https://doi.org/10.31925/farmacia.2025.1.6 **ORIGINAL ARTICLE**

DESIGN AND EVALUATION OF A DUAL-COATED CAPSULE FOR CONCOMITANT COLON DELIVERY OF LIVING BACTERIA AND SMALL MOLECULE MODEL COMPOUNDS

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Manuscript received: October 2024

Abstract

Live biotherapeutic products, alone or in combination with other medicines, are emerging as promising and novel therapeutic modalities aimed to prevent and treat various types of health conditions and meet the unmet treatment needs. The colon represents one of the major sites of action of living bacteria when applied orally and thus designing a robust and precise delivery system is of crucial importance. Within this research a colon targeting formulation platform has been developed for concomitant delivery of a model living bacteria and model small molecule substance. Dual coating of capsules based on inner dextrin/pH dependent polymer layer enabling precise colon delivery and outer layer based on pH dependent polymer enabling acid-protection and minimal release in the gastric segment has been designed. Variations in the amount of polymer/dextrin in the inner layer tended to affect product performance - disintegration, and dissolution while applying the outer layer provided extensive strain protection and prevention of premature drug release. The formulation containing dextrin:polymer ratio of 20%:80% in the inner coating layer tended to provide the most optimal product performance and proved that the applied approach of dual capsule coating triggering pH- and enzymatic-dependent release is viable manner to precisely target the colon.

Rezumat

Produsele bioterapeutice vii, singure sau în combinație cu alte medicamente, apar ca modalități terapeutice promițătoare și noi, menite să prevină și să trateze diferite tipuri de afecțiuni și să răspundă nevoilor de tratament actuale. Colonul reprezintă unul dintre locurile principale de acțiune a bacteriilor vii atunci când se folosește calea orală și, astfel, proiectarea unui sistem de cedare robust și precis este de o importanță crucială. În cadrul acestei cercetări a fost dezvoltată o formulare pentru cedare concomitentă la nivelul colonului a unei bacterii vii model și a unei substanțe model cu moleculă mică. Acoperirea dublă a capsulelor a inclus un strat interior de dextrină/polimer care permite livrarea precisă în colon dependent de pH și stratul exterior bazat pe polimer, pH dependent, care permite protecția față de acțiunea acidului clorhidric și eliberarea minimă în segmentul gastric. Variațiile cantității de polimer/dextrină din stratul interior au avut tendința de a afecta performanța produsului - dezintegrarea și dizolvarea, în timp ce stratul exterior a asigurat o protecție extinsă la deformare și prevenirea eliberării premature a API. Formularea care conține dextrină:polimer în raport de 20%:80% în stratul de acoperire interior a avut tendința de a oferi cea mai bună performanță a produsului și a demonstrat că utilizarea stratului dublu este o modalitate viabilă de a viza cu precizie colonul.

Keywords: colon-targeting, live-biotherapeutic products, coating, capsules

Introduction

Live biotherapeutic products (LBPs) are an innovative class of biologics composed of live microorganisms, such as bacteria, yeasts or their components, designed to treat, cure, or prevent various type of conditions [1-3]. Unlike the classical probiotics, LBPs aim to affect and modulate specific disease pathways, addressing conditions linked to dysbiosis in the human microbiome, such as certain infections, gastrointestinal inflammatory states (inflammatory bowel disease, irritable bowel syndrome) metabolic disorders (diabetes, obesity), neurological conditions (including autism and depression), and even certain cancers [4]. This therapeutic approach aims to restore or enhance health by positively tailoring microbiome composition and activity [1]. Combining live bacteria with small molecules may further enhance therapeutic outcomes, providing synergistic effects, minimizing side effects, and simplifying treatment regimens for various conditions and in that regard meet the unmet treatment needs [5-8]. The colon is considered as one of the key sites of action for LBPs due to its diverse microbial population and its vital role in various physiological processes [9, 10]. Targeting LBPs to the colon maximizes the therapeutic efficacy by modulating beneficial interactions

of the applied living organisms with the local microbiota while avoiding the harsh conditions of the upper gastrointestinal tract, such as acidic pH in the stomach and digestive enzymes in the small intestine [11, 12]. However, achieving precise colon targeting requires navigating multiple challenges, such as stomach acidity, presences of enzymes and bile salts, fluctuating gastro-intestinal transit times and juices composition, peristaltic movements etc [13, 14].

To address these challenges, various advanced colon-targeting formulation approaches have been developed. pH-sensitive systems use polymers that remain un-dissolved in stomach and proximal small intestine but dissolve at the higher pH of the distal small intestine and colon, protecting and preventing release of the active ingredient while transiting and deliver it to the colon. However, inter- and intra-subject variations in the gastro-intestinal pH values can affect their release precision and thus effectiveness [15-17]. Time-dependent systems are based on various hydrophilic and hydro-phobic polymers whose dissolving and/or eroding enable release after predefined lag time corresponding to the gastro-intestinal transit time up to the colon. However, as in case of the pH values variations in individual transit times and rates may impact the accuracy of these systems targeting the large intestine [16, 18-21]. Microbial-triggered systems rely on utilization of certain substrates (mainly polysaccharides) metabolised by colonic bacteria to trigger drug release, enhancing local delivery at this site of action. Despite using highly specific substrates, variations in microbiota may affect release consistency within and between different subjects [22-24]. Combined colon-targeting systems improve precision and reduce variability by integrating multiple delivery approaches within a single formulation. These hybrid systems can use pH-sensitive polymers addressing the pH values of the gastro-intestinal juices, time-dependent release to account for the transit times, and microbial substrates for enzymatic degradation by the colon microbiota. This multi-faceted approach helps mitigate the variations in the gastro-intestinal pH, transit time, and microbiota composition, enhancing colon targeting and therapeutic performance and outcome [15, 25, 26].

This research aims to develop a dual-coated, colon-targeted capsule formulation containing model live bacteria and a small molecule drug. The first coating layer combines a pH-sensitive polymer (Eudragit FS30D) and microbial-degradable dextrin (Nutriose FB06) for targeted colon release. The second layer, made from another pH dependent polymer (Eudragit L30D55), protects against gastric acidity and premature release, ensuring bacterial viability and effective colon delivery. This study systematically evaluates the effects of coating formulation and processing on capsule performance, with final goal effective and precise delivery of the two model compounds in the large intestine.

Materials and Methods

Materials

Bifidobacterium spp. (BS) based drug substance was produced in Bacthera A/S, Denmark. Tartrazin was produced by Arcos Organics and purchased from VWR, Switzerland. Dibasic calcium phosphate anhydrous (Emcompress Anhydrous Coarse Powder) and magnesium stearate (LubriPrez 2) were kindly provided by JRS Pharma, Germany. Sodium hydrogen carbonate was purchased by VWR, Switzerland. HPMC based hard capsule (VCaps plus) was purchased from Capsugel, Lonza, Belgium. Wheat dextrin (Nutriose FB06) was kindly provided by Roquette, France. Polymetacrylates (Eudragit L30 D55 and Eudragit FS30 D), plasticizer mixtures (PlasAcryl T20 and PlasAcryl HTP20) and colloidal silicon dioxide (Aeropearl 300) were kindly donated by Evonik, Germany. Talc was produced by JT Baker and purchased from VWR, Switzerland. Polysorbate 80 (Tween 80) was purchased from Merck, Germany. Glycerlyl monostearate (Imwitor 900 (F) P) was kindly provided from IOI Oleo, Germany. The enzyme mixture of glucoamylase and pullulanase (CANDY WLE3000) has been purchased from White Labs, Denmark. All other reagents were of analytical grade.

Uncoated capsule preparation

The composition of the capsule filling is given in Table I.

Table I

Composition of the capsules filling blend

Component	Amount (%)
***Bifidobacterium spp.* (BS) drug substance**	30.00
Tartrazine	3.00
Dibasic calcium phosphate anhydrous	54.00
Sodium hydrogen carbonate	10.00
Colloidal silicon dioxide	2.00
Magnesium stearate	1.00

All components were weighed separately and dispensed in a HDPE bottle where they were submerged to 1 min manual pre-mixing and subsequently screened through 1.18 mm sieve to break down potential agglomerates. Sieved premix was mixed for 5 min at 49 rpm using a turbular blender (Turbula T2F, WAB, Switzerland). Obtained mixtures were used for capsule filling using a manual device (ProFill 100, Torpac, USA). Filled capsules were primarily assessed for the filling weight as well as for the uniformity of dosage units as *per* Ph. Eur. 11.0, 20940 (04/2017) [27].

Capsule coating

Coating experiments described in this article were conducted using a Labcoat™ Benchtop system from O'Hara Technologies with a perforated pan (diameter: 216 mm). The coater was equipped with a SCHLICK Nano nozzle spraying system featuring a 0.5 mm bore. To create a tailored functional double-layer coating, capsules were coated with two aqueous film-forming

suspensions. The inner coating layer aimed to provide the colon targeting action consisted of dextrin and Eudragit FS30D, while the outer layer aimed to provide the primary acid protection was based on Eudragit L30 D55 as the film-forming polymer. Coating dispersions have been prepared in accordance with the good practices for Eudragit FS30D and Eudragit L30 D55 provided in the Eudragit Application Guidelines [28]. A water-based dispersion of dextrin and talc have been mixed with the Eudragit FS30D dispersion in order to obtain the dispersion for the inner colon targeting layer. Three different coating experiments were conducted by varying the ratio of dextrin to Eudragit FS30D in the inner layer (50:50, 35:65 and 20:80), while keeping the outer layer constant. All experiments aimed to achieve a target inner layer of 80 mg/capsule of dry polymer/dextrin and a target outer layer of 100 mg/capsule of dry polymer. The detailed formulations for each coating layer are provided in Table II.

Table II

Formulation composition of the inner and outer coating layers

Dextrin:Eudragit ratio		50:50	35:65	20:80
Layer	**Component**	**Dry amount (mg/capsule)**		
Inner	Eudragit FS30D	40.00	52.00	64.00
	Nutriose FB06	40.00	28.00	16.00
	PlasAcryl T20	12.00	12.00	12.00
	Talc	14.00	9.80	5.60
	Tween 80	8.00	8.00	8.00
	GMS	4.00	4.00	4.00
Outer	Eudragit L 30 D55	100.00		
	PlasAcryl HTP20	17.00		

The film coating process parameters were adjusted to fit the properties of the used polymers and also to provide minimal thermal and mechanical effect on the capsules. The main parameter values are summarised in Table III.

Table III

Process parameter values for coating processes

Step	**Parameters**								
	Supply temp (°C)	**Product temp (°C)**	**Exhaust temp (°C)**	**Air flow (m^3/h)**	**Pan differential pressure (mbar)**	**Pan speed (rpm)**	**Solution flow rate (g/min)**	**Atomizing pressure (bar)**	**Pattern pressure (bar)**
Heating	50*	r.t. - 39	r.t. - 39	100*	-0.25*	5	n/a	n/a	n/a
Coating (inner layer)	37 - 40	31 - 36	34 - 37	120*	-0.25*	20	1.0 - 1.5	1.20*	1.20*
Coating (outer layer)	40 - 45	32 - 38	35 - 39	120*	-0.25*	20	3.0	1.20*	1.20*
Drying	35*	34 - 38	34 - 38	85*	-0.25*	5 - 20	n/a	n/a	n/a

*: set point values (actual values varied slightly around set point values); r.t.: room temperature; n/a: not applicable

Media uptake

The media uptake was assessed by measuring the weight and the moisture of the content of coated capsules as well as visually before and after immersion in 0.1 M HCl (pH 1.2) for 2 hours in a standard disintegration set up and apparatus (DisiTest 50, SOTAX AG, Aesch, Switzerland). Capsule weight was assessed through a precise balance (XPR226CDR, Mettler Toledo, GmbH, Greifensee, Switzerland) while the moisture content was measured through the Loss on Drying Method (LOD) using halogen oven coupled with a precise balance (HX204, Mettler Toledo, GmbH, Greifensee, Switzerland). The media uptake test was performed on 6 coated capsules.

Biorelevant disintegration and dissolution tests

In order to reproduce the passage and faith (capsule integrity, drug release and strain viability) of the product through the gastrointestinal tract under fasting conditions, a biorelevant disintegration and dissolution tests were established taking into consideration the different pH values and residence times in the different segments of the GIT as described in the literature [29]. This concept has been equally applied for assessing the faith of the capsule integrity, BS and tartrazine and its general outline is given in Table IV. Yet some alterations in the set-up of the test and sample handling had to be applied to fit the specificity of the disintegration, bacterial strain viability and tartrazine release respectively.

Table IV

Biorelevant disintegration and dissolution tests conditions

Step	**Simulated GUT segment**	**Media**	**Residence time**
1	**Stomach**	0.1 M HCl pH 1.2	2 h
2	**Duodenum**	Phosphate buffer solution pH 5.5	15 min
3	**Jejunum**	Phosphate buffer solution pH 6.8	1 h
4	**Ileum**	Buffer solution pH 7.4	1 h and 30 min
5	**Colon**	Buffer solution pH 6.6 without/with 0.5 g of enzymes	Until decayed - for the disintegration; Until dissolved – for BI; 4 h for tartrazine (sampling points: 1, 2, 3 and 4 h)

Biorelevant disintegration

The biorelevant disintegration has been conducted in an accordance with previous published data [30-32] through the test B designated for units larger than 18 mm following the set up described in Table IV and without usage of discs (DisiTest 50, SOTAX AG, Aesch, Switzerland). 720 mL of medium has been prepared and tempered at 37°C prior each disintegration step was started. Capsules were immersed in the media and the test has been monitored through parameters such as visual appearance of capsules, defect formation as well as media coloration. The end point has been described as state in which the capsules completely opened, and the filling has been completely exposed to the media.

Biorelevant dissolution – tartrazine

The biorelevant dissolution set-up shown in Table IV has also been used to investigate the release of tartrazine as a model of a small molecule drug substance. The dissolution tests were performed in USP II dissolution apparatus (SOTAX AT XtendTM, SOTAX AG, Aesch, Switzerland). Capsules were maintained on the bottom of the dissolution vessel with sinkers. Each vessel was filled with 500 mL media pre-heated in a water bath at 37°C. The biorelevant dissolution tests was conducted at 37°C, with paddle speed of 100 rpm. At each time point sample of 20 mL is withdrawn from the media filtered through 0,41 µm pore size polyethersulfone membrane, (Millipore Stericup; Merck, Germany). Filtered samples were analysed to determine the concentration of tartrazine using a UV-vis spectroscopy (UV7, Mettler Toledo, GmbH, Greifensee, Switzerland) at wavelength of 425 nm. The UV-VIS method was adapted according Grzelj, 2011 [33].

Biorelevant dissolution - Bifidobacterium spp. (BS)

After preparation, the relevant media were sterile filtered (0,22 µm pore size polyethersulfone membrane, Millipore Stericup; Merck, Germany). 50 mL of each medium was aliquot into 50 mL Falcon tubes. During the experiment, the capsules were incubated in each medium at 37°C in a shaking incubator (Thermo-Fisher Scientific MaxQ 6000, 50 rpm) to mimic the peristaltic movements in the digestive system. For each formulation, capsules were tested in accordance with the set up outlined in Table IV with manual transfer from one medium to the consecutive one.

At the end of step 1, 2, 3 and 4, release of BI was evaluated by quantifying total cell count (TCC) using flow cytometer (NovoCyte Advanteon coupled with the NovoSampler Q for 96-well microtiter plates, (Agilent technologies, USA). The instrument is equipped with 488 nm excitation laser, and both a 530/30 nm and a 725/40 nm band pass. Light scatter was measured by forward side scatter (FSC) and side scatter (SSC). For the assay, the media were centrifuged for 15 min at 4600 rpm (Sigma 3-18KS, Sigma Laborzentrifugen GmbH, Germany). Supernatant was discarded and pellet was resuspended in sterile-filtered anaerobic phosphate saline buffer. The permeant nucleic acid dye SYBR green I (stock 10000X, ThermoFischer, USA) was applied for total cells count assay [34].

Impact of the transition through the different media on the bacterial membrane integrity was assessed as intact cells count (ICC) by flow cytometry on dissolved capsules (end of step 1 and step 5). Intact cells count was quantified by applying a double-staining procedure where bacterial cells were stained with both SYBR green I and propidium iodide (PI, 1 mg/mL in water, Sigma-Aldrich, Germany). The former dye penetrates all the cells and binds to the DNA, the latter is a membrane impermeant dye which can only penetrate cells that have lost membrane integrity, binding to their DNA. The double staining procedure allows to discriminate bacterial cells with intact cell membranes from non-intact cells [35]. The staining protocol was based on ISO method 19344:2015, protocol B with adaptations [36].

For each capsule formulation, viability control was included to assess the initial potency of BS in the capsule (ICC/cap).

All data were acquired as two-parameters density plots and processed using the NovoExpress software.

Results and Discussion

Capsule properties

The uncoated capsule properties are shown in Table V. From the data one could conclude that the proposed formulation together with the process are suitable in terms of obtaining robust and uniform product.

Table V

Uncoated capsules average weight, weight variability and acceptance value as parameter describing the uniformity of dosage units

Average weight of uncoated capsule (mg)	Average weight of capsule shell (mg)	Average weight of capsule filling (mg)	RSD (%)	Acceptance value
523	98	425	2.23	5.3

The film coating process was governed in a suitable way for both inner and outer layer. The thickness of the coating layer deposited on the capsules was determined by comparing the average weights of uncoated and coated capsules. The achieved mean weights of the inner and outer coating layers are presented in Table VI, along with the difference in loss on drying (LOD) of the capsule contents before and after the coating process. These results indicate that the moisture uptake of the capsule contents during the coating process was

low, suggesting that the process parameters are well-suited for film coating applications involving moisture sensitive and hygroscopic drug substances such as ones containing living bacteria and/or being processed by lyophilization. Additionally, the capsules have been analysed for the intact cell count and compared to the corresponding amount of pure drug substance. Results demonstrated minimal drop in the ICC inside the tested formulation in comparison with the pure DS. This suggest that both the formulation as well as the coating process fit the purpose in retaining the appropriate level of intact cells within the product.

Table VI

Average coating layers weights gain, LOD difference and intact cells count change *per* formulation (before/after coating processes)

Dextrin:Eudragit ratio	Weight gain (mg) – inner layer	Weight gain (mg) – outer layer	Weight gain (mg) – overall layer	LOD difference (%) – overall process	ICC (cells/128 mg DS)	ICC (cells/cps)	Δ ICC DS *vs.* Cps
50:50	125 (24%)	114 (17%)	240 (46%)	0.42	2.39E+10	1.46E+10	0.21
35:65	117 (22%)	136 (21%)	252 (48%)	0.57		8.28E+09	0.46
20:80	110 (21%)	149 (23%)	259 (49%)	1.13		5.87E+10	0

Capsule resistance towards acid intrusion

The media uptake test was conducted in order to evaluate the effectiveness of the double coating layer in protecting the content of the capsules towards the intrusion of the acidic media simulating the gastric juice in fasted state. Results in the Table VII have demonstrated that all three formulations provided adequate protection towards the intrusion of the 0.1 M HCl with the formulation containing highest portion of Eudragit FS30D showing lowest weight gain and lowest LOD difference. This trend could be explained with the fact that the coating formulation containing highest portion of Eudragit FS30D and lowest of dextrin is the one with most dominant acid-resistant and hydrophobic nature which enables establishing of most resistant barrier towards the media.

The effectiveness of the double coating layer in protecting the bacterial strain from the harmful effect of the 0.1 M HCl has been also assessed through the cell counting method as described in the materials and methods chapter for the biorelevant dissolution of BS. Results in Table VIII demonstrate that all three coating formulations enable minimal (and similar) loss in the strain viability. This data fits well with the results for the weight gain and LOD describing the tested coating formulations as highly suitable to resist media intrusion and thus protect the content form over-wetting and loss in viability (when bacterial strains are present).

Table VII

Acid intrusion values expressed as weight gain and difference in the LOD

Dextrin:Eudragit ratio	Weight gain (mg)	LOD difference (%)
50:50	28 (4%)	1.30
35:65	28 (4%)	1.77
20:80	25 (3%)	0.23

Table VIII

Strain viability after exposure to 0.1M HCl for 2 h

Dextrin:Eudragit ratio	ICC prior acid exposure		ICC after acid exposure		Δ pre-after acid exposure
	ICC/cap	STDV	ICC/cap	STDV	
50:50	5.87E+10	5.99E+09	3.61E+10	1.66E+09	0.21
35:65	1.46E+10	4.58E+08	7.23E+09	2.29E+08	0.31
20:80	8.28E+09	2.21E+08	4.72E+09	4.14E+07	0.24

Biorelevant disintegration testing outcomes

The outcome of the biorelevant disintegration testing is shown in Table IX. As one could see the results are suggesting that all three formulations provided adequate performance without opening, major deformation and material release in the simulated gastric, duodenal and jejunal segment. The turbidity of the media in the jejunal part could be connected to the extended dissolution of the outer coating layer which is mainly based on the Eudragit L30 D55. As for the ileal segment all three formulations failed to retain their integrity and intactness and reached out their end point. A trend is seen in the disintegration pattern in the simulated ileal segment with the formulation containing 50% dextrin in the inner coating layer providing fastest disintegration followed by the formulation containing 35% and 20% dextrin respectively. This trend could be connected with the higher hydrophilicity of the inner coating layer when the portion of the water-soluble dextrin is higher. This leads to faster pore formation and increase in membrane tortuosity followed by membrane collapse especially under more pronounced mechanical motion such as seen during disintegration testing. The usage of the conventional disintegration apparatus in simulating biorelevant conditions in general is not considered as optimal approach due to the continuous frequent occurrence of high hydrodynamic forces. Still in the context of the current research work its application pointed the high robustness and resistance of the tested formulations

when exposed to the harsh disintegration testing up to the jejunal segment. As for the behaviour of the formulations in the ileal and colonic segment it is the dissolution testing giving more accurate insights of the formulation performance compared to the disintegration itself.

Table IX

Outcome of the biorelevant disintegration tests

Simulated gastro-intestinal part	Dextrin:Eudragit ratio		
	50:50	35:65	20:80
Stomach	• No opening • Swollen body-cap junction • Clear medium	• No opening • Swollen body-cap junction • Clear medium	• No opening • Swollen body-cap junction • Clear medium
Duodenum	• No opening • Swollen body-cap junction • Clear medium	• No opening • Swollen body-cap junction • Clear medium	• No opening • Swollen body-cap junction • Clear medium
Jejunum	• No opening • Swollen body-cap junction • Slightly turbid medium	• No opening • Swollen body-cap junction • Slightly turbid medium	• No opening • Swollen body-cap junction • Slightly turbid medium
Ileum	• Complete capsule opening and content exposed to media at 22 min.	• Complete capsule opening and content exposed to media at 42 min.	• Complete capsule opening and content exposed to media at 54 min.
Colon	• Not performed	• Not performed	• Not performed

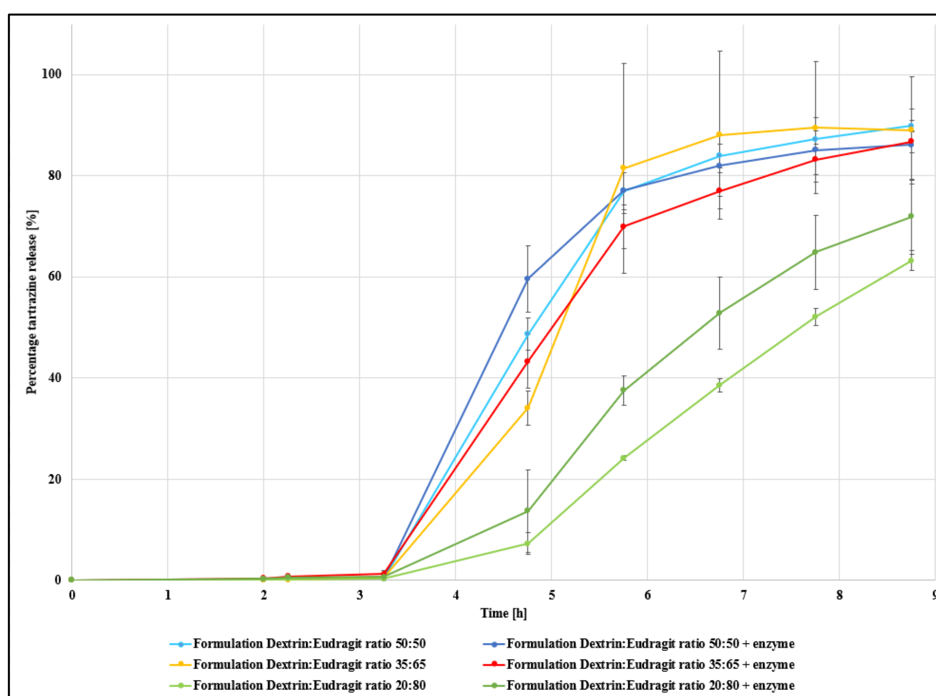

Figure 1.

Tartrazine release as result of the different dextrin: Eudragit FS30D inner layer ratio and presence/absence of enzymes

Biorelevant dissolution testing outcome - Tartrazine release

The release of tartrazine from the tested formulation has been evaluated from several aspects and the outcome of the dissolution tests are given in Figure 1. All formulation being under consideration in the scope of the work showed tendency not to release the active compound within the time and conditions simulating the stomach, duodenum and jejunum (up to 3 h and 15 min). These results confirm the selected approach of double coating and the chosen materials to fit their purposes. Namely the outer layer of the dual membrane which is composed on Eudragit L30 D55 provides the adequate delay in the release of the tartrazine due to its minimal/borderline solubility in the simulated stomach and duodenal conditions respectively. As for the jejunal segment despite the fact that the afore-mentioned polymer is soluble in the phosphate buffer pH 6.8 (through ionization) it is the dissolution rate of it which is driving the possible release of tartrazine within the media. Applying the outer layer in amounts of solid content approximately 120 mg (corresponding

to 100 mg dry polymer) provides robust and thick barrier whose dissolving is lasting longer than the standard acid-resistant coatings and thus prevent any release of the tartrazine in the jejunal-simulating media.

The inner layer of the coating barrier is designed to target the colon through two mechanisms - pH dependent solubility of the polymer Eudragit FS30D and enzymatic degradation of the dextrin. From the results shown in Figure 1 one could notice that by varying the amount of the polymer and the dextrin a variation in the dissolution profile is present. Namely increasing the amount of the dextrin and decreasing the amount of the pH dependent polymer leads into faster release of the tartrazine within the simulated ileal and colonic environment. This phenomenon is also in accordance with the observations in the disintegration patterns and could be related to the water-soluble nature of the dextrin whose increased presence leads into faster pore formation, disturbance of the membrane integrity and consequently faster liberation of the model drug substance. From the obtained results it could be seen that it is the formulation containing 80% of Eudragit FS30 and 20% of dextrin which provided lowest drug release up until the colonic segment suggesting that it is this combination providing optimal outcome for the targeting purpose in this part of the gastro-intestinal tract.

The concept of promoting more robust and reproducible drug release in the colon through enzymatic degradation of a polysaccharide incorporated in the film coating formulation has also been evaluated in the current work. Introduction of the enzyme mixture of glucoamylase and pullulanase in the dissolution medium simulating the colonic juice tended to give faster drug release in the formulation containing 80% of Eudragit FS30D and 20% of dextrin compared to the case when the formulation was tested in a dissolution set up without enzymes in the medium. These results suggest that the designed system is also enzyme-sensitive and that in this regards it could be applied as a more robust solution for colon targeting avoiding the drawbacks of formulations based exclusively on pH- or time-dependent polymers emerging from the high variability of the gastro-intestinal juice composition and transit times.

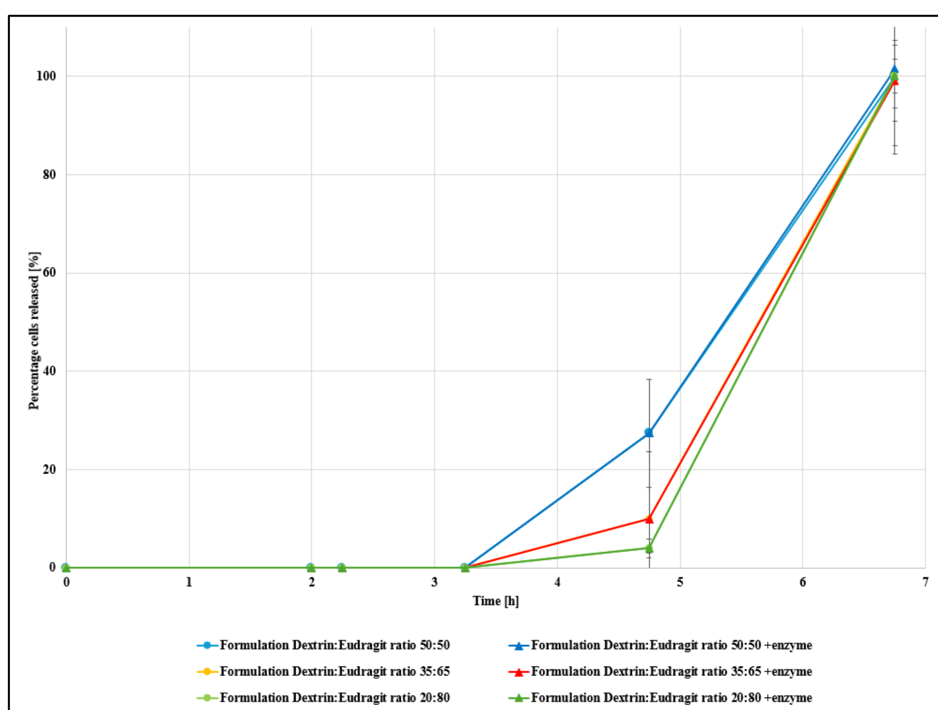

Figure 2.

BS release as result of the different dextrin: Eudragit inner layer ratio and presence/absence of enzymes

Biorelevant dissolution testing outcome - BS release and cell membrane integrity

Total cells count (TCC/mL) was used to monitor the release of BI during the transition in the different media simulating the different compartments of the gastro-intestinal tract. Despite the differences in the inner layer composition all three capsule formulations provided events rate which was below the optimal operational range (< 500 events/µL) up to the jejunal segment (Figure 2). Values in this operational range indicate that minimal/negligible strain release from the capsules to the outer environment/media occurred. Higher levels of strain release have been observed in the segment simulating the ileum, which is also in accordance with the dissolution results obtained for the tartrazine. Yet the level of strain released in this

segment is lower compared to the level of colour release for the same type of formulation. This might be connected to the fact that tartrazine is readily soluble in water-based media and thus when in a form of molecular solution, it could easily transit membranes whose pore size is even in nano scale. On the other hand, the bacterial cells are not prone to classical dissolving and are usually transiting membranes in their actual size (for the current strain usually around 2 - 5 µm). This might lead to conclusion that it is the pore size formed in the coating layer during dissolution that dictates the number of cells able to be detected in the media. As for the influence of the inner coating layer composition on the release of the bacterial strain same pattern has been observed as for the tartrazine. Increasing the portion of dextrin to 50% led into highest release in the ileal segment with approx. 27% of cells being detected in the media. The formulations containing 35% and 20% of dextrin in the inner layer provided strain release of 10% and 3%, respectively and thus could be seen as viable option in delivering bacterial based drug substance in the colon. No difference in the dissolution test outcome has been seen when enzyme has been added to the media compared to the tests when it was not used. Still due to the experimental setup total cell count has been performed only when the capsule completely dissolved which hinders any particular effect of the enzymatic presence on the dissolution rateApart for the total cell count measured to assess the dissolution rate, at the test end point samples were evaluated also for the intact cell count. No differences in viability (cell membrane integrity) were observed between the capsules treated with and without the addition of the enzyme mixture, as summarised in Table X. Compared to the results of the intact cell enumeration after capsules were exposed to acid (Table VIII) only slight drop in the viability could be detected which suggest that the all three tested formulations provided delivery of adequate amount of viable cell in the colon region.

Table X

Intact cell counts (ICC/cap) with corresponsive standard deviation measured on dissolved capsules in buffer pH 6.6 with and without addition of enzyme

Dextrin:Eudragit	Buffer sol. pH 6.6 - enzyme		Buffer sol. pH 6.6 + enzyme	
	ICC/cap	STDV	ICC/cap	STDV
50:50	3.56E+09	1.02E+09	3.96E+09	2.45E+09
35:65	3.93E+09	1.30E+08	3.17E+09	1.19E+09
20:80	4.04E+09	6.37E+08	3.77E+09	2.48E+08

Conclusions

A novel colon-targeting formulation platform based on dual coating of inert hard capsules was developed for concomitant delivery of living bacteria and small molecule-based drug substances. Application of an inner coating layer based on a pH sensitive polymer (Eudragit FS30D) and a dextrin (Nutriose FB06) enabled adequate delivery of both model drug substances in the simulated colon segment through pH dependent solubility and enzymatic degradation respectively. Variation in the ratio of the main film forming components in the inner coating layer led into variation of in the disintegration and dissolution pattern, with coating composition containing 20% dextrin and 80% pH sensitive polymer providing best fit for purpose – colon targeting of both model compounds. Deposition of an outer coating layer based on pH sensitive polymer (Eudragit L30 D55) enabled transit of the capsules to the simulated ileal segment in an intact form which consequently prevented premature drug release as well as loss in strain viability in the harsh acidic environment due to media intrusion. The proposed formulation platform represents a viable option for more robust and precise colon targeting of living bacteria and/or small molecules minimizing the influence of inter- and intra-subject variability in gastro-intestinal pH values and transit times.

Conflict of interest

The authors declare no conflict of interest.

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