GMS				1.11
Aluminium oxide				1.11
Ethanol		√		√
Isopropanol			√	
Water			√	√

% of coating	**3.19**	**7.05**	**31.93**

* The percentages of water in the coating dispersions were 13% and 6% for the second and third coating layers, respectively.

It is important to underline that the coating process used for the production of the multi-layered sustained-release pellets is much simpler and faster than that used in a previous paper for obtaining omeprazole delayed-release pellets (three coating layers versus five coating layers). This is because in his case, there is no need to apply two additional isolating layers to protect the active ingredient from the enteric polymer (which is acidic) or to avoid interaction between the modified-release and protective coating layers (both are cationic polymers, in contrast to the anionic and cationic polymers used for omeprazole).

3.2 Physicochemical characterization of coated pellets

The particle-size distribution of both budesonide formulations was evaluated at the end of each coating step. Film coating is a complex process because many variables are involved, and the altering of these parameters is generally restricted [33]. The critical process variables that influence the coating efficiency include the following: the inlet air temperature and humidity; the air pressure used for atomization; the flow rate of coating suspension sprayed and of the drying air; and the possible subsequent curing process [34-36]. Coating experiments were performed to find the right balance among all these parameters to avoid some common problems met during the coating of small particles. Such problems can be agglomeration phenomena, spray-drying effects, occlusion of the nozzle or filter and the non-uniform application of coating dispersion droplets. The flow rate of the coating suspension and the air pressure defined the spray frequency and the size of droplets sprayed. Moreover, good ventilation guaranteed a homogeneous flow of particles into the Würster over a number of applications until the desired coating weight was deposited. Factors such as a higher coating suspension flow rate, higher humidity or lower drying temperature in comparison to the optimal values lead to an over-wetting of pellets that can potentially cause agglomeration

425 phenomenon. The generation of electrostatic charges during the coating process is another critical
426 parameter that can alter the formation of a homogeneous layer on the particles. This is because the
427 particles remain attached at the wall of the main chamber, and so avoid receiving the sprayed
428 coating suspension, which dries and flows away through the filter.

429 The generation of electrostatic charges was particularly high with the outermost protective coating,
430 which contained Eudragit® E100. This effect has most influence on this polymer in comparison to
431 the other polymers. However, as observed from the data shown, the particle size increased during
432 the coating procedure (Tables 5 and 6, from 277 µm for the neutral pellets to 350-370 µm for the
433 sustained-release and colon-targeted multi-layered pellets, respectively) thanks to the coating
434 deposited. However, it remained low enough not to create a swallowing impairment for patients
435 (D(50) < 500 µm) [19]. The low standard deviation values obtained for each coating step give a
436 good indication of the homogeneity of the coating on the pellets, underlining an effective dispersion
437 of coating on the particles.

438 At the end of the whole coating process, the mean budesonide content inside the coated pellets of
439 both formulations was around 90% w/w in comparison to the theoretical loaded drug. This
440 relatively low drug content is probably due to the loss of a fraction of the sprayed suspension by
441 spray-drying. The results presented in Tables 5 and 6 showed that the production could be
442 considered satisfactory for lab-scale coating equipment for such small particles.
443 High levels of residual solvent in the inner structure of coated pellets may compromise their
444 stability over time during storage. The production process could be considered as satisfactory as the
445 value of residual solvent reached was limited after the entire coating process and remained below
446 5% w/w. The coated pellets were analysed by TGA by heating them to temperatures in the range of
447 between 30°C and 150°C, at which solvents used in the preparation of the coating suspensions were
448 fully removed, considering their boiling temperatures (78.4°C for ethanol, 100°C for water and
449 82.5°C for isopropanol) (Table 5 and 6). The obtained results for the residual solvent content (i.e.

between 2.4 and 4.5%) demonstrate that these values can be limited once the right parameters are set during the whole coating process, reducing the possibility of poor stability of the multi-layered pellets during storage in the reconstitutable syrup.

Table 5: Percentage of agglomerates, particle size distribution, yield and residual solvent content results obtained after each step of the coating procedure for a representative budesonide colon-targeted multi-layered particle batch produced with the selected formulation (D(50) of uncoated pellets®: 277.2 ± 1.8)

Colon-targeted formulation	% of agglomerate (n = 1)	Mean diameter D(50) (µm) (n = 3, mean ± s.d)	% of yield (n = 5, mean ± s.d)	% residual solvent (n = 1)
Budesonide 1st coating	0.0	278.2 ± 0.6	95.2 ± 1.0	4.5
Budesonide 2nd coating	0.2	315.6 ± 0.3	91.2 ± 2.6	2.4
Budesonide 4th coating	0.5	371.0 ± 1.5	90.1 ± 4.9	2.9

Table 6: Percentage of agglomerates, particle size distribution, yield and residual solvent content results obtained after each step of the coating procedure for a representative budesonide sustained-release multi-layered particle batch produced with the selected formulation (D(50) of uncoated pellets®: 277.2 ± 1.8)

Sample: sustained-release formulation	% of agglomerates (n =1)	Mean diameter D(50) (µm) (n = 3, mean ± s.d)	% of yield (n = 5, mean ± s.d)	% residual solvent (n = 1)
Budesonide 1st coating	0.0	281.1 ± 0.3	92.2 ± 2.2	4.5
Budesonide 2nd coating	0.4	291.2 ± 0.5	91.9 ± 4.2	4.4
Budesonide 3rd coating	0.6	350.5 ± 6.8	91.8 ± 1.8	2.8

3.3 Microstructure evaluation of coating layers

Generally, uncoated pellets presented a rough and irregular surface [33]. From observation, the uncoated surfaces (Figure 1) were smooth but irregular and became more rounded, with no visible cracks, when coating polymers were applied to the surface of the particle using appropriate experimental parameters. Structural imperfections, such as porosity or discontinuity of the coating layer and lower layer thickness, could be observed from the cutting of pellets. Such imperfections should be avoided by optimizing the coating procedures to obtain a modified release of the drug, with good reproducibility. The surfaces and inner structures of the coated pellets were evaluated by SEM after the whole coating procedure for both formulations (Figures 1 and 2).

As can be seen in Figure 1, the neutral pellets used presented quite smooth surfaces. The multilayered pellets obtained at the end of each coating step maintained their regular aspect with no apparent porosity or defects, which indicated that the coating processes were performed appropriately. Moreover, it should be pointed out that the film formation process in organic solvent-based systems is fundamentally easier in comparison to that with aqueous-based systems. The polymer solutions undergo a sol to gel transition upon solvent evaporation to eventually form the polymeric film [37]. Once the solutions are sprayed onto the pellet surfaces, the organic solvent evaporates and the polymer chains approach each other to form a thin homogeneous film. As a consequence of the easier film formation and low initial viscosity presented by the coating dispersions, a smooth film on particles was provided. Indeed, all the sprayed suspensions presented relatively low initial viscosities. Consequently, the surface of the film coated pellets was not porous and the agglomeration phenomena were very limited when spraying the suspension in the fluidized bed equipment (Figure 1; Tables 5 and 6, agglomeration below 1%).

The low percentage of coating sprayed on and the low amount of drug loaded reduced the whole coating procedure time (< 10 hours). Indeed, the coating time was much shorter to that used for the preparation of omeprazole pellets (i.e. 5 coating layer, coating time around 24 hours) [22]. This reduction limited the number of particle-particle and particle-wall collisions, which are normally

489 responsible for small cracks on the particle surface [38]. The major problem related to the coating
490 process was the generation of electrostatic charges when the polymer employed was Eudragit®
491 E100. These charges were mainly critical for the external protection coating layer. During this step,
492 pellets tended to remain attached to the walls of the central chamber. Consequently, they avoided
493 the appropriate application of the coating droplets onto their surface and their size increased, both
494 of which facilitated the spray drying effect. The generation of electrostatic charges was partially
495 solved thanks to the addition of water in the coating dispersions, which increased the humidity
496 inside the main chamber. Imperfections such as film porosity, resulting from spray drying
497 phenomenon, and cracks, resulting from multiple particle-particle and particle-wall collisions or
498 from the formation of repetitive particle-particle sticking detachment [38], can be observed as
499 shown in Figure 2. For these small particles, the presence of very small imperfections could
500 potentially make the protective film much more sensitive to the liquid vehicle during storage, which
501 may potentially alter the release profiles during the dissolution test.
502
503
504 3.4 *Dissolution test*
505 Dissolution tests were performed on both budesonide formulations to demonstrate the achievement
506 of the release targets, using the methods described in Section 2.3.6.

507 *3.4.1 Budesonide colon-targeted formulation*

508 The requirements imposed by European Pharmacopoeia 9th Edition for solid delayed-release
509 formulations allow a maximum of 10% w/w of API to be released during the two-hour acidic step
510 and a minimum of 85% w/w of API to be released during the 45-minute buffer step [39]. As can be
511 seen in Figure 3, the release of budesonide from the colon-targeted formulation after 2 hours in
512 acidic medium was below the LOQ, demonstrating that the gastro-resistance of the coating was
513 preserved at pH 1.2. Moreover, around 95% of budesonide was released at pH 7.5 after 45 min.

(Fig. 3). This demonstrated that the last protective layer was dissolved in acid medium during the first two-hour acidic step and that the colonic layer was properly dissolved at pH 7.5. Indeed, the last protective layer maintained the stability of the multi-layered microparticles once suspended in the syrup at pH higher than 5. However, it dissolved quickly at acidic pH. This exposed the layer responsible for the colonic release, which, once the pH was brought to 7.5, dissolved and released the active substance. As described above, the pH of the buffer medium was chosen to allow the dissolution of the polymer selected for the colonic targeting, which happens at pH higher than 7.0. The same colon-targeted formulation with two different percentages of second coating (17.2% and 24.9% total solids, equivalent to 12.4% and to 18.1% of Eudragit S100) was evaluated to verify the influence of the coating thickness on the release. In both cases, the targeted release was reached with similar results (% budesonide released: < LOQ in the acidic step and around 95% in the buffer step). However, the formulation with the lower percentage of sprayed coating was selected due to its lower processing time, which is more compatible with a future scaling up.

Moreover, similar percentages of released budesonide were obtained when the dissolution test was performed from the multi-layered pellets dispersed in the syrup, immediately after its reconstitution (Figure 3). The amount of drug released, in both acid and phosphate buffer medium, corresponded to the results obtained for dry budesonide multi-layered particles (% budesonide released: < LOQ in the acidic step and around 95% in the buffer step). This demonstrated that the fourth protective coating layer presented an appropriate barrier against the external aqueous vehicle.

3.4.2 Budesonide sustained-release formulation

For the sustained-release formulation, the aim was to evaluate the ability of such small coated pellets to sustain the release of the drug and to modulate the dissolution profile. Modification would be due to different ratios of the two insoluble polymers (Eudragits® RS and RL), as the polymers have different permeability characteristics. As a first step, different batches were produced up to the

second coating, which is responsible for the sustained release. This was done to determine the appropriate RS-RL ratio to control the budesonide release up to around 24 hours, by which time most of the API would be released (Fig. 4).

Maximal percentages (100%) of both RS PO and RL 100 were used to evaluate the two extreme dissolution profiles to obtain the lowest and the highest amounts of drug released. To determine the suitable polymer ratio from the ones produced, dissolution tests were done at a constant buffered pH of 7.5. This avoided the starting acidic step, as the permeability of both insoluble polymers is pH-independent and there was no protective coating layer. The dissolution tests were performed over 24 hours even though the residence in the colon after the transit in the stomach and in the small intestine is within a variable period of time (between 7 to 48 hours) [40]. Indeed, it seemed reasonable to consider a maximum dissolution test duration of 24 hours as the GI transit times are generally shorter for small particles, especially when using a liquid dosage form.

The examination of the dissolution results obtained in Figure 4 showed that the drug release was very low when the pellets were coated with Eudragit RS alone (around 5% release within 24h, showing that the film permeability was too low). On the other hand, the budesonide release was around 80% within 24h for the pellets coated with Eudragit RL alone. As expected, when the ratio of the high-permeability polymer RL was increased in the polymer blends, the drug release profiles increased from around 40% (RS:RL 70:30) to around 78% (RS:RL 30:70) of drug released within a 24h dissolution test. When the polymers were present in an equal blend ratio (RS:RL 50:50), the drug release was around 55% within 24h.

The RS:RL 30:70 ratio was selected because its ability to release the API was similar to that obtained with the high permeability polymer alone (RL 100%) and consequently higher in comparison the other ratios. Moreover, the presence in this ratio of 30% of the low-permeability polymer (RS) guaranteed a better protection of multicoated pellets once dispersed in the syrup, during storage.

Two different percentages of the second coating layer (7.1% and 9.5% total solids, equivalent to 4.3% and to 5.8% of polymer blend), using a RS:RL ratio of 30:70, were then evaluated to verify the influence of the coating thickness on the drug release. It was demonstrated that the increase in the thickness of the second coating layer from 7.1% to 9.5% provoked a decrease in the release of budesonide within the 24h dissolution test from 78% to 68%, respectively (data not shown). Consequently, two batches with the two different percentages of the second coating (i.e. 7.1% and 9.5% of total solids) were produced until the last protective coating layer. These were analysed in terms of dissolution profile and stability after dispersion in the syrup. Once the final batches were produced, dissolution tests involving two hours of acidic step at pH 1.2 and 22 hours of buffer step at pH 7.5 (with withdrawals after 1, 2, 4, 6, 8 and 22 hours from the beginning of the buffer step) were performed.

In both cases, as the progressive release of the drug was effective from the beginning of the dissolution test, the outermost layer was quickly dissolved without an initial lag time within the first two hours of the acidic step. As soon as the last coating was dissolved, the sustained-release layer started to control the release of budesonide (Fig. 5). Moreover, the release of the drug continued after the pH changed (from 1.2 to 7.5), following the two-hour acidic step. The release of budesonide from the coating consisting of two insoluble polymers, was pH-independent.

The comparison of the release profiles obtained from the multi-layered pellets before (dry) and after dispersion in the syrup permitted the observation that slightly higher percentages of budesonide were released when the dissolution test was performed on the multi-layered pellets dispersed in the syrup, immediately after its reconstitution (Fig. 5). The amount of drug released from the formulation with the two different coating percentages (2^{nd} coating equal to 7.1% and 9.5%) occurred in similar quantities, reaching values higher than 90% w/w in 24 hours. This result demonstrated that the fourth coating was an appropriate barrier against the external aqueous vehicle

in both cases. As such a thick coating layer is more compatible with a future scaling up in industry, the formulation with a lower percentage of coating (2^{nd} coating equal to 7.2%) was selected.

The similarity factor f_2 was calculated for this formulation for the different time points (1 and 2 hours in the acidic step and 1, 2, 4, 6, 8 and 22 hours in the buffer step). This value was in the range between 50 and 100. This showed that no significant modification in terms of the sustained-release properties of the second coating were observed after dispersion of multicoated pellets in a neutral pH aqueous medium.

3.5 Storage stability test

The reconstitutable syrup exhibited its capability to provide a stable system for at least 2 weeks at ambient temperature for both formulations of budesonide multi-layered particles. As shown in Table 7, after a storage period of 2 weeks in the syrup, a minimum of 89% w/w of the budesonide nominal dose was released from the colon-targeted coated pellets in the 45 minutes of buffer stage. Indeed, it was observed that the release of BUD in the buffer medium decreased slightly during the storage. Release went from 97.8% immediately after dispersion to 89.3% after 2 weeks of storage at 25°C. To verify the reason for the decrease in BUD release after storage, the dissolution test was prolonged to 4 hours in the buffer medium. From this analysis, it was clear that the drug release was not completed within 45 min in the buffer stage, or when the storage time was increased. This could be due to the progressive swelling and diffusion of the polymers used in the different coating layers when the pellets were dispersed in the reconstituted syrup. Indeed, an interaction between the cationic and the anionic polymer (E100 and S100) can occur during storage, forming a less permeable coating that can alter the diffusion of the dissolved drug. The results in Table 7 showed that the decreasing effect observed during storage in the reconstituted syrup was higher after 2 weeks of storage and greater release of the drug after 4h dissolution in the buffer stage.

The *p* was calculated between the average of the dissolution points after 45 minutes of buffer step, (the point to be considered in accordance with the European Pharmacopoeia 9th Edition) at time zero and at the stability at 1 week, using the Student t-test. The results demonstrated that the two sets of data were not significantly different from each other (p=0.07). When the *p* was calculated between the average of dissolution points, after 45 minutes in the buffer step, at time zero and at the stability at 2 weeks, the results showed a significant difference between the two sets of data (p=0.003). Nevertheless, the drug release values obtained for up to 2 weeks' storage satisfied the common requirements for delayed-release dosage forms. Less than 10% of BUD was released in the 2 hours of the acidic stage and more than 85% of BUD was released in the first 45 minutes of the buffer stage, considering all the storage periods up to 2 weeks.

Table 7: Percentages of budesonide released from colon-targeted multicoated pellets dispersed in the reconstituted syrup at the predetermined time points of storage at 25°C (time zero, 1 and 2 weeks): 2-hour acidic stage (pH 1.2), followed by a pH 7.5 buffer stage at different time points: 45 minutes, 2 and 4 hours (n=6, mean ± s.d.)

Time points:	% BUD released after 2 h in the acidic stage	% BUD released after 45 min. in the buffer stage	% BUD released after 2h in the buffer stage	% BUD released after 4h in the buffer stage
Time zero	0.96 ± 0.11	97.8 ± 1.8	101.3 ± 1.2	100.5 ± 1.5
1 week of storage	2.81± 0.24	95.5 ± 4.2	101.8 ± 5.0	101.6 ± 3.6
2 weeks of storage	4.25 ± 0.37	89.3 ± 4.2	90.8 ± 3.7	96.5 ± 3.6

In the case of the sustained-release formulation, the reconstituted syrup also exhibited its capability to provide a stable system for budesonide multi-layered particles with the selected formulation, with 7.1% second sprayed coating layer. Indeed, after a storage period of 2 weeks in the syrup, the drug release obtained at the end of the dissolution test in the buffer medium was limited and decreased from 90.2% to 87.2% w/w of budesonide's nominal dose (Table 8). A deeper evaluation of the dissolution results obtained during storage showed that the decrease in drug released is higher during the first hours of the dissolution test (e.g. budesonide release values of 60.0%, 52.9% and 49.7% after 2 h in the buffer medium for the coated pellets immediately after reconstitution, and after 1 and 2 weeks of storage, respectively). These slight modifications in the release profiles could

be explained by the potential diffusion of the aqueous medium through the different coating layers and their progressive swelling during the storage in the syrup. Both the compositions of the protective layer (i.e. addition of hydrophobic compounds) and the syrup (i.e. addition of high concentrations of sorbitol and of viscosifying agents) were chosen to try to limit the penetration of water into the pellets and to guarantee the stability of the pellets after reconstitution.

The similarity factor (f_2) was calculated for all the dissolution results generated to verify the similarity of the dissolution profiles. The f_2 values obtained were 65% and 60% (in comparison to the initial dissolution profiles), after 1 week and 2 weeks, respectively. Therefore, the dissolution profiles obtained during the 2-week storage at 25°C could be considered similar to that at time zero. The stability of the dissolution was also tested for the multilayered batch with the higher percentage of second coating (9.5%). In this case, a good stability of the coated pellets was provided after their dispersion in the syrup for the two weeks of storage, although with a lower percentage of budesonide released in a 24 h dissolution test (maximum release in the 24h of buffer step equal to 91.0 ± 1.4 at time 0, 89.1 ± 1.7 at 1 week and 81.4 ± 1.1at 2 weeks).

Table 8: Percentages of budesonide release from sustained-release multicoated pellets with 7.1% of second coating total solids (equivalent to 4.3% of polymers blend), dispersed in the reconstituted syrup at predetermined time points of storage at 25°C (time zero, 1 and 2 weeks): 2-hour acidic stage, followed by a 24-hour pH 7.5 buffer stage (n=6, mean ± s.d.)

Samples	% drug release		
	Time zero	1 week of storage	2 weeks of storage
1 h acid pH 1.2	35.0 ± 1.8	27.6 ± 0.7	24.9 ± 0.7
2 h acid pH 1.2	48.4 ± 2.7	39.6 ± 1.7	36.8 ± 1.9
1 h buffer pH 7.5	51.7 ± 1.9	40.3 ± 2.5	37.2 ± 1.9
2 h buffer pH 7.5	60.0 ± 1.9	52.9 ± 0.9	49.7 ± 1.4
4 h buffer pH 7.5	70.9 ± 2.6	62.0 ± 3.3	56.6 ± 0.9
6 h buffer pH 7.5	78.3 ± 3.2	64.0 ± 1.6	61.3 ± 1.8
8 h buffer pH 7.5	81.9 ± 3.1	72.7 ± 1.5	69.5 ± 1.5
24 h buffer pH 7.5	90.2 ± 4.2	89.4 ± 2.9	87.2 ± 2.1

4. Conclusion

In this study, the oral system based on a multi-layered particle technology showed an innovative process that will permit the production of reconstitutable liquid dosage forms with modified-release properties. The multi-layered microparticles obtained using a coating procedure in a bottom-spray fluid-bed coater maintained, at the end of the process, the smaller size which has been highly awaited to address the need in patients with swallowing impairment, such as paediatric, elderly or critically ill patients. Such small particles dispersed in a syrup could improve patients' compliance and consequently the effectiveness of their therapy.

In this study, it was demonstrated that the multi-layered particle technology is a flexible manufacturing process that is suitable for different kinds of release, such as colon-targeted or sustained release. In both the developed formulations, the multicoated pellets were able to provide an appropriate control of the drug release in both dry and liquid dispersed states. Moreover, this new approach showed a good stability of the drug release profiles for at least 2 weeks when the multi-layered pellets were suspended in a syrup and stored at ambient temperature. From an industrial perspective, it could be interesting to apply this kind of technology to other drugs.

Role of the funding source

This work was financially supported by BePharBel Manufacturing. The sponsor of this study approved the study design, but was not involved in the interpretation of the data or in the writing of the article. The sponsor has read and approved the content of this article before its submission.

679 **References**
680
681 1) Pinto, J.F., 2010, Site-specific drug delivery systems within the gastro-intestinal tract: from
682 the mouth to the colon, Int. J. Pharm.395. 44-52.
683 2) Gaur, P.K., Mishra, S., Bhardwaj, S., Kumar, S.S, Bajpai, M., Verma, A., and Verma N.
684 (2014). "Recent Developments for oral time-controlled drug delivery by pelletization
685 techniques: an overview". *J. Pharm. Sci. Pharmacol.* 1, 283-295.
686 3) Gaur, P.K., Mishra, S., Gautman, R., Singh, A.P., and Yasir, M. (2014a). "Film coating
687 technology: Past, present and future". *J. Pharm. Sci. Pharmacol*. 1, 57-67.
688 4) Gaur, P.K., Mishra, S., Kumar, A., and Panda, B.P. (2014b). "Development and
689 optimization of gastro-retentive mucoadhesive microspheres of gabapentin by Box-Behnken
690 design". *Artif Cells Nanomed. Biotechnol*. 42, 167-77.
691 5) Chien, Y.W. (2001), Novel Drug Delivery System: Fundamentals, Development Concept,
692 Biomedical Assessments, Marcel Dekker, Inc., New York.
693 6) Maroni, A., Del Curto, M.D., Zema, L., Foppoli, A., Gazzaniga, A., Film coatings for oral
694 colon delivery, Int. J. Pharm. 457 (2013), 372-394.
695 7) Debotton, N. and Dahan, A., (2017). "Application of Polymers as Pharmaceutical Excipients
696 in solid oral dosage forms". *Medicinal Research Reviews*, 37, No. 1, 52-97.
697 8) Dressman, J.B., Bass, P., Ritschel, W.A., Friend, D.R., Rubinstein, A. and Ziv, E.,
698 Gastrointestinal parameters that influence oral medications. J. Pharm. Sci., 82 (1993) 857-
699 872.
700 9) Gazzaniga, A., Giordano, F., Sangalli, M.E., Zema, L., 1994. Oral colon- specific drug
701 delivery: design strategies. S.T.P. Pharma Prat. 4, 336-343.
702 10) Gazzaniga, A., Maroni, A., Sangalli, M.E., Zema, L., 2006. Time-controlled oral delivery
703 systems for colon targeting. Expert. Opin. Drug Deliv. 3, 583- 597.
704 11) Aleksovski, A., Dreu, R., Gasperlin, M., Planinsek, O., "Mini-tablets: a contemporary
705 system for oral drug delivery in targeted patient groups". *Expert Opin. Drug Deliv.* 12 (1)

(2015) 65-84.

12) Zhu, X., Qi, X., Wu, Z., Zhang, Z., Xing, J., Li, X., "Preparation of multi-unit floating bioadhesive cooperative minitablets for improving the oral bioavailability of famotidine in rats", *Drug Deliv.* 21 (6) (2014) 459-466.

13) Newton, J.M., "Gastric emptying of multi-particulate dosage forms", Int. J. Pharm., 395 (1-2) (2010) 2-8.

14) Chen, T., Li, J., Chen, T., Sun, C., Zheng, Y., "Tablets of multi-unit pellet system for controlled drug delivery", *J. Control. Rel.* 262 (2017), 222-231.

15) Liu, F., Ghauffur, A., Bains, J., Hamdy, S., Acceptability of oral solid medicines in older adults with and without dysphagia: A nested pilot validation questionnaire based observational study, Int. J. Pharm. 512(2016) 374- 381.

16) Strachan, I., Greener, M., 2005. Medication- related swallowing difficulties may be more common than we realize", Pharm. Pract. 15, 411- 414.

17) Nunn, T., Williams, J., Formulation of medicines for children, Br. J. Clin. Pharmacol. 59:6 (2004), 674-676.

18) Liu, F., Ranmal, S., Batchelor, H.K., Orlu-Gul, M., Ernest, T. B., Thomas, I. W., Flanagan, T., Kendall, R., Tuleu, C., Formulation factors affecting acceptability of oral medicines in children, Int. J. Pharm., 492 (2015) 341- 343.

19) Schiele, J.T., Quinzler, R., Klimm, H.D., Pruszydlo, M.G., Haefeli, W.E., Difficulties swallowing solid oral dosage forms in a general practice population: prevalence, causes, and relationship to dosage forms, Eur. J. Clin. Pharmacol. (2013) 69:937- 948.

20) Mc Gillicuddy, A., Crean, A.M., Sahm, L.J, Older adults with difficulty swallowing oral medicines: a systematic review of the literature, Eur. J. Clin. Pharmacol. (2016) 72: 141-151.

21) Wright, D. (2002), Tablet crushing is a widespread practice but it is not safe and may not be legal, Pharm. J. 269 (7208):132.

22) Ronchi, F., Sereno, A., Paide, M., Sacré, P., Guillaume, G., Stéphenne, V., Goole, J., Amighi, K., "Development and evaluation of an omeprazole-based delayed- release liquid oral dosage form", submitted to Int. J. Pharm.

23) Lin-Y. Kuo, L.Y., Hung, C.H., Tseng, H.I., Wu, J.R., Jong, Y.J., Chu, Y.T., "Effects of Budesonide and Fluticasone Propionate in Pediatric Asthma Patients", *Pediatr. Neonata*l 2010; 51(1): 31- 36.

24) Skin Diseases and Conditions - Lichen Planus; Findings from Mayo Clinic Reveals New Findings on Lichen Planus (Oesophageal lichen planus: The efficacy of topical steroid-based therapies). (2017). Obesity, Fitness, & Wellness Week., 4774.

25) The Regents of the University of California; Patent Issued for Viscous Budesonide for the Treatment of Inflammatory Diseases of the Gastrointestinal Tract. (2015). Biotech Week, 1460.

26) Łodyga, M., Eder, P., Bartnik, W., Gonciarz, M., Klopocka, M., Linke, K., Malecka-Panas, E., Radwan, P., Rydzewska, G., "New pharmaceuticals in inflammatory bowel disease", Prz Gastroenterol. 2015; 10 (2): 57- 60.

27) Langholz, E., Munkholm, P., Krasilnikoff, P.A., Binder, V., "Inflammatory bowel diseases with onset in childhood. Clinical features, morbidity, and mortality in a regional cohort". *Scand. J. Gastroenterol.* 1997: 32: 139.47.

28) Fleury J., dans : Introduction à l'usage des méthodes statistiques en pharmacie. Editions médecine et hygiène, Genève (1987).

29) Shah, V. P., Tsong, Y., Takeuchi, H., & Yamamoto, H. (2004), In vitro dissolution profile comparison- statistics and analysis of the similarity factor f_2, Pharm. Res., 15, 889- 896.

30) Edwards, C., 1997, Physiology of the colorectal barrier" Adv. Drug delivery Rev. 28, 173-190.

31) Nugent, S.G., Kumar, D., Rampton, D.S., Evans, D.F., 2001, Intestinal luminal pH in

757 inflammatory bowel disease: possible determinants and implications for therapy with amino
758 salicylates and other drug, Gut 48, 571- 577.
759 32) Patra, C.N., Priya, R., Swain, S., Kumara Jena, G., Panigrahi, K.C., Ghose, D.,
760 Pharmaceutical significance of Eudragit: A review, Future J. Pharm. Sc. (2017), doi:
761 10.1016/j.fjps.2017.02.001.
762 33) Albanez, R., Nitz, M., Taranto, O.P., "Enteric coating process of diclofenac sodium pellets
763 in a fluid bed coater with a Würster insert: influence of process variables on coating
764 performance and release profile", "Adv. Powder Technol. 24 (2013) 650-666.
765 34) Dewettinck, K., Huyghebaert, A., "Fluidized bed coating in food technology", Trends in
766 Food Sci. Technol., 10 (1999), 163-168.
767 35) Yang, S.T., Vansavage, G., Weiss, J., Ghebresellassie, I., The effect of spray mode and
768 chamber geometry of fluid-bed coating equipment and other parameters on an aqueous-
769 based ethylcellulose coating, Int. J. Pharm. 86 (2- 3) (1992) 247- 257.
770 36) Lorck, C.A., Grunenberg, P., Jünger, H., Laicher, A., Influence of process parameters on
771 sustained-release theophylline pellets coated with aqueous polymer dispersions and organic
772 solvent- based polymeric solutions, Eur. J. Pharm. Biopharm. 43 (1997) 135-140.
773 37) Siepmann, F., Siepmann, J., Walther, M., MacRae, R.J., Bodmeier, R., Polymer blends for
774 controlled release coatings, J. Control. Rel. 125 (2008) 1-15.
775 38) Perfetti, G., Aubert, T., Wildeboer, W.J., Meesters, G.M.H., Influence of handling and
776 storage conditions on morphological and mechanical properties of polymer-coated particles:
777 characterization and modelling, Powder Technol., 206 (2011) 99-111.
778 39) European Pharmacopoeia, 9th edition (2016), Dissolution test for oral dosage forms,
779 chapter 2.9.3, 302-309.
780 40) Davis, S.S., 1985, The design and evaluation of controlled release systems for the
781 gastrointestinal tract, J. Control. Rel. 2, 27- 38.

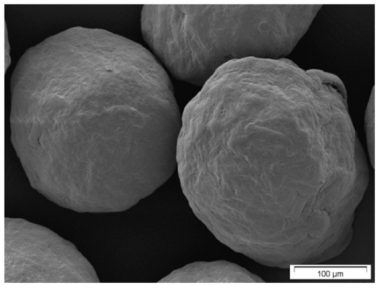
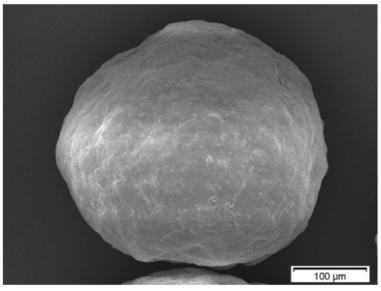
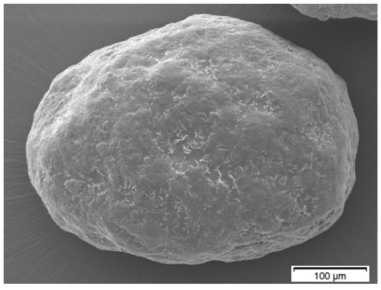
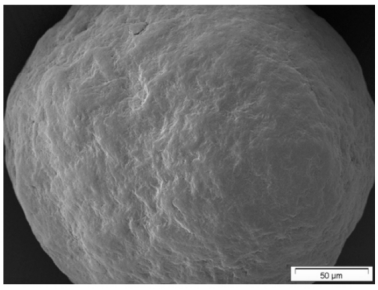
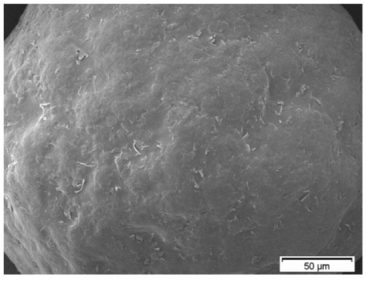
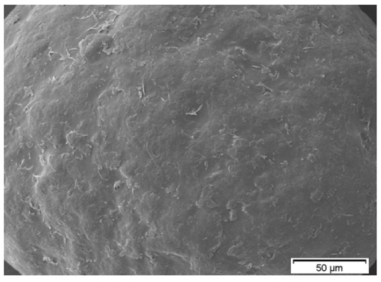
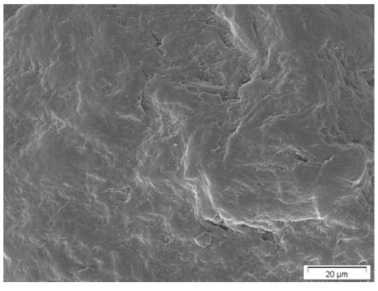
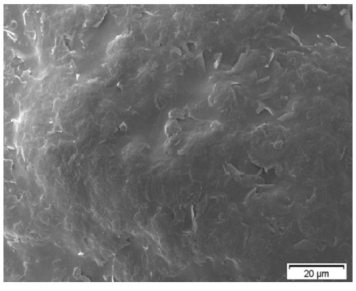
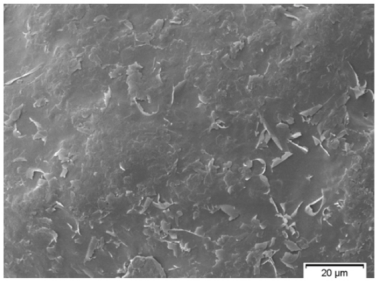
Fig. 1: SEM pictures of budesonide colon-targeted and sustained-release batches surface at the end of a coating procedure, taken following the formulation and coating parameters presented in Tables 1-4, respectively. The microstructure of the coated pellets was compared with the neutral pellets (Cellets® 263), and analysed at different magnification (250x, 500x, 1000x).

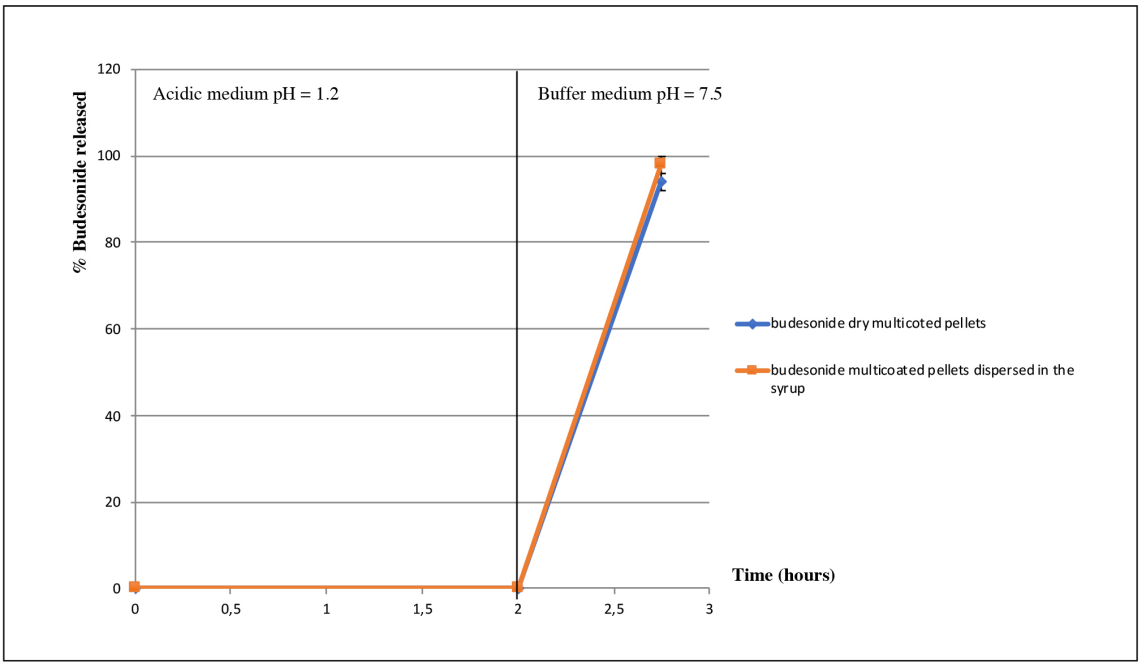
Fig. 2: SEM pictures of the cross section of budesonide colon-targeted and sustained-release batches at the end of a coating procedure, taken following the formulation and coating parameters presented in Tables 1-4, respectively. The coated pellets were analysed at different magnification (500x, 1000x). Imperfections such as porosity and small cracks are shown.

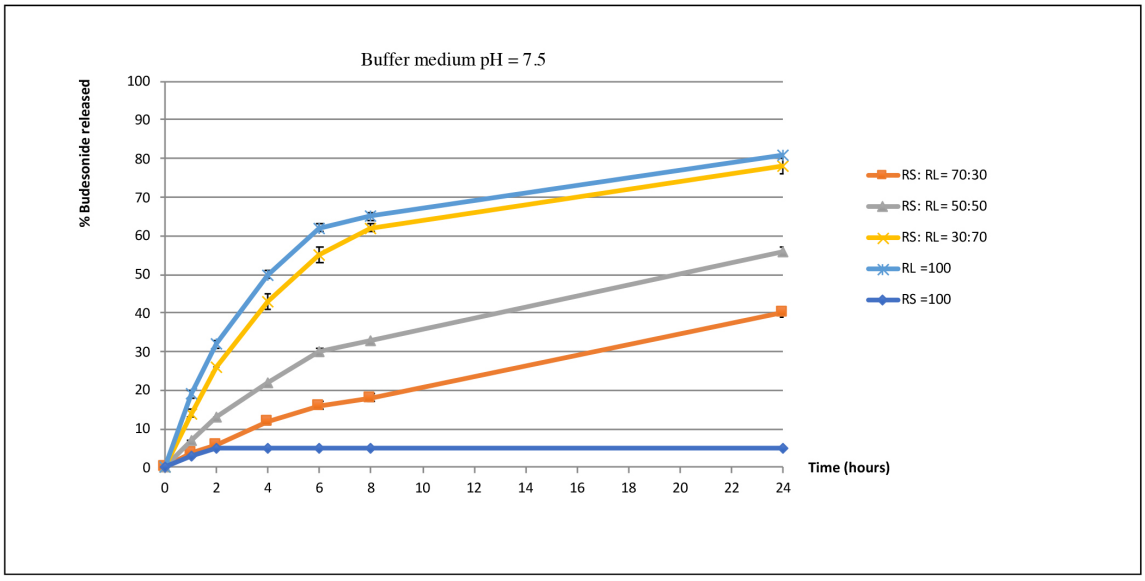
Fig. 3: Dissolution profiles of the colon-targeted multi-layered pellets (17.2% of 2nd-coating total solids, equivalent to 12.4% of Eudragit S100) before and after dispersion in a conventional syrup, after 2 hours in the acidic stage and after 45 minutes in the pH 7.5 buffer stage (mean values +/- s.d., n=6).

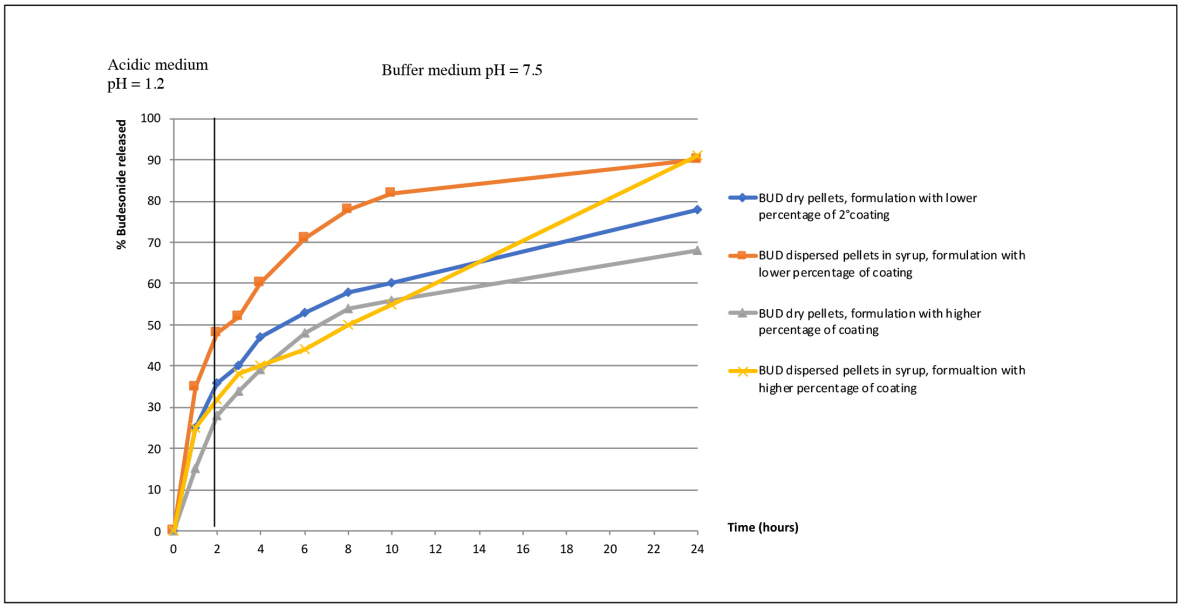
Fig. 4: Influence of the different ratios of insoluble polymers (RS:RL) in the modified-release layer on the dissolution profiles of budesonide, done in a pH 7.5 buffered medium. The test was performed on the dry sustained-release pellets coated up to the second layer, which consisted of 9.5% of total solid, equivalent to 5.85% of insoluble polymer blends (mean values +/- s.d., n=6).

Fig. 5: Dissolution profiles of the dry sustained-release multi-layered pellets before and after dispersion in a conventional syrup after 2 hours in the acidic stage pH 1.2 and 22 hours in a pH 7.5 buffer medium (mean values, n=6). The coated pellets tested were constituted of the same formulation as shown in Table 4 but with two different percentages of 2nd coating layer: 7.1% and 9.5% of total solids, equivalent to 3.4% and to 5.8% of polymer blends using an RS/RL ratio of 30:70.

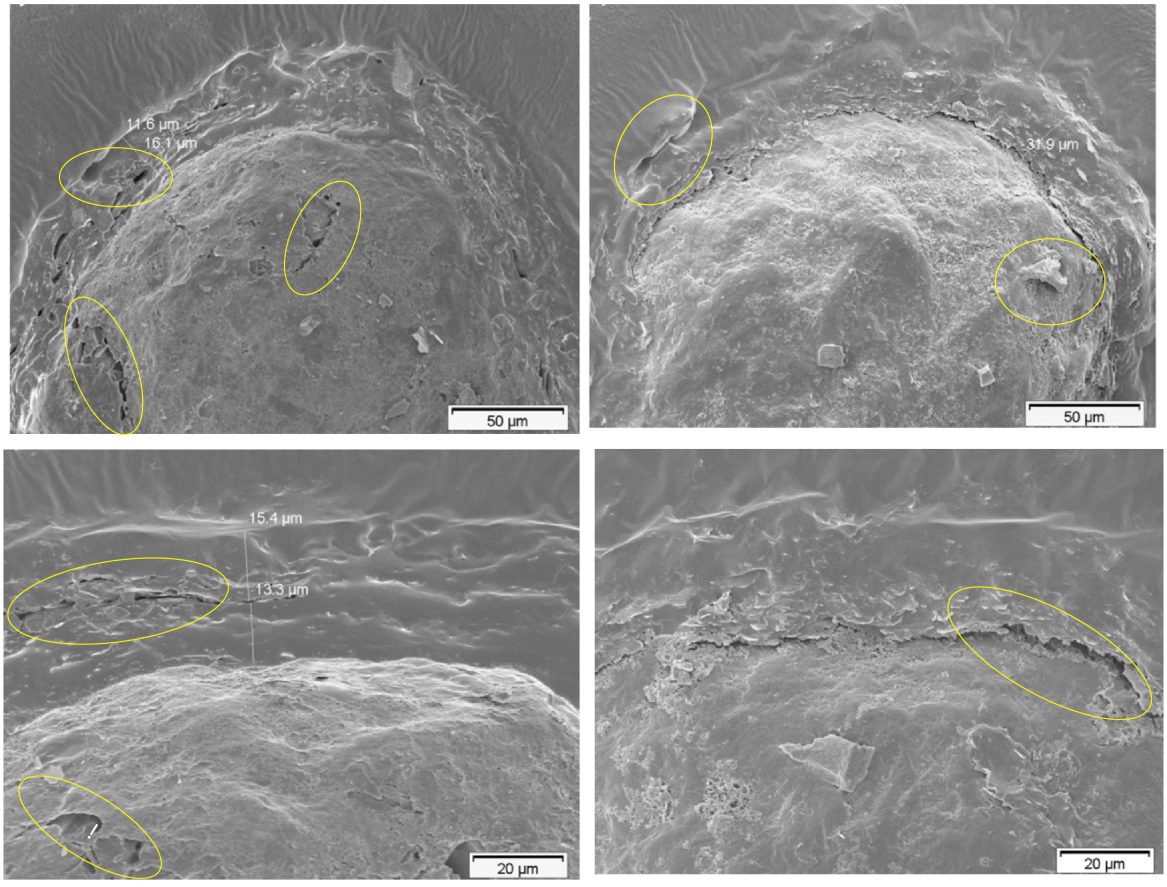
Neutral pellets (Cellets® 263)	Budesonide colon-targeted formulation	Budesonide sustained-release formulation
		
		
		






Acidic medium
pH = 1.2
Buffer medium pH = 7.5
% Budesonide released
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (hours)
BUD dry pellets, formulation with lower percentage of 2°coating
BUD dispersed pellets in syrup, formulation with lower percentage of coating
BUD dry pellets, formulation with higher percentage of coating
BUD dispersed pellets in syrup, formualtion with higher percentage of coating

Budesonide colon-targeted formulation	Budesonide sustained-release formulation



Declaration of interests

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

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

This work was financially supported by BePharBel Manufacturing. The sponsor of this study approved the study design but it was not involved in the interpretation
of the data or in the writing of the article. The sponsor has read and approved the content of this article before its submission.